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## ANALYSIS OF THE PHYLOGENETIC SEQUENCE RELATIONSHIPS BETWEEN PLANT ALPHA AMYLASES

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## ABSTRACT

Enzymes are highly valuable in the industry, such industries are food, beverage industry and the biofuel sector. Amylases are very important in starch processing such as liquefaction, hydrolysis and saccharification. Alpha amylase has liquefying ability by hydrolysing the glycosidic bonds at internal positions. This paper aims to present a brief overview of the sequence comparison between plant alpha amylases. The protein sequences were obtained from the NCBI, aligned using the clustal W tool and the phylogenetic trees were constructed using the Mega 7.0 tool. The proteins can be classified into three distinct families; one, two and three. Each family has some peculiar features, family one amylases are proteins of about 450 amino acids long with signal peptides at their N-terminus, family two proteins are similar in size to those of family one but are cytosolic with no targeting peptide. In contrast, family three amylase are proteins targeted to the chloroplast with transit peptides at their N-terminus, however, they are 900 amino acids long. In addition, the last family of protein has an additional domain that is yet to be fully characterized. Across the three families, there is a high homology and thus conservation of the catalytic domain which is at the C-terminus. This implied that the proteins are evolutionary related and perform similar functions. The unknown domain in family three amylases is also conserved across members of this family. The domain may be a starch binding domain that is required for the family three amylases to digest storage starch in the plastid.

Key words: Alpha amylases, Cytosol, Plastid, Family one, Unknown domain

### INTRODUCTION

The amylases are a group of proteins also referred to as glycoside hydrolases or hydrolases (E. C. 3.1) The proteins have a wide range of application such as starch conversion and processing; thus occupy more than thirty percent of the enzyme market worldwide. They are used in food and beverage industries, breweries and the biofuel sector (Kirk, *et al.*, 2002). Structurally, hydrolases differ in their sequence of amino acids, secondary and other higher levels of the proteins, thereby creating variability and dynamism in the protein properties and functions (Henrissat, 1991; Henrissat, *et al.*, 2001; Stam, *et al.*, 2006). Typical examples of the above are the reaction

mechanisms, pH and isoelectric points of the amylases (McCarter and Withers, 1994).

Hydrolases can be classified as endoamylases and exoamylases. The endoamylase break a-1,4glycosidic bonds in internal positions of the glycans; a-amylase belongs to this class. It hydrolyses the a-1,6 glycosidic bonds in polysaccharides such as starch, thus referred to as liquefying enzyme (Kirk, *et al.*, 2002)bonds at the external part of the starch molecule (Hehre, *et al.*, 1979; Lao, *et al.*, 1999). In contrast, glucoamylase hydrolyses both a-1,4- and a-1,6glycosidic bonds from the external positions of the starch molecule (Kim and Robyt 1999; Sauer, *et al.*, 2000). Thus, it is referred to as a saccharifying enzyme due to its ability to convert dextrins to simple sugars.

Various researches have focused on the revealing the mode of action of amylases due to their roles in starch processing (Hehre, et al., 1979; McCarter and Withers, 1994). The above in conjugation with the amino acid sequence of the amylases are important in improving the protein functions (Lopez-Casado, et al., 2008; 1999; Richardson, *et al.*, 2002). Reilly Knowledge of the primary structure of the protein is significant in determining protein activity. Elucidating the amino acids at the active site as well as those that affect protein properties such as stability are very paramount (Bessler, et al,. 2003; Cherry and Fidantsef, 2003; Johannes and Zhao 2006; Richardson, et al. 2002). Consequently, the effect of this will facilitate the engineering of the proteins for desirable properties and activities (Eijsink, et al., 2004; Eijsink, et al., 2008; Kelly, et al., 2009).

Amylases have two distinct mechanisms of action; retention and inversion modes. In retention mechanism, the product of hydrolyses retains the a-configuration of starch. Here a single amino acid serves as an acid and a base while another one serves as a nucleophile and a leaving group (McCarter and Withers, 1994). In contrast, the a-configuration of starch is inverted in the second mechanism hence the product has a β-configuration. An amino acid serves as a general acid and another one acts as a base. Examples of amylases that use retention and inversion mechanisms are a-amylase and beta amylase respectively (McCarter and Withers, 1994). Glu186 and Glu380 residues of β-amylase from soy bean act as the general acid and base respectively (Kang, et al., 2004).

Alpha-amylase (1,4-a-D-glucan-4glucanohydrolase, EC 3.2.1.1) belongs to the glucosylhydrolase class 13, the proteins have three domains A, B and C (Kumari, et al., 2010; Kuriki and Imanaka, 1999). The name alpha refers to the configuration at carbon one of the reducing unit of the oligosaccharides generated by the action of the amylase (Chao and Serpe, 2010). It is found in microbes (bacteria and fungi), plants and the archaea; it has has been purified and characterized from В. amyloquefaciens (Demirkan, et al., 2005), apple (Stanley, et al., 2002; Wegrzyn, et al,. 2000), malted finger millet (Nirmala and Muralikrishna 2003), banana (Junior, et al., 2006) and soybean (Kumari, et al., 2010) among others. In plants, a-amylase may be produced and secreted by the aleurone cells (in rice) or scutellum (in maize and sorghum) or both into the starch endosperm (do Nascimento, et al.,

2006; Ranki and Sopanen 1984; Warner and Knutson, 1991).

The protein properties such as stability and activity are affected by certain factors. These are high and low pI forms of alpha amylase found on chromosomes 6 and 1 respectively (Mitsui and Itoh 1997). AMY1 and AMY2 of H. vulgare are low and high pI of 4.9 and 5.9 forms respectively; thus the optimum pH of the aamylases is from 4.5 to 5.5 (Robert, et al., 2003; Tibbot, et al., 2002). The optimum temperature of the amylases is between 40 to 55°C however in mature seed germinated at much lower temperatures therefore thermal stability is not important (Prakash and Jaiswal, 2010). High temperatures above 60°C may lead to inactivation of these proteins with a few exception as observed in the brewing variety of barlev a-amylase which can withstand temperature of 65°C (Prakash and Jaiswal, 2010). Some a-amylases require calcium for their stability, and activity (Tanaka and Hoshino, 2002; Tanaka and Hoshino, 2003).

Glycosylation which is the addition of glycans, a process that takes place in the endoplasmic reticulum (Vitale and Denecke, 1999). It affects protein activity, stability and functions (de Barros, *et al.*, 2009; Motyan, *et al.*, 2011). O-glycosylation involves glycan addition at hydroxyl groups of serine and threonine residues; while in N-glycosylation the addition is on asparagine residues of the sequence Asn-X-Ser/Thr. The role of the glycosylation is not clear *(Motyan, et al.,* 2011; Vitale and Denecke, 1999). This work is aimed at establishing the relationships between plant amylases.

## METHODOLOGY

### Retrieval of a-amylase Sequence

The protein sequences of various plants **a**amylases were obtained from the NCBI databases (<u>http://www.ncbi.nlm.nih.gov/</u>). Amino acids sequences of the following plants aamylases were retrieved: Hv (*H. vulgare*), In (I. nil), Pv (*P. vulgaris*), Gm (G. max), Vm (*V. mungoculata*), Sb (*S. bicolor*), Md (*M. domestica*), Ma (*M. acuminata*), Me (*M. esculanta*), At (*A. thaliana*), Vv (*V. vinifera*), St (*S. tuberasum*), Ot (*O. tauri*), Ac (*A. chinensis*), and Rc (*R. communis*). The sequences were subject to in silico and bioinformatics analysis as described below.

## Alignment of amino sequences of plant aamylases

The alignments of the proteins were performed using publicly available database at EBI. The sequences were aligned using the BLOSUM62 algorithm with the ClustalW alignment tool. The full protein sequences, the C-terminal (domain) sequences only and N-terminal (domain) sequences only in some instances were aligned to determine the homology and relationships between the respective plant amylases

### **Phylogenetic Analysis**

Phylogenetic trees of plant alpha amylases showing the evolutionary relationship between plant a-amylases were constructed using protein sequences obtained from the publicly available data (http://www.ncbi.nlm.nih.gov/). The sequences were aligned using the ClustalW alignment tool. The aligned sequences were assembled into a phylogenetic tree using the boot-strapped neighbor-joining algorithm (Saitou and Nei 1987) and the Jones-Taylor-Thornton amino acid substitution model (Jones, et al., in MEGA 7.0 with 1000 1992) trials (<u>http://www.megasoftware.net/</u>) (Tamura, et al., 2011). Bootstrap values are indicated as percentages of the 1000 trials at their respective node.

#### **RESULTS AND DISCUSSION**

## Classification of Plant α-amylases and Phylogenetic Relationship

In order to review the relationship between plant a-amylases; In silico and bioinformatic tools were used. The protein sequences of the amylases belonging the three families were retrieved from the National Centre for Biotechnology (NCBI) database. In order to understand in greater details, the sequence homology between the amylase, one representative of each family was chosen. The sequences were subjected to multiple alignment using ClustalW at EBI. The alignment is shown in Figure 1, different homologies were observed

between the different families. The family I and II a-amylases are more closely related to each other, but more distantly related to family III a-amylases.

The alignment indicated that certain amylases have longer sequences compared to the others. These sequences are in between the N-terminus and C-terminus of the proteins of about 450 amino acids, hence the need to understand the relationship between the proteins. Members of the three families were used to construct a phylogenetic tree. The dendogram is shown in Figure 2 below, it indicated closer relationship between family one and two amylases compared to family three as was shown by the alignment (Figure 1). Family two α-amylase may likely be the ancentral origin of plant alpha amylases from which other types evolved.

There are various types of classification for the plant a-amylases based on the tissues they are found (Huang, et al., 1992; Mitsui and Itoh 1997). A popular classification for plant aamylases is based on the cellular localisation of the proteins. The amylases are grouped into three distinct families; one, two and three (Janecek 2002; Stanley, et al., 2002). Family one a-amylases contain signal peptides that target the proteins to endoplasmic reticulum. The second family are the cytosolic a-amylases that do not have any targeting peptide (Janecek, 2002; Stanley et al., 2002). Family three amylases have transit peptide and are localised to the plastid (chloroplast) (Stanley, et al. 2005; Stanley, et al., 2002).. The three families differ in the size of proteins, family one and two aamylases are smaller while family three amylases are twice size.

BAJOPA	AS Volume 11 Number 2 December, 2018
Azamy3 Na my2 Azamy2	M 8 T V P I E 8 L L H H 8 H L R D N 8 K I Y R G T R 8 F F I P C 8 L N L P 8 H F T 8 N K L L H 8 I R T 8 V G A 8 8 K H R R 8 V A I R - A
Azamy3 Hva my2 Azamy2	8880TAVVETAQ8DDVIFKENFPVQRIEKAQQKIYVRLKQVKEK - NWEL8VG881PGKWILHWGV8YVG
4zamy3 Hva my2 Azamy2	DTG8EWDQPPEDMRPPG8IAIKDYAIETPLKKL 8EGD8FFEVAINLNLE88WAALNFVLKDEETGAW
Azəmy3 Hvə my2 Azəmy2	YQHKĞRDFKVPLVDDVPDNĞNL GAKKGFGA GQL 8NIPLKÖDE 88AEVKKK8K 888D 8TK ERKÖL
4zamy3 Hva my2 Azamy2	QEFYEEMPISKRVADDN SÜSÜTÂRKCSETSKNIVSIETDLPGDVTVHWĞÜCKNĞÜKKWEIPSEPYPEDTS
Azamys Hva my2 Azamy2	EFKNKALRTKLWRKDDGNGSFGLFBLDGNLEGGEDFYVPFLIT8888LVGTEAT.
Plantalnamy VvamyUD Roamy Atamy3 Hvamy2 Atamy2 Atamy2	IVHEQQAGTEIHDVRN&L&PNN&YPLQNK&&LEA&DPQNIN&LPMKPQGPEELIEAVAYTDEIIKEIRH Q&EGWGK&ERVV&VPTEI&GKTAGENELV&DAAYTDGIINDIRN GKDAEGNEEV&RTAYTDEIINDIRN GKDAEGNEEV&RTAYTDEIIDEIRN GKDAEGNEEV&RTAYTDEIIDEIRN 
Azamys Hva my2 Azamy2	LA ID IH 8H KN QKTNVKE VQEN I LQE I EKLAAEAY 8 I FR 8T T PT F 8E E 8 I LAEAE - KPD I K I 88 GT G 8 GF VIL GII 8A 8 DQT DI GRVI R
4zamy3 Hva my2 Azamy2	EILCQGFNWESHKSÖR - WYLELQEKADELASLÖFT VLWLPPPTESVSPEGYMPKDLYNLN - SRYGTTÖEL QVLFQGFNWESWKHNGGWYNFLMGKVDDIAAAGITHWULPPASQSVAEQGYMPGRLYDLDASKYGNKAQL EV LQAYNWESHKYD WWRNLDGKVPDIAKSGFTSAWLPPPSQSLAPEGYLPQDLYSLN - SAYGSEHLL
4zamy3 Hva my2 4zamy2	KDTVR KFH KVGTRVLGDAVLNH RCAHFKN GNGVWNLFGGR EN WDDRAVVADDP.HFGGRGNK 88G K8LIGALHGKGVKATADIVINH RTAHKDGRGIYCTFEGGTPDARLDWGPHMICRDDR PMADGRGN 80 K8LLRKMKGYKVRAMADIVINH RVGTTRGHGGMYN RYDGI8LPWDEHAMT®CTGGLGN R81G
4zamy3 Hva my2 4zamy2	DNFHAAPNIDH SQUFYKKDIK EWLCWMMEEV GYDGWREDFYRGFWGGYYKDYMDA SRPYFAV GEYWD 8E S ADFGAAPDIDHLNLRVQKELVEWLNWLKADIGFDGWRFDFAKGYSADVAKIYIDR SEP SFAVAEIWT SLA DNFNG VPNMDHTQHFVRKDIIGWLRWLRNTVGFQDFRFDFARGYSANYVKEYIGAAKPLFBVGECWD SCN
4zamy3 Namy2 Azamy2	YT. YG EMDYN QDAHR QR I VDWI NATB. GAF GAF DYTTKGIL HTALQKCE YWRL 8D PKGKPPGY YGWWP 8 YGGDGKPNLN QDQHR QELVNWYD KYGGKGPATTFDTTKGIL NVA VEG - ELWRL 8D DKAPGMIGWWP 8 YN - GHGLDYN QDBHR QR II 8WTDATG QIBAAFDFTTKGIL QEAVKG - QYWRL CDAQGKPPGVMGWWP 8
4zamy3 Hva my2 Azamy2	RAVTFIENHDTG 8 TQ GHWR FPEGKEMQ GY AY ILTHPGT PAV FFDHIF 8 DYHPEIAALL 8 LRNR GKLH KAVTFVDNHD TG 8 TQ HMWP FP 8 DRVMQ GY AY ILTHPGT PC IFY DHFFDWG LKEEID RLV 8 VRTRHGIH RAVTFLDNHD TG 8 TQ AHWP FP 8 HHVMEGY AY ILTHPG IPC VFY DHFY DWG 8 8 IHDQ IVKLTD IR RR GD IH
Aramy3	CR SEVNIDK BERDVYAAIIDDKVAMKIGPGHYEPPNG SKNWSVAVEGRDYKVWETS

Azamy3	CRSEVN	IDKSERDVYAAII	IDDKVAMKIGPGHYEPPNG	SKNWSVAVEGRDYKVWETS
Namy2	NE 8 KLQ	ILEADADLYLAEI	DGKVIVKLGPRYDVGNLI	POGEKVAAHONDYAVWEKI

**Figure 1** An alignment of representative of the three families. Protein sequences were obtained using publicly available data (http://www.ncbi.nlm.nih.gov/), aligned using the BLOSUM62 algorithm with the ClustalW alignment tool. Family I is represented by Hvamy2, family II represented by Atamy2 and family three represented by *Atamy3.* Grey = conserved residues.

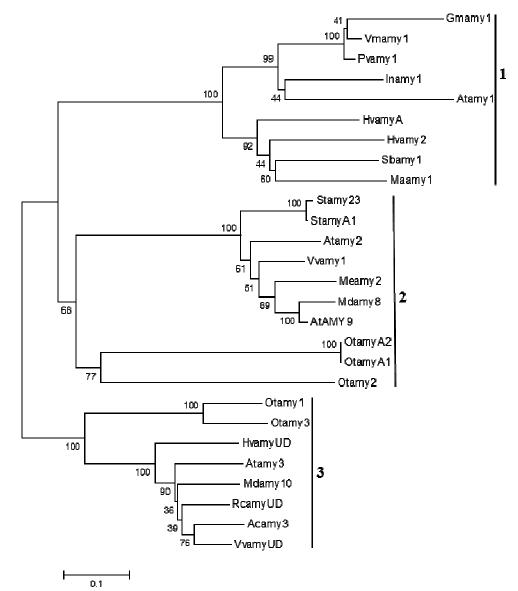


Figure 2. Phylogenetic tree of plant alpha amylases showing the evolutionary relationship between plant q-amylases. Protein sequences were obtained usina publicly available data (http://www.ncbi.nlm.nih.gov/), aligned using the ClustalW alignment tool. The aligned sequences were assembled into a phylogenetic tree using the boot-strapped neighbor-joining algorithm (Saitou and Nei 1987) and the Jones-Taylor-Thornton amino acid substitution model (Jones, et al. 1992) in MEGA 7.0 with 1000 trials (http://www.megasoftware.net/) (Tamura, et al. 2011). Bootstrap values are indicated as percentages of the 1000 trials at their respective node. Abbreviatios: Hv (H. vulgare), In (I. nil), Pv (P. vulgaris), Gm (G. max), Vm (V. mungoculata), Sb (S. bicolor), Md (M. domestica), Ma (M. acuminata), Me (M. esculanta), At (A. thaliana), Vv (V. vinifera), St (S. tuberasum), Ot (O. taurii), Ac (A. chinensis), Rc and (R. communis). 1, 2 and 3 represents family one, two and three respectively.

# Establishing Sequence Homology between Family One and Two Amylase

Following the alignment shown in Fig. 1 and the dendogram in Fig. 2, a close relationship between family one and two amylases was

observed. To analyse and confirm the homology between the proteins; amylases from the two families were aligned using ClustalW and is shown in Figure 3.

BAJOPAS	Volume 11 Number 2 December, 2018
H. vulgare V. mungoculata M. acuminata G. max I. nil P. vulgaris S. bicolor	MANKHLSLSLFLVLLGLSASLASGQVLFQGFNWESWKHNGGWYNFLMGKVDDIAAAGITHVWLPPASQSVAEQGYMPG
H. vulgare V. mungoculata M. acuminata G. max I. nil P. vulgaris S. bicolor	RLYDLDASKYGNKAQLKSLIGALHGKGVKAIADIVINHRTÄEHKDGRGIYCIFEGGTPDARLDWGPHMICRDDRPYADGT RLYDLDASKYGSKNELKSLIAAFHEKGIKCLADIVINHRTAERKDGRGIYCIFEGGTPDSRQDWGPSFICRDDTAYSDGT RLYDLGASKYGNQDELKALIGAFHDKGVKCVADIVINHRTAERKDGRGIWCIFEGGTDDARLDWGPHMICRDDTQYSDGT RLYDLDASKYGTKDQLKSLIAAFHDKGIKCLADIVINHRTAERKDGRGIYCIFEGGTPDARLDWGPSFICKDDNTYSDGT RLYDLDASKYGNKQQLQALVAALHDKGIKCLADIVINHRTAERKDGRGIYCIFEGGTPDARLDWGPGLICKDDNTYSDGT RLYDLDASKYGNKQLALVAALHDKGIKCLADIVINHRTAERKDGRGIYCIFEGGTPDARLDWGPSFICKDDTYSDGT RLYDLDASKYGNKQLALVAALHFKGIKCLADIVINHRCADYKDSRGIYCIFEGGTPDARLDWGPSFICKDDTYSDGT RLYDLDASKYGTHAELKSLIAAFHEKGIKCLADIVINHRCADYKDSRGIYCIFEGGTPDSRLDWGPSFICKDDTYSDGT
H. vulgare V. mungoculata M. acuminata G. max I. nil P. vulgaris S. bicolor	GNPDTGADFGAAPDIDHLNLRVOKELVEWLNWLKADIGFDGWRFDFAKGYSADVAKIYIDRSEPSFAVAEIWTSLAYGGD GNNDSGEGYDAAPDIDHLNPQVQRELSEWMNWLKTEIGFDGWRFDFVKGYAPSISKIYMEQTKPDFAVGEKWDSISYGQD GNLDTGEGFAAAPDIDHLNTQVOHELTDWLNWLKTDIGFDGWRLDFAKGYSSSIAKIYVEOTOPNFVVAEIWSSLAYRND GNLDSGEPYDPAPDIDHLNPQVQRELSEWMNWLKTEIGFDGWRFDYVKGYAPSITKIYMEQTRPDFAVGEKWDSLSI GNADTGMDFGGAPDIDHLNPQVQRELSEWMNWLKTEIGFDGWRFDFVKGYAPSITKIYMEQTRPDFAVGEKWDSLSI GNNDSGESYDAAPDIDHLNPQVQRELSEWMNWLKSEIGFDGWRFDFVKGYAASLTKIYMEQTRPDFAVGEKWDSLSI GNNDSGESYDAAPDIDHLNPQVQRELSEWMNWLKSEIGFDGWRFDFVKGYAPSISKIYMEQTRPDFAVGEKWDPLSY-EN GNNDSGESYDAAPDIDHLNPQVQRELSEWMNWLKSEIGFDGWRFDFVKGYAPSISKIYMEQTRPDFAVGEKWDPLSY-EN GHRDTGADFGAAPDIDHLNPQVQRELSEWMNWLKSDLGFDGWRLDFAKGYSAAVAKVYVDNTAPTFVVAEIWSSLHYDGN
H. vulgare V. mungoculata M. acuminata G. max I. nil P. vulgaris S. bicolor	GKPNLNQDQHRQEL VNWVDKVGGKGPATTFDFTTKGILNVAVEGELWRLRGTDGKAPGMIGWWPAKAVTFVDNHDTGSTQ GKPNYNQDSHRGALVNWVESAGGAITAFDFTTKGILQAAVQGELWRLIDPNGKPPGMIGVKPENAVTFIDNHDTGSTQ GKPTYDQNGNRQGLVNWVQQVGGPVTAFDFTTKGILQAAVEGELWRMRDPQGKAPGMNGWWPEKAVTFVDNHDTGSTQ DNYDGHRGALVNWVESAGGAITAFDFTTKGILQAAVQQLWRLKDSNGKPSGMIGVKPENAVTFIDNHDTGSTQ GKPDYNQDNHRLSQWVONGGGAVTAFDFTTKGILQAAVQGLWRLKDPNGKPPGLIGISPKNAVTFIDNHDTGSTQ GKPTYNQDSHRGALVNWVESAGGAITAFDFTTKGILQAAVQGELWRLKDPNGKPFGLIGISPKNAVTFIDNHDTGSTQ GKPTYNQDSHRGALVNWVESAGGAITAFDFTTKGILQAAVQGELWRLKDPNGKPSGMIGVKPENAVTFIDNHDTGSTQ GEPSNNQDADRQELVNWAQAVGGPAAAFDFTTKGILQAAVQGELWRMKDGNGKAPGMIGWLPEKAVTFVDNHDTGSTQ
H. vulgare V. mungoculata M. acuminata G. max I. nil P. vulgaris S. bicolor	HMWPFPSDRVMQGYAYILTHPGTPCIFYDHFFDWGLKEEIDRLVSVRTRHGIHNESKLOIIEADADLYLAEIDG····KV RLWPFPSDKVMQGYAYILTHPGTPSIFYDHFFDWGLKEQIAKLSSIRLRNGINEKSTVKIMASEGDLYVAKIDN····KI KLWPFPSDKVMQGYAYILTHPGVPSIFYDHMFDWGLKEKITRLAKTRTRNRIHSGSSLMILASDADLYMAMIDG····KI RIWPFPSDKVMQGYAYILTHPGTPSIFYDHFFDWGLKEQIAKLSSIRVKHGINEKSSVNILAAEADLCCKDRQQDLFED SMWPFSKDKVIQGYAYILTHPGTPSIFYDHFFDWGLKEQIAKLSSIRVKHGINEKSTVEILAADADAYVAKIDD····KV RLWPFPSDKVMQGYAYILTHPGTPSIFYDHFFDWGLKEQIAKLSSIRVRNGISETSNVEILAADADAYVAKIDN····KI NSWPFPSDKVMQGYAYILTHPGTPSIFYDHFFDWGLKEQIAKLSSIRVRNGINEKSTVEIMAAEGDLYVAKIDN····KI
M. acuminata G. max I. nil P. vulgaris S. bicolor	IVKLGPRYDVGNLIPGGFKVAAHGNDYAVWEKI MVKIGPKMDLGNLIPSNLHVATSGQDYAVWE LTKLGSRYDVGNLVPSNFHVVASGNDYCVWEKR RAKDGPWKPYSPKFPRCYLWPRLCRVGVTQFITLQ IMKIGSKYDVGNLIPPNFNLVTSGQDYAVWEKKI MVKIGPKMDLGKLIPSNFHVATSGQDYAVWEKKI IVKIGSRYDVGNLIPPSNFHVATSGQDYAVW IVKIGSRYDVGNLIPSDFHAVAHGNNYCVWEKSGLRVPAGRHH

**Figure 3** Sequence conservation of Family One & two amylases. Shows the alignment of amino acid sequences of a-amylases from, Hv= *Hordeum vulgare*, Vm= *Vigna mungo*, Ms= *Musa* specie, Gm= *Glycine max*, In= *Ipomea nil*, Pv= *Phaseolis vulgaris*, *Sb*= *Sorghum bicolour*. Grey= conserved residues. No conservation in the signal peptide, but some areas with good sequence conservation were observed.

# Conservation in the Catalytic Domain across Plant Alpha Amylases

It was observed that the amylases have lots of variability in the N-terminus, but high homology in the C-terminus, where the protein activity resides (also referred to as catalytic or amylase domain). In order to investigate the conservation in this domain, the C-terminus of

amylases were subject to alignment using clustalW. Figure 4 shows the alignments, the homology in the C-terminus is quite striking with little variation in the amino sequences. More than 80% homology was observed between the plant amylases in the catalytic region or C-terminus.

BAJOPAS ReamvUD	S Volume 11 Number 2 December, 2018 EISSLGFTVIWLPPTESVSPEGYMPKDLYNIN - SRYGSIDELKDLVKSLHRVGLKVLGD
VvamyUD VvamyUD	ELSSLGFT VVWLPPPTASVSPEGYMPT DLYNLN - SRYGSSDELKVLVKSFHEVGVKVLGD
Atamy3 Mdamy10	E LASLGFT VLWLPPPTE SVSPEGYMPKDLYNLN - SRYGT I DE LKDT VRKFHKVG I KVLGD E LSSLGFT VIWFPPTDSVSPOGYMPRDLYNMN - SRYGNMDE LKET VKTFHDAGLKVLGD
Hvamv2	DIAAAGITHVWLPPASOSVAFOGYMPGRLYDLDASKYGNKAOLKSLIGALHGKGVKAIAD,
Atamy 1	DIANAG IT HLWLPPPS QS VAPE GYLPGKLYDLNSSKYGSE AE LKSLIKALNQKGIKALAD
Atamy2 Mdamy8	D I A K S G F T S A WL P P P S Q S L A P E G Y L P Q D L Y S L N - S A Y G S E H L L K S L L R K M K Q Y K V R A M A D D I G R S G F T S A WL P P A T H S F A P E G Y L P Q D I Y S L N - S K Y G S E N L L T S L L H K M K Q H K V R A M A D
ReamvUD	AVLNHRČAHFONONGVWN I FGGK LNWDDR - A I VADDPHFOGRUSKSSGDNFHAAP
VvamvUD	VVLNHRCAQYQNQNGIWNIFGGR LNWDDR - AIVADDPHFQGRGNKSSGDNFHAAP
Atamy3 Mdamy10	AV LNHR CAHFKNONG VWN LFGGR LNWDDR - AV VADDPHFOGRGNKS SGDNFHAAP AV LNHR CAE YON ONG VWN IFGGR LNWDER - AV VADDPHFOGRGNKS SGD SFHAAP
Hvamy2	IVINHRTAEHKDÖRGIYCIFEGGTPDARLDWGPHMICRDDRPYADGTGNPDTGADFGAAP
Atamy 1	IVINHRTAERKDDKCGYCYFEGGTSDDRLDWDPSFVCRND-PKFPCTGNLDTGGDFDGAP
Atamy2 Mdamy8	IVINHRVGTTRGHGGMYNRYDGISLPWDFH - AVTSCTGGLGNRSTGDNFNGVP IVINHPVGTTRGHGGKYNRYDGISLSWDFR - AATSCTGGLGNPSTGDNFHGVP
RcamvUD -	NIDHSODFVRODLKEWLCWLRDE IGYNGWRLDFVRGFWGGYVKDYMEATEPYFAVGEYWD
VvamvUD	NIDHSODFVREDIKEWLCWLRKE IGYDGWRLDFVRGFWGGYVKDYMDASEPYFAVGEYWD
Atamy3 Mdamv10	N I D H S Q D F V R K D I K E WL CWMME E V G Y D G WR L D F V R G F WG G Y V K D Y MD A S K P Y F A V G E Y WD N I D H S O D F V R K D I R E WL CWL R DD I G Y D G WR L D F V R G F WG G Y V K D Y MD A S E P Y F A V G E Y WD
Hvamy2	DIDHLNLRVQKELVEWLNWLKADIGFDGWRFDFAKGYSADVAKIYIDRSEPSFAVAEIWD
Atamy 1	DIDHLNPRVOKELSEWMNWLKTEICFHGWRFDYVRGYASSITKLYVQNTSPDFAVGEKWD
Atamy2 Mdamv8	NVDHT QHF VRKD I I GWL RWL RNT VG F QDF RF DF ARG YS AN YVKE Y I G AAKP LF S VGE CWD NI DHSOL F VRKD I T GWL OWL RNN VG F ODF RF DF ARG YS AKYVKE Y I E GAKP I F S VGE YWD
RcamvUD	S L S YT Y - GEMDHNODAHROR I IDWINATNG TAGAFDVTTKGILHSALDRCEYWRL S DO
VvamvUD	S L S YT Y - GEMDHNOD AHROR I IDWINATNG AAGAFDVTTKGILHSALGRCE YWRL S DQ
Atamy3 Mdamv10	S L S YT Y - GEMD YN OD AHROR I VDWIN AT SG AT G AF DVT T KG I LHT A LQK CE YWRL SD P S L S YT Y - GEMDHN OD AHROR I VDWIN AT NG T CG AF DVT T KG I LH AA LE R CE YWRL SD E
Hvamy2	SLAYGGDGKPNLNODQHRQELVNWVDKVGGKGPATTFDFTTKGILNVAVEG-ELWRLRGT
Atamy 1	DMKYCCDCKLDYDONEHRSCLKQWIEEACG - GVLTAFDFTTKGILQSAVKC - ELWRLKDS
Atamy2 Mdamv8	S C N YN G - HG L D Y N OD S HR OR I I S WI D AT G Q I S A A F D F T T KG I L Q E A V KG - Q Y WR L C D A S C N YN G - HG L D Y T OD S HR OP I V N WI N G T G O L S T A F D F T T KG I L O E A V KG - O L WR L R D P
	Section nothing sound in the section of the section
ReamvUD	KGKPPG VVGWWPSRAVTFIENHD
VvamvUD	KRKPPGVVGWWPSRAVTFIENHD
Atamy3 Mdamv10	KGKP PG VVGWWP S RAVT FIE NHD KGKP PG VLGWWP S RAVT FIE NHD
Hvamy2	DGKAPGMIGWWPAKAVTFILNHD
Atamy I	QGKP PGM IG IMPGN AVT F IDN HD-
Atamy2 Mdamy8	QGKPPGVMGWWPSRAVTFLDNHD, OGKPPGVVGWWPSRSVTFLDNHD
	Alignment of C-terminus of some amylases clustalw alignment of amino acid sequence of a-

**Figure 4** Alignment of C-terminus of some amylases clustalw alignment of amino acid sequence of aamylases from the three families. Family 3: *RcamyUD* (*R. communis*), *VvamyUD* (*V. vinifera*), *Atamy3* and *Mdamy10*, family 2: *Atamy2* and *Mdamy8* (apple) and family 1: *Hvamy2* (*H. vulgare*) and *Atamy1* (Arabidopsis); Grey areas represent conserved regions.

The amino acid residues in the catalytic region are highly conserved among the plant aamylases. This implied that the proteins carry similar and related function. In addition to the homology observed in Figure 4 above, it became imperative to subject the C-terminus of the proteins from the three families to phylogenetic analysis. Figure 5 is a dendogram constructed using amino acid sequence of the C-terminus alone. The tree showed similar pattern and relationship to that shown in Figure 2, it further confirmed the classification of amylases into three families and that family one and two proteins are closer to each other, than to amylases of family three.

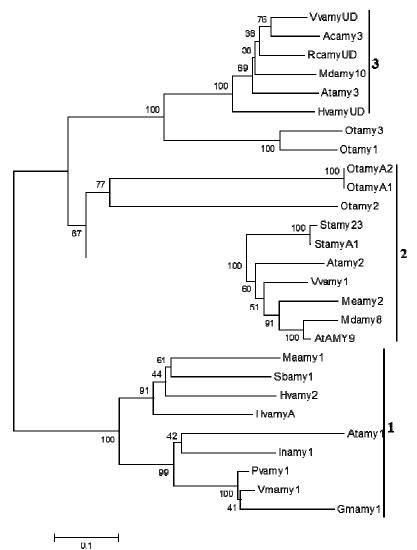


Figure 5 Phylogenetic tree of C-terminus of plant alpha amylases showing the evolutionary relationship using only the catalytic domain for comparison. All other details are as described in Figure 2.1, 2 and 3 represents family one, two and three respectively.

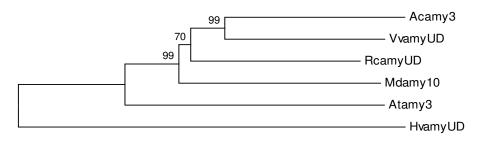
# Conservation in the Unknown Domain of family 3 amylases

The family three amylases have a large Nterminal domain of yet to be established function, the domain starts after the transit peptide and ends with a linker region (GTGSG). The linker connects the unknown domain to the rest part of the protein which is the amylase domain. There may be conservation of amino sequence of this regions between the proteins. In order to obtain evidence, protein sequences corresponding to the 500 amino acids from six different type 3 a-amylases were aligned using clustalW2. Figure 6 reveals the degree of conservation in the N-terminal unknown domain of family 3 proteins. Significant conservation between the different proteins in the additional domain was observed. However, there are some regions in the domain that are not conserved across the group. This is not uncommon as it is often the case in large family of proteins.

BA IOP	AS Volume 11 Number 2 December, 2018
VvamvUD	MSTVCIEPLFQRCR-RENPFRLK-SLATKPSSLNYSPKPLRNGGSFCNFKSLHGVRP-LGAAS-
RcamyUD	MSTITUEPLIFURCE-REFARTER SLATESSLATSTRTLERAUGSFURTESLEUVEFLUGARS- MSTLTVEPLIRFSG-REK-SLPIGSRKILKPSSLNFSKKLLLRNUGSFURTESENTWRSENTWRASST
Atamy3	MSTVPIESLLHISHLRDNSKIYRGTRSFIPCSLNLPSHFTSNKLLHSIRTSVGASKHRRSVAIRASS
Mdamv10	MSTYLIEBLEHHYR - ROKPSHREPPSKHPIKLSSSFTAFPKK-LVVSNGRSFCNFOPP-TLSVRAS-
Acamy10	MPTVTLEPLLINTR-RQKFSHRLFFSKIKAKASSIAFFKK-LVSNUKSFCNFQFF-ILSVKAAS- MPTVTLEPLRYOFR-REILGFHSN-FRKAKAFSLNYAORPUSHGSSFCNFRPOPLSVKAAS-
Acamys HvamvUD	METALEFLETER I QFR - REIL GFR SN - FRRAKAFSLA I AQRF LSHOSSFCN FRFQFLS VRASS- MSAASWS - I PA I PR AVPPPRAAPPGEAFMVPAR PRAAWCR AAPR VRLARGGGGGG GV VAR AGA
HVamyUD	MSAASWS - IFAIFKAVFFFKAAFFGEAFMVFAKFKAAWCK AAFKKVKLAKGGGGGGV VARAGA
VvamyUD	I D T A L F E T T D V F F K E T F I L K R T E V V E G K I S I R L D P G - K N G E N WOL T V G C N I P G S W V L H W G V S Y I D D V
RcamyUD	TD T A L I E T F K S A D V L F K E T F S L S R T E T I E G K I F V R L D K E E K D Q O R WOL S V G C S L P G K W I L H W G V S Y V G D V
Atamy3	SD TAVVETAOSDD VI FKEN F PVOR I EKAOGKI YVRLKOV - · KEKNWELSVG SSI PGKWI LHWGVSYVGD T
Mdamy10	TD T A T V E A T E F A D A F Y K E T F P L K R T E V V E G K M I V K L D NG - K D A K N W V L T V G C N L P G K W V L H W G V N Y V D D V
Acamy3	AD TA V VET SD S VD VL FKET FALKR I EK VEGH I S I KLDNG - KERENWOLS VG CN L PG KWVLHWG VN Y I ND I
HvamyUD	A E A V P V A D S G E A S V V F S E K F P L R R C O T V E G K AWVR VE A E P D A D G K C K V V I G C D V E G K W L L H W G V S Y H G E T
<b>VvamyUD</b>	G S EWD Q P P L EMR P P G S V A I K D Y A I E T P L K K L S S A S E R D T L H E V T I D F S P N S E I A A I R F V L K D E D Y G A W Y Q
RcamyUD	G S EWD Q P PKNMR P R G S I S I KD Y A I E T P L E K S S E A D M F Y E V K I D L D P N S S I A A I N F V L K D E E T G A W Y Q
Atamy3	G S EWD Q P PEDMR P PG S I A I KD YA I ET PLKKL S EGD S F FE VA I N LN LE S S VAALN F VLKD EE TG AWY Q
Mdamy10	G S EWD Q P P S EMR P AG S V S I KD Y A I E T P L K E S L S P V G G D T S H E V K I D V T P N S A I A A I N F V L KD E E T G A W Y Q
Acamy3	G S EWD Q P P V EMR P P G S V P I K D Y A I E T P L K K S S A V V E G D L Y Y E L K I D F S T D K D I A A I N F V L K D E E T G A W Y Q
HvamyUD	G S EWD Q P P S E I R P P G S V P I K D S A I E T P L E I S P N - S D G H I L H E V Q I K F D K D T P I A A I N F V L K E E G T G A W F Q
VvamyUD	HR GRD F EVLLMD YL CEG TN T VGAKEG FG I WP G PL G QL S N ML LKAE G S H PK G QD S S S V S G D L I T G
RcamyUD	HKGRDFKVPLVDYLLEGGNVVGAKRGFSIWPGSL LSNMLLKTETLPSKDEDNNSETKDVKQDSGQLKG
Atamy3	HKGRDFKVPLVDDVPDNGNLIGAKKGFGAIGQLSNIPLKQDESSAEVKKKSKSSSDSTKERKGLQE
Mdamy10	HR GRD F K V PF V G YL QDDDN V V G A T R A L G AWS G T L G KL SN V F V K A E T SN SK D Q E S S S E S R D P Q Q K T M R L E G
Acamy3	R R G R D F K V X L I D X L H E D G N K L G A K K G L G V X P G P F E Q L S S L L L K S E E A H P K G E D S S D - S R D P S K T T K C L E A H K G G D F R I P L G G S L - E G G D P L G A K O G A K P E G P S A Q L K E T V P G D K G P S T K C I S K
HvamyUD	HRGGDFRIPLGGSL-EGGDPLGARQGARPEGPSAQLREIVPGDRGPSIRCISK
VvamyUD	FYEEHSIVKEVPVDNSVNVSVKKCPETARNLLYLETDLIGDVVVHWGVCRDDSKTWEIPAAPHPPETKLF
RcamyUD	FYEEOPITKOVTION SATVS VTKCPKTAKYLLYLETDLPGEVVLHWGVCRDDAKNWEIPS SPHPPETTVF
Atamy3	FYEEMPISKR VADDNSVSVTARKCSETSKNIVSIETDLPGDVTVHWGVCKNGSKKWEIPSEPYPEDTSLF
Mdamy10	FYEELPIAKEIAVNHSATVSVRKCPETTKNLLYLETDLPDHAVVHWGVCRDDAKRWEIPAAPHPPETVVF
Acamy3	FYEEHS I VREVLINN SVS VS ARKCPKTAKN LLH I ETD I PGD V V HWGLCKDDGENWE I PAK PYPAET I VF
HvamyUD	FYEEYPILKSEYFEHSVSVAVRENSEKDKSLVEFYTDITGDVIIHWGVCKDNTMTWEIPPEPHPPNTKVF
-	
VvamyUD	KKKALRTLLQSKEDGHGSWGLFTLDEELEGFLFVLKLNENTWLR CMGNDFYIPLLGSSSLPAQSRQGQ
RcamyUD	KNKALQTMLQPNDGGNGCSGLFSLDEEFAGFLFVLKLNEGTWLK CKGNDFYVPLSTSSSLPTQPGQGQ
Atamy3	KNKALRTRLQRKDDGNGSFGLFSLDGNLEG GEDFYVPFLTSSSSLVGTEATE
Mdamy10	KDKALRTRLQQREDGNGCSGLFTLEEGLAGFLFVFKLNETMWLN CVGNDFYIPLLSSNNSIAVQNEVQ
Acamy3	KNKALRTLLKXKEGGKGGWSLFTLDEGYAGFVFVLKINENTWLN YMGNDFYIPLSSSSVLPAQPRHDQ
HvamyUD	R Q K A L Q T L L E Q K T D G T G N A V S F L L D A D Y T G L V F V L K L N E H TWL R N L E N G F D F Y V P L T R • • • • • • • V E Q V D
VvamyUD	SEGWGKSER VVSVPTE I SGKTAGENE I VSD AAYTDG I IND IRNL VSD I SSEKROKTKTKOAOES I LOE I E
RcamyUD	SEGVLAS
Atamy3	AAQLSKH
Mdamy10	A AQUSKIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
Acamy3	SEGAV OVETDOEVS PAAYTOGI INDIR SLVSDISS EKSROKKSKE AQUI ILQUI E
Acamy5 HvamyUD	SEGRA
11vaniy0D	
VvamyUD	KLAAEAYSIFRSSIPTFSEDAVLET LKP - PEKLTSGTGSG
RcamvUD	KLAAEAYSI FRSSI PTFTEESVLESEVEKAPPAKI CSGTGTG
Atamy3	KLAAEAYSIFRSTTPTFSEESILAEAEKPD IKISSGTGSG
Mdamy10	K L A A E A Y S I FR T T V P T L P E I I A E T E K V K V A P A K I C S G T G T G
Acamy3	K L A A E A Y S I F R S S I P T Y X E D VM V E S E E V E P - P A K I S S G T G S G
HvamvUD	R L A A F A Y S I F R S P T I D A VE G S V Y I D G P E T V K P A C S G T G S G
Figure	<b>6.</b> Conservation of the unknown domain in family three g-amylases, an alignment of the some

**Figure 6**. Conservation of the unknown domain in family three a-amylases, an alignment of the some of the family three a-amylases is shown. Areas in grey indicate amino acids that are conserved in the family three proteins.

Furthermore, in order to have conclusive evidence, a phylogenetic tree, Figure 7 shows the dendogram produced of the unknown domain only and the full proteins respectively. The trees further established and confirmed the relationships revealed by the alignment in Figure 6.





**Figure 7.** Phylogenetic tree of family three amylases showing the evolutionary relationship between them. The full protein sequence of about 900 amino acids were. Protein sequences were obtained using publicly available data (http://www.ncbi.nlm.nih.gov/), aligned using the ClustalW alignment tool. The aligned sequences were assembled into a phylogenetic tree using the boot-strapped neighbor-joining algorithm (Saitou and Nei 1987) and the Jones-Taylor-Thornton amino acid substitution model (Jones, et al. 1992) in MEGA 7.0 with 1000 trials (http://www.megasoftware.net/) (Tamura, et al. 2011). Bootstrap values are indicated as percentages of the 1000 trials at their respective node. Abbreviatios: Ac (A. chinensis), Vv (V. vinifera), Rc (R. communis), Md (M. domestica), At (A. thaliana) and Hv (H. vulgare).

### **Perspectives and Conclusion**

The a-amylases are proteins of invaluable functions in plants, they are involved in the hydrolysis of starch. This is in addition to their significant roles in industrial starch processing (Adam, et al., 2017). Lots of experimental evidence support this view, the function of the amylases were deduced by generating mutants. In germinating cereal endosperm, a secreted saamylase converts starch to linear and branched glucans (Beck and Ziegler, 1989; Kotting, et al., 2010). The oligosaccharides are hydrolysed further by the actions of enzymes; limit dextrinase attacks the a-1,6 linkages and while β-amylase hydrolyse the linear oligosaccharides from the ends. Maltose and glucose are released; and are exported to the scutellum. Similarly, the same process occurs in ripening fruits. Maltose may be converted to sucrose through the glucose 1-phosphate pathway. This is also supported further by the fact that only trace amounts of maltose are found in ripening fruit (Fioravante Bernardes Silva, et al., 2008; and Aprees 1994; Prabha Hill and Bhagyalakshmi, 1998).

High activity of a-amylase has been reported during seed germination. This indicates its role in starch mobilization in germinating seeds where starch reserves are used for energy (Irving, et al., 1999; Zeeman, et al., 2010). The synthesized a-amylase in the aleurone and scutellum is secreted into the endosperm to degrade starch. Thus, a-amylase plays a principal role in starch hydrolysis in the endosperm during germination (James, et al., 2009; Kumari, et al., 2010). Although a-amylase plays an important role in storage starch hydrolysis in the endosperm, it may not be involved in transitory starch hydrolysis in the chloroplasts of leaves. An Arabidopsis mutant (designated as sex4 mutant) with low a-amylase activity showed normal trend of starch metabolism compared to the wild type. It is also evident that mutation in AMY3 that is present in the chloroplast does not change the rate of starch hydrolysis in leaves (Yu, et al., 2005; Zeeman, et al., 2007a &b). Thus alpha-amylase may be less significant in transitory starch breakdown.

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Adam, I. K., et al. (2017) Purification of an Alpha amylase like Protein from Plantains. Journal of Advances in Biology and Biotechnology 14(4): 1-8 Family one amylase such as Hvamy2 are secreted proteins with signal peptide for translocation across the ER membrane. In the cytosol, the SP of this soluble protein is recognised by signal recognition particle (SRP) and is cleaved by the signal peptide peptidase (SPP) (Lyko, et al., 1995; Walter and Blobel, 1981; Weihofen, et al., 2002). They are found in cereals and seeds of dicot plants. This family of enzymes has also been described to be involved in the degradation of extracellular starch and can be found in microbes. The presence of this type of protein in seeds of higher plants may be due to the need of the enzyme to translocate across membranes to the specialised starch tissues such as the endosperm in cereals (Stanley, et al., 2005; Stanley, et al., 2002). The second family of alpha amylase consists of proteins that localise to the cytoplasm due to the absence of any characterised targeting peptide. They are found in leaves of monocots and dicot plants as well as gymnosperms. This group of enzymes degrade cytosolic a-glucan or heteroglycan (Stanley, et al., 2005; Stanley, et al., 2002). Family three a-amylases have a chloroplast transit peptide with a large Nterminal domain in addition to the a-amylase domain considered to be starch binding domain (Stanley, et al., 2005; Stanley, et al., 2002). In conclusion, starch is semi-crystalline in nature

of starch hence enzymes of starch degradation have properties to enable effective and strong binding to the substrate leading to hydrolysis. This can be a specific substrate binding site in the catalytic domain of the enzyme or a carbohydrate binding module (CBM) or starch binding domain (SBD) ) (Chou, et al., 2010; Machovic and Janecek 2006b; Rodriguez-Sanoja, et al., 2005) (Glaring, et al., 2011; Janecek, et al., 2011; Machovic and Janecek 2006). This implied that the proteins are evolutionary related and perform similar functions. The unknown domain in family three amylases is also conserved across members of this family. The domain may be a starch binding domain that is required for the family three amylases to digest storage starch in the plastid.

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