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In silico characterization of ripening proteins in *Musa accuminata*

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Abstract- *Musa accuminata* is the member of family Musaceae. The fruits of *M. accuminata* are harvested in unriped green in order to better withstand transportation and to slow down the natural ripening process because of several weeks travel from the production areas to the end markets. The ripening process is a multi step reaction involves a number of complex enzymes. The present work is aimed to computational characterization of these ripening enzymes in order to understand the ripening mechanism. In this work identification of the enzymes/proteins involved in ripening process are characterized by their conserved domains (conserved, 3-dimension region). The domains are identified by using the computational tool, CDART (Conserved Domain Architecture Retrieval Tool). By using this applied approach a number of conserved domains were identified, out of these enzymes, the domain pattern of Aspartate amino-transferase (AAT_Like), Glycosyl hydrolase (Glyco_Hydr) and oxoglutarate (20G_Fe) were reported the most conserved followed by Ethylene insensitive (EIN3), GT1, DPBB & Pollen in ripening enzyme complexes. The data, thus, obtained provide new insights in order to understand the role of the proteins playing in different stages of ripening process. These results provide a basis for further studies on the molecular mechanism of ripening process and food technological applications for delayed ripening in fruits, where ripening proteins plays an important role in.

Key words: Musa accuminata; Banana ripening; Domains; CDART; Polygalacturonase; pyridoxal phosphate; Aspartate aminotransferase.

Introduction

Musa acuminate is a tree-like plant (though strictly an herb) of the genus Musa in the family *Musaceae*. The genus was originated from South East Asia, Malasia. The fruits (*Pisang Jacky*) are eaten locally in New Gunea and Java Islands. It is a large herbaceous plant. The other varieties of the genus like *Musa paradisiaca* (Adam's Banana), *M. sapientum* (Banana) and other edible species are derived from *M. accuminata*, which ate triploid in chromosomal organization. The derivative forms of *M. accuminata* higher starch contant in the fruit. The higher starch content of the fruits and its slightly bitter flavour make it unpalatable to eat raw, but they are eaten widely when roasted or boiled after peeled. The flour from the fruit (*Guiana Arrowroot*) is an excellent binder used as food additives. The ripened banana contains a high level of easily digestible natural, which it releases quickly in the blood stream. It has immense nutritional value and medicinal properties apart from being effective cosmetics. Any edible part of the plant, be it fruit, flower or stem, provides energy, vitamins and minerals. Cholesterol and fat content is minimal. The ripened fruit has the ability to correct acidity, gastritis and peptic ulcer. It is claimed that it stimulates mucus production by stomach lining. The ripened fruit contains about 74% water, 23% carbohydrates, 1% proteins, 0.5% fat, and 2.6% fiber (these values vary between different cultivars, degree of ripeness and growing conditions). In an unripe fruit the carbohydrates are mostly starches. In the process of ripenening the complex starches are converted in to simplest sugars. A fully ripe fruit has only 1-2% starch. Besides being a good source of energy, it is a rich source of potassium, and hence is highly recommended for patients suffering from high blood pressure. It is claimed that fruits of banana have beneficial effect in the treatment of intestinal disorders, including diarrhoea. They contain mucilaginous bulking substances and are easy to digest. Being iron rich, bananas can easily combat iron deficiency. Low levels of iron cause lethargy and weakness. Iron deficiency is common among women and vegetarians. The use of bananas has been found beneficial in the treatments of several medical conditions such as intestinal disorders, constipation, arthritis, gout, anemia, kidney stones, tuberculosis and urinary disorders. Bananas (*Musa acuminata*) are generally yellow when ripe and have a soft yellow flesh with a sweet taste. Ripe fruit demonstrates a wide range of diversity in form, texture, pigmentation, aroma, flavour, and biochemical as well as nutrient composition. Fruits of various species undergo modification of cell wall ultra structure and texture, conversion of starch to sugars, increased susceptibility to post-harvest pathogens, alterations in pigment biosynthesis/accumulation, and increased levels of flavour and aromatic volatiles during the course of maturation and ripening. Like other fruits, there are certain factors which influence the rate at which they ripen. Major among them is the presence of ethylene, it is an abundant hormone produced by most fruits. Ethylene plays a crucial role in the ripening process. It is produced by bananas in natural conditions, however in much smaller quantities that are supplied in the chambers. When a fruit is exposed to ethylene, it ripens at the rate faster as compared to that without

ethylene [1]. Oxygen is mandatory for this reaction as well [2]. The affinity and reactivity towards ethylene varies from fruit to fruit. An increased dose of ethylene accelerates the ripening process, which is very important for trading. Ripening process consists not only in changing the color of the peel, but also in breaking the starch into plain sugars which in turn influences the taste of the fruit. In green bananas starch and plain sugars are in the ratio of 20 to 1 whereas in yellow fruits the proportion is reversed and is 1:20. An average process lasts 4-8 days depending on the program chosen by the reopener. There are also other factors that affect the level at which all fruit ripen, viz, temperature [3, 4], light exposure etc. During banana fruit ripening ethylene production triggers a developmental cascade that is accompanied by an enormous conversion of starch to simpler sugars [5], a coupled explosion of respiratory activity, and an enhancement in the senescence-specific protein synthesis [6]. Banana starch disappearance during ripening originated at the central portion of the fruit radiating, afterwards, to the surface; the amylose/amylopectin ratio remained constant during the process. The surface of starch granules was smooth, and the unique modification observed during ripening was the reduction of the granule dimensions; at advanced ripening stages, some striations were detected on the surface of both small and large granules. Several amylolytic enzymes were followed during banana ripening [7]. These enzymes can be studied by computational tool in order to understand their 3-dimensional conserved region (domains). These conserved regions (domains) are the 3-dimensional packing of amino acids, which play an important role in the activity of proteins. The functions and evolution of proteins can be understood using genomic, structural and proteomic data [8-10] when the three-dimensional level of protein structure viewed, it was found that a domain is a compact arrangement of secondary structures connected by linker polypeptides [12, 13]. It usually folds independently and possesses a relatively hydrophobic core. The importance of domains is that they cannot be divided into smaller units they represent a fundamental building block that can be used to understand the evolution and function of proteins [14]. Detecting domains from sequence can be a laborious process with many pitfalls. The present study was aimed to identify the domains present in different proteins accumulated in ripening process of banana.

Materials & Methods

In the present study a total of 111 protein sequences were found for banana (*Musa acuminata*) from NCBI web server, www.ncbi.nlm.nih.gov. These protein sequences belong to different families, which catalyzed the ripening process are listed along with their domains (Table 1). The sequences are filtered on the basis of their length/ molecular weight and submitted to the CDART in FASTA format. The e-value is set 0.01, CDART performs similarity searches of the NCBI Entrez Protein Database based on domain architecture, defined as the sequential order of conserved domains in proteins [15]. The algorithm found protein similarities across significant evolutionary distances using sensitive protein domain profiles rather than by direct sequence similarity. Proteins similar to a query protein were grouped and scored by architecture. Relying on domain profiles allows CDART to be fast, and, because it relies on annotated functional domains, informative. Domain profiles were derived from several collections of domain definitions that include functional annotation [16, 17, 18]. Searches can be further refined by taxonomy and by selecting domains of interest. CDART is available at http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi.

Results & Discussion

In present studies, out of 111 sequences found for the fruit ripening in banana, 67 sequences were filtered (considering the sequences having length >100 aa) for the domain analysis using CDART.

Domain organization of the Banana ripening protein

These 67 sequences (Musa acuminata) were submitted to CDART in FASTA format, the e-value is set 0.01 and the domain analysis is performed. The CDART reported a number of domains (Table 1). Analyzing these domains, it was found that only few of them like AAT_like, 20G_FE, Glyco_hydr, GT1,DPBB, Pollen & EIN3 were most conserved (Fig 1).

The domains AAT_like, 20G_FE, Glyco_hydr, GT1,DPBB, Pollen & EIN3 identified in 67 sequences were further examined, it shown that AAT_like domains were present in 11 sequences, 20G_FE & Glyco_hydro domains were present in 8 sequences and DPBB, Pollen, GT1 & EIN3 were present in 4 sequences each (Fig 2). Analyzing the functionality of the domains AAT_like, 20G_FE, Glyco_hydr, GT1, DPBB, Pollen & EIN3, it was found that each domains has some specific activity and they play an important role to decide the functionality of the ripening proteins. The functionality of these domains are summarised as;

Glycosyl hydrolases family 28

Glycosyl hydrolase family 28 includes polygalacturonase EC: 3.2.1.15 as well as rhamnogalacturonase A (RGase A), EC: 3.2.1.-. These enzymes are important in cell wall metabolism [19].

AAT_Like

Aspartate aminotransferase family belongs to pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily (fold I). Pyridoxal phosphate combines with an alpha-amino acid to form a compound called a Schiff base or aldimine intermediate, which depending on the reaction, is the substrate in four kinds of reactions viz
Transamination (movement of amino groups),
Racemization (redistribution of enantiomers),
Decarboxylation (removing COOH groups), and
Various side-chain reactions depending on the enzyme involved.

Pyridoxal phosphate (PLP) dependent enzymes were previously classified into alpha, beta and gamma classes, based on the chemical characteristics (carbon atom involved) of the reaction they catalyzed. The availability of several structures allowed a comprehensive analysis of the evolutionary classification of PLP dependent enzymes, and it was found that the functional classification did not always agree with the evolutionary history of these enzymes. The major groups corresponds to Aspartate aminotransferase a, b and c, Tyrosine, Alanine, Aromatic-amino-acid, Glutamine phenylpyruvate, 1-Aminocyclopropane-1-carboxylate synthase, Histidinol-phosphate, gene products of malY and cobC, Valine-pyruvate aminotransferase and Rhizopine catabolism regulatory protein.

2OG-Fe (II) oxygenase superfamily

This family contains members of the 2-oxoglutarate (2OG) and Fe (II)-dependent oxygenase superfamily. This family includes the C-terminal of prolyl 4-hydroxylase alpha subunit.
The holoenzyme has the activity EC: 1.14.11.2 catalysing the reaction:
Procollagen L-proline + 2-oxoglutarate + O2 <=> procollagen trans-4-hydroxy-L-

proline + succinate + CO2.
The full enzyme consists of an alpha2 beta2 complex with the alpha subunit contributing most of the parts of the active site. The family also includes lysyl hydrolases, isopenicillin synthases and AlkB [20].

Rare lipoprotein A (RlpA)-like double-psi beta-barrel

Rare lipoprotein A (RlpA) contains a conserved region that has the double-psi beta-barrel (DPBB) fold. The function of RlpA is not well understood, but it has been shown to act as a prc mutant suppressor in Escherichia coli. The DPBB fold is often an enzymatic domain. The members of this family are quite diverse, and if catalytic this family may contain several different functions [21]. Another example of this domain is found in the N terminus of pollen allergen.

Pollen allergen

This family contains allergens lol PI, PII and PIII from Lolium perenne.

Ethylene insensitive 3

Ethylene insensitive 3 (EIN3) proteins are a family of plant DNA-binding proteins that regulate transcription in response to the gaseous plant hormone ethylene, and are essential for ethylene-mediated responses including the triple response, cell growth inhibition, and accelerated senescence.

Characteristic of domains were analyzed and it was found that ripening process in banana involved a number of protein domains and these domains have some specific functionality viz. The domain AAT_like (Aspartate aminotransferase) belongs to pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily (fold I). PLP combines with an alpha-amino acid to form a compound called a Schiff base or aldimine intermediate, which depending on the reaction, is the substrate in four kinds of reactions transamination (movement of amino groups), racemization (redistribution of enantiomers), decarboxylation (removing COOH groups), and various side-chain reactions depending on the enzyme involved. The domain Glyco_hydr (Glycosyl hydrolase) is important in cell wall metabolism. Similarly, the domain EIN3 (Ethylene insensitive 3) found in a family of plant DNA-binding proteins that regulate transcription in response to the gaseous plant hormone ethylene. 20G_FE-dependent oxygenase superfamily includes the C-terminal of prolyl 4-hydroxylase alpha subunit. The holoenzyme has the activity EC: 1.14.11.2 catalysing the reaction.

Conclusion

These ripening enzymes studied by computational tool in order to understand their 3-dimensional conserved region (domains). These conserved regions (domains) are the 3-dimensional packing of amino acids, which play an important role in the activity of proteins. It was found that a domain is a compact arrangement of secondary structures connected by linker polypeptides. The domains cannot be divided into smaller units they represent a fundamental building block that can be used to understand the evolution and function of proteins. Detecting domains from sequence is a complex process with high inputs. The present study was aimed to identify the domains present in different proteins participated in ripening process of banana. A number of protein domains participating in ripening reactions have some specific functionality. The different domains are important in different cell metabolism and their regulation mechanism. The domain EIN3 (Ethylene insensitive 3) was found as a DNA-binding protein that regulate transcription in response to the gaseous plant hormone ethylene. 20G_FE-dependent oxygenase superfamily.

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Table 1- Domains identified in Banana (*Musa acuminata*) using the CDART

Protein Domains	Protein Acc. No.	Protein	Protein Acc. No.	Protein
Glyco_hydro	CAE51357.1	putative polygalacturonase	AAF08679.1	beta-1,3-glucanase
	CAE51356.1	putative beta-galactosidase	AAO27531.1	Polygalacturonase
	CAE51355.1	putative beta-galactosidase	AAZ94622.1	beta-amylase
	AAB82772.2	beta-1, 3-glucananse	AAT74603.1	Polygalacturonase
AAT_LIKE	CAD44267.2	putative aminocyclopropane carboxylic acid synthase	CAE53271.1	1-aminocyclopropane-1-carboxylate synthase
	AAR00513.1	1-aminocyclopropane-1-carboxylate synthase	AAR00512.1	1-aminocyclopropane-1-carboxylate synthase
	AAL82597.1	aspartate aminotransferase 2	AAL82596.1	aspartate aminotransferase 1
	CAA75749.1	1-aminocyclopropane-1-carboxylate synthase	AAU09672.1	ACC synthase
	BAA84947.1	ACC synthase	BAA84945.1	ACC synthase
	AAC31571.1	1-aminocyclopropane-1-carboxylate synthase		
20G_FE	AAR00511.1	1-aminocyclopropane-1-carboxylate oxidase	AAB68602.1	1-aminocyclopropane-1-carboxylate oxidase
	CAD44265.2	putative aminocyclopropane carboxylate oxidase	ABW20470.1	ACC oxidase
	CAE53174.1	1-aminocyclopropane-1-carboxylate oxidase	ABV45543.1	1-aminocyclopropane-1-carboxylate oxidase
	AAB00556.1	1-aminocyclopropane-1-carboxylate oxidase	AAC31967.1	1-aminocyclopropane-1-carboxylate oxidase
DPBB,POLLEN	AAN31756.1	expansin1	AAM08930.1	expansin 1
	ABN09942.1	expansin A5	ABN09939.1	expansin A4
GT1	CAD44260.1	putative sucrose-phosphate synthase	CAD44259.1	putative sucrose-phosphate synthase
	AAB82780.1	ripening-associated protein	AAC23914.1	sucrose-phosphate synthase
AMBALL	AAF19196.1	AF206320_1 pectate lyase 2	AAF19195.1	AF206319_1 pectate lyase 1
Polyprenyl	AAL82595.1	AF470318_1 farnesyl pyrophosphare synthase	ABI73983.1	farnesyl pyrophosphate synthase

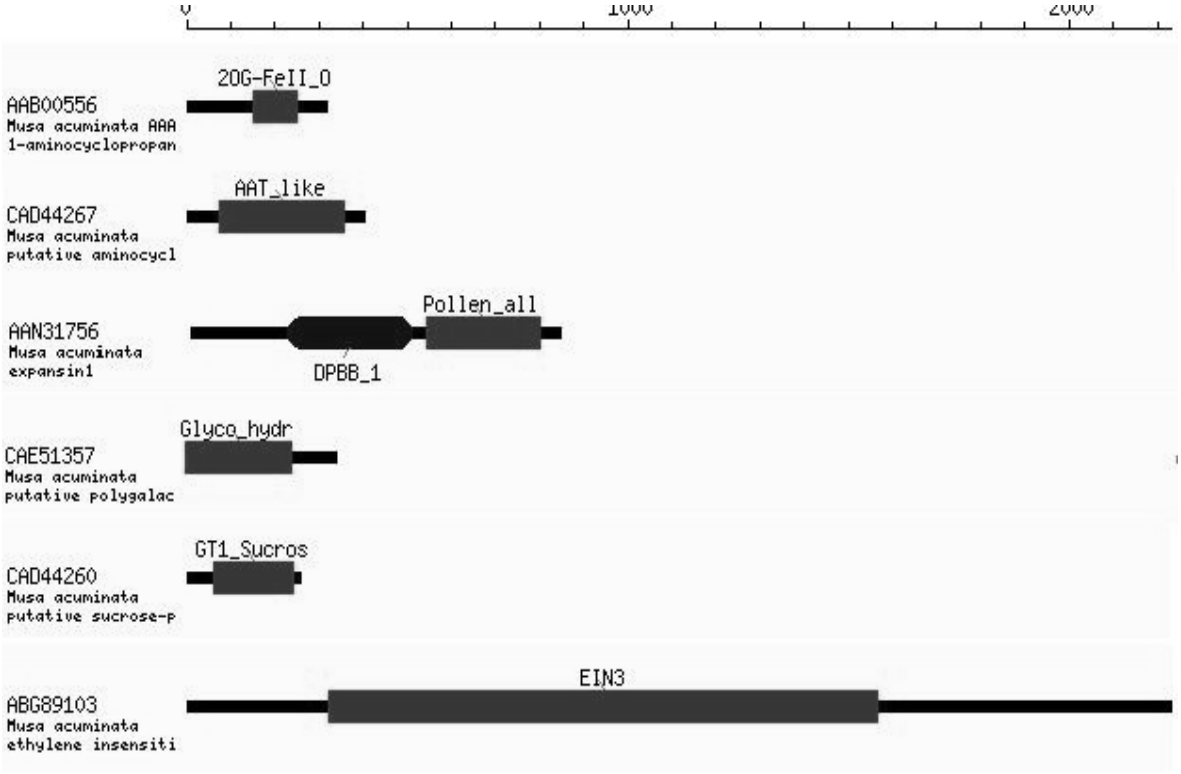

Fig 1- Conserved domains identified in the banana (*Musa acuminata*) protein

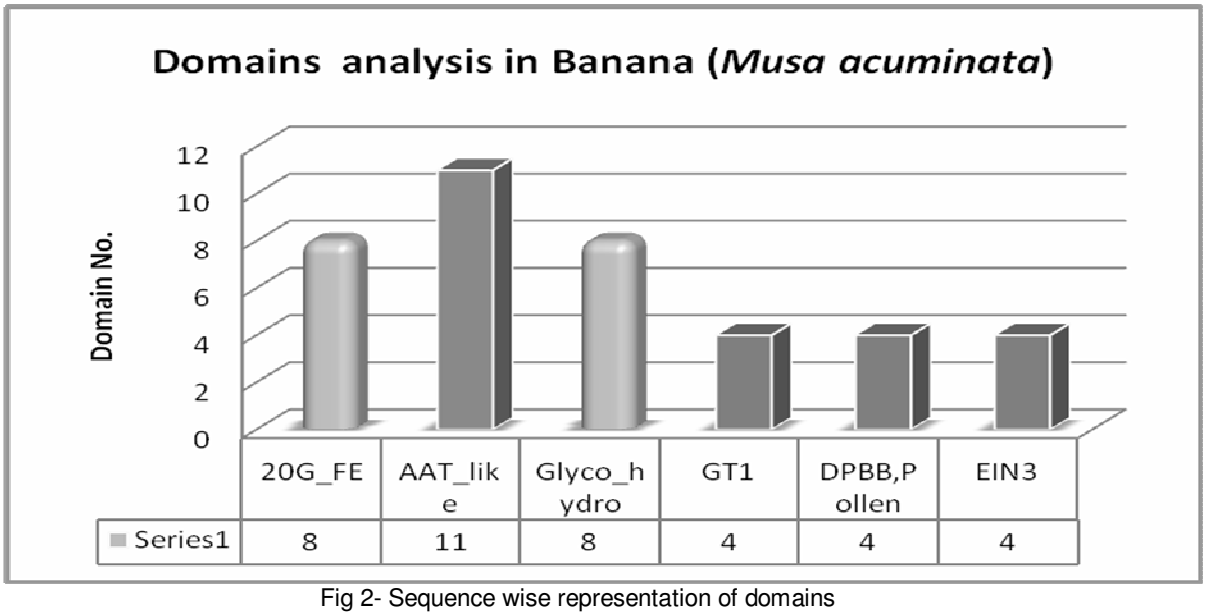

Fig 2- Sequence wise representation of domains