PHOTOSYNTHESIS AND ENZYME ACTIVITIES REGULATING STARCH BIOSYNTHESIS IN DIFFERENT VARIETIES OF CASSAVA (*Manihot esculenta* Crantz)

by

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THESIS

Submitted in partial fulfilment of the

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KERALA, INDIA

2016

DECLARATION

I hereby declare that this thesis entitled **"Photosynthesis and enzyme activities regulating starch biosynthesis in different varieties of Cassava (*Manihot esculenta* Crantz)"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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GEETHU KRISHNA P.R.

Dedicated

To

My beloved

Parents

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LIST OF ABBREVIATIONS AND SYMBOLS USED

g	Gram
Kg	Kilo gram
cm	Centimetre
Min	Minute
Hr	Hour
Sec	Seconds
rpm	Rotation Per Minute
nm	Nanogram
OD	Optical density
A_{340}	Absorbance at 340
µg	Micro gram
µl	Micro litre
ml	Milli litre
b	Breadth
l	Length
Fig	Figure
°C	Degrees centigrade
M	Molarity
Mm	Milli molar
PVP	Polyvinyl pyrollidine
Ect	Etcetera
et al.	And other co workers
N	Normality
%	Percentage
PEG	Poly Ethylene Glycol

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EGTA	Ethylene Glycol Tetra Acetic acid
DNS	Dinitro salicylic acid
UV	Ultra violet
U	Unit
µmol	Micro molar
m	Metre
L	Litre
BSA	Bovine Serum Albumin
HEPES	HydroxyethylPiperazine Ethane Sulfonic Acid
DTT	Dithiothretiol
AGPase	ADP-Glucose pyrophosphorylase
SS	Starch synthase
SuSy	Sucrose synthase
SPS	Sucrose phosphate synthase
PGM	Posphoglucomutase
G6PD	Glucose 6 phospho dehydrogenase
PEP	PhosphoEnol Pyruvate
PPi	Inorganic pyrophosphate

INTRODUCTION

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with edible root grown in tropical and sub-tropical regions of the world. The cassava belongs to the family of Euphorbiaceae and genus of Manihot. Cassava is popularly called tapioca, yucca or manioc. It is originated in South America. Portuguese explorers introduced cassava in Africa during the 16^{th} and 17^{th} centuries through their trade and cassava becomes their staple food. Then cassava spread further by Africans to almost all parts of tropical countries. Over 50% of global production is concentrated in Africa. In tropics, cassava is the third most important source of calories after rice and maize. More than a millions of people depend on cassava in Africa, Asia and Latin America. The bulk of world trade in cassava is in the form of pellets and chips for feed (70%) and the balance mostly in starch and flour for food processing and industrial use. Very little is traded in the form of fresh root, given the product's bulkiness and perishable nature. Thailand is a dominant supplier to world markets, accounting for 80% of global trade.

Cassava has good health benefits. It is rich in nutrients like carbohydrate, calcium, vitamin B and C and other essential minerals. It contains high amount of dietary fibres. It may also help in reducing unhealthy cholesterol levels. In sub-Saharan Africa, cassava is grown mainly for food by small scale farmers since it provides both food security and income generation. It grows easily in poor soils with limited labour requirements (IITA, Research to nourish Africa). It can be grown intercropped with vegetables and plantation crops. Small scale farmers have the advantage of cassava for its limited application of fertilizers due to its high price and lack of availability.

Cassava is highly drought resistant and grown in many parts where rainfall is low and unreliable. Cassava tuber has a versatile use.

Many types of products can be made such as food, confectionary, sweeteners, glues, paper, biodegradable products, textiles, drugs and its feedstock for the production of ethanol biofuel. Now, it has also been found that cassava has also a special utility in the production of synthetic rice.

The importance of cassava in agriculture has changed dramatically. Between 1980 and 2011, the global harvested area of cassava expanded by 44 percent, from 13.6 million to 19.6 million hectares. The cassava production in Asia has grown from 45.9 million tonnes in 1980 to 76.6 million tonnes in 2011 (Howeler, 2013). "According to the Horticulture statistics at 2015, the area of production of cassava was increased from 228000 ha to 253000 ha and the production also increased from 8139000 tonnes to 8542000 tonnes". In India, the major cassava producing states are Tamilnadu, Puducherry, Kerala, Punjab, Andaman Nicobar islands, Odisha, Andhra Pradesh, Nagaland, Mizoram, Karnataka, Assam, Meghalaya, and Lakshadweep. In India, cassava is grown mainly in Tamilnadu about 43.22 tonnes/ha. In Kerala, the production of cassava is 28.80 tonnes/ha.

Starch is the main constituent of cassava. Cassava is a high starch producer with levels varying between 73.7 and 84.9% of its total storage root dry weight (Baguma, 2004). It is mainly used as food, but it can be readily converted into many other useful products. It can be used to produce diverse products such as food, paper, textiles, adhesives, beverages, confectionary, pharmaceuticals, and building materials.

Starch is a glucose polymer of α- D-glucose linked by α-1, 4 linkage and branched at α- 1, 6 positions. Starch is synthesized in leaves as transitory starch from photosynthetically fixed carbon during the day and in heterotrophic organs as storage starch. The process of starch accumulation is very complex and it involves various key enzymes. ADP-

glucose pyrophosphorylase, starch synthase, branching enzymes and debranching enzymes (Li *et al.*, 2016). The major enzymes involved in the starch biosynthesis has been well studied.

Starch is a storage compound found in many plant tissues. Sucrose is synthesised in leaves from the assimilated CO_2 during photosynthesis. During day time, the rate of sucrose synthesis is much greater than the rate of its export and translocation to other organs. Therefore, the excess sucrose formed in the leaves is converted to starch, what is known as "transient starch". This transient starch is then hydrolyzed to sucrose and translocated during night time to the heterotrophic storage tissues where starch is synthesized and stored (Hostettler and Carmen Elisa, 2014).

In cassava, there are long and short duration varieties. Long duration varieties are cultivated for 10 to 12 months whereas short duration varieties are cultivated for 7 months. The starch content of tubers of long duration varieties vary between 20 and 30% whereas it varies between 20 and 25% for short duration varieties. Prevailing agroclimatic conditions such as rainfall (soil moisture), soil nutrients like nitrogen and potassium and temperature influence the starch content of tubers (Ravi and Mohankumar). When agroclimatic conditions are favourable, the starch content of tubers steadily increases and platues during 8 to 10 months in long duration varieties and during 5 to 7 months in short duration varieties. The present study was contemplated to understand the relation between photosynthesis, enzymes involved in sucrose and starch biosynthesis in leaves and tubers of long and short duration varieties of cassava.

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REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Cassava- an important starch crop

Cassava (*Manihot esculenta Crantz*) is an important food crop which belongs to the family of Euphorbiaceae. It is one of the five important starch crops *viz.,* rice, wheat, potato and maize. It is commonly known as cassava or tapioca. It is the third most important source of calories in the tropics, consumed by some 600 million people on a daily basis in Africa, Asia, and Latin America. It provides a cheap source of dietary carbohydrate energy (720.1 x 1012 KJ day^{-1}) ranking fourth after rice, sugarcane and maize, and sixth among crops in global production. In recent years, its commercialization increased markedly. "World market assessment revealed a 5% increase in cassava productivity and reached 291 million tonnes in 2014 (www.fao.org/giews; Food outlook, 2015)".

The cassava production in Asia has grown from 45.9 million tonnes in 1980 to 76.6 million tonnes in 2011 (Howeler, R.L, 2013). "According to the Horticulture statistics at 2015, the area of production of cassava was increased from 228000 ha to 253000 ha and the production also increased from 8139000 tonnes to 8542000 tonnes". In India, the major cassava producing states are Tamilnadu, Puducherry, Kerala, Punjab, Andaman Nicobar islands, Odisha, Andhra Pradesh, Nagaland, Mizoram, Karnataka, Assam, Meghalaya, and Lakshadweep. In India, cassava is grown mainly in Tamilnadu about 43.22 tonnes/ha. In Kerala, the production of cassava is 28.80 tonnes/ha.

Cassava is a drought tolerant staple food crop grown in tropical and subtropical areas of the World. It is grown by small holding farmers in more than 100 tropical and subtropical countries. Cassava may represent the future food security crop in some developing countries because it is drought tolerant and its mature storage root can maintain their nutritional value for a long time without water. As a crop it shows several good

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characteristics like stress tolerance, tolerance for limited soil-nutrient and, can be cultivated annually with harvesting time ranging between 7 to 12 months. It can be cultivated with other crops in intercropping systems.

2.2. Cassava production

The world cassava production reached to 289 million tonnes in 2015 just over 500000 tonnes more than 2014. "According to FAO estimates in 2015, Thailand is the Asia's largest producer of cassava and the production is about 34 million tonnes".

2.3. Cassava Physiology

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. It grows to a height about 2-4 m. It is a root crop belonging to section Fructicosae of family Euphorbiaceae, Dicotyledonae (Baguma, 2004). It originated from South America, most likely from Brazil. The tubers and leaves are the essential parts of the cassava.

2.3.1. Morphology

Cassava storage roots are long, tuberous and tapered with homogenous flesh encased in a detachable rind. Storage roots of commercial varieties can be 5cm to 10cm in diameter at the top and around 15cm to 30 cm long. The flesh can be white, cream or yellowish. Cassava has two types of roots; fibrous roots involved in nutrient uptake and thick storage roots containing high starch levels (up to 80% of dry weight). During the first two months, the cassava plant mainly develops shoot and a fine root system (fibrous root). Formation of storage root occurs during the 4^{th} week after planting. The starch deposition in storage

roots occurs at about 30- 40 days after planting. The leaves are large and palmate and it have five to nine lobes borne on a long slender petiole. Leaves are dark green above and light green below. Male and female flowers are arranged in loose plumes and are found on the same plant. The fruit is a globose capsule, 1-2 cm in diameter with six winged ribs. Each capsule contains three seeds.

2.3.2. Climatic requirements

The optimum conditions for high cassava productivity are mean day temperature of 28°C, atmospheric humidity of 70% and solar radiation of about 22MJ m^{-2} day^{-1} (El-Sharkawy, 2003). Since it is a typical tropical plant, it is most productive between 15 degrees North and 15 degrees South latitudes. In general, it requires a warm humid climate. The highest production can be expected in the tropical low lands, below an altitude of 150 m where temperature is between 25°C to 29 °C. Cassava requires fairly abundant rainfall of 1500mm, but it can be grown where the annual rainfall is as low as 500mm. Therefore, the plant can withstand prolonged period of drought than most other crops. This makes it a valuable cropin region where there is low rainfall. If moisture availability becomes low, the plant will stop its growth and shed older leaves and reduce the rate of respiration. When the moisture is available it will continue its growth and produce new leaves. The plant grows best on light sandy loams which are moist and fertile, but it also grows well on soils of relatively low fertility. In practice, it is grown on wide range of soils, provided the soil texture is friable enough to allow the development of tubers. Cassava can produce tubers where soils depleted by repeated cultivation to the extent that it is unsuitable for other crops.

2.3.3. Propagation

Cassava is vegetatively propagated from woody stem cuttings. They can be planted horizontally, vertically, or inclined on flat or ridged soils. Seeds are used mainly in breeding programs (El-Sharkawy, 2003).

2.3.4. Planting

The cassava is grown either as a single crop or may be intercropped with other crops. Mixed cropping reduces the danger of crop loss due to unfavourable conditions. For selection purpose cassava is grown from seed only. Seed produces plants with fewer and smaller tubers than that of parent plant and upto 50% of the seed may fail to germinate. It is planted at spacing of 90×90 cm.

2.3.5. Fertilizers

The application of fertilizers increases the yield of storage roots as well as starch content. Potassium salts favour the formation of starch while nitrogen and phosphorus favours growth. If the assimilated nitrogen in the soil increases, then there is a large increase in the production. Small scale farmers use different kinds of manures such as cattle or poultry manure. The kinds and quantities required by a plant depend on the soil nutrient status. The recommended dose of N: P: K is at 100: 50: 100 Kg per ha respectively.

2.3.6. Weed control

Weeding is required when the cassava plants are 20 to 25 cm tall that is four to six weeks after planting. Second time weeding is needed at two or three months after first weeding.

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2.3.7. Harvesting

Storage roots are generally harvested between the periods of seven to twelve months after planting depending on the variety. However, fresh roots need to be processed immediately due to rapid post-harvest physiological deterioration (PPD). This process happens within 24 -72h and after that storage root become unsavoury. PPD is a major drawback as harvested roots have a short shelf life. Hence, harvested storage roots are often quickly processed to chips or flour.

2.3.8. Leaf area

The leaf area determines the plant productivity (Weraduwage, *et al.*, 2015). Leaf area index is the ratio of total leaf area to land area. It reflects the changes in leaf number and size. In some cultivars, that have early branching habit and dense canopy, 90% of the land area is covered with foliage by 45 days after planting (DAP), while other cultivars, regardless of their branching habit, may take 75- 90 days to achieve the same foliage cover over the land area.

2.3.9. Storage root yield

Yield is one of the factors determining the deposition of starch in the storage organs. Starch yield varies between 5 and 17 t/ha when harvested 10-12 MAP. Storage roots contain more than 80% starch on a dry weight basis at temperatures less than 24˚C but starch content decreases at high temperatures.

2.4. Starch : an important raw material

Starch is the chief storage form of carbohydrates in plant tubers and seed endosperm where it is found as granules. It is an important raw material used for the diverse applications in the starch industry. It is used in the manufacture of paper, textiles, pharmaceuticals biodegradable polymers and as an additive in foods. Two kinds of starches can be distinguished in plants which fulfil different storage requirements. Transient starch in photosynthetic leaf undergoes a diurnal-nocturnal cycle of synthesis and degradation. In non-photosynthetic tissues (i.e. potato tubers, cassava storage roots, maize kernels) carbohydrate sucrose translocated from the photosynthetic tissue, imported into the amyloplast and converted to starch. Starch in amyloplasts is built-up and stored over a long-term period (Geigenberger, 2003., Sonnewald and Kossmann, 2013).

Cassava produces both transient and storage starch. Starch originating from different botanical sources behave in a different physico-chemical way. The tissue as well as the species it is extracted from defines the properties of starch. The granule size and the amylose to amylopectin ratio both contribute to defining the starch properties. In pharmaceutical industries, starch is used as a filling material in tablets, for which starch with a small granule size is ideal. In the food industry, starch is used as a binding agent in processed foods in addition to being a carbohydrate source. For this, starches with low amylose contents are often preferred for their stable gelling properties when heated in water (i.e. gelatinised). Native starches are often pre-treated either chemically or physically in order to improve or deliver the properties required by the various branches of the food industry. In the paper industry, starch is used as a coating agent.

2.5. Structure

Starch is a polysaccharide composed of simple sugar glucose. Starch is an inert polyglucan consist of two molecules, amylase (normally 20-30%) amylopectin (normally 70-80%). Amylose consisting of essentially linear α-1,4 linked glucose chains with a low proportion of α-1,6 linkages (branch points) thus glucose molecules are arranged in a linear fashion. The estimated molecular weight of amylose is 105 -106 Daltons (Perez and Bertoft, 2010). Amylopectin has an estimated molecular weight of 107 -109 Daltons (Yoo and Jane, 2002). It consists of high amount of α-1,6-branches and of shorter linear α-1,4 linked chains (Perez and Bertoft, 2010). The crystalline structure of starch granules is due to amylopectin. Each amylopectin molecule contains up to two million glucose residues. The molecules are oriented radially in the starch granule and as the radius increases, the number of branches fill up the space, with consequent formation of alternating amorphous and crystalline structure. Amorphous layers alternate with crystalline layer that contain mainly linear α-1,4 linked glucan chain segments. These linear chains form double helices then pack in an ordered pattern. This arrangement gives starch its insoluble, semi-crystalline properties. These alternating layers make up the starch granules can be viewed as a ring-like structure. Depending on the starch type and plant species difference in the formation of helices and their arrangement. The helix exists in either A-type or B-type crystallinity.

In an A-type starch, typically found in cereals, the helices are packed together densely in a monoclinic unit cell. In a B-type starch, characteristic for tuberous starch, is arranged in an open hexagonal way with water-filled space between the helices (Blennow and Engelsen, 2010). In some species, a mixture of A- and B-type packing is observed. This so-called C-type starch is found in pea and cassava (Damager *et al.*, 2010).

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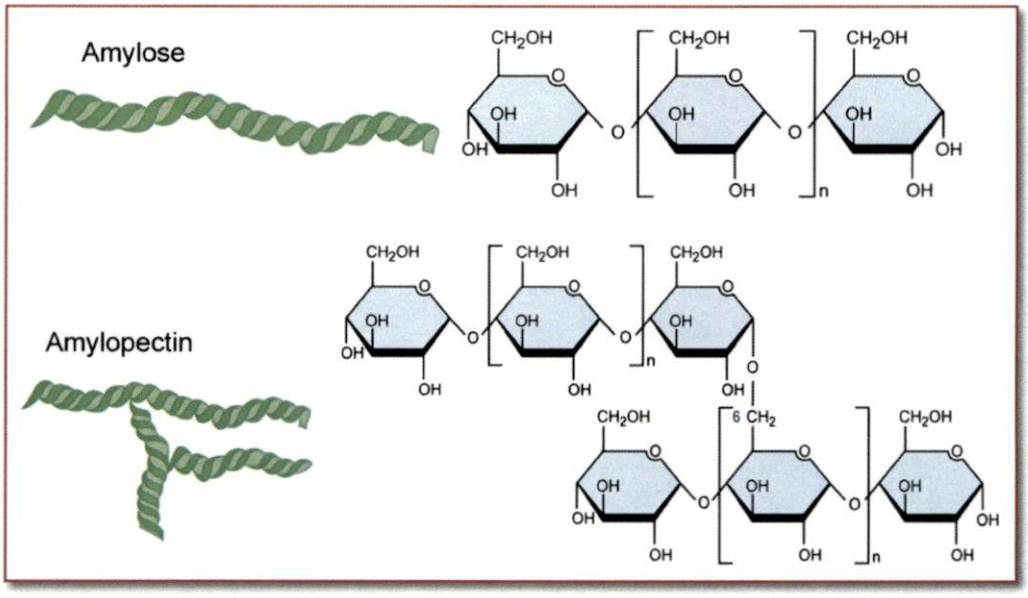

Fig.1. Structure of Amylose and Amylopectin

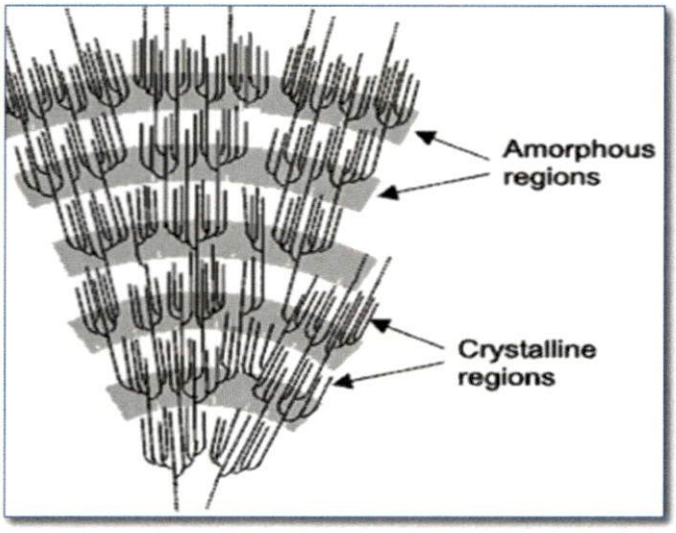

Fig.2. Structure of starch molecule

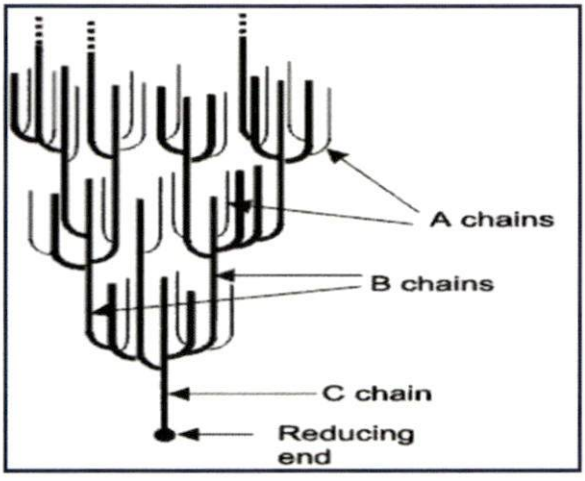

Fig.3. Helical structure of starch

2.6.Carbon assimilation and storage: from source to sink tissue

Photosynthesis is a process through which plants, algae and some prokaryotes directly use sunlight to synthesize organic compounds. This makes it the most important biological process on the earth because it provides biomass which is directly used as food by all living organisms. In higher plants, carbon assimilation during photosynthesis is followed by partitioning of newly fixed carbon within the cells. The process of carbon assimilation and partitioning is believed to be a major determinant of crop yield.

In higher plants, carbon synthesised during photosynthesis is transported mainly in the form of sucrose to other organs for their growth and development or to provide assimilates for the synthesis of storage compounds. Therefore, the organs in higher plants are classified into source and sink organs. Carbon fixed during photosynthesis is either utilized for the starch synthesis in the chloroplast for temporary storage (transient starch) and sucrose synthesis in the cytosol for export from the leaves.

2.7.Carbon assimilation by photosynthesis

Photosynthesis is the major source of energy for all plants living in sunlight. It is a highly regulated multistep process. It is through the process of photosynthesis plants convert energy from sunlight to produce sugar (Balino 2012). The light reactions synthesis ATP and NADPH while the dark reactions are the synthesis part of the photosynthesis which is involved in carbon fixation. Plants use light energy to produce glucose from carbon dioxide. The glucose is stored mainly in the form of starch granules, in plastids such as chloroplasts and amyloplasts. A series of enzymatic reactions involved in this carbon assimilation process (Tanaka and Makino, 2009).

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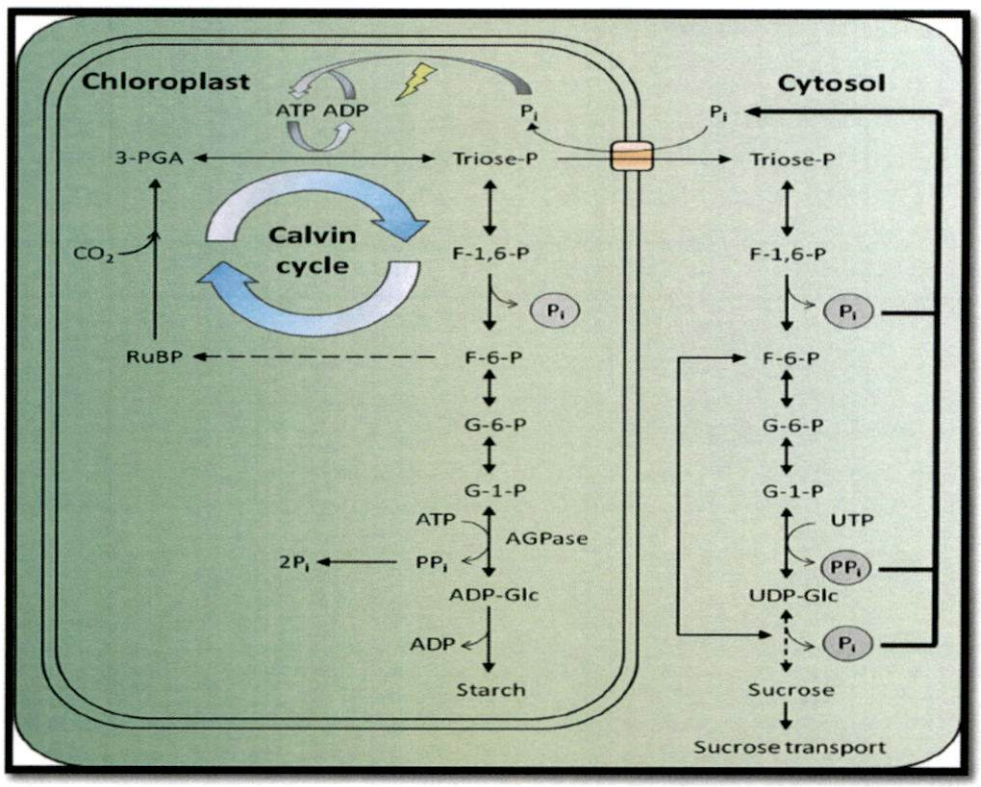

Fig.4. Carbon assimilation and storage

In the light reaction of photosynthesis, the water molecules are split and the NADP coenzyme picks up the freed hydrogen and electrons and transforms them into NADPH. This stage of photosynthesis takes place at the thylakoid membrane there found stacked disks called grana. Assimilated carbon is partitioned between starch and sucrose synthesis. Sucrose is the primary product, synthesized in the cytosol of leaves and exported to heterotrophic tissues via the phloem. Starch in the chloroplast is transiently stored during the day and degraded during the subsequent night, when no photosynthesis occurs. The extent of partitioning into starch depends on the need of the plant and on the species.

During day time, as the rate of sucrose synthesis much greater than the rate of translocation, the leaves sucrose is temporarily converted into starch as transient starch in leaves. During night, the transient starch is hydrolyzed into sucrose and translocated into sink tissues.

2.8. Starch biosynthesis in storage tissues

The overall pathway

The starch biosynthesis involves the conversion of sucrose into ADP glucose, and the subsequent conversion of this soluble precursor into insoluble polyglucan. The sucrose synthesised in the photosynthetic tissue is transpoted into the cytosol through sucrose transporter. Sucrose is converted into glucose and fructose by the invertase or fructose and UDPglucose (UDPG) by the Sucrose synthase enzyme (SuSy). At the same time Sucrose Phosphate Synthase (SPS) synthesize sucrose from the Frcutose 6 phosphate (F-6-P).

Then fructose and Glucose is converted into Glucose -1 - Phosphate (Glc-6-P) and it is converted into Glc-1-P by phopho glucomutase (PGM). Then it is converted into ADPglucose (ADPG), the reaction catalysed by ADP-glucose pyrophosphorylase. Later starch is

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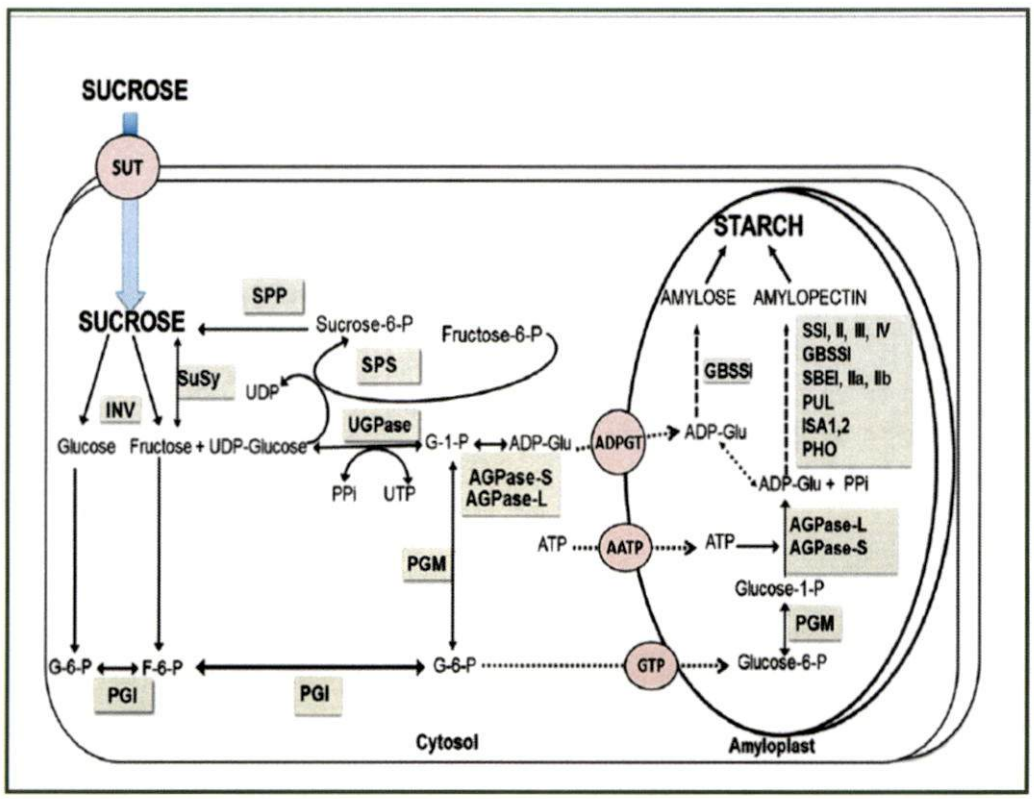


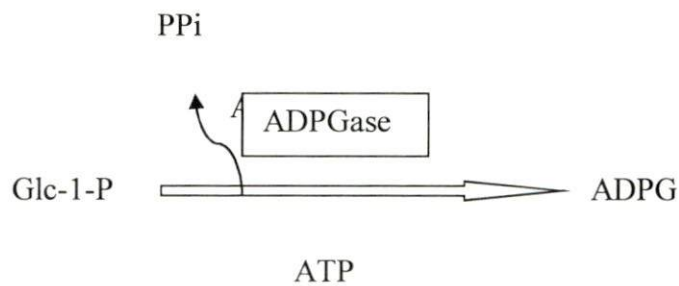
Fig.5. Starch biosynthesis in the Amyloplast

linearized by the enzyme Starch synthase (SS). Then Starch branching enzyme modifies the structure by providing branches. Thus starch synthesis occurs in the storage organs.

2.9. Enzymes involved in starch biosynthesis

2.9.1. ADP_Glucose pyrophosphorylase

AGPase catalyze the first committed step in the starch synthesis in the plastid. It converts Glc-1-P and ATP to ADP-glucose and PPi. ADP glucose is the subsequently used for starch biosynthesis.



AGPase assay was conducted in barley enosperm (Emes *et al.*, 2003) to study the pathway of ATP production, its subcellular compartmentation and the role of metabolite transporters in mediating its delivery to the site of starch synthesis.

Kolbe *et al.*, 2005 reported that AGPase catalyze the first commited step of starch biosynthesis in the plastid converting glucose-1-phosphate and ATP to ADP-glucose and PPi. ADP-glucose is subsequently used by starch synthases and branching enzymes to elongate the glucan chains of the starch granule. The study was conducted in potato tubers.

Changes in ADPG pyrophosphorylase during the development of pea seed and wheat grain revealed that maximum rate of starch synthesis coincided with the maximum activity of ADPG pyrophosphorylase (Turner, 1969). In developing pea seed and wheat grain, sucrose content

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and ADPG pyrophosphorylase activity declined in parallel with an increase in starch content towards maturation /later period of seed growth.

In potato tubers, starch synthesis decreased when AGPase activity decreased (Müller-Röber *et al.,* 1992; Geigenberger *et al.,* 1999). In potato tubers, the sucrose content correlated strongly with AGPase activity (Tiessen *et al.,* 2002).

Denyer *et.al.* 1996 reported that AGPase provides ADP glucose for starch synthesis. There is evidence that AGPase is exclusively plastidial. In the case of maize it was not clear. The study shows only a small amount of AGPase activity in maize endosperm is plastidial. Most of the activity is extraplastidial and is likely to be located in the cytosol.

Dickinson and Preiss, (1969) reported that AGPase was first found in the wheat flour. The presence and role of the enzyme was subsequently reported in other non-photosynthetic plant tissue. This study resulted that low amount of starch in the maize endosperm of the maize mutant is due to the deficiency of ADP-glucose pyrophosphorylase.

Smith *et al.* (1989) reported that the low activity of ADP glucose pyrophosphorylase in pea embryos account for their low starch contents. Activity of ADP glucose pyrophosphorylase done through AGPase assay. It was shown that during the development of embryos the maximum catalytic activity of this enzyme is considerably less than or only equal to the rate of starch synthesis in embryos. Thus, the low starch contents of embryos can be attributable to their low activities of ADP glucose pyrophosphorylase.

2.9.2. Starch synthase

SS catalyzes three processes in starch biosynthesis: initiation, chain elongation and branching (Preiss 1988., Baba *et al.,* 1993). It exists in two

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major forms: soluble SS(SSS) and granule bound SS (GBSS). Baba *et al.* (1993) conducted SS enzyme assay in rice and the study concentrated the identification and gene expression of soluble starch synthase.

Starch synthases (SS) involved in starch (amylase and / or amylopectin) biosynthesis exists in multiple forms in starch producing tissues. At least four distinct SS isoforms have been defined. All plants possess the granule bound isoform GBSSI, whereas the SSI, SSII, and SSIII isoforms are located either partially or entirely in the soluble phase and their occurrence in plant tissues may vary depending upon plant species (Myers et al., 2000). GBSS is primarily involved in synthesis of amylase whereas SS is involved in synthesis of amylopectin (Jeon *et al.*, 2010).

Denyer, *et al.* (1993) provided evidence that the major soluble SS II of wild type pea embryos is the same protein as the major granule bound starch synthase, GBSSII. SSSII and GBSSII are of the same molecular mass and are strong antigenically related.

The SS isozymes in higher plants such as rice are divided into five isozyme types: SSI, SSII, SSIII, SSIV, and GBSS (Hirose and Terao 2004., Fujita, *et al.*, 2006).

Edwards *et al.* (1999) studied interactions between roles of starch synthases during the synthesis of storage starch. It was reported that multiple isoform of starch synthase were occurred in storage organs and it can be divided into distinct classes on the basis of similarities in their primary amino acid sequences. The isoforms of starch synthase II is similar in sequence to each other than to other isoforms, have been reported from pea, potato, cassava and maize (Dry *et al.*, 1992., Marshall *et al.*, 1996., Munyikwa *et al.*, 1997., Harn *et al.*, 1998., Edwards *et al.*, 1999).

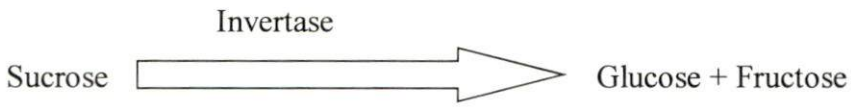
Studies of pea, maize and *Chlamydomonas* mutants show that the loss of specific isoforms of starch synthase results in changes in the branch-length distribution of amylopectin, thus understood that these

isoforms make distinct contributions to amylopectin synthesis (Craig *et al.,* 1998).

It was studied that the starch synthase activity in the soluble fraction of potato tubers is contributed by two isoforms, SSII and SSIII, which account for about 10–15% and 80% of the activity respectively (Abel *et al.,* 1996., Edward *et al.,* 1995., Marshall *et al.,* 1996). The study concluded that different isoforms of starch synthase make distinct contributions to the synthesis of amylopectin in potato tubers. Although the rate of starch accumulation is unaffected by reductions in either SSII or SSIII, both reductions have marked and had distinct effects on starch structure and properties.

2.9.3. Invertase

Invertase enzyme hydrolyzes sucrose and related sugars thereby providing hexoses (glucose and fructose) which can be utilized for the energy and carbon requirements of the cell.



Early research suggested that invertase was the key enzyme regulating sucrose accumulation. Later research investigated that other enzymes such as SuSy and SPS play an important role in the regulation of sucrose accumulation.

Invertase enzymes are key regulators of accumulation of Suc in sugarcane stem storage parenchyma (Zhu *et al.,* 1997., Hatch and Glasziou 1963., Sacher *et al.,* 1963., Gayler and Glasziou, 1972).

It was reported that varieties within the Saccharum group that retain high levels of SAI activity in mature stem storage tissue do not store high levels of Suc (Zhu, *et al.,* 1997., Hatch and Glasziou, 1963).

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Zhu et al. (1997) identified that clones of saccharum had lowest activity of sucrose acid invertase associated with high levels of sucrose in the mature internodes and highest activities of SAI associated with low levels of sucrose in the immature sugarcane stem internodes.

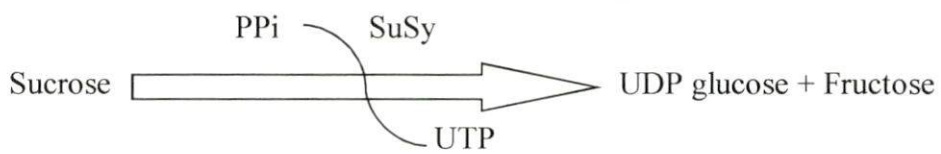
It was found that SPS activity can be correlated with invertase activity. The Sucrose synthesis enzymes SuSy and SPS were not by themselves correlated with sucrose accumulation. Sucrose acid invertase can be significantly correlated with sucrose concentration.

Matsushita and Uritani (1974) reported that in sweet potato root tissue, the increase in respiratory activity in response to wounding is accompanied by decrease in sucrose content. Increase in acid invertase activity resulted in decrease in sucrose content. In cut tissue, there was a disappearance in rate of sucrose content and invertase activity was sufficient.

High invertase activity in potato tubers reduced sucrose content over 95% and significantly increased glucose, increase in the metabolic intermediates of glycolysis, catalytic activity of enzymes in respiratory pathway, CO_2 production and a reduction in starch biosynthesis suggesting that invertase diverts sucrose towards glycolytic pathway (Trethway *et al.*, 1998).

2.9.4. Sucrose synthase

Sucrose degradation in plants through two irreversible hydrolysis: first one is sucrose is degraded into glucose and fructose by invertase and second one is unique to plants and involves UDP –dependent cleavage of sucrose into UDP glucose and fructose catalyzed by sucrose synthase (Bologa, 2003., Huber and Akazawa, 1986., Kruger, 1997).



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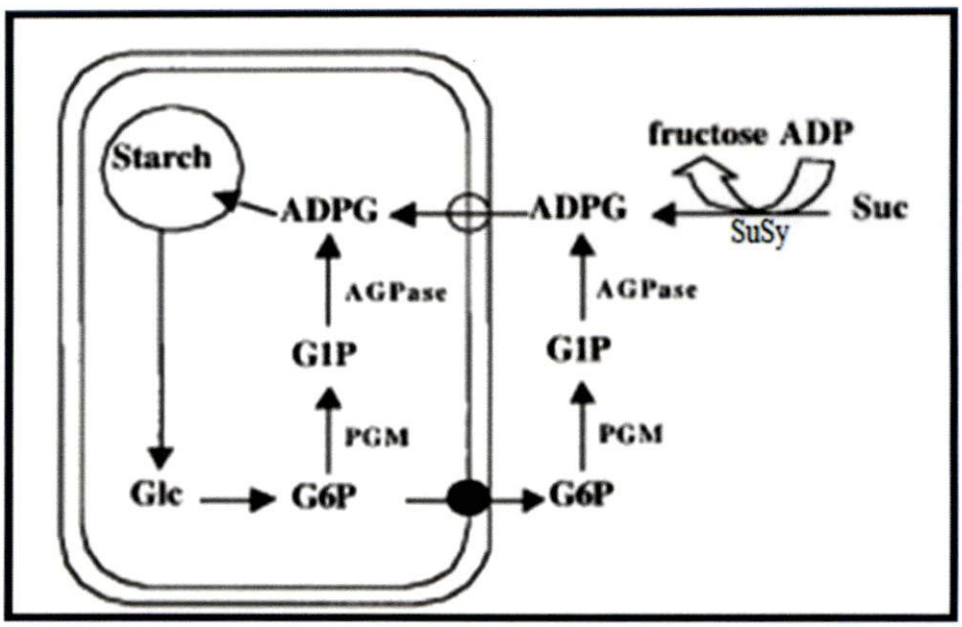

Fig.6. SuSy involved in the direct conversion of sucrose to ADPG

Bologa (2003) investigated that sucrose degradation through SuSy allows maintenance of increased oxygen levels and improved storage metabolism in tubers. It was found that the overexpression of invertase or Suc phosphorylase to bypass the energetically less expensive SuSy route leads to a strong decrease in internal oxygen tensions in growing tubers, a marked decrease in their energy state, and an inhibition of starch synthesis.

Sucrose synthase (SuSy) activity consistently remained much greater than the invertase activity which did not change appreciably during endosperm development in wheat (Riffkin *et al.,* 1995).

In musk melon increasing sucrose concentration may be due to increased sucrose synthase activity accompanied by decrease in acid invertase activity during fruit growth. It was also studied that there is an inverse relation between invertase and sucrose accumulation in fruits as well as sink tissues such as roots of carrots and sugar beet. The reduction in acid invertase would be essential for sucrose accumulation (Hubbard, 1989., Shaffer *et al.,* 1987., McCollum *et al.*, 1996).

Baroja-Fernández (2003) was conducted SuSy assay in barley seed and potato tubers and explored that the extent of SuSy responsible for the direct conversion of sucrose to ADPG linked to starch biosynthesis in heterotrophic tissues. The synthesis of ADPG by two different mechanisms: (1) denovo catalysis by SS using the newly imported Suc (2) scavenging glucose units derived from starch breakdown.

2.9.5. Sucrose Phosphate Synthase

Sucrose Phosphate synthase is a key enzyme in the regulation of sucrose synthesis (Geigenberger, 1999., Huber and Huber, 1992., Huber and Huber, 1996., Huber *et al.,* 1992., Stitt *et al.,* 1987). SPS is also present in growing tissues, including potato tubers (Geigenberger and Stitt, 1993; Geigenberger *et al.* 1997), sugar cane stems (Zhu *et al.* 1997), heterotrophic Ricinus cotyledons (Geigenberger and Stitt, 1991), kiwi

fruits (MacRae *et al.* 1992) and ripening bananas (Hubbard *et al.* 1990). SPS is involved in a regulatory cycle in which sucrose is simultaneously degraded and resynthesised (Geigenberger et al., 1997). This cycle had been shown by potato tubers and other plant tissues.

In musk melon fruit, the SPS activity was increased during the ripening and the increase was generally correlated with the sucrose accumulation. The study investigated the metabolic changes associated with the accumulation of sucrose within the developing musk melon fruit (Hubbard, 1989).

Huber (1983) investigated the variation in leaf starch accumulation were observed among four species wheat, soybean, tobacco and red beet. It was found that the activity of SPS in leaf extracts was correlated negatively with leaf starch accumulation but positively with leaf sucrose content. It was also reported that leaf sucrose phosphate synthase activity was significantly increased by the low-temperature treatment (Guy, 1992).

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MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "Photosynthesis and enzyme activities regulating starch biosynthesis in different varieties of cassava (*Manihot esculenta* Crantz) was conducted at the ICAR-Central Tuber Crops Research Institute during October 2015 to June 2016. Details regarding the experimental materials used and methodology adopted for various experiments are presented in this chapter.

3.1. Cultivation of Cassava

Four varieties of Cassava (*Manihot esculenta* Crantz) viz., Sree Vijaya, Sree Athulya, H165 and H226 were cultivated in the field with three replications each consisting of 25 plants each. The crop was planted and grown under irrigated field conditions in Block I of Central Tuber Crops Research Institute (CTCRI) during October 2015.

3.2. Sample Collection

Fresh Cassava leaves and tubers were harvested for studying the activity of enzymes involved in regulating the starch biosynthesis. Cassava leaves of different varieties were collected and kept in hot air oven for drying at 75°C for 2-3 weeks. Tubers were collected, washed in tap water and sliced into small cube shaped. Then keep for drying in hot air oven at 75°C for 2-3 weeks. After drying, the leaf and tuber samples were powdered using homogenizer. These powdered samples were used for the starch and sucrose estimation.

3.3. Morphological Parameters

3.3.1. Number of leaves per plant

Cassava (*Manihot esculenta* Crantz) varieties viz., Sree Vijaya, Sree Athulya, H165 and H226 leaves were counted from 2nd month to 8th month after planting (November to June).

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Plate.1. Field View of Experimental Plot

3.3.2. Leaf Area (cm^2) per plant

The Leaf Area of four varieties viz., Sree Vijaya, Sree Athulya, H165 and H226 were calculated from 2nd month to 5th month after planting. It can be calculated as:

Leaf area =l×b×0.44×no: of lobes×total no. of leaves/plant

Where,

l = length of the leaf

b = breadth of the leaf

3.3.3. Tuber yield

The tuber yield was recorded after the harvest of four varieties of cassava at 8th months after planting.

Biochemical parameters

3.3.4. Quantitative estimation of sucrose

Sucrose estimation of leaf and tuber samples were done by the Dinitrosalicylic method (Wang, 1993 Sadasivam and Manickam, 1992). It is a colorimetric reaction test for the presence of reducing sugars present in the sample.

3.3.1.1.Extraction of sugar

- Weigh 250mg powdered leaf and tuber sample.
- For leaf samples 80% acetone was added for removing the chlorophyll content by kept in boiling water bath for 15 min.
- The supernatant (green coloured) was decanted and repeated the above step for twice.
- 10ml of 80% ethanol was added to the residue and kept in boiling water bath until most of the alcohol is evaporated.

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- The ethanol extraction of sugar was repeated thrice.
- Pooled the sugar solution and made up to 25ml with distilled water in a standard flask.
- The above procedure was done for tuber samples, except the acetone treatment.

Estimation of sugar

For hydrolysis of sugar

- To 1ml of extracted sugar taken in a test tube, 20µl of 11.9 N HCl was added.
- The test tube containing sugar were incubated at 90°C for 5min.
- 50µl 5N KOH was added to stop the reaction and follow DNS method.

DNS Method

- Hydrolysed sample was made up to 3ml with distilled water and 3ml DNS reagent was added to it.
- Incubated at 90°C for 5min.
- After an orange brown colour developed, 1ml of 40% Rochelle salt solution was added (the contents are still warm).
- The content was mixed well.
- Cooled the test tubes under running tap water
- Measured the absorbance at 510 nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer. (Three replicate were taken for each sample).
- Also read the absorbance of non hydrolysed sample.
- The sucrose content can be calculated by taking difference in absorbance of hydrolysed and non hydrolysed sample using glucose standard curve.

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3.3.2. Quantitative Estimation of Starch

The starch content was estimated according to the method of Yoshida *et al.,* (1976). The residue left in the tubes after alcoholic extraction of sucrose estimation was used for determining the starch content. The dried residue was treated with Perchloric acid and the starch was hydrolysed into simple sugars. This sugar was estimated by the method of phenol sulphuric acid. Simple sugars give an orange yellow colour when treated with phenol and conc.H_2SO_4. The reaction is sensitive and colour is stable, and the OD was recorded. The estimated sugar is then converted into starch content by multiplying with the factor 0.9.

3.3.2.1. Standard curve

- Standard glucose: 100 mg glucose was dissolved in 100 ml distilled water.
- Working standard: 100 ml stock was diluted to 100 ml with distilled water.
- Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard into a series of test tubes. Made up the volume in each tubes to 1ml with distilled H_2O.
- Set a blank with 1 ml of distilled water.
- 1ml of 5% Phenol was added to each tube.
- After that 5 ml of 96% Sulphuric acid was added gently to the sample and shake well.
- The tubes were incubated at Room temperature for 20 min for cooling.
- The orange colour was developed
- Read the absorbance at 490nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer.
- Calculated the amount of total carbohydrate present in the sample solution using the standard graph.

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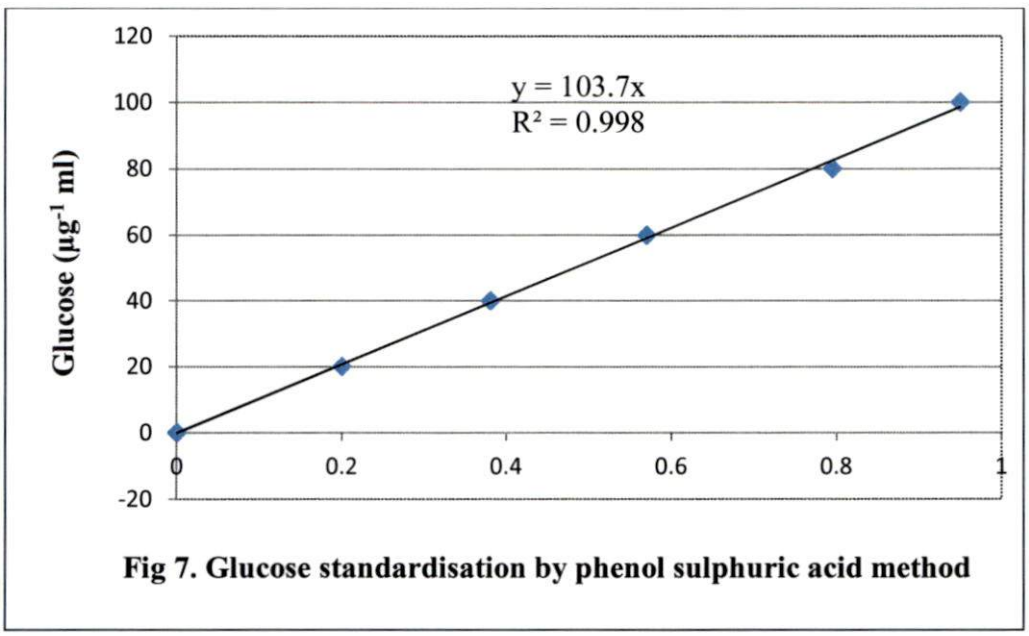

Fig 7. Glucose standardisation by phenol sulphuric acid method

3.3.2.2. Starch Estimation

- Dried the residue left in the centrifuge tubes after alcoholic extraction of sugars.
- Grind the dried residue with 5ml dis. H_2O using mortar and pestle.
- Placed the tube in boiling water bath for 15 min and allowed to cool.
- Then 2ml of 9.2 N Perchloric acid was added to each sample and incubated for 15 min.
- Suspension was made up to 10ml with distilled water.
- Centrifuged at 10,000 rpm for 10 min.
- Removed the pellet and collected supernatant in a 25ml standard flask.
- Then added 2ml of 4.6 N Perchloric acid to the pellet and kept for 15 min and stir occasionally.
- Suspension was diluted to 10ml with distilled water and again centrifuged at 10,000 rpm for 10 min.
- Combined the supernatants and made up the volume to 25ml with distilled water in a standard flask.
- The sugar content was analysed by the method according to Dubois *et al.* (1956). Three replicates were taken for each sample.

3.4. Assay of Enzymes involved in Starch Biosynthesis

3.4.1. ADP Glucose pyrophophorylase (EC 2.7.7.27)

The AGPase enzyme assay was done by the method of Nakamura *et al.* (1989).

3.4.1.1. Enzyme extraction

- Weighed 0.5 g fresh leaf and tuber samples.
- Grind in pestle and mortar with 1.5 ml AGPase extraction buffer.
- Centrifugation was done at 10,000rpm for 10 min at 4°C.

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- The supernatant was collected and stored at -20°C as the crude extracts of AGPase and starch synthase.

3.4.1.2. Enzyme assay

- For AGPase assay, 20µl of enzyme preparation was mixed with 110µl of Reaction buffer taken in an Eppendorff tube.

- Allowed to Incubate at 30°C for 20 min.
- Reaction was terminated by incubating for 30s in a 100°C waterbath.
- The reaction solution was cooled rapidly in ice waterbath.
- Centrifuged the mixture at 10,000 rpm for 10 min and transferred the supernatant in eppendorff tube.
- 100µl of supernatant was transferred to another eppendorff tube and added 5.2 µl $NADP^+$ and mixed thoroughly.
- 1µl of Phosphoglucomutase (0.4 units) and Glucose-6-phosphodehydrogenase (0.35 units) were added to the reaction medium.
- Glucose-1-phosphate production was measured by the increasing absorbance at 340nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer. Three aliquots from each sample were used for absorbance reading.
- The enzyme activity was expressed as Units/ min/100g fresh weight.
  (1 unit = change in absorbance at 340 nm by 100g fresh tissue/ min)

3.4.2. Starch synthase (EC 2.4.1.21)

SS activity was assayed by the method of Nakamura *et al.* (1989). The same enzyme extraction procedure of AGPase enzyme was done for starch synthase enzyme.

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Enzyme assay

- For enzyme assay, 20µl enzyme extract was mixed with 36µl of reaction buffer I taken in an eppendorff tube.
- Incubated for 20 min at 30°C to start the reaction.
- Mixture was placed in a boiling water bath for 30 Sec.
- Cooled rapidly by placed in ice water bath.
- Mixed the solution with 100µl of Reaction buffer II.
- Incubated the contents at 30°C for 20 min in water bath
- Reaction terminated by treated with 100°C for 30s.
- Cooled to 0°C in an ice waterbath.
- Then subjected to centrifugation at 10,000 rpm for 5 min at 4°C.
- From this 60µl supernatant was mixed with 43µl of Reaction buffer III
- Allowed to react for 10 min in 30°C water bath.
- Then 1µl of Hexokinase (1.4 units) and 1µl of Glucose-6-phosphodehydrogenase (0.35 units) were added to the above mixture.
- The enzymatic activity was measured as the increasing absorbance at 340 nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer. Three replicates were taken for absorbance reading.

3.4.3. Invertase (EC 3.2.1.26)

The Invertase assay was carried out as described by Tsai *et al.*, (1970) and Sadasivam and Manickam (1992). Glucose liberated by the hydrolytic activity of invertase on sucrose was measured colorimetrically.

3.4.3.1. Standard curve

- 25 mg BSA was dissolved in 0.15M NaCl and make up the volume to 25ml with distilled water (1mg/ml) (Standard protein solution).
- Pipette out 0.01, 0.02, and 0.04 to 0.1ml of standard protein solution into a series of test tubes.

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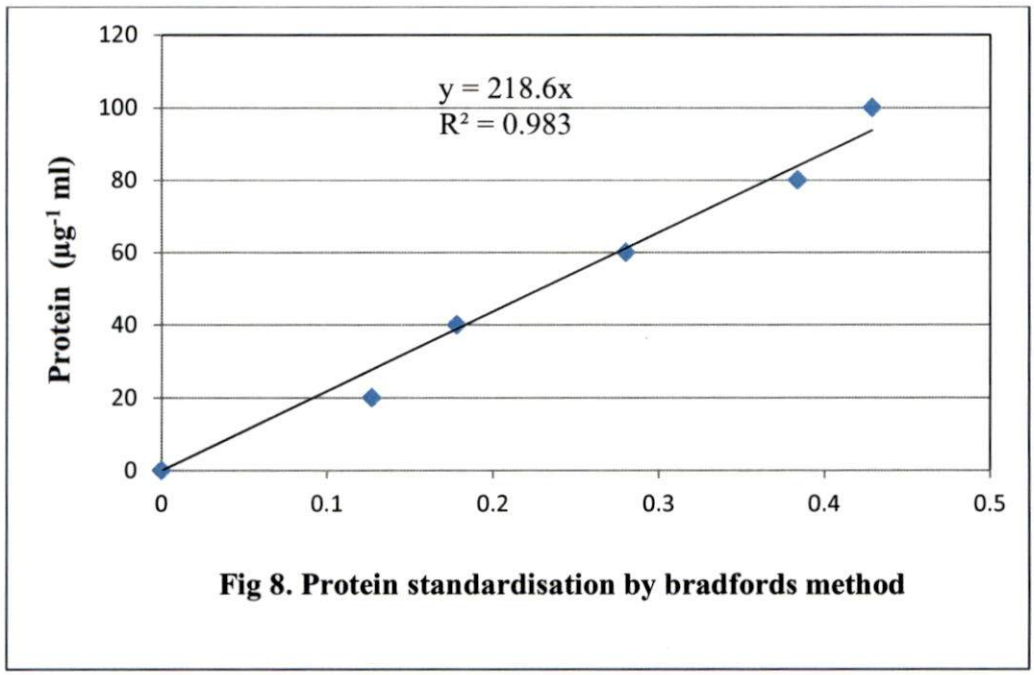

Fig 8. Protein standardisation by bradfords method

- Made up the volume in each tube to 0.1ml with 0.1M phosphate buffer (pH-7.4). 0.1ml buffer alone served as the blank.
- 5ml of 0.01% protein reagent was added and mixed thoroughly by inversion.
- The absorbance was measured at 595nm using a Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer.

3.4.3.2. Enzyme extraction

- Weigh 0.5g of fresh leaf and tuber samples of four varieties of cassava
- Homogenized with 10 ml of 20% glycerol using mortar and pestle.
- The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C.
- Collected the supernatant and made up the volume to 25ml with 20% glycerol and used for enzyme assay.

3.4.3.3. Enzyme assay

- For the enzyme assay, pipette out 5ml enzyme solution , 10ml sodium acetate buffer and 5ml of 5% sucrose solution in a beaker.
- Incubate at 37°C for 24 hrs.
- After incubation, 1ml of reaction mixture was taken and the reaction terminated by adding 1ml DNS reagent.
- Then reducing sugar present in the reaction mixture was estimated by method of Dubois *et al.* (1956).
- Protein content in the enzyme extract was determined by protein standard curve from the method of Bradford (1976).
- Expressed the enzyme activity as mg glucose released/hr/mg protein.

3.4.4. Sucrose synthase Enzyme (EC. 2.4.1.13)

Susy activity was measured by the method of Wardlaw *et al.*, (1988).

Enzyme extraction

- Weighed 0.5g of fresh leaf and tubers of four varieties of cassava.

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- Homogenized with 8ml Extraction buffer.
- Centrifuged the sample at 10,000 rpm for 10 min at 4°C.
- Supernatant was collected and used for measuring SuSy activity.

3.4.4.1. Enzyme assay

- 90µl of enzyme preparation was taken in test tube.
- The reaction medium containing 50µl of 200mM Hepes-NaoH (pH-7.5), 20µl of 5mM $Mgcl_2$, 20µl of 100mM UDP-Glucose and 20µl of 50mM fructose were added and the contents mixed thoroughly.
- Incubated at 30°C for 30 min.
- After incubation, the reaction was terminated by adding 200µl of 1N NaoH.
- Boiled the contents for 10 min and 0.5 ml of 1% resorcinol and 1.5 ml of 30% HCl were added and incubated the contents at 80°C for 8 min.
- Reaction was stopped by keeping on ice.
- Measured the absorbance at 520nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer. Three replicates of each samples were taken for reading.
- Expressed the enzyme activity as mg of sucrose synthesized/mg fresh weight of leaf or tuber using pre-established standards.

3.4.5. Sucrose phosphate synthase (EC 2.4.1.13)

Activity of SPS was measured according to the method of Wardlaw *et al.* (1988). The enzyme extraction procedure of SPS was same as that of SuSy.

3.4.5.1. Enzyme assay

- 90µl of enzyme preparation was taken in test tube.
- The reaction medium containing 50µl of 200mM Hepes-NaoH (pH-7.5), 20µl of 5mM $Mgcl_2$, 20µl of 100mM UDP-Glucose and 20µl of 50mM fructose were added and the contents mixed thoroughly.

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- Incubated at 30°C for 30 min.
- After incubation, the reaction was terminated by adding 200µl of 1N NaoH and boiled the contents for 10 min.
- 0.5 ml of 1% resorcinol and 1.5 ml of 30% HCl were added and incubated the contents at 80°C for 8 min.
- Reaction was stopped by keeping on ice.
- Measured the absorbance at 520nm using Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer. Three replicates of each sample were used for reading OD.
- The enzyme activity was represented as mg of sucrose synthesized/mg fresh weight of leaf or tuber using pre-established standards.

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RESULTS

4. RESULTS

The present study entitled the "Photosynthesis and enzyme activity regulating starch biosynthesis in different varieties of cassava (*Manihot esculenta* Crantz)" was conducted during the period 2015-2016 at the Division of Crop Production, Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram and the Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The results obtained from the study are summarized below.

4.1. Morphological parameter

4.1.1. Changes in the number of leaves of four varieties of cassava during the growth period

The no. of leaves were recorded monthly for the four varieties of cassava *viz.*, SreeVijaya, H165, Sree Athulya, and H226 and the leaf number was compared among different varieties (Fig 9). The no. of leaves per plant was steadily increases between the 2nd and 8th month. The maximum mean no: of leaves was observed in the variety Sree Vijaya (194.57± 20.63) and minimum mean no: of leaves was observed in the variety H226 (97.28±35.07). The mean no. of leaves over the period of 8 months of four varieties was given in (table 1).

Variety/Genotype	No. of leaves
SreeVijaya	194.57±20.63
H165	99.85±31.12
Sree Athulya	100.57±25.17
H226	97.28±35.07

Table.1. Mean number of leaves per plant over the period of 8 months of varieties of cassava

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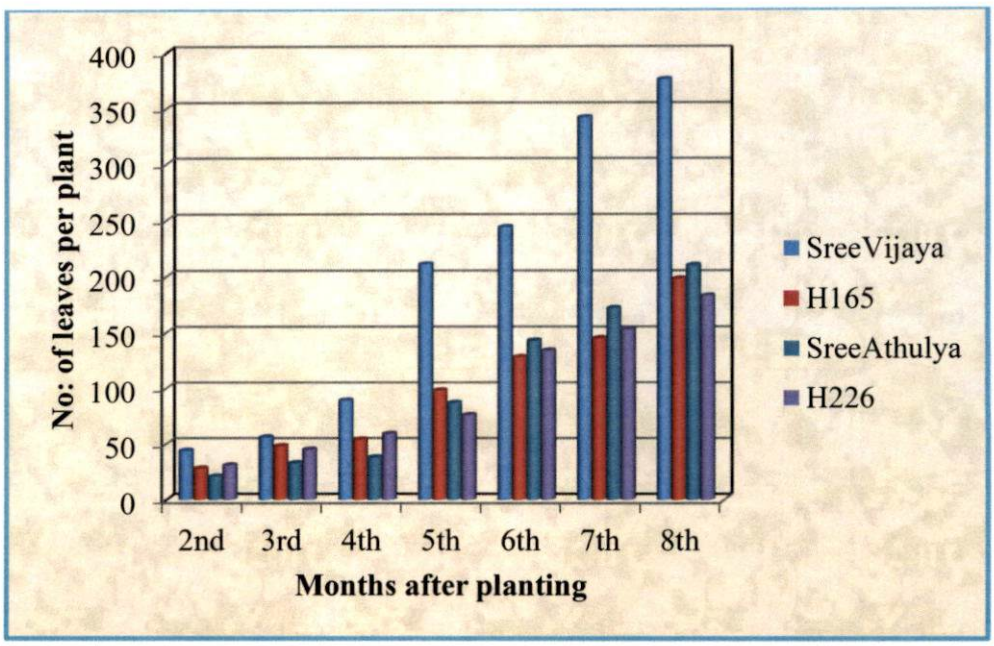

Fig.9. Changes in the no: of leaves of four varieties of cassava

4.1.2. Changes in the leaf area of four varieties of cassava during the growth period

Leaf area of four varieties of cassava was recorded (Fig 10) and the highest mean value for leaf area was observed in Sree Vijaya (59197.51cm²) and the lowest leaf area was found in the variety H165 (34753.66cm²).

Leaf area index of four varieties was calculated (Fig 11) and the maximum mean LAI was observed in SreeVijaya (7.28cm) and lowest mean value was observed in the variety H165 (4.00cm) (Table 2).

Variety	LA (cm²)	LAI
SreeVijaya	59197.51±93.73cm²	7.28cm
H165	34753.66±109.42cm²	4.00cm
Sree Athulya	42521.72±95.96cm²	4.89cm
H226	37501.56±155.23cm²	4.6cm

Table. 2. Mean value for LA and LAI of Cassava varieties

4.1.3. Changes in the tuber yield of four varieties of cassava

Four varieties of cassava were harvested at the end of the growth period. Tubers were collected and weighed. The yield was expressed in kg (Table 3). The tuber yield was highest in H226 with 3.623±0.26 kg^{-1} plant and lowest yield in H165 with 1.318±0.41 kg^{-1} plant.

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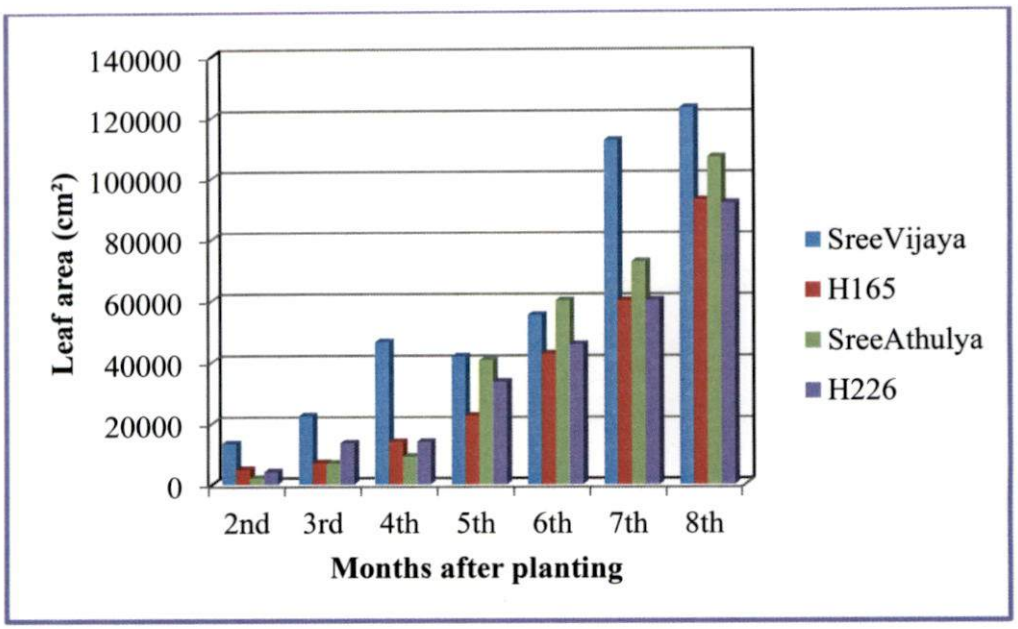

Fig.10. Changes in leaf area per plant of four varieties of cassava during the growth period

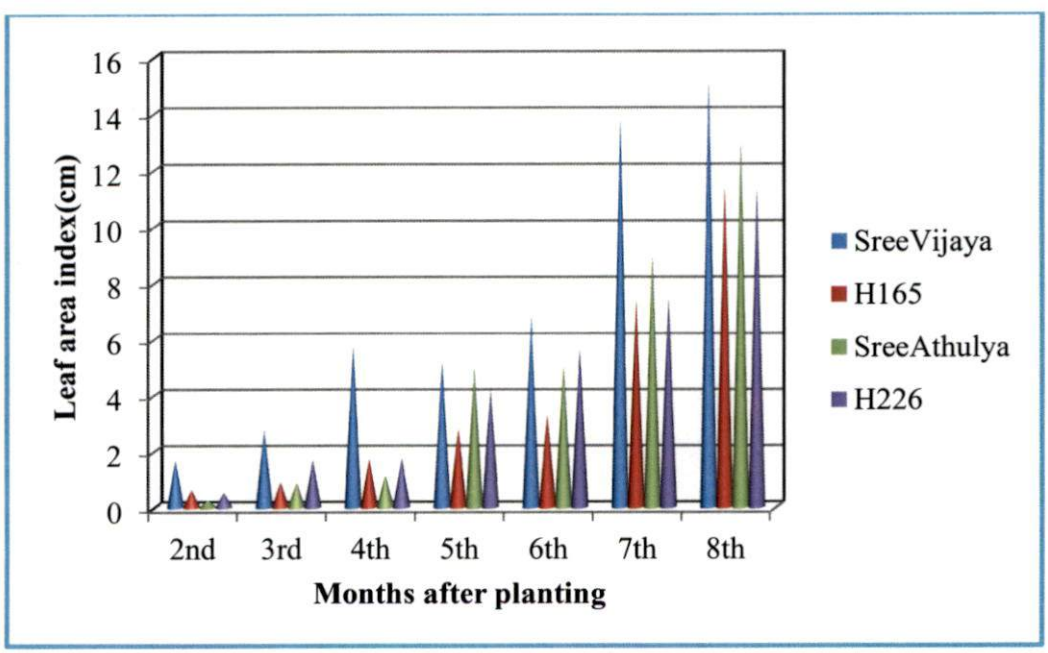

Fig.11. Changes in leaf area index per plant of four varieties of cassava during the growth period

Sree Vijaya

H165

Sree Athulya

H226

Plate.2. Tubers of four varieties of cassava *viz.*, Sree Vijaya, H165, Sree Athulya, H226

Variety Name	Tuber Yield/Plant(Kg)
Sree Vijaya	1.96 ±0.12Kg
H165	1.318±0.41 Kg
SreeAthulya	3.31 ±0.39Kg
H226	3.623±0.26 Kg

Table.3. Changes in tuber yield of four varieties of cassava

4.2. Biochemical Parameters

4.2.1. Changes in sucrose content in leaf and tubers of four varieties of cassava

The sucrose content in the tubers of four varieties of cassava was recorded monthly. The sucrose content in leaves and in tubers are represented in Fig 12 and Fig 13 respectively. The highest mean sucrose content was found in the leaves of SreeAthulya (2.5±0.093) and the lowest in SreeVijaya (1.63±0.08).

In tuber samples, the maximum mean sucrose content was observed in the variety SreeAthulya (3.6±0.091) and H165 (2.7±0.131) with lowest mean sucrose (Table 4).

Variety	Sucrose content (mg^{-1} 100 mg^{-1}) dry weight	
	Leaf	Tuber
SreeVijaya	1.63±0.089	3.32±0.105
H165	1.84±0.039	2.7±0.131
Sree Athulya	2.51±0.093	3.6±0.091
H226	2.12±0.102	3.06±0.081

63

Table. 4. Mean sucrose content of cassava varieties

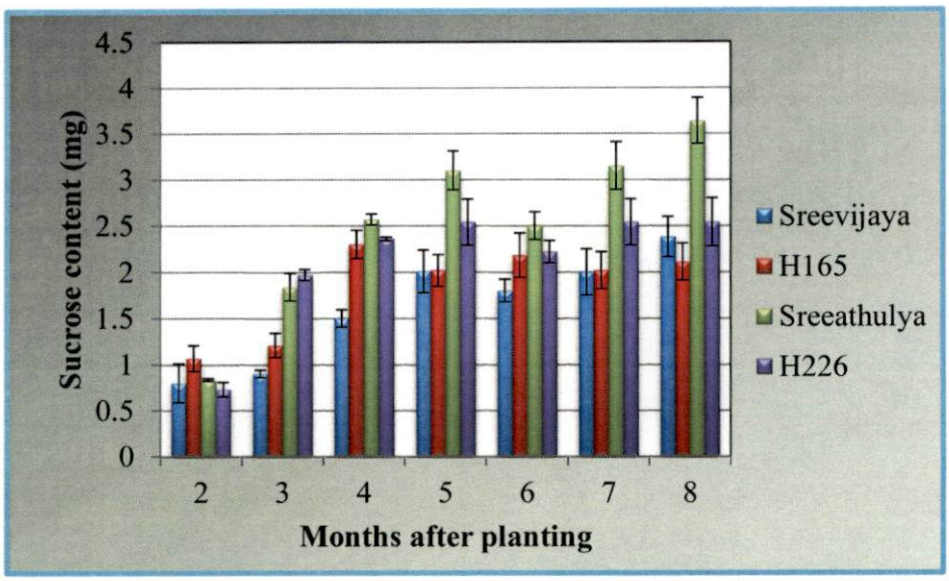

Fig. 12. Changes in the sucrose content in the dry leaves of four varieties of cassava during the growth period

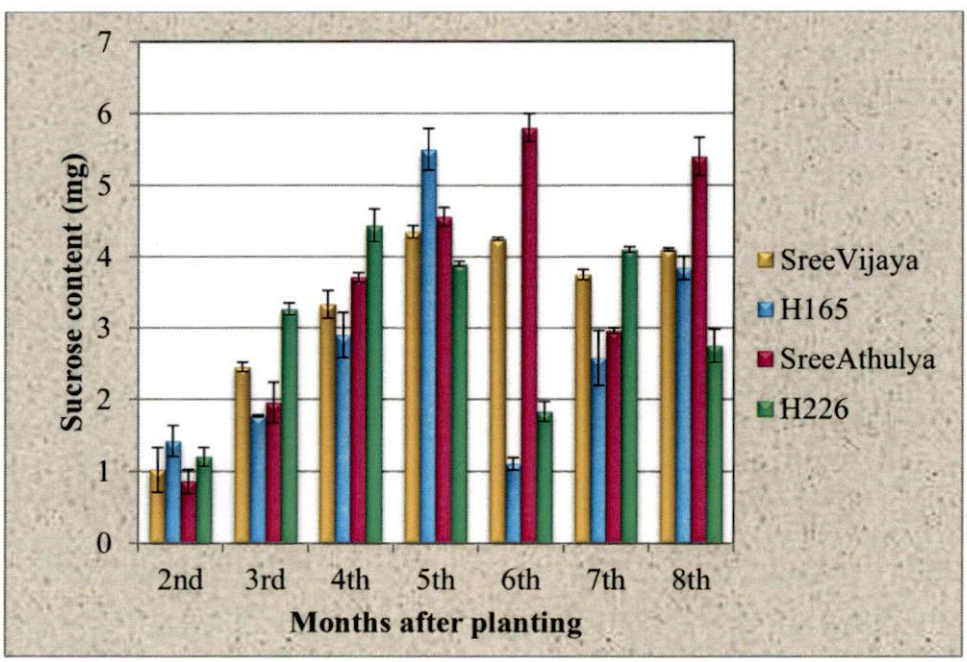

Fig. 13. Changes in the sucrose content in the dry tubers of four varieties of cassava during the growth period

4.2.2. Changes in percentage of starch in leaves and tubers of four varieties of cassava during the growth period

The starch content in the leaves (Fig14) and in the tubers (Fig15) of all the four varieties were recorded at monthly intervals. The highest starch content was found in SreeAthulya (3.5% ± 0.10) and the lowest in H165 (2.52% ± 0.049) (Table 5).

In tuber samples, the starch content was higher in SreeAthulya (29.10%±0.06) and lowest in the variety H165 (22.12% ± 0.05). By comparing leaves and tubers, the starch content was maximum in tubers.

Variety	Starch content (%)	
	Leaf	Tuber
SreeVijaya	3.09±0.10	23.80±0.06
H165	2.52±0.049	22.12±0.09
Sree Athulya	3.1±0.079	29.10±0.05
H226	2.6±0.069	26.44±0.11

Table.5. Mean starch content of cassava varieties

4.3. Measurement of photosynthetic activity

4.3.1. Changes in photosynthetic activity of all the four varieties of cassava

Photosynthetic rate in leaves was measured using portable photosynthetic analyzer (Fig 16). The highest photosynthetic rate was observed in SreeVijaya (21.59±0.15 CO_2 $\mu mol^{-1} m^{2-1} s$) and the lowest rate in the H226 (19.45±0.19CO_2 μmol^{-1} $m^{2-1}s$) (Table 6).

65

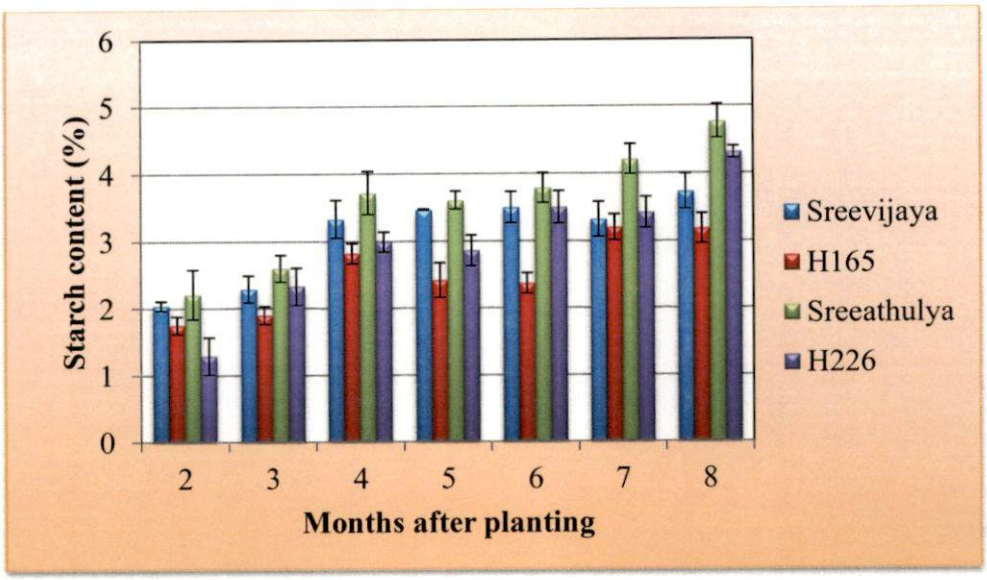

Fig.14. Changes in starch content in dry leaves of cassava during the growth period

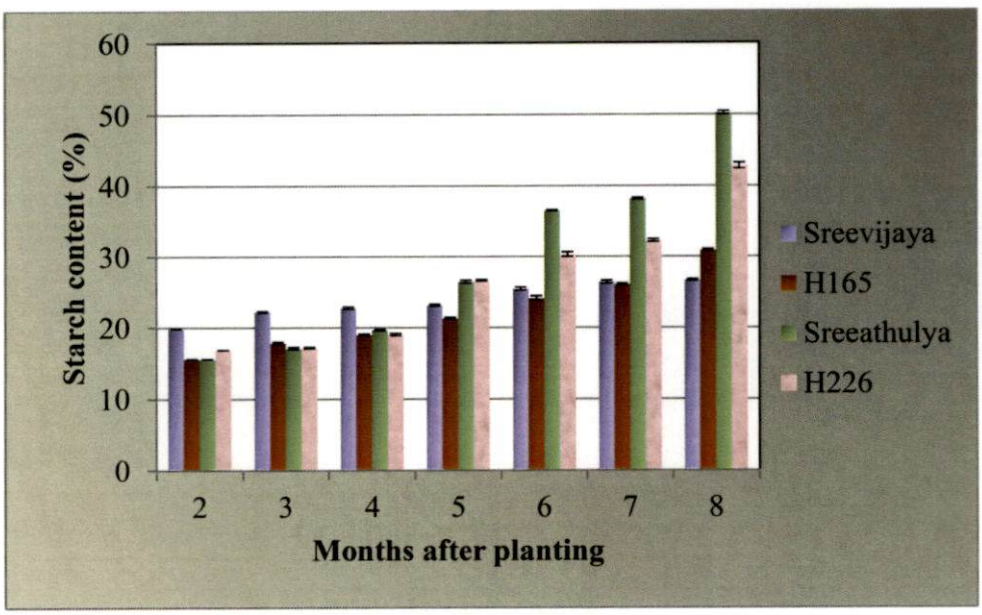

Fig.15. Changes in starch content in dry tubers of cassava during the growth period

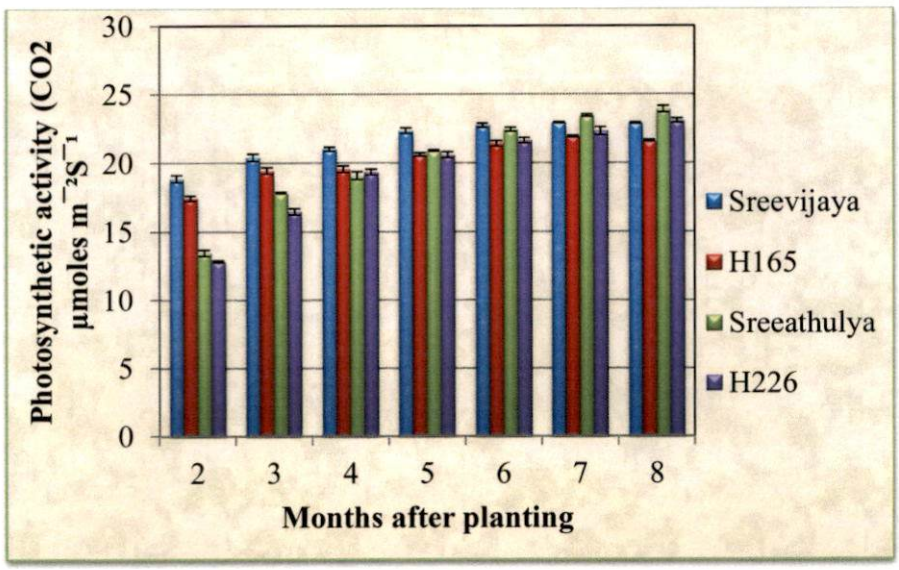

Fig.16. Photosynthetic rate during the growth period of four varieties of cassava

Variety/Genotype	P_N rate (CO_2 μmol^{-1} $m^2 s^{-1}$)
SreeVijaya	21.59±0.15
H165	20.27±0.152
Sree Athulya	20.15±0.155
H226	19.45±0.19

Table. 6. Mean Photosynthetic rate of cassava varieties

4.4. Enzyme Assay

4.4.1. Changes in Invertase activity in leaves and tubers of all the four varieties of cassava

The invertase activity was assayed in both leaves (Fig17) and in tubers (Fig 18) of cassava. Changes in the activity were recorded every month.

In leaves, H165 (2.79±0.077 mg glucose released^{-1} hr^{-1} mg protein) was found to have highest mean invertase activity and SreeVijaya (2.25±0.080 mg glucose released^{-1} hr^{-1} mg protein) had lowest activity. The mean invertase activity was found higher in H165 (4.86±0.12 mg glucose released^{-1} hr^{-1} mg protein) tubers while lowest activity was observed in SreeAthulya (4.36±0.15mg glucose released^{-1} hr^{-1} mg protein) (Table 7).

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Variety/Genotype	Invertase activity (mg glucose released^{-1} hr^{-1} mg protein)	
	Leaf	Tuber
Sree Vijaya	2.25±0.80	4.6±0.09
H165	2.79±0.077	4.86±0.15
Sree Athulya	2.72±0.100	4.36±0.12
H226	2.32±0.088	4.44±0.26

Table.7. Changes in the invertase activity in the leaves and tubers of cassava varieties

4.4.2. Changes in Sucrose Synthase activity in leaves and tubers of all the four varieties of cassava

SuSy enzymatic activity was studied in leaves and tubers of four varieties of cassava. SuSy activity was found higher in tubers than in leaf tissues. Changes in leaves (Fig 19) and in tubers (Fig 20) were recorded at monthly intervals.

The mean SuSy was observed maximum in the leaves of H165 with 2.95±0.033 mg sucrose synthesized^{-1}, and observed minimum in the variety SreeAthulya (2.46±0.011 mg sucrose synthesized^{-1} 100 mg fresh weight. Highest mean SuSy activity was observed in the tubers of SreeAthulya (3.15±0.064 mg sucrose synthesized^{-1} 100 mg fresh weight) and lowest activity in tuber was observed in the variety SreeVijaya (2.49±0.03 mg sucrose synthesized^{-1} 100 mg fresh weight). The mean average sucrose synthase activity in leaves and tubers of four varieties are given in (table 8).

69

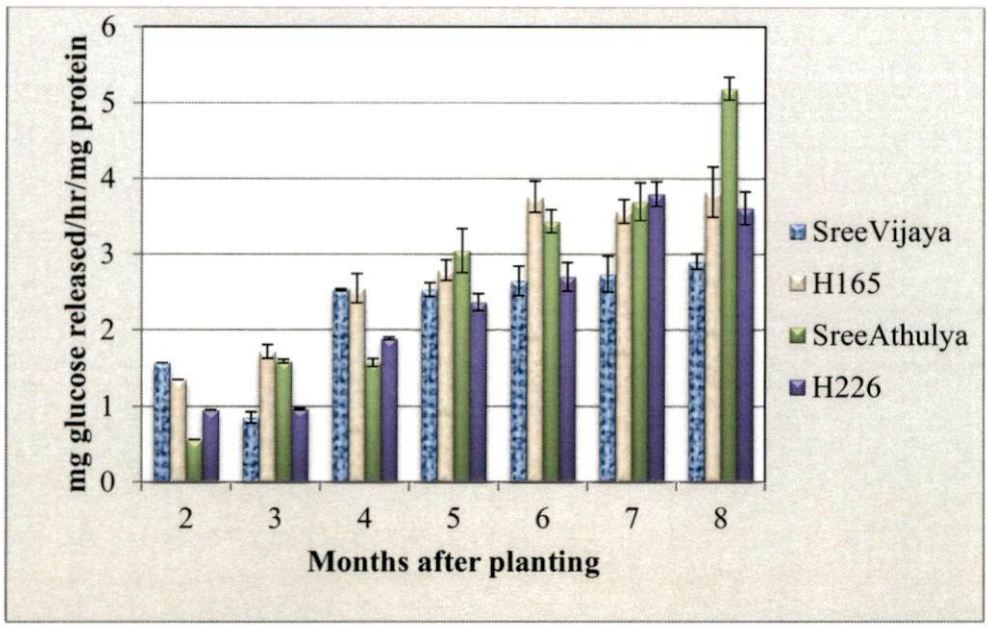

Fig.17. Invertase activity in fresh leaves of cassava varieties during the growth period

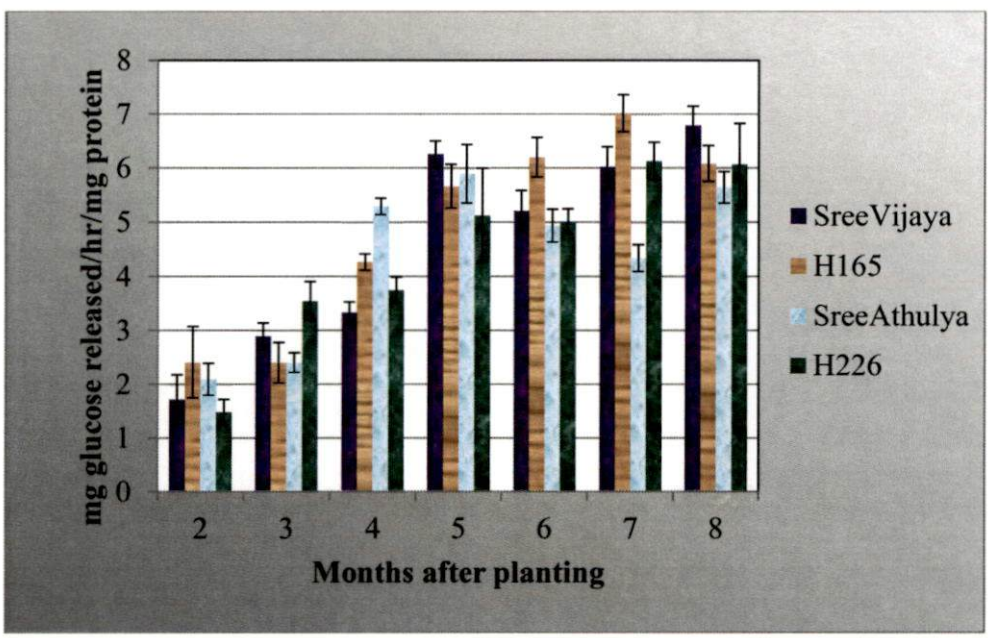

Fig.18. Invertase activity in fresh tubers of cassava varieties during the growth period

Variety/Genotype	Sucrose synthase activity (mg sucrose synthesized^{-1} mg fresh weight)	
	Leaf	Tuber
Sree Vijaya	2.49±1.37	2.89±0.072
H165	2.65±1.52	2.95±0.033
Sree Athulya	1.91±0.67	2.46±0.011
H226	2.23±1.07	2.78±0.067

Table. 8. Mean value for SuSy activity in the leaves and tubers of cassava varieties

4.4.3. Changes in Sucrose Phosphate synthase activity in leaves and tubers of all the four varieties of cassava

The SPS activity was found higher in tuber samples than in leaf samples. SPS activity was changed during the growth period and those changes in leaf (Fig 21) and tuber (Fig 22) were recorded monthly.

In leaves, the mean SPS activity was higher in H165 (2.65±0.069 mg sucrose synthesized^{-1} 100 mg fresh weight) while lower in SreeVijaya (1.825±0.077 mg sucrose synthesized^{-1} 100 mg fresh weight). In tubers, the mean SPS activity was found higher in SreeVijaya (4.25±0.10 mg sucrose synthesized^{-1} 100 mg fresh weight and the lowest activity was found in H165(3.45±0.102 mg sucrose synthesized^{-1} 100 mg fresh weight (Table 9).

71

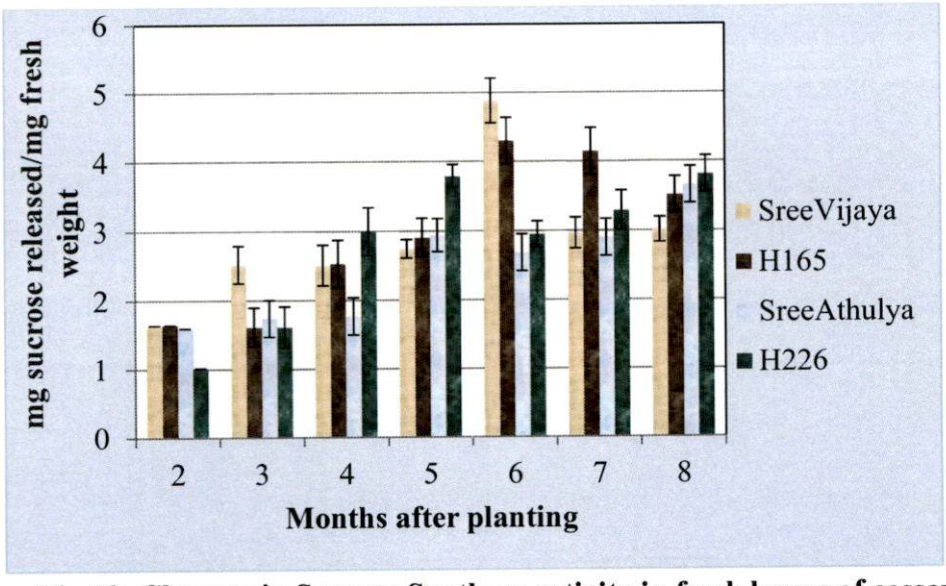

Fig 19: Changes in Sucrose Synthase activity in fresh leaves of cassava varieties during the growth period

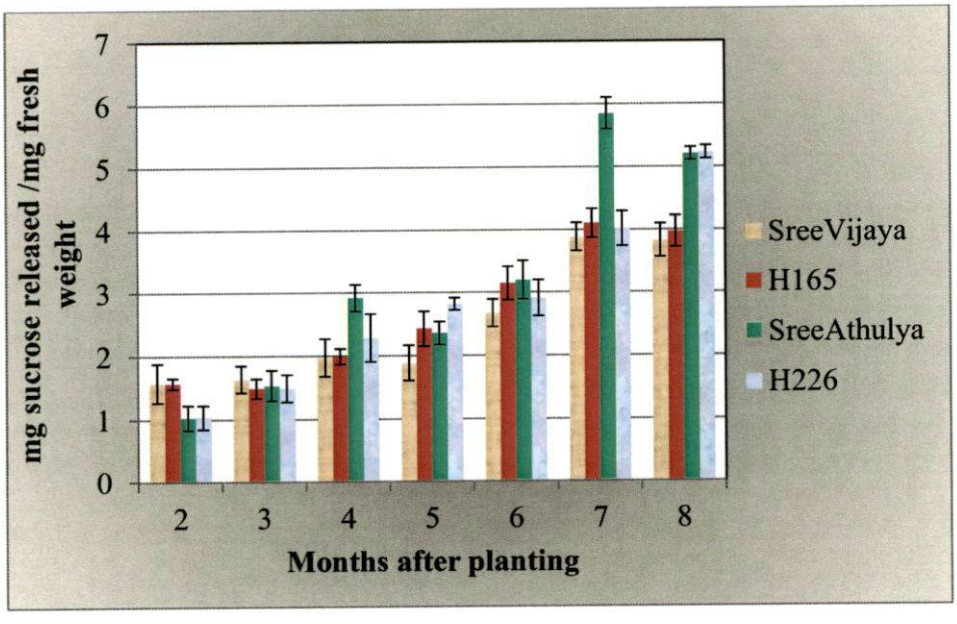

Fig 20: Changes in Sucrose Synthase activity in fresh tubers of cassava varieties during the growth period

Variety/Genotype	Sucrose phosphate synthase activity (mg sucrose synthesized^{-1} mg fresh weight)	
	Leaf	Tuber
Sree Vijaya	1.82±0.077	4.25±0.1
H165	2.65±0.069	3.45±0.102
Sree Athulya	2.22±0.049	3.53±0.09
H226	2.47±0.060	3.80±0.066

Table.9. Changes in the SPS activity in the leaves and tubers of cassava varieties

4.4.4. Changes in ADPGlucose Pyrophosphorylase activity in leaves and tubers of all the four varieties of cassava

AGPase activity was assayed in leaf and tubers of four varieties of cassava. Changes occurred during the growth of cassava were recorded at monthly intervals and compared. Changes in activity of leaf and tuber are represented in Fig 23 and Fig 24 respectively.

AGPase activity in leaves was measured. H165 (389.09±2.19 Units^{-1} minute) showed the highest activity while lowest activity was measured in SreeVijaya (278.95±1.22 Units^{-1} minute). In tubers, SreeAthulya (524.44±1.63 Units^{-1} minute) has the highest AGPase activity and H165 (359.76±1.2 Units^{-1} minute). The mean AGPase activity of four varieties of cassava was given in the (table 10).

73

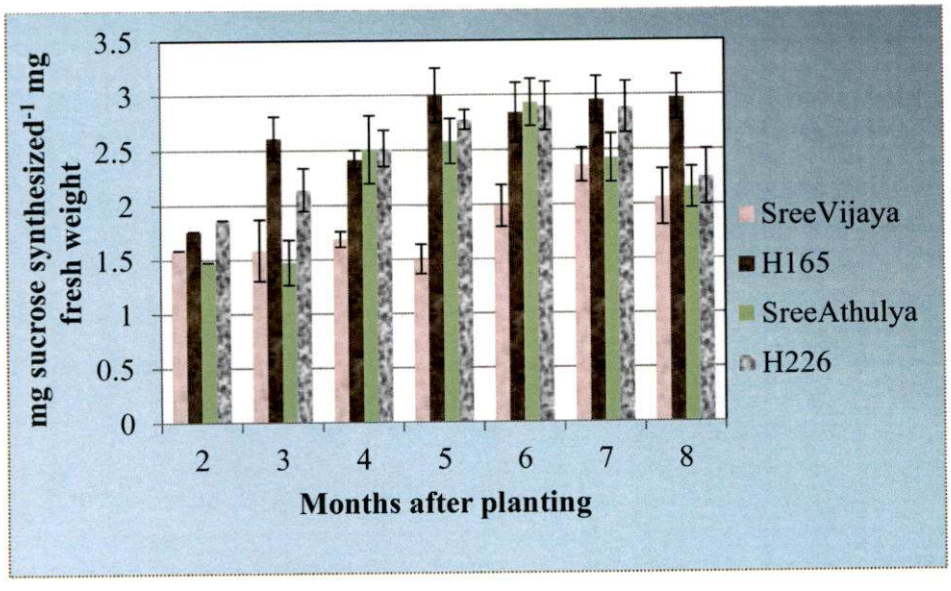

Fig.21.Changes in Sucrose Phosphate Synthase activity in fresh leaves of four varieties of cassava

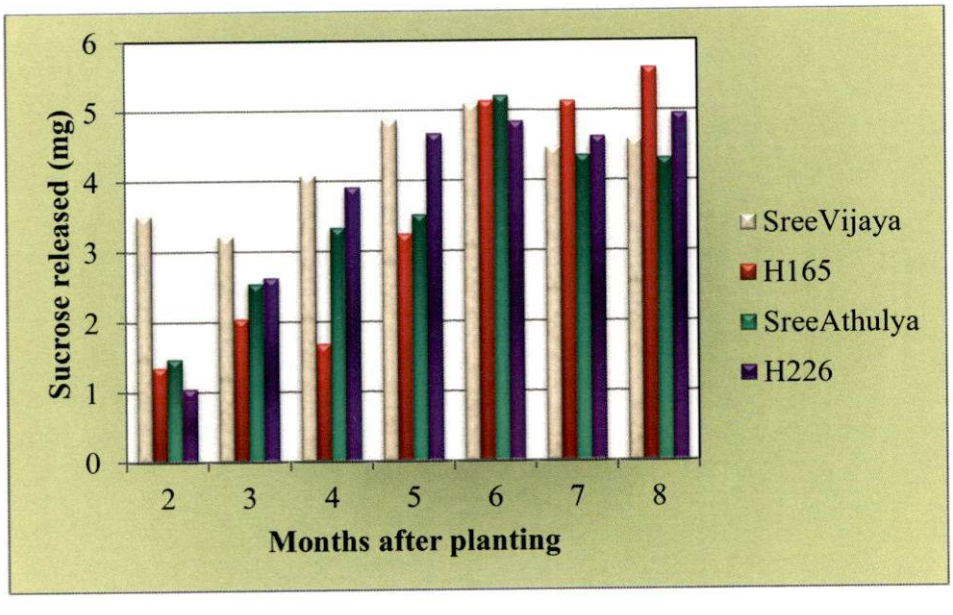

Fig.22. Changes in SPS activity in tubers of four varieties of cassava

Variety/Genotype	AGPase activity (Units^{-1} minute^{-1} mg fresh weight)	
	Leaf	Tuber
SreeVijaya	278.95±1.22	359.76±1.2
H165	389.09±2.19	387.91±2.2
Sree Vijaya	353.78±1.3	524.44±1.63
H226	281.82±0.77	402.66±1.41

Table. 10. The mean AGPase activity in the leaves and tubers of cassava varieties

4.4.5. Changes in Starch synthase activity in leaves and tubers of all the four varieties of cassava

Changes in the activity of SS were recorded monthly in leaves (Fig 25) and tubers (Fig 26) of four cassava varieties. In leaves, the SS activity was higher in the variety H226 (245.95±1.70 Units^{-1} minute) while lowest activity was found in variety H165 (185.94±0.94 Units^{-1} minute).

In tubers, the highest activity of SS was observed in variety SreeVijaya (541.55±2.05 Units^{-1} minute) and lowest activity was observed in variety H165 (410.61±1.55 Units^{-1} minute. The mean SS activity was given in the table 11.

75

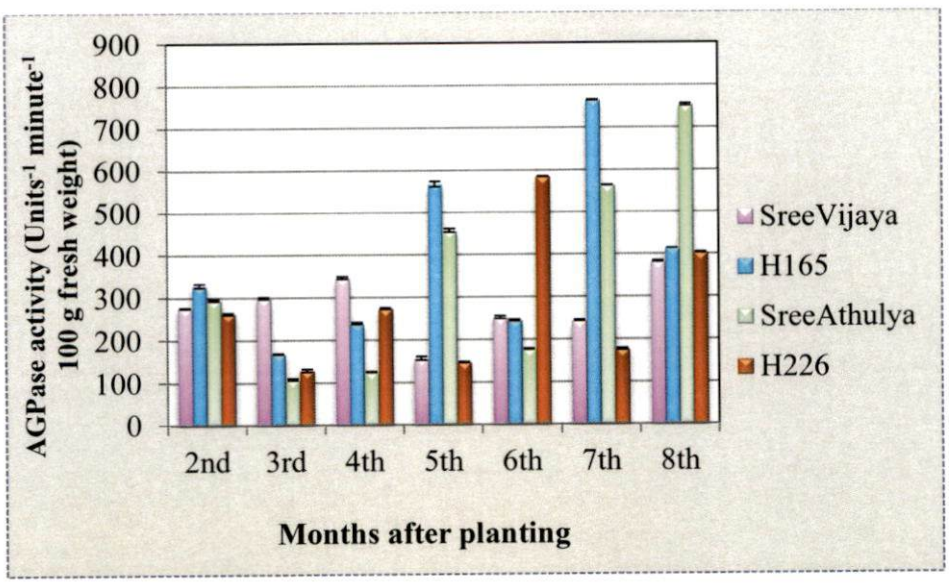

Fig. 23: AGPase activity in fresh leaves during the growth period of cassava varieties

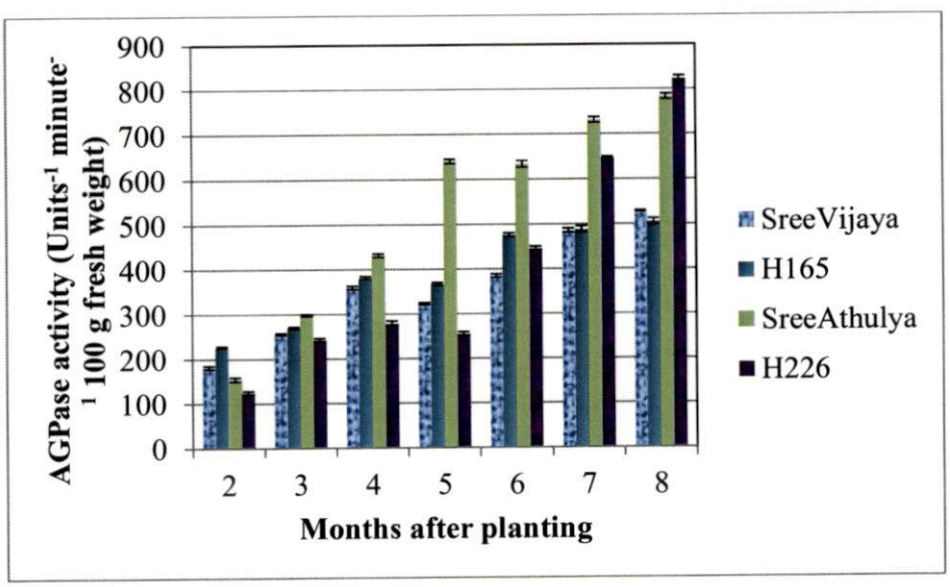

Fig.24. AGPase activity in fresh tubers during the growth period of cassava varieties

Variety/Genotype	Starch synthase activity (Units^{-1} minute^{-1} mg fresh weight)	
	Leaf	Tuber
SreeVijaya	196.63±1.60	541.55±2.05
H165	185.94±0.94	410.61±1.55
Sree Athulya	206.66±1.37	520.99±2.99
H226	245.95±1.70	480.42±1.35

Table. 11. The mean SS activity in the leaves and tubers of cassava varieties

4.4.6. Correlation of the activities of starch synthesis enzymes and starch accumulation rate

The correlations between leaf area, photosynthetic rate and starch regulating enzymes in tubers and leaves were analyzed. The activities of AGPase, Starch synthase, photosynthetic rate and leaf area in tubers were positively correlated with starch accumulation rate. The activity of sucrose synthase was varied in different varieties during the growth period. Sucrose phosphate synthase activities were varied with different varieties. In leaves, invertase enzyme shows positive correlation with starch accumulation. The correlation between starch accumulation and other parameters were shown in the following figures.

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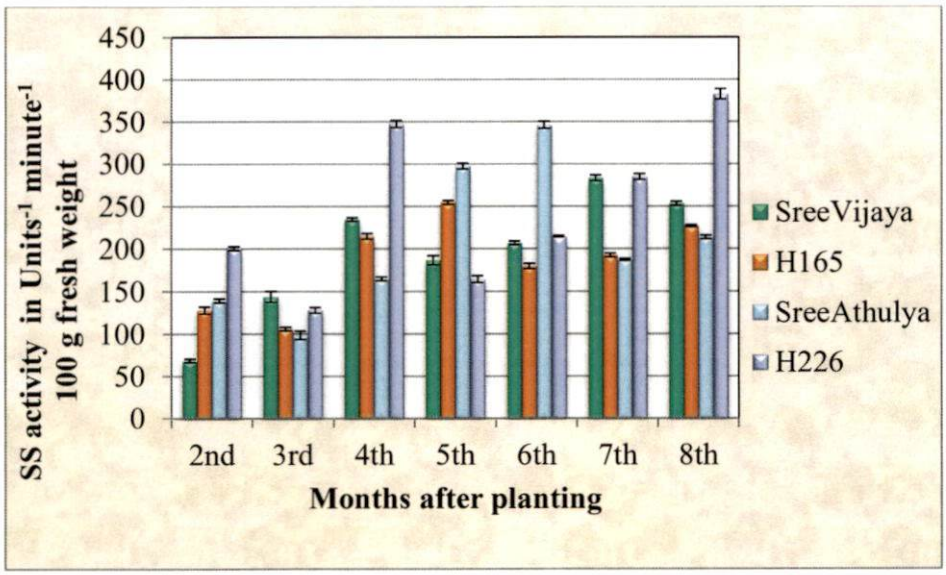


Fig.25. Changes in Starch synthase activity in fresh leaves of four varieties of cassava

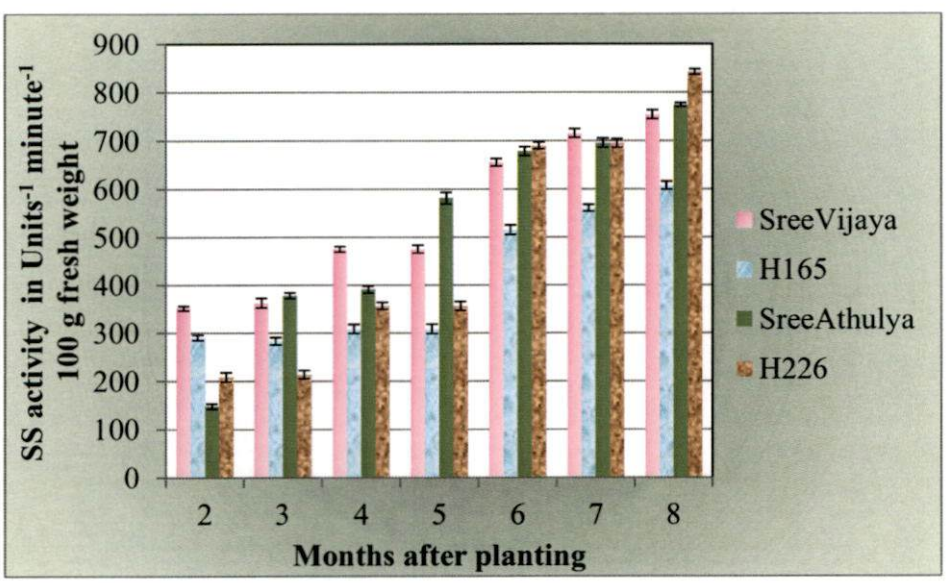
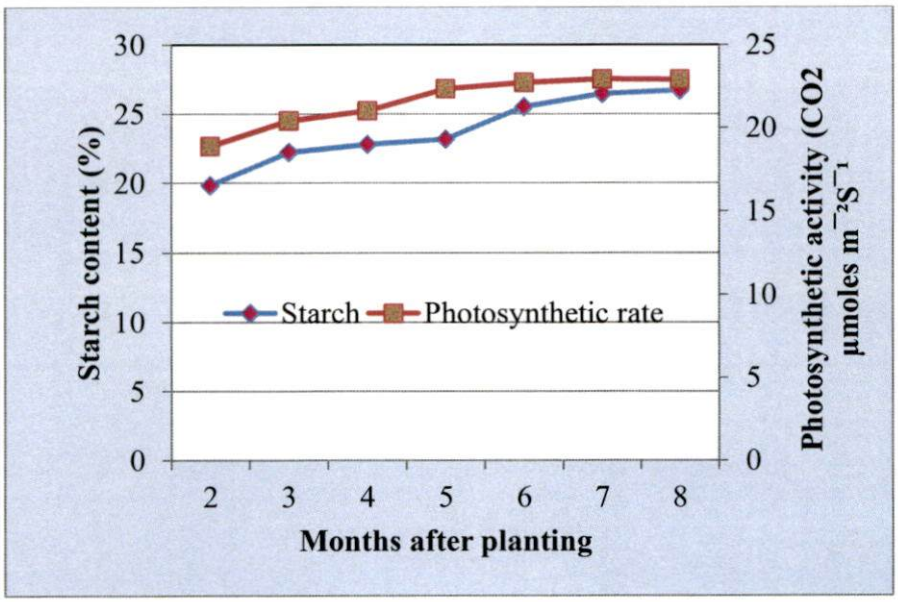
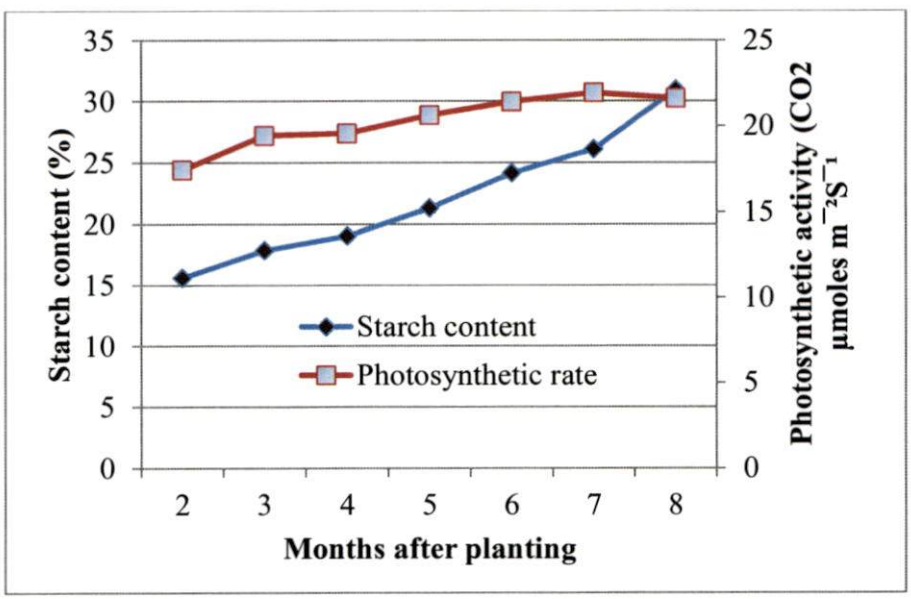
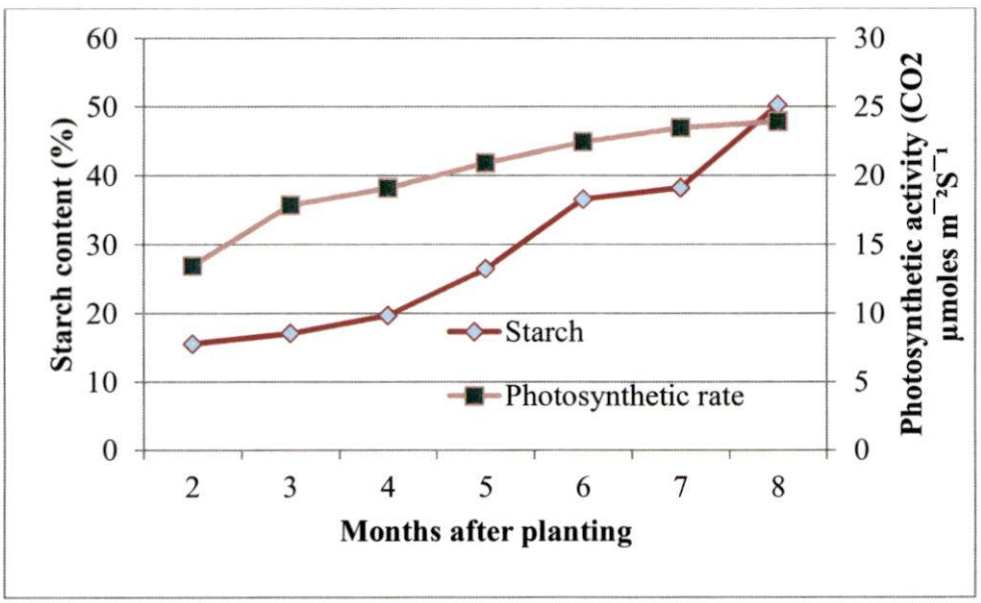


Fig.26. Changes in Starch synthase activity in fresh tubers of four varieties of cassava


Starch content (%)
Photosynthetic activity (CO2 µmoles m⁻²S⁻¹
Starch
Photosynthetic rate
Months after planting


Starch content (%)
Photosynthetic activity (CO2 µmoles m⁻²S⁻¹
Starch content
Photosynthetic rate
Months after planting



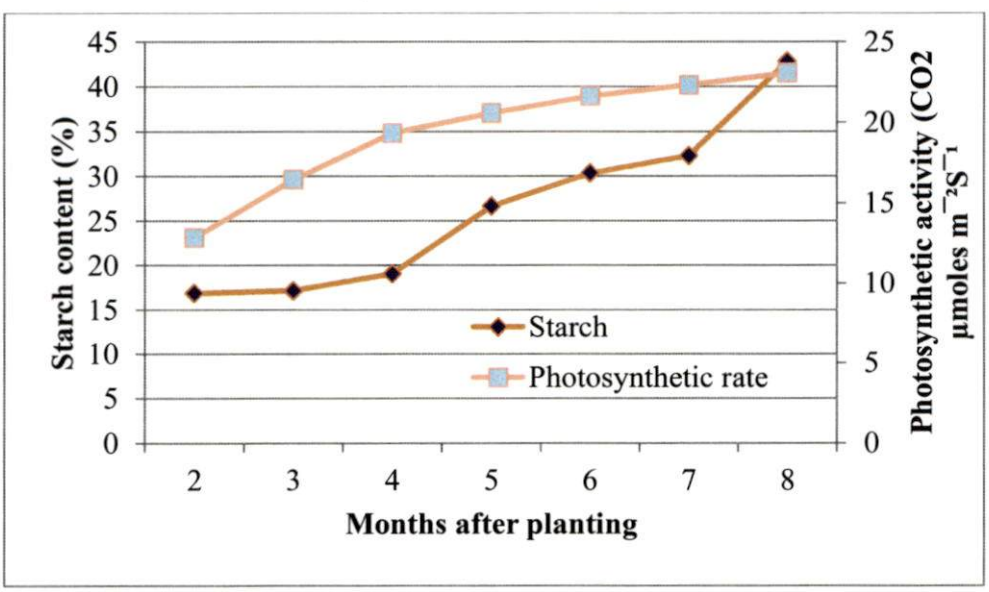
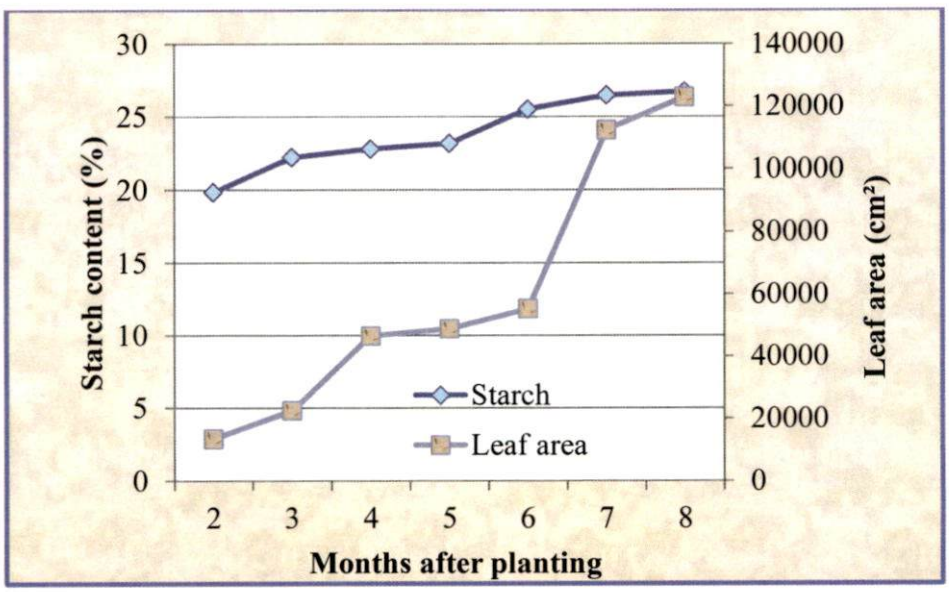
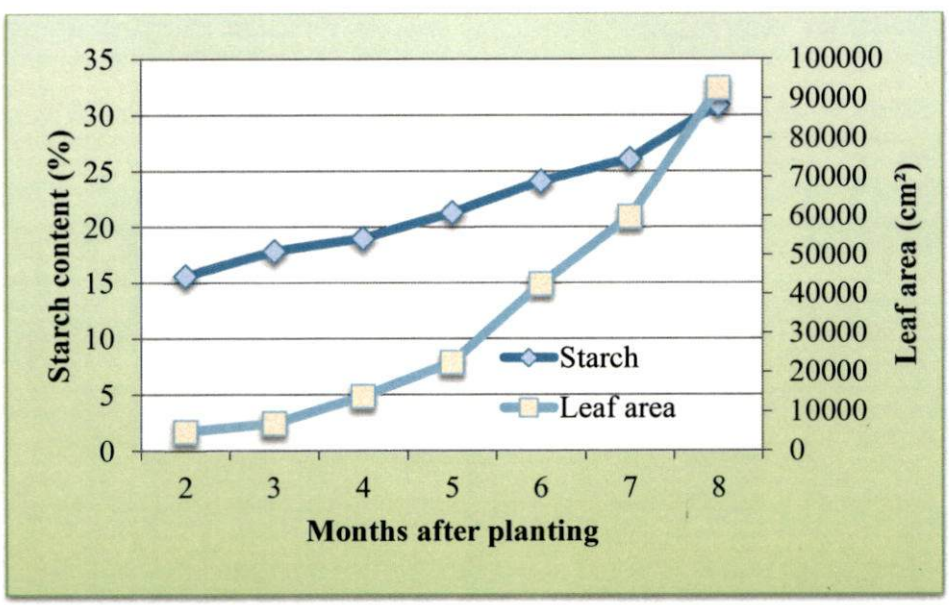
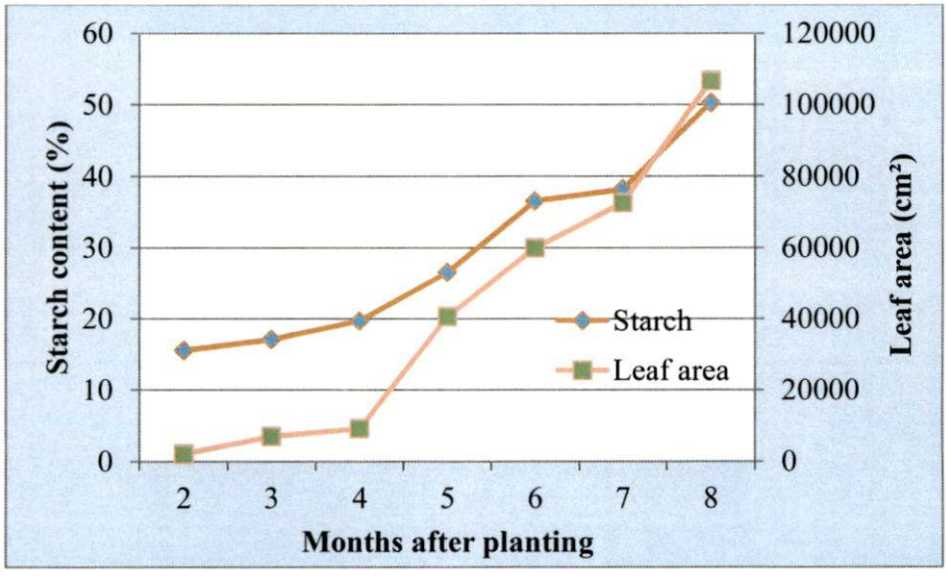


Fig.27. Correlation between starch content and photosynthetic rate of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period


30
25
20
15
10
5
0
Starch content (%)
140000
120000
100000
80000
60000
40000
20000
0
Leaf area (cm²)
Starch
Leaf area
2
3
4
5
6
7
8
Months after planting


35
30
25
20
15
10
5
0
Starch content (%)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Leaf area (cm²)
Starch
Leaf area
2
3
4
5
6
7
8
Months after planting



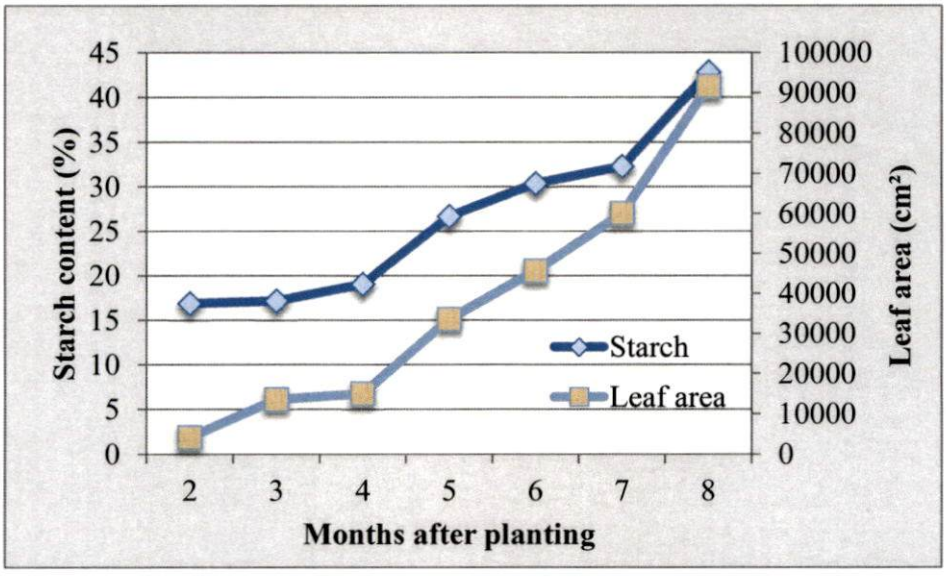
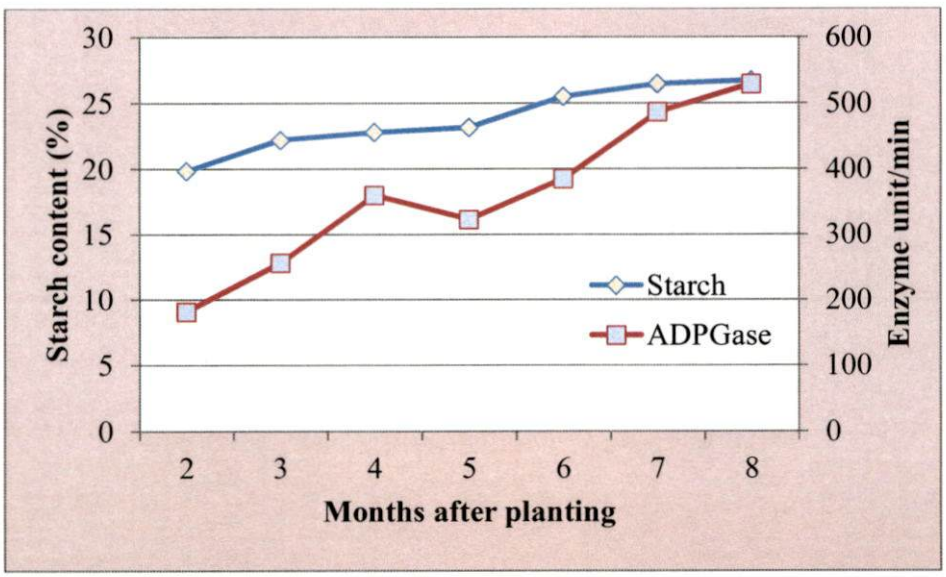
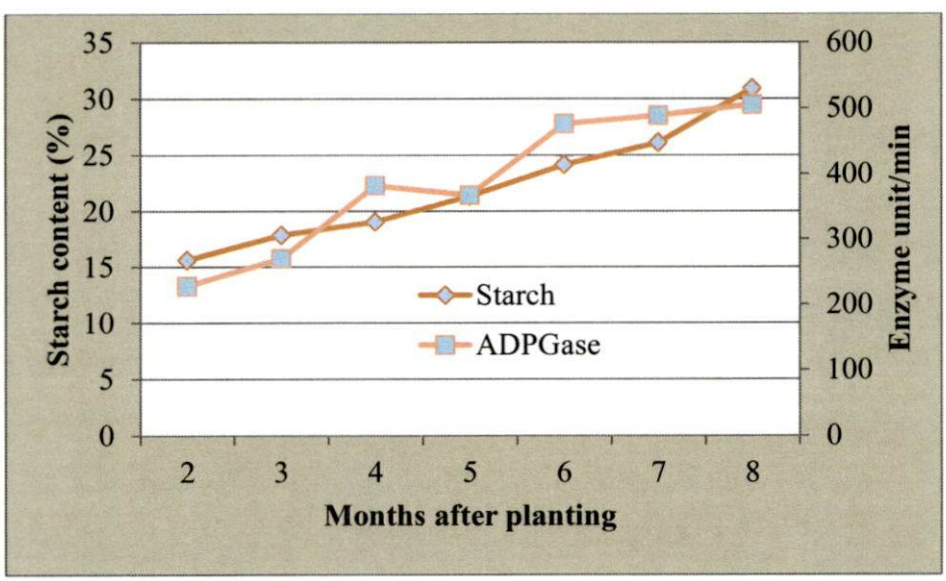
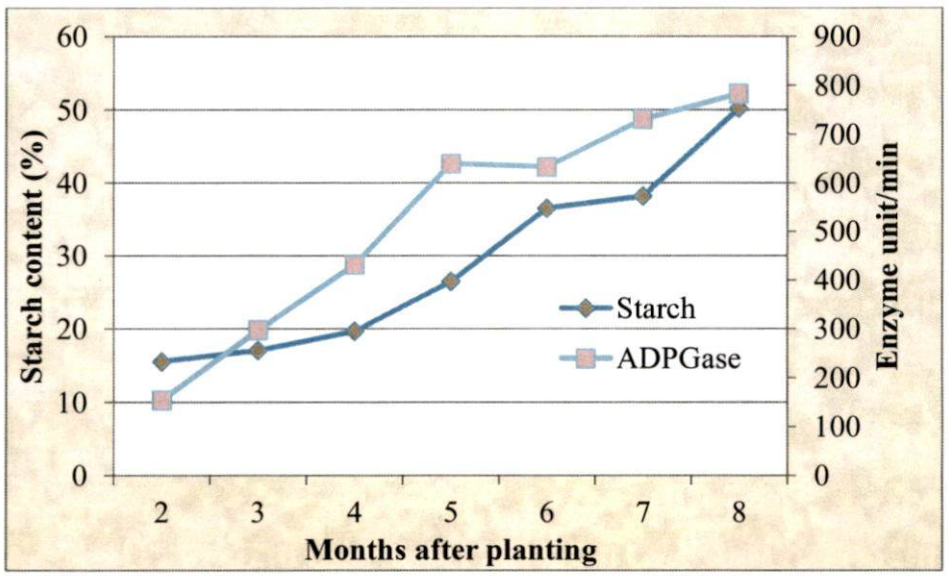


Fig.28. Correlation between starch content and Leaf area of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period


Starch content (%)
Enzyme unit/min
Months after planting
Starch
ADPGase
0
5
10
15
20
25
30
0
100
200
300
400
500
600
2
3
4
5
6
7
8


Starch content (%)
Enzyme unit/min
Months after planting
Starch
ADPGase
0
5
10
15
20
25
30
35
0
100
200
300
400
500
600
2
3
4
5
6
7
8



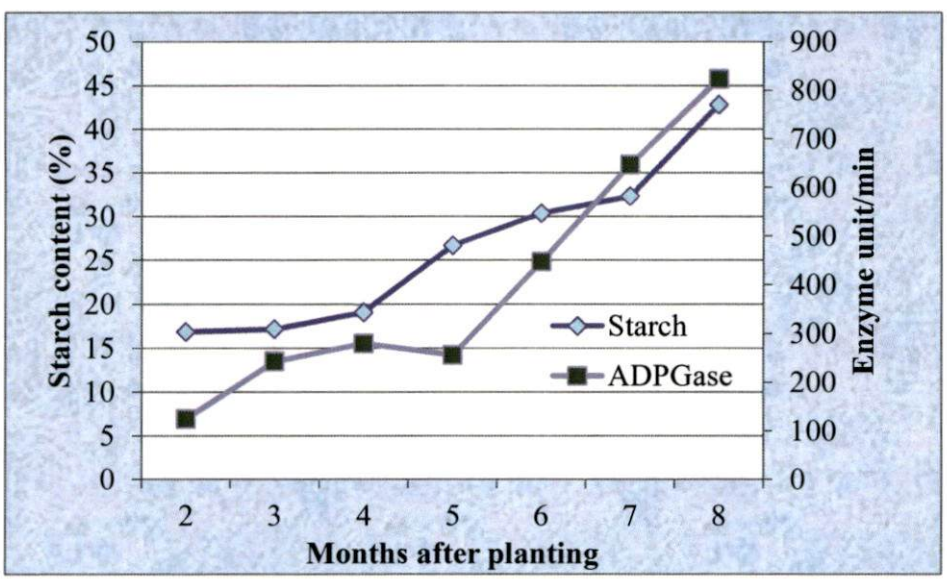
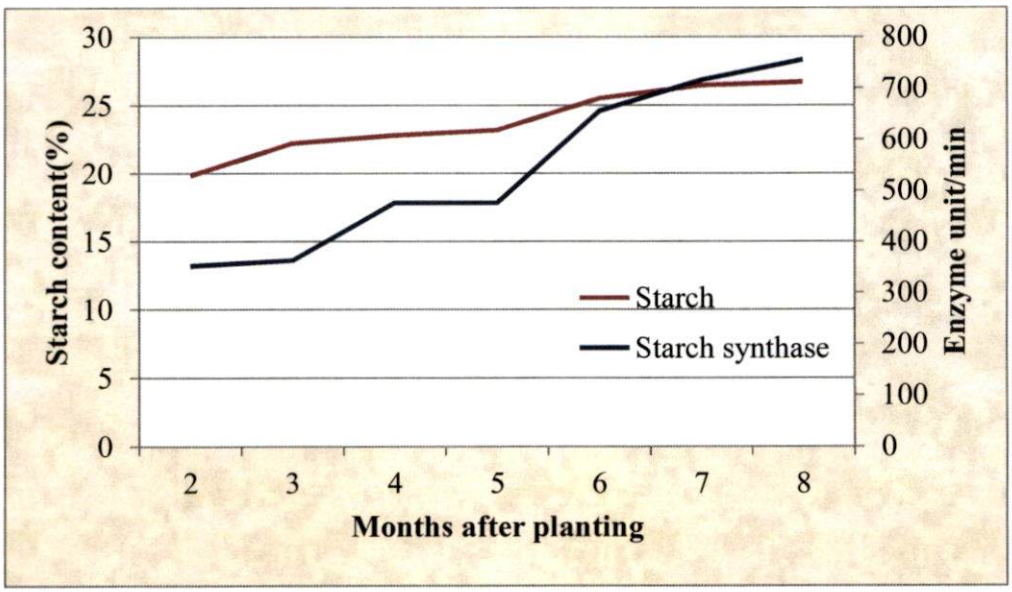
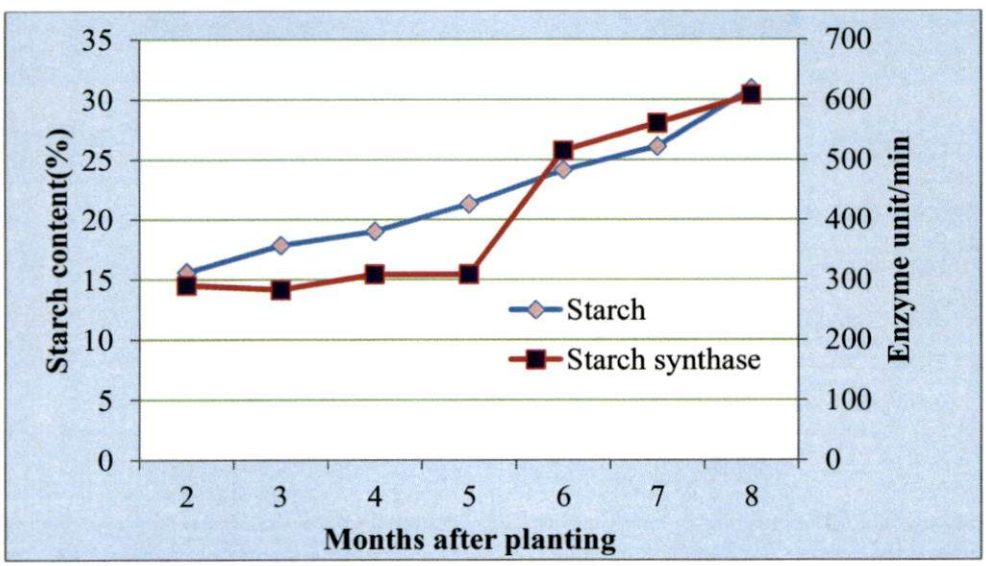
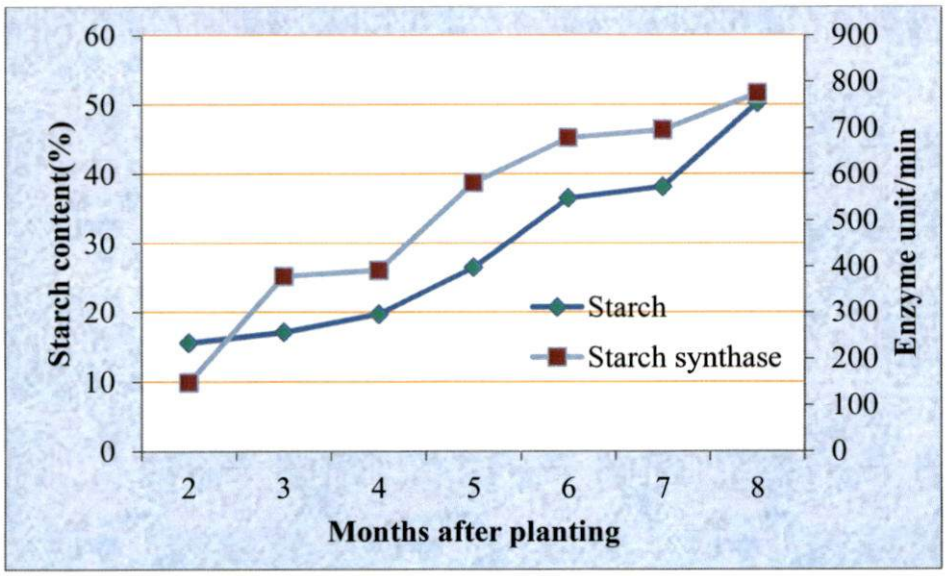


Fig.29. Correlation between starch content and AGPase in tubers of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period


Starch content(%)
Enzyme unit/min
Months after planting
Starch
Starch synthase
0
5
10
15
20
25
30
0
100
200
300
400
500
600
700
800
2
3
4
5
6
7
8


Starch content(%)
Enzyme unit/min
Months after planting
Starch
Starch synthase
0
5
10
15
20
25
30
35
0
100
200
300
400
500
600
700
2
3
4
5
6
7
8



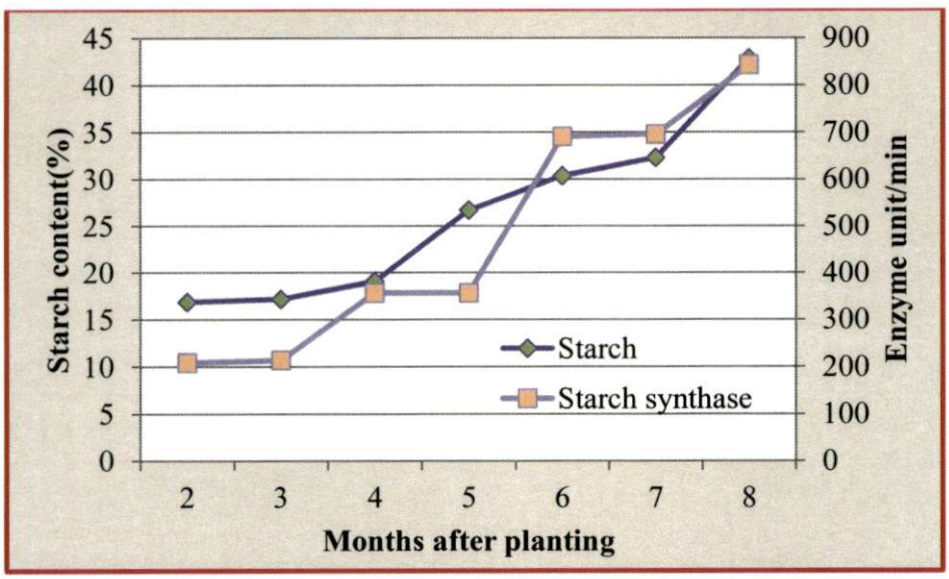
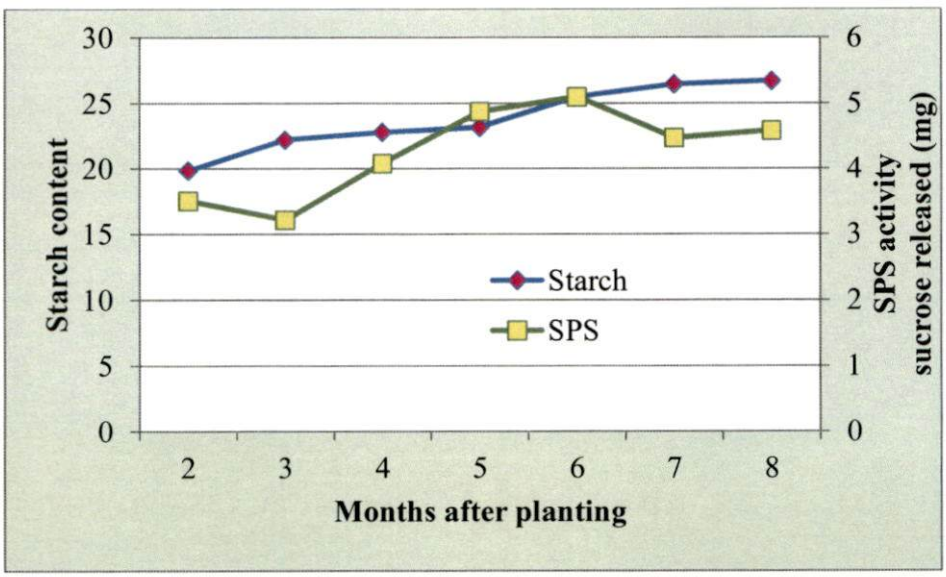


Fig.30. Correlation between starch content and Starch synthase in tubers of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period



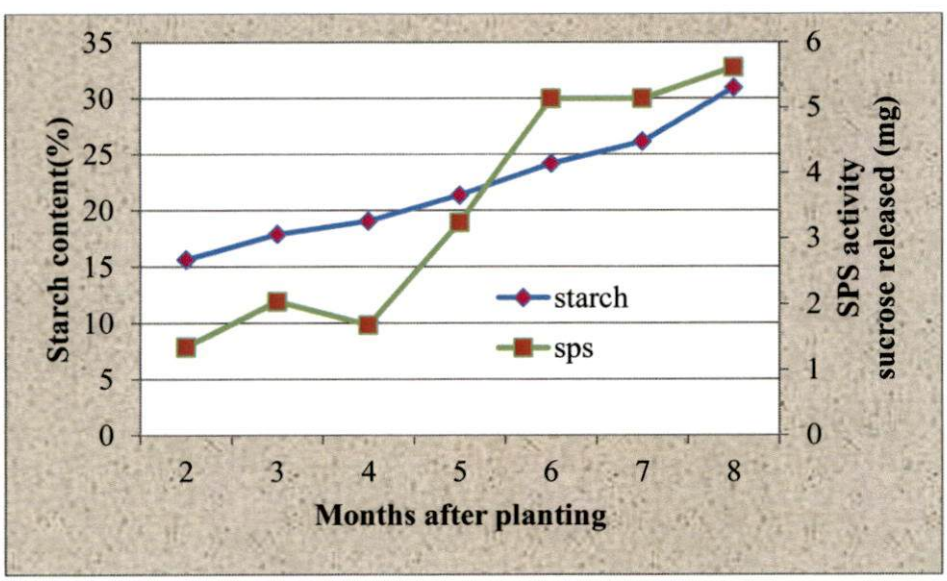
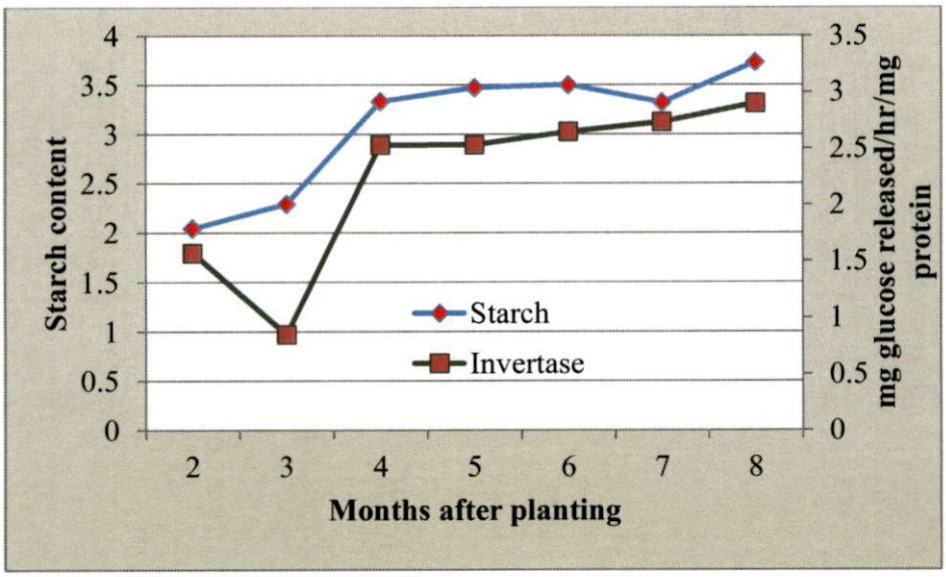
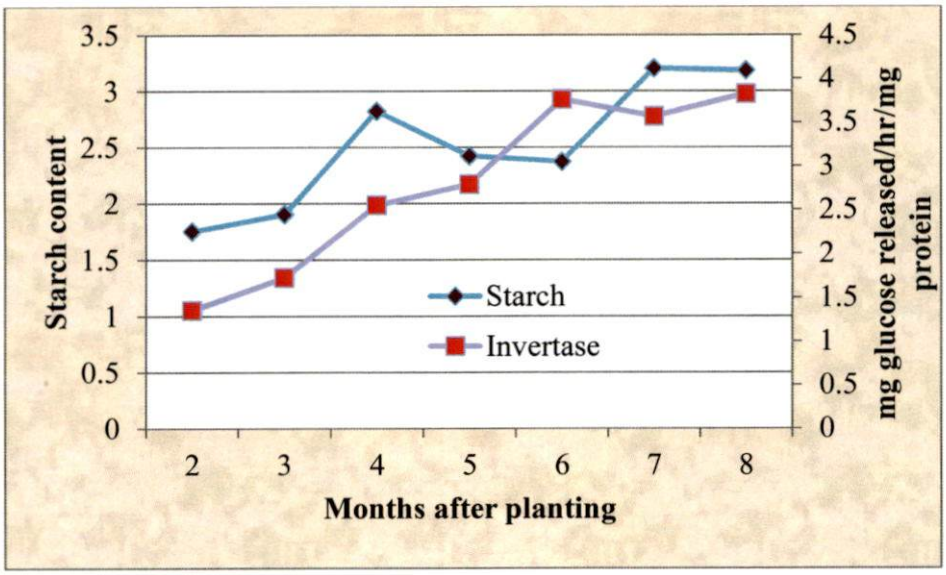
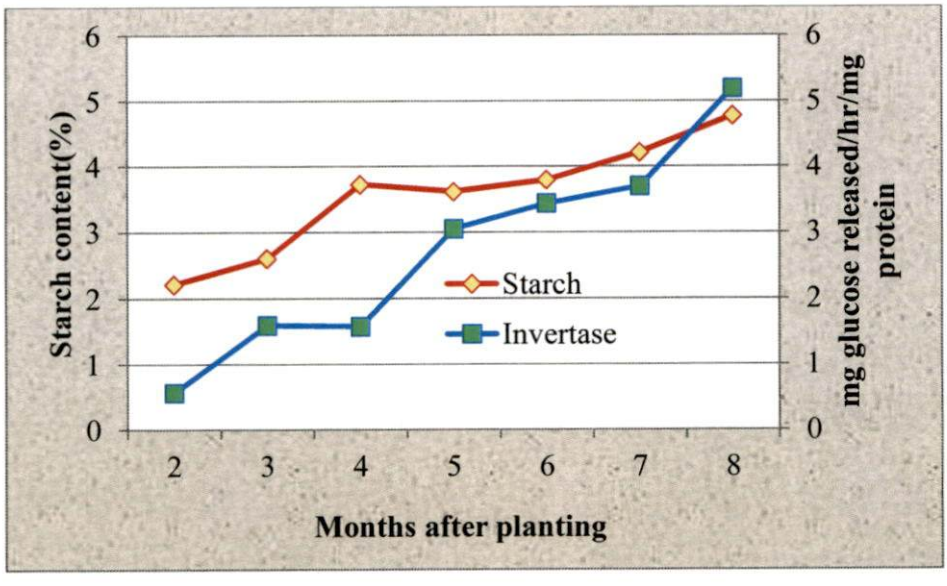


Fig.31. Correlation between Starch content and Sucrose phosphate synthase activity in tubers of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period


Starch content
mg glucose released/hr/mg protein
Starch
Invertase
Months after planting
0
0.5
1
1.5
2
2.5
3
3.5
4
2
3
4
5
6
7
8


Starch content
mg glucose released/hr/mg protein
Starch
Invertase
Months after planting
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
2
3
4
5
6
7
8



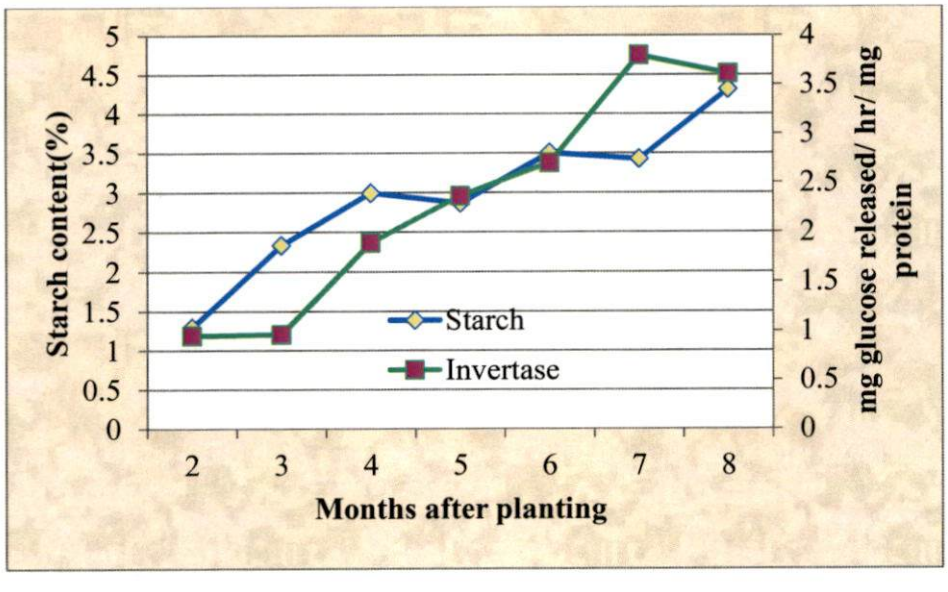


Fig.32. Correlation between Starch content and Invertase activity in leaves of four varieties of cassava- Sree Vijaya, H165, Sree Athulya, H226 during the growth period

DISCUSSION

5. DISCUSSION

Cassava is an important tropical crop, grown mainly for its starchy root which is an important source of food. Cassava ranks very high among crops that convert the greatest amount of solar energy into soluble carbohydrates per unit of area. Starch is the main constituent of cassava determined as carbohydrates. (Pandey *et al.,* 2000). The starch content varies between 73.7 and 84.9% of its total storage root dry weight (Baguma 2004). Cassava starch has many important characteristics high paste viscosity, high paste clarity, and high freeze-thaw stability, which are advantageous to many industries. It is mainly used as food, but it can be readily converted into many other useful products. It can be used to produce diverse products such as food, paper, textiles, adhesives, beverages, confectionary, pharmaceuticals, and building materials.

The present study was conducted out to find out the relation between photosynthesis, enzymes involved in sucrose and starch biosynthesis in leaves and tubers of long and short duration varieties of cassava. Long duration varieties selected for this study were SreeAthulya and H226 and short duration varieties selected for this study were H165 and SreeVijaya. The enzymes namely ADPglucose pyrophosphorylase, Starch synthase, Invertase, Sucrose Phosphate synthase and Sucrose synthase were studied.

The metabolic pathways for biosynthesis of starch had been well studied in endosperm of rice, wheat, maize, pea, sorghum and tuber of potato. It sink tissue starch is synthesized from sucrose. The cytosolic sucrose must be translocated into the amyloplasts for the starch biosynthesis. Sucrose is converted into hexose phosphates which are then transported into the amyloplast. The enzymes involved in the conversion of sucrose to hexose phosphates are invertase and SuSy. The SPS enzyme involved in the reversible synthesis of Suc from the hexose phosphate. The key enzymes involved in starch biosynthesis are AGPase, Starch synthase. Then branching enzyme involved in the further modification of starch molecule. Hence in the present study understanding the enzymatic activity of different enzymes was important (Munyikwa *et al.*, 1997).

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Photosynthesis is the most important biological process, because it provides biomass which is directly used as food by all living organisms. In higher plants, carbon fixed during photosynthesis is either utilized for starch biosynthesis in chloroplast and sucrose synthesis in the cytosol for export from the leaves. Glucose synthesized during photosynthesis is temporarily stored as transitory starch during the day time in source tissues. During night, it is mainly transported in the form of sucrose to other storage organs. Thus the photosynthetic rate is influences the starch biosynthesis. Therefore in the present study photosynthetic activity of four genotypes of cassava is important.

5.1. Effect of morphological parameters on starch biosynthesis

The leaf production, leaf area and leaf area index are the different morphological parameters were recorded for four varieties of cassava during the 2^{nd} to 8^{th} month after planting. During the growth period, an increasing trend in no: of leaves was seen in four varieties of cassava. The maximum leaf production, leaf area and leaf area index was observed in the variety SreeVijaya. The minimum leaf production was observed in the variety H226 but the minimum leaf area index and minimum leaf area were observed in the variety H165. The leaf production and leaf area were significantly affected the starch biosynthesis. Starch content of varieties increases with the increase in leaf area, leaf area index and no. of leaves.

In cassava varieties the cumulative number of leaves, regardless of their branching habit continue to increase up to 6-8 months. (Lian and Cock, 1979., Ramanujam and Indira, 1983). The rate of leaf formation and LAI decreased at temperature less than 24°C (Irikura *et al.*, 1979., Manrique 1990)

The tuber yield was observed maximum in the H226 variety and minimum in the H165 variety. In cassava the optimum LAI was 3.0 to 3.5 for maximum light interception and tuber yield.

Cassava storage root contains 20-41% starch on a fresh weight basis (Maini *et al.*, 1970., Obigbesan and Agboola 1973., CTCRI 1998., Ravi 2001) or

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50- 86% on a dry weight basis(Maini 1970., Kurian *et al.*, 1973). Storage root contains more than 80% starch on a dry weight basis at temperature less than 24°C but starch content decreased at higher temperatures (Irikura *et al.*, 1979).

5.3. Effect of biochemical parameters on starch biosynthesis

Among the four varieties the sucrose content and starch content in tubers was maximum in SreeAthulya. The starch content was observed minimum in the variety H165. It was observed that the starch content was increased with increase in sucrose content. An increasing in trend of sucrose and starch content was observed during the growth period.

5.2. Effect of photosynthetic activity on starch biosynthesis

The photosynthetic activity in leaves was measured in four varieties of cassava during the 2-8 months after planting. However an increasing trend in photosynthetic activity had seen in four varieties of cassava. The photosynthetic rate amplified by the leaf area were significantly affected the starch biosynthesis. The photosynthetic activity was observed maximum in SreeVijaya. It was observed that the photosynthetic activity among tubers was minimum in H165, the sucrose and starch content in tubers was minimum in the variety H165.

Similar studies were conducted in soybean leaves (Chatterton and Silvius 1980), revealed certain relationships of CER to leaf morphology and carbohydrate accumulation and the relationships among starch synthesis, translocation, and plant growth strategies.

Plaut (1987) studied the effect of altered sink: source ratio on photosynthesis of source leaves. Seven crop species were grown under identical environmental conditions, decreased sink: source ratio led to a decreased photosynthetic rate within 1 to 3 days in *Cucumis sativus* L., *Gossypium hirsutum* L., and *Rapwaaus sativus* L, but not in *Capsicum annuum* L., *Solanum melongena* L., *Phaseolus vulgaris* L., or *Ricinus communis* L.

5.4. Effect of enzymes on starch biosynthesis

5.4.1. Effect of ADPglucose pyrophosphorylase activity on starch biosynthesis

The ADPglucose pyrophosphorylase in leaves and tubers were checked for four varieties of cassava for 2-8 months after planting. There was a difference in enzyme activity has been seen among the varieties. The maximum enzymatic activity in tuber was shown by the variety SreeAthulya. The starch content and sucrose content were observed maximum in SreeAthulya. It was also observed that the starch content increases with the increase in ADPGase. The increase in both ADPGase and starch synthase have been correlated with increased rate of starch accumulation. leaves the enzymatic activity was shown highest by the variety H165. SPS activity was also maximum for H165 in leaves.

Studies on AGPase activity studied in potato (Sowokinos, 1975) revealed that the potato tuberization was accompanied with a marked rise in ADP-glucose pyrophosphorylase activity and the onset of rapid potato tuber starch biosynthesis may be closely related to the simultaneous increase in ADP-glucose pyrophosphorylase activity.

The increases in both ADPglucose pyrophosphorylase and starch synthase have been correlated with increased rates of starch accumulation in barley, peas, wheat, maize endosperm, and potatoes during development (Sowokinos and Preiss 1982). The maize mutant shrunken-2 synthesized low starch because it completely lacks AGPase activity in both endosperm and embryo tissue (Tsai and Nelson, 1966).

5.4.2. Effect of Starch synthase activity on starch biosynthesis

The starch synthase activity in leaves and tubers were assayed for four varieties of cassava during 2-8 months after planting. The SS activity of tuber was maximum in SreeVijaya. Photosynthetic activity, no: of leaves, leaf area were also observed high for SreeVijaya. The SS activity was observed lowest for H165 in tubers and leaves. The photosynthetic activity, leaf area, leaf area index, tuber

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yield, sucrose and starch content were also observed minimum for H165. It was observed that starch synthase activity of all the four varieties were positively correlated with the starch accumulation.

In leaves SS activity was maximum in H226 and minimum in H165. The starch content and sucrose content were also minimum for H165. It was observed in leaves there is a difference in enzyme activity for all the four varieties during the growth period and SS activity in leaves cannot be correlated with the starch content.

Starch synthases (SS) involved in starch (amylase and / or amylopectin) biosynthesis exists in multiple forms in starch producing tissues. At least four distinct SS isoforms have been defined. All plants possess the granule bound isoform GBSSI, whereas the SSI, SSII, and SSIII isoforms are located either partially or entirely in the soluble phase and their occurrence in plant tissues may vary depending upon plant species (Myers et al., 2000). GBSS is primarily involved in synthesis of amylase whereas SS is involved in synthesis of amylopectin (Jeon et al., 2010).

5.4.3. Effect of Sucrose synthase activity on starch biosynthesis

The SuSy activity in leaves and tubers were assayed for four varieties of cassava from 2- 8 months after planting. In leaves and tubers the sucrose synthase activity was different. In tubers, the SuSy activity was maximum in SreeAthulya and minimum in SreeVijaya, while the SPS activity was observed highest in SreeVijaya. In leaves the SuSy activity was highest in H165 and lowest in SreeAthulya.

The SuSy activity in tuber was minimum in H165 but highest in leaves while the SuSy activity in tuber is maximum for SreeVijaya and minimum in leaves. It was observed that in tubers there was a difference in sucrose synthase activity for all the variety during the growth period.

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The sucrose synthase activity can be positively correlated with the fruit growth and starch content in the pericarp tissue. It was also found that acid invertase cannot be positively correlated with the starch content(Wang *et al.*, 1993)

Sucrose synthase (SuSy) activity consistently remained much greater than the invertase activity which did not change appreciably during endosperm development in wheat (Riffkin et al., 1995).

5.4.4. Effect of Sucrose phosphate synthase activity on starch biosynthesis

The sucrose phosphate synthase (SPS) activity in leaves and tubers were checked for four varieties of cassava from 2- 8th months after planting. In leaves the SPS activity was highest in H165 and lowest in SreeVijaya. The SPS activity in tubers was maximum in SreeVijaya and lowest in H165.

Hubbard et al., 1989 reported that sucrose synthase activity was low in orange fleshed and green fleshed musk melon while invertase activity decreases and SPS activity increased during fruit growth.

5.4.5. Effect of invertase activity on starch biosynthesis

The invertase activity in leaves and tubers were assayed for four varieties of cassava from 2-8 months after planting. The invertase activity in leaves was observed maximum in SreeAthulya and minimum in SreeVijaya. In tubes, the invertase activity among the four varieties was observed maximum in H165 and minimum in SreeAthulya. It was observed that starch content was increased in SreeAthulya while the invertase activity decreased in this variety. H165 had highest invertase activity than SreeAthulya but had lowest starch accumulation.

High sucrose acid invertase activity prevent sucrose accumulation but not all, while low level of sucrose acid invertase activity was not sufficient for the sucrose accumulation (Zhu, *et al.*, 1997).

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High invertase activity in potato tubers reduced sucrose content over 95% and significantly increased glucose, increase in the metabolic intermediates of glycolysis, catalytic activity of enzymes in respiratory pathway, CO_2 production and a reduction in starch biosynthesis suggesting that invertase diverts sucrose towards glycolytic pathway (Trethway *et al.* 1998).

From the present study it was observed that the variety Sree Athulya had relatively higher AGPase enzyme activity, SuSy activity, moderate SS activity and SPS activity and high starch content with high yield. The variety H226 has maximum tuber yield and moderate starch content due to decreased SPS activity, moderate AGPase activity and SS activity. The variety H165 had the minimum starch and sucrose content due to decreased SuSy, AGPase activity and SS activity and increased invertase activity. The variety Sree Vijaya had the moderate starch content in tuber as it is a short duration variety due to the highest photosynthetic rate, SS activity and moderate AGPase activity.

The variety Sree Athulya was identified as the high yielding one with high activities of AGPase, SS and SuSy. The variety is a triploid and high doses of genes encoding these enzymes might have attributed for the results. Low starch content varieties can be improved by manipulating the activity of these enzymes.

SUMMARY

6. SUMMARY

A study on photosynthesis and enzyme activities regulating starch biosynthesis in cassava (*Manihot esculenta* Crantz) was conducted at CTCRI (Central Tuber Crops Research Institute) Sreekariyam, Thiruvananthapuram and Department of Biotechnology, College of Agriculture, Vellayani during the period of 2015 – 2016.

The study focused on four varieties/genotypes of cassava *viz.,* Sree Vijaya, H165, Sree Athulya, H226 in a field trial with three replications, each replication with 25 Plants. Plants were cultivated under irrigated conditions. Observation had been made for morphological parameters, enzyme activities and photosynthetic rate and tuber yield of four varieties of cassava.

Morphological parameters such as total number of leaves, leaf area and tuber yield were recorded between 2nd and 8th months after planting (MAP) at monthly intervals. Biochemical parameters such as sucrose and starch content of all the four varieties were estimated monthly. Photosynthetic activity was measured monthly using portable CO_2 analyser. Enzyme activities of ADP glucose pyrophosphorylase, starch synthase, sucrose synthase, sucrose phosphate synthase, invertase were assayed in leaves and tubers of all the four varieties.

The present study revealed that cassava varieties had variation in the morphological and biochemical parameters. Morphological parameters such as number of leaves, leaf area and leaf area index were maximum in the variety Sree Vijaya and minimum in the variety H165. The tuber yield was observed maximum in the variety H226 and minimum in the variety H165.

Biochemical parameters such as sucrose content and starch content were recorded monthly between 2nd and 8th MAP. The starch content among four varieties showed gradually increased in each interval of time.

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Starch content and sucrose content were observed maximum in the variety Sree Athulya and minimum in the variety H165. While comparing leaves and tubers, the starch and sucrose content was observed maximum in tubers.

Enzyme assay of AGPase, SS, SuSy activity was observed maximum in the variety Sree Athulya. SPS activity, SS activity and photosynthetic activity was observed maximum in the variety Sree Vijaya. H165 variety had the highest invertase activity and lowest SuSy activity and SS activity. H226 had moderate starch content and maximum tuber yield, moderate AGPase activity and SS activity.

From the study it was observed that the variety Sree Athulya had relatively higher AGPase enzyme activity, SuSy activity, moderate SS activity and lower SPS activity and high starch content in tuber with a total yield of 3.31 Kg/plant. The variety H226 has maximum tuber yield and moderate starch content due to decreased SPS activity, moderate AGPase activity and SS activity. The variety H165 had the minimum starch and sucrose content due to decreased SuSy, AGPase activity and SS activity and increased invertase activity. The variety Sree Vijaya had the moderate starch content in tuber as it is a short duration variety due to the highest photosynthetic rate, SS activity and moderate AGPase activity.

High starch varieties (varieties having 30% starch on fresh weight basis) are preferred by industries for starch extraction for various purposes. High starch varieties are also important to meet the calorie requirement of people and food and nutrition security. Therefore by modification of AGPase, SS and SuSy can improve the starch content of tubers of cassava varieties. Sometimes high starch varieties may have bitterness due to the presence of cyanoglucosides. Hence such varieties are not suitable for culinary purpose. On the other hand low starch content. So manipulation of AGPase, SS, SuSy can help in enhancing the starch

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content of such variety. This study can also help in identifying genotypes from the germplasm for genetic improvement of cassava for high starch content.

The variety Sree Athulya was identified as the high yielding one with high activities of AGPase, SS and SuSy. Low starch content varieties can be improved by manipulating the activity of these enzymes.

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APPENDICES

7. APPENDIX

1. REAGENTS FOR SUCROSE ESTIMATION

a. **Dinitrosalicylic Acid Reagent**
   - Dinitrosalicylic acid
   - Phenol
   - Sodium sulfite
   - Sodium hydroxide

b. **Potassium sodium tartarate solution** 40%

c. **HCl, concentrate** (37.3%, 11.9 N)

d. **KOH** , 5N solution

2. REAGENTS FOR STARCH ESTIMATION

a. **5%phenol:** dissolve 50g of redistilled (reagent grade) phenol in water and dilute to one litre.

b. **96% sulphuric acid**

c. **Standard glucose:** stock- 100 mg in 100ml of water.

d. **Working standard** – 10 ml of stock dilute in 100ml with distilled water.

2. REAGENTS FOR ENZYME ASSAY

2.1. Reagents for assay of Invertase

a. 2.5% sucrose solution

b. 1M sodium acetate buffer, pH 5.0

c. 20% glycerol

d. Toluene

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e. **5%phenol:** dissolve 50g of redistilled (reagent grade) phenol in water and dilute to one litre.

f. **96% sulphuric acid**

2.2. Reagents for assay of Starch Synthase

a. 50mM Hepes NaOH

b. 1.6 mM ADP Glucose

c. 0.7 mg Amylopectin

d. 15 mM DTT

2.3. Reagents for assay of AGPase

Extraction buffer

a. 100mM Tricine NaOH

b. 8mM MgCl2

c. 2mM EDTA

d. 50mM 2- Mercaptoethanol

e. 12.5% Glycerol

f. 5% PolyVinyl Pyrolidone

Assay

a. 100mM Hepes NaOH

b. 1.6 mM ADP Glucose

c. 3mM Inorganic Pyrophosphate

d. 5mM Magnesium chloride

e. 4mM DTT

2.4. Reagents for assay of Sucrose Synthase

a. Extraction Buffer

- 50mM Hepes buffer, pH 7.5
- 75mM MgCl2
- 2mM EGTA

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- 5mM MgCl2
- 2% PEG
- 2% PVP

b. 200mM Hepes buffer, pH 7.5

c. 50mM MgCl2

d. 100mM UDP- Glucose

e. 100mM Fructose

f. NaOH, 1N

g. Resorcinol, 1%

h. 30% HCl

2.5. Reagents for assay of Sucrose phosphate Synthase

a. Extraction medium

- 50mM Hepes buffer, pH 7.5
- 75mM MgCl2
- 2mM EGTA
- 5mM MgCl2
- 2% PEG
- 2% PVP

b. 200mM Hepes buffer, pH 7.5

c. 50mM MgCl2

d. 100mM UDP- Glucose

e. 100mM Fructose 6 Phosphate

f. NaOH, 1N

g. Resorcinol, 1%

h. 30% HCl

ABSTRACT

PHOTOSYNTHESIS AND ENZYME ACTIVITIES REGULATING STARCH BIOSYNTHESIS IN DIFFERENT VARIETIES OF CASSAVA (*Manihot esculenta* Crantz)

by

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study on Photosynthesis and enzyme activity regulating starch biosynthesis in different varieties of cassava (*Manihot esculenta* Crantz) was conducted during the period of 2015-2016 in the Division of Crop Production, ICAR-CTCRI, Thiruvananthapuram.

The objective of the study was to study the relation between photosynthesis, leaf area, crop duration and enzymes activities regulating starch biosynthesis in four varieties of cassava (*Manihot esculenta* Crantz) and to determine limiting factor(s) for low starch content of tubers in low starch varieties. Four varieties selected for the study are viz., Sree Vijaya, Sree Athulya, H165, H226. Sree Athulya, a triploid long duration variety had the maximum starch and sucrose content whereas H165, a short duration variety, had the minimum starch content.

The different parameters such as morphological, biochemical, enzyme activity, and photosynthetic activity were recorded / assayed for four cassava varieties. Morphological parameters are number of leaves, leaf area, leaf area index and tuber yield. Maximum noumber of leaves and leaf area was observed in the variety Sree Vijaya. Photosynthetic activity was observed maximum in the variety Sree Vijaya. The minimum number of leaves and leaf area were observed in the varieties H226 and H165. The maximum tuber yield was observed in the variety H226 and minimum tuber yield was observed in the variety H165. Biochemical parameters like sucrose content and starch content were maximum in in the variety Sree Athulya and minimum in the variety H165.

The enzymes activities involved in starch biosynthesis assayed in the present study are viz., AGPase, SPS, SS, SuSy and invertase. Sree Athulya had the highest AGPase activity and SuSy activity and high tuber yield. Sree Vijaya had the highest SPS activity. H165 had the lowest tuber yield due to decrease in leaf area, sucrose and starch content, photosynthetic activity, lowest enzymatic activities of SuSy, SS. Generally the photosynthetic activity the activities of

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AGPase, SS and SuSy enzymes were high in high starch variety Sree Athulya. The present study reveals low activity of enzymes to be the rate limiting for low starch content of varieties H165. Low starch content varieties can be improved by manipulating the activity of these enzymes.

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