PHOTOSYNTHESIS AND ENZYME ACTIVITIES REGULATING STARCH BIOSYNTHESIS IN DIFFERENT VARIETIES OF SWEET POTATO (*Ipomoea batatas* Lam.)

by

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THESIS

Submitted in partial fulfilment of the

requirement for the degree of

MASTER OF SCIENCE IN (INTEGRATED) BIOTECHNOLOGY

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2016

DECLARATION

I hereby declare that this thesis entitled "**Photosynthesis and Enzyme activities regulating starch biosynthesis in different varieties of Sweet potato (*Ipomoea batatas* Lam.)**"is a bonafide record of research work done by me during the course of research and that thethesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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ACKNOWLEDGMENT

Right from the outset, I praise the Almighty God for helping me in successfully undertake the course work, field, laboratory and library research, and to compile this project.

I would like to express my deepest gratitude and appreciation to my major advisor, ***Dr. V. Ravi,*** *Principal scientist, ICAR-CTCRI, for his invaluable advice and stimulating guidance throughout my research work.*

My sincere thanks goes to ***Dr. James George,*** *Director, ICAR-CTCRI, who provided me an opportunity as a CTCRI project fellow, and who gave access to the laboratory and research facilities at the institute. I would also like to thank* ***Dr. A. N. Jyothy*** *(Principal Scientist),* ***Dr. Saravanan Raju*** *(Senior Scientist) and* ***Dr. Padmaja*** *(Principal Scientist) of ICAR-CTCRI for their timely help and support in completion of my thesis work.*

I am also extremely indebted to rest of my advisory committee: ***Dr. B. R. Reghunath, Dr. Roy Stephen*** *for their insightful comments, guidance and encouragement.*

I would never have been able to finish my thesis without the guidance and help from Ms. Aju, Ms. Pallavi and Ms. Vidhya, Manjima and Rijoy. Heartfelt thanks to all the members of Division of Crop production, ICAR-CTCRI. I thank Ms. Geethu for the stimulating discussions, help and support during my project. I would also like to extend my special thanks to all members of CTCRI for providing me with the necessary services and facilities.

Words are short to express my deep sense of gratitude towards my following friends, Geethu, Deepthy, Lechu and all other batchmates who cared and helped me overcome setbacks and stay focused on my work. I greatly value their friendship and I deeply appreciate their belief in me.

I express my deep sense of gratitude to my family members, my Father Krishnan Kutty, my mother Nirmala and My sisters for the unfailing support and continuous encouragement throughout my years of study and research. This accomplishment would not have been possible without them.

Last but not the least, I would extend my deepest gratitude to dear God in helping and strengthening me in all aspects of my life. Your presence has been a constant source of love, concern, support and strength all these years.

Aswini Krishna N. K.

DEDICATED TO MY FAMILY

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LIST OF ABBREVIATIONS AND SYMBOLS USED

°C	:	Degree Celsius
%	:	Percentage
µg	:	micro gram
µl	:	micro litre
µmol	:	micro molar
AGPase	:	ADP-Glucose pyrophosphorylase
BSA	:	Bovine Serum Albumin
cm	:	Centimetre
DNS	:	Dinitro Salicylic Acid
DW	:	Dry Weight
DTT	:	Dithiothretiol
EGTA	:	Ethylene Glycol Tetra Acetic acid
et al.	:	And other co workers
FW	:	Fresh Weight
G6PD	:	Glucose 6 Phospho Dehydrogenase
HEPES	:	Hydroxyethyl Piperazine Ethane Sulfonic Acid
hr	:	Hour
kg	:	kilo gram
rpm	:	rotation per minute
sec	:	Seconds
MAP	:	Month After Planting
ml	:	milli litre
mM	:	milli Molar
min	:	Minute
M	:	Molarity
nm	:	nano molar
N	:	Normality
OD	:	Optical Density
PEG	:	Poly Ethylene Glycol
PEP	:	Phospho Enol Pyruvate
PGM	:	Phospho Gluco-Mutase
PPi	:	Inorganic Pyrophosphate
PVP	:	PolyVinyl Pyrrolidone
SS	:	Starch Synthase
SuSy	:	Sucrose Synthase
SPS	:	Sucrose Phosphate Synthase
U	:	Unit
UV	:	Ultra Violet

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INTRODUCTION

Chapter 1

INTRODUCTION

Sweet potato (*Ipomoea batatas* Lam.) belongs to Convolvulaceae family. It is grown as a starchy food crop throughout the tropical, subtropical and frost-free temperate climatic zone(Bourke, 1982). Sweet potato is the seventh most important among the food crops of the world (Nedunchezhiyan and Ray, 2010). India is one of the leading producers of this crop. It plays an important role in food and nutritional security of poor and tribal farmers and has potential to substitute cereals owing to their high carbohydrate and calorie content. In addition to its nutritional value, sweet potato is remarkable for its ability to produce high yields of edible tubers in a relatively short growing season (Vries *et al.*, 1967). In India, the annual productivity of Sweet potato is 12.1 tonnes/ha (Indiastat, 2015).

Sweet potato is considered as the major staple crop and produces large quantities of dietary energy for the populations in the developing world. Yield of sweet potato is determined by many factors such as soil fertility, varietal selection, weather conditions, weed, resistance to diseases and crop management practices. Soil fertility increases the growth and yield performance of the crop (Onunka *et al.*, 2012). The plant is tolerant to a wide range of soil conditions but it is sensitive to water logging. A major cause of production loss arises from infestation of the tubers with sweet potato weevil and related pests. It is very difficult to control the continuous reproduction of the weevil throughout the year.

Sweet potatoes have a wide variability in tuber flesh color which ranges from white, yellow and purple to orange flesh. The purple-fleshed sweet potato is rich in anthocyanins with a strong anti-oxidative, anti-mutagenic, anti-hyperglycemic, hepatoprotective and antihypertensive effects. The orange-fleshed sweet potato is rich in beta-carotene and can alleviate the problem of vitamin A deficiency. Mitra, 2012 reported that increased consumption of orange-fleshed sweet potato in either fresh or cooked form can alleviate the dietary deficiency of Vitamin A and thereby combating night blindness which is a major public health concern.

The light intensity is an important factor for photosynthesis, growth and the yield or quality of tuber crops. In the case of sweet potato, the photosynthetic rate is highest during early growth and declines towards the end of growth. The net photosynthesis has a direct relation with the crop yield (Zelitch, 1982). Yield also depends upon leaf area, which means that the tuber yield increases with the increase in leaf area. The rise in temperature between 5 and 35°C increases the rate of photosynthesis more than 5-fold (Cen and Sage, 2005). Sweet potato leaves show maximum photosynthetic rate between 10.00-11.00hr followed by stomatal closure and significant decrease in P_N rate during mid-day (Ravi, 2003).

Starch is one of the most abundant polysaccharide and it is mainly used as food. It has many other applications in various industries. At present, starch is extracted commercially from a number of sources. Genetic engineering may allow the modification of tuber crops for increasing the content and quality of starch. The engineering of starch biosynthesis may also lead to the modification of starch granule shape, amylase to amylopectin ratio, amylopectin chain length, crystallinity, gelling properties, phosphorylation and lipid content. These changes may significantly alter the chemical and physical properties of starch.

Starch synthesis mainly takes place as a result of photosynthesis in chloroplast. Storage organs committed primarily to starch production are called amyloplasts. The dynamic changes of the activities of enzymes involving in starch biosynthesis including invertase (IT), ADP-glucose pyrophosphorylase (AGPase), starch synthases (SS), sucrose synthase (SuSy) and sucrose phosphate synthase (SPS) were studied in different crops (Lu *et al.*, 2008; Nakamura *et al.*, 1989)

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

2.1.Sweet Potato - An introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a member of the Convolvulaceae family (Purseglove, 1972). It ranks seventh among the food crops of the world. Sweet potato is considered as a hexaploid. Most sweet potato cultivars are self-incompatible. Nishiyama (1971) and Martin and Jones (1972) suggested Mexico as a centre of diversity of the *batatas* section of Ipomoea. Sweet potatoes are cultivated in tropical and warm temperate regions where sufficient water is available for their growth. It is used as a food security crop in wide population.

The sweet potato plant consists of branching creeping vine with spirally arranged lobed, heart shaped leaves on stem, white or lavender flowers and roots, which stores starch as the major carbohydrate. It also produces few to numerous branches depending on the branching characteristics. Generally, fully-grown sweet potato plants have primary, secondary and tertiary branches. Sweet potato vines can reach 4 m in length and the plant is usually grown for 90-120 days.

The canopy of sweet potato is a thin layer of leaves held parallel to the soil surface because of its prostrate nature. Leaf size is strongly modified by the sunlight and thereby increases the total leaf number. The sweet potato plant produces one to several tuberous roots at its maturity and it may vary between different cultivars. In addition to cultivar difference, the total number of tuberous roots can be affected by potassium fertilization, shading and low temperature.

Depending on the tuber flesh of the sweet potato, it can be white, cream, yellow, orange, pink or deep purple. Among these, the most common are white or cream and yellow-orange flesh. The intensity of the Sweet potato's yellow or orange flesh colour is directly correlated to its beta-carotene content. Our bodies can produce vitamin A by consuming the beta-carotene of orange-fleshed Sweet potato. Therefore, this nutrient is referred to as "Pro-vitamin A" (Dincer *et al.*,

2011). Purple-fleshed sweet potatoes are the source of anthocyanins and have high anti-oxidant activity. Even the leaves of the sweet potato plant have high important anti-oxidant properties (Steed and Truong, 2008).

2.2.Origin and History

The Sweet potato was originated in Central or Tropical South America. It entered into cultivation in 3000 B. C. (O'Brien, 1972). The extensive cultivation of sweet potato in Europe, Africa and Asia occurred in times that are more recent. Today, sweet potato is cultivated in all parts of the tropical and sub-tropical world and in temperate regions also. It was introduced into china in different places at different times where Fujan historically had excellent conditions for obtaining the higher yield and close trading links with many other provinces (Gitomer, 1996).

2.3. Economic importance of Sweet potato

Sweet potato is an important food crop in large sectors of population in the tropics, subtropical and temperate regions of the world. The bulk production was now concentrated in Asian countries especially in developing countries. This accounts for 93% of the global production. From these, china is the largest producer of sweet potato and it accounts for more than 83% of world sweet potato. A reason for promoting the production of sweet potato is because of the increased income and all parts of the sweet potato plant used for consumption purpose and the associated demand for a more diverse diet.

Sweet potato is regarded as the most important root crop of the tropics. It can be planted and harvested at any time of the year, especially in frost free areas. It has short cropping season (3 to 4 months) and usually vines are used as the planting material.

Sweet potato produces more edible than any other major food crop. It is more productive within short periods on marginal lands and plays an important role in the economy of poor households (Motsa *et al.*, 2015). The most important edible sweet potato parts are the storage roots and immature leaves, which are used for human consumption and as animal feed (Placide *et al.*, 2013)

Storage roots can be consumed after boiling, baking or roasting but some people preferring to eat them raw. The leaves can also be consumed as a green leafy vegetable and the vines are widely used as a fodder for livestock. Sweet potato parts such as leaves, stalks and stems have a high nutritive value and all parts are rich in dietary fibre (Ishida *et al.*, 2000). Mineral contents and vitamins such as A, B2, C and E are high in leaves in comparison with other vegetables (Bovell-Benjamin, 2007). Storage roots and vines are used as a protein and vitamin source for animals (Chen *et al.*, 1977).

It was reported that sweet potato leaves have some medicinal properties. Polyphenol rich leaf extracts of sweet potato play an important role in reducing prostate cancer (Karna *et al.*, 2011). In South America, the plant sap or juice of red sweet potato has been used for producing dye for cloth was reported. To a limited extent, they can be used as starch source in industrial processes and alcohol production.

2.4. Soil type and texture

Sweet potato shows good adaptability to diverse soil conditions. It grows well on a different soil type. However, excels on fine sandy loam with good internal drainage. The ideal soil pH value ranges from 5.8-6. It grows well in high rainfall regions and well-irrigated conditions. Water stress conditions can affect the yield reduction. Sweet potato is quite sensitive to water logging conditions. Ridging to promote optimum drainage is important during the later stages of growth and development of storage roots.

2.5. Weed control

Weeds are the major problem in the Sweet potato field during the first two months of the growth. Sweet potato vines grow quickly in six to seven weeks, cause rapid and effective coverage of the ground surface and prevent the over growth of weeds. For this reason, traditional farmers do hand weeding in every three weeks after planting. The use of herbicides to control weeds in sweet potato

is widely practiced in various parts of the world. Herbicides have been found not to affect the storage root quality or processing quality.

2.6. Fertilizers

Sweet potato often considered as a crop grown in poor soils. Therefore, the fertilizer use varies with the soil genesis, rainfall regions. It is well suited to sandy soils and the yield lowers in heavily fertilized soils. Potassium is the necessary element for the development of storage roots because leaves promote the translocation of potassium to the root system. Sweet potato responds to phosphorous fertilizer also because this crop adapted to the less available soils. Many growers have developed NPK ratios for their own fertilizers program.

2.7. Harvesting

The growth and harvesting period of Sweet potato varies with cultivars and the environmental conditions and growing locations. The tubers were harvested only after its full maturity. Yellowing of the leaves and senescence indicates that the tubers of sweet potato are matured and the crop is ready for harvesting. Sometimes visible signs of maturity of crop will not occur. Mature tubers are recognized by cutting and it does not readily darken. Sweet potato is harvested by clearing the vines, earthed up the tubers and stored for further usage. These leaves can use as an animal feed. Traditionally Sweet potato cultivation and harvesting is done for the food.

2.8. Curing and storage

After harvesting, the storage roots are not sold directly from the field. Tubers are subjected to curing to promote rapid healing of wounds inflicted during harvesting and to increase the strength of the storage root. Curing must be done immediately after harvesting. It is effective for reducing the post-harvest water loss and to minimizing microbial infection and keeps well in storage for long time. Storage roots should be subjected for curing process within 4-5 days after harvest and it is often carried out in farm with low cost. Lower humidity

prolongs the curing time. High humidity promotes the healing process and it reduces the shrinkage of the storage roots. It was reported that the curing process can be enhancing the culinary property by increasing the amylase activity and improves the cooking quality and sweetness (Walter *et al.*, 1975). Some researches refer to the green flavor of uncured sweet potato that disappears after curing (Hamann *et al.*, 1980). After curing process, the sweet potato tubers can be stored at conditions of 12.8-15.6°C.

2.10. Morphological parameters

Crop growth parameters are important trait in crop physiology such as total number of leaves per plant, leaf area (cm^2) per plant and tuber yield per plant. The total dry matter production by crops depends on the size of the leaf canopy, the rate of leaf production and duration of the canopy persists. Leaf area per plant is a function of the total number of leaves per plant and the size of the leaves per plant.

According to Kays (1985) differences in leaf size arises due to effects on cell division and expansion. Some morphological parameters were determined in cassava. Several studies were conducted on the effects of elevated atmospheric CO_2 on potato plants (Sicher and Bunce, 1999; Schapendonk *et al.*, 2000). The P_N rate is measured in plants of potatoat weekly intervals throughout their growth in a controlled environment. Leaf area and dry weight of sections of the plant were also determined and discussed in relation to existing theories that photosynthesis can be limited by carbohydrate accumulation in leaves, and stimulated by the initiation of tubers (Frier, 1977).

2.11. Biochemical parameters

Sweet potato carbohydrate fraction consists of starch, sugars, pectin, cellulose and hemicelluloses. Starch is the major form of carbohydrate reserve in higher plants. Transient starch is synthesized in leaves at day time and consumed at night for plant respiration and translocation to sink tissues. Storage starch is the

energy reserve for the reproduction of plants and is an important energy source for humans and animals.

In Sweet potato, starch is the major carbohydrate and accounts for 65-80% of the total dry matter and it vary among varieties. The major sugar in storage root is sucrose followed by fructose and glucose whereasmaltose is the major sugar after cooking in storage roots.

A significant variation in sugar content was noticed among the raw as well as baked Sweet potatoes and the study assumed that sweetness observed in baked tubers may be due to the initial sugar content, hydrolysis of starch or else a combination of both (Morrison *et al.*, 1993).

Very young tubers have the highest levels of reducing sugars and decreases considerably towards the end of the growing season (Harris, 1992). Similarly, high levels of sucrose is found in the young tubers and this may differ by decrease of sucrose at the later phase of growth and increase in sucrose level until flowering (Kolbe and Stephan-Beckmann, 1997).

Sweet potato roots continue to respire after harvest. Glucose is the most likely substrate for respiration. It can be postulated that starch is slowly degraded to dextrin, which is rapidly degraded to glucose.

According to Tsou *et al.* (1989) the sugar and starch content in sweet potato vary between 8- 28% and 44- 78% respectively. Based on the exhaustive review of Tian *et al.* (1991), starch content of Sweet potato starch ranges from 83.5% in commercial samples to 98.8% in those produced under research conditions. Non-starch components associated with the starch were residual ash, lipids, fiber and proteins. The phosphate content of sweet potato ranged from 9-22 mg per 100g (Madamba *et al.*, 1975).

2.12. Rate of photosynthesis

Photosynthesis is a process for assimilating carbon to provide energy for plant growth. In most plants, the assimilated carbon is partitioned between sucrose which is immediately available for growth and transient starch which accumulates temporarily during the day in leaf chloroplasts. The leaf starch is degraded during

the night to provide a continued supply of sugar to sustain metabolism and for export to reserve tissues such as the developing sink throughout the night. A rise in the photosynthetic productivity is essential for root formation in sweet potato (Enyi, 1977) which may be achieved by increasing the amount of PAR (Photosynthetically Active Radiation) intercepted by the photosynthetic tissues.

An optimal level of light capturing is required for the efficient growth of crop plants and for attaining maximum dry matter yield. This suggests the significance of higher rate of of photosynthesis (P_N) per unit leaf area. The substantial genetic increase in grain yield observed in most of the crop plants is associated with change in P_N rate. Thephotosynthesis yield relationships are described in terms of single leaf photosynthesis.

The measurement of Photosynthesis at saturate radiation level by maintaining a defined leaf position for short durations may not represent crop production potential. The photosynthetic rate of leaf tissue depends greatly on age of leaf, leaf position, sink strength and time of the day. LeCain *et al.* (1989) have found that size of the leaf is inversely related to P_N, photosynthetic rate. The study reported that smaller size of leaf is a desirable trait which tends to reduce/enlarge its size with increased photosynthetic activity per unit leaf area and result in less mutual shading in a crop canopy. This briefly implies that increased P_N rate result in subsequent decline in in leaf area. But certain wheat varieties combine both traits of high $P_N$and large leaf area. Sharma and Ghildiyal (2005) have suggested the usefulness of such genotypes in crop breeding programmes as a different source of germplasm for increasing P_N rate.

The measurement of leaf area at different stages of plant is essential for the quantification of several growth analysis parameters involving photosynthesis and respiration. Aase (1978) have described the association between leaf area and dry weight of leaf and estimation of leaf area using leaf weight.

As the leaf area estimation relates to dry matter variation, this relationship can efficiently be represented in terms of leaf area per unit land area, i.e., leaf area index (LAI) than referring it as leaf area (cm^2) per plant (Watson, 1952). Stern and Donald (1961) have found an optimum LAI corresponding to maximum

growth rate for any intensity of radiation. It was reported that the total dry weight of the plant and total grain yield correlated with the leaf dry weight which depended on the total leaf area produced.

In several crops like cassava, rice, corn, peanut and tobacco plant, regression analysis have been used to elucidate prediction equations for leaf area estimation (Tejwani *et al.*, 1957; Spencer, 1962; Vivekanandan *et al.*, 1972).

One of the fastest methods for leaf area estimation was reported by Pearce *et al.* (1975). This method offers the use of length and width measurements for only a single leaf per plant which can be used for experimental treatment with several plants per genotype. In maize, the area of leaf number 8 from the top of the plant was highly depended on the total leaf area of the plant which obtained a large mean area over several other genotypes. On computation of leaf area, the regression coefficient, b = 9.39, was considered as a reliable leaf-area coefficient for the estimation of total plant leaf area in maize.

Recent studies have focused on photosynthate accumulation in low and high yielding sweet potato varieties and found increased level of accumulation in different cultivars which maximized the tuber yield (Bhagsari and Ashley, 1990) and biomass production (Hai and Kubota, 2001). In addition, some studies have proved that dry matter of tubers is positively correlated with the photosynthetic (P_N) rate (El-Sharkawy *et al.*, 1990; Huang *et al.*, 2012).

Liu (2015) have studied the photosynthate accumulation and transport among low and high yielding varieties of sweet potato. The authors reported that high yielding varieties showed maximum tuber yield with strong transport capacities and exhibited higher mean accumulation rate. In the early growth stage of high yielding cultivars, the net P_N rate was 8.9% which increased to 11.4% in the middle phase of growth and after the growth peak, the leaf area index (LAI) decreased to an optimum range (2-3).

The development of storage roots originates at cellular level and includes cell expansion which is associated with increase in size, cell number and weight as a result of accumulation of photosynthetic metabolites (Ravi *et al.*, 2009). The increase in dry matter content is largely depended on the ability of storage root to

receive metabolites from photosynthetic organs. For instance, Li (2008) have stated the transportation of photosynthesized sucrose from leaves towards underground parts of storage roots *via* stem.

2.13. Starch biosynthesis in plants

Starch is the major storage component present in barley, cassava, maize, potato, rice and sweet potato and serves as the primary carbohydrate rich diet of humans and livestock, and also has important industrial applications. Starch is synthesized in plastids of photosynthetic and non-photosynthetic tissues and the storage organs committed for starch synthesis is called as amyloplasts.In the leaf chloroplast, it isin the form of transitory starch and as storage starch in amyloplasts (Ball *et al.*, 1996). During the day, the mature chloroplasts provide energy in the form of ATP and photosynthetically fixed carbon for starch biosynthesis (Streb and Zeeman, 2012) and mobilization of starch take place during night to support non-photosynthetic metabolism. The biochemical pathway of starch synthesis from sucrose is represented in Fig 1.

The transient biosynthesis of starch is observed in storage organs like seeds, fruits, tubers and storage roots. Neuhaus *et al.* (2005) have found that such type of transient synthesis is different from the long-term storage synthesis in case of non-storage organs.

Starch is chemically made up of two types of glucose polymers: amylose (15-30%) and amylopectin (70-85%). Amylose consists of predominantly linear chains of glucose residues linked via α-(l,4)-glucosidic bonds(length of each chain is about 1000 glucose units). It is usually branched at a low level by α-(1-6) linkages and forms 30% of starch. This estimated fraction varies with plant species and was assumed to range between 11% and 37% of storage starch when surveyed in about 51 plant species (Deatherage *et al.*, 1955) and ranged from 20 to 36% in a survey of 399 maize varieties. Once the starch is obtained from plants, amylose forms inter molecular hydrogen bonding resulting in rigid gel formation. However, it may lead to retrogradation/shrinkage and crystallization of solid gels

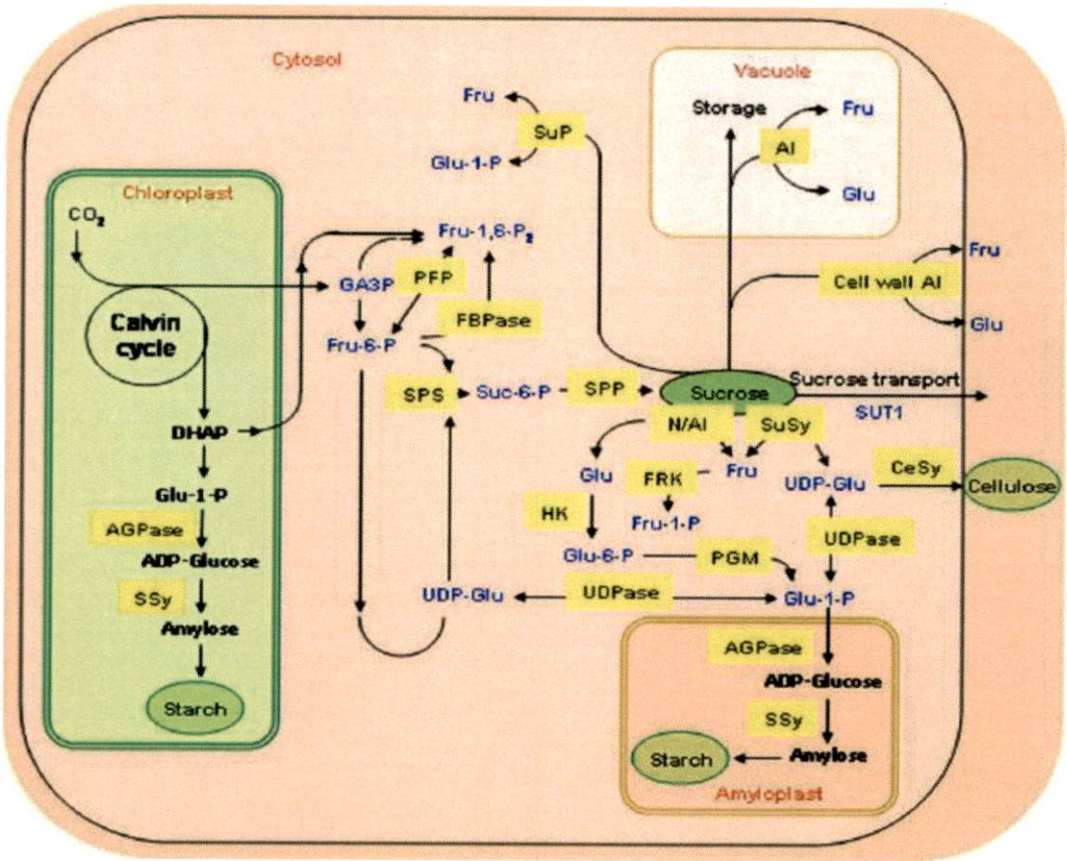


Fig 1: Schematic representation of sucrose to starch metabolism in higher plants

NB:

AGPase	:	ADP-glucose pyrophosphorylase
AI	:	Acid Invertase
N/AI	:	Neutral or Alkaline Invertase
SPS	:	Sucrose Phosphate Synthase
SS	:	Starch Synthase
SuSy	:	Sucrose Synthase

on melting and therefore it depends on concentration, degree of polymerization and temperature (Shewmaker and Stalker, 1992).

Amylopectin, accounts for 70-90% starch granule weight and consists of highly branched glucose units, which makes up the matrix where amylose is synthesized. These amylopectin molecules are radially arranged with around 20 α-(l,4) linked glucan chains joined by α-(1,6) chains with heavy branching. The amylopectin chains are categorized into A (external α-(1,6) chains of 6-16 glucan units carrying no branch points), B (longer chains with one or more branch point) and C chain (single B-chain with a free reducing end).An average estimate on length of amylopectin molecule ranges between 200 and 400nm (i.e., 20-40 clusters) and has a width of 15nm (Martin and Smith, 1995).

The first committed step in starch biosynthesis involves the conversion of Glc-1-P and ATP to ADP-G (ADP Glucose) and inorganic pyrophosphate (PPi), catalyzed by ADP-Glc pyrophosphorylase (AGPase), also called as alpha-D-glucose-1-phosphate adenyl transferase (Greene and Hannah 1998).

Once activated, starch synthase catalyzes the transfer of ADPG to the non-reducing end of an α-1,4 glucose residue resulting in the formation of linear α-1,4 glucose unit. This linear α-1,4 glucan product is used by starch branching enzyme (SBE or Q-enzyme) for introducing α-1,6 interlinear chain linkages to form amylopectin molecules. As a result, the starch debranching enzymes (DBE) together with phosphorylase (P-enzyme) and glucanotransferase (D-enzyme) crystallizes the amylopectin into starch (Ball *et al.*, 1996). It was reported that another enzyme called UDP-glucose: protein glucosyl transferase or amylogenin (38 or 45 kDa) was assumed to function in the initial priming process of starch biosynthesis.

2.14. Enzymes involved in starch synthesis

a) ADP-Glucose pyrophosphorylase (EC 2.7.7.27)

The Starch biosynthesis pathway commences with sucrose synthesis *via* photosynthesis and ADP-Glc pyrophosphorylase represent the rate limiting step during this process (Nakatani and Komeichi, 1992).The Sucrose produced is used

for different metabolic process such as wound/stress signaling, assimilate partitioning, osmoregulation and is otherwise stored depending upon the plant's developmental status.

This enzyme belongs to transferases group of enzymes which are involved in transferring phosphorus containing nucleotide groups. AGPase is having 220kDa size with catalytic and allosteric regulatory function (Stark *et al.*, 1992). In case of bacteria, the AGPase is a homo-tetrameric unit encoded by a single gene and is activated by glycolytic intermediates such as Fru-6-P, Fru-1,6-bisP, pyruvate and inhibited by AMP. But in higher plants and blue green algae, AGPase is activated by CO_2 assimilatory intermediates of C3 pathway such as 3-phosphoglycerate (3PGA) and inhibited by Pi, inorganic phosphate (Preiss *et al.*, 1966).The ratio of 3PGA to Pi is supposed to be the key regulator of starch accumulation in many plant species and isozymes.

Perez *et al.* (1975) reported that AGPase activity accelerated during starch accumulation to a high rate within 8-18 days after flowering period. Substantial evidence was given by Joseph *et al.* (2001) that AGPase activity directly increased the starch and sucrose content in soybean (*Glycine max*) grown in controlled conditions of elevated CO_2 and supra-optimal temperature. Increased activity of AGPase and UGPase with simultaneous increase in starch content was observed in stolon tissues and developing potatoes during the tuberization, maturation and storage of potato (Sowokinos, 1976). The loss of starch producing ability was reported in maize as a result of nonfunctional AGPase enzyme in shrunken and collapsed kernel phenotype of maize endosperm (Dickinson and Preiss, 1969). The level of starch deposition and AGPase activity was studied in non-embryogenic cells and developing embryos of induced cultures of carrot (Keller *et al.*, 1988).

A recent study notified a 3-fold decrease in ADP-G activity with simultaneous inhibition of starch synthesis during the detachment of potato tubers from the mother plant (Geigenberger *et al.*, 1994). MacDonald and Strobel (1970) studied the activity of AGPase enzymes at different intervals after pollination in wheat endosperm and found the increased activity of AGPase in controlling starch

biosynthetic pathway. The role of AGPase activity in heterotrophic tissues of plants was stated by Fernandez *et al.* (2003). Genetic evidence has been provided on the redox regulation of starch synthesis in transgenic *Arabidopsis* leaves expressing mutated AGPase (Neuhaus and Stitt, 1990) and the study proved that complete control of biosynthesis was catalyzed by AGPase.

As we know, AGPase donates ADP-G for starch synthesis, but under certain conditions, AGPase becomes a key regulator in determining the extent of starch deposition. This evidenceis more convincing for treatments that modify partitioning between sugar and starch in *Arabidopsis* leaves (Glaring *et al.*, 2012). Fluctuations in AGPase during the development of pea seed and wheat grain revealed that maximum rate of starch production coincided with the maximum AGPase activity. In these developing plants, sucrose content and AGPase activity declined in parallel with an increase in starch content towards the maturation/later phase of seed growth (Turner, 1969a,b). In potato tubers, starch synthesis decreased with AGPase activity (Geigenberger *et al.*, 1999) while strong dependence was observed between sucrose content and AGPase activity (Tiessen *et al.*, 2002).

b) Starch Synthase (EC 2.4.1.21)

Starch synthase (SS) is the major enzyme involved in starch synthesisin plant tissues. Starch synthase uses ADP-G to form linear glucan chains by forming α-1,4 linkages to the non-reducing end of glucan. The storage endosperm contains atleast five distinct isoforms of SS which are classified based on the amino-acid sequence characteristics and reaction patterns. Similar attempts for characterizing starch synthases was reported in soluble fraction of potato tubers (Baba *et al.*, 1990; Hawker *et al.*, 1972).

SSI, SSII, SSIII and SSIV are four soluble isoforms primarily involved in amylopectin synthesis and the fifth isoform, SSV called granule bound starch synthase (GBSS) present in all plant species is responsible for amylose synthesis. The occurrence of four soluble forms in plant tissues may vary depending upon plant species. The enzymatic assay of SS synthase was determined

spectrophotometrically by measuring the ADP-G-dependent production of ADP at A340 nm.

Vrinten and Nakamura (2000) identified a new GBSS isoform, GBSSII, from waxy wheat which is expressed only in the non-storage tissues, but not in the cereal endosperms. The activity of SS bound to the starch granule also increased progressively with increased starch content of the grain (Perez *et al.*, 1975). In wheat grains, the high temperature-sensitive soluble starch synthase is found to be more important in regulating starch synthesis (Pravin *et al.*, 2004).

The solubulization of starch synthase by urea and pullulanase treatments resulted in the interpretation that bound SS on storage granules specific for ADP-G or UDP-G were different and was localized in the starch granules (Sasaki and Kainuma, 1980).The study on SS activity by Ozbun *et al.* (1973) on normal kernels and on mutant waxy maize endosperm revealed that the waxy mutation brought changes in pattern of enzyme activity that can be correlated with the different types of SS isozymes. The type I enzyme showed higher activity with glycogen as primer than with amylopectin (MacDonald and Preiss, 1983). Two soluble SS isozymes such as SS I and SS II were detected in developing kernels of maize (*Zea mays*).

c) Sucrose Synthase (EC. 2.4.1.13)

Sucrose synthase (SuSy) is a highly regulated enzyme of cytosol that catalyzes the reversible chemical reactionconverting sucrose and a nucleoside diphosphate (UDP is the preferred substrate) into fructose and the corresponding nucleoside diphosphate glucose which is shown below:

UDP-glucose + D-fructose <------->UDP + sucrose

The sucrose synthase is having significant importance in studying sink strength and starch production (Ruan *et al.*, 2003) in heterotrophic plant tissues. The genetic evidence for the importance of SuSy in sink strength determination was explained by applying QTL analyses in endosperm of maize and in cotton (Thevenot *et al.*, 2005; Rong *et al.*, 2005). Studies citing similar reports included biomass production in transgenic cotton expressing SuSy (Xu *et al.*, 2012), starch

level reduction in maize endosperm mutants having less SuSy activity (Chourey and Nelson, 1976) and transgenic potato (Zrenner *et al.*, 1995) and carrot (Tang and Sturm, 1999) exhibiting altered SuSy activity.

The correlation between the activities of SuSy and sink strength and/or dry weight accumulation was recently studied among different crop plant such as radish (Usuda *et al.*, 1999), sweet potato (Yatomi *et al.*, 1996) and tomato (Wang *et al.*, 1993). Li and Zhang (2003) have identified that SuSy activity was predominantly active during the developmental stage of storage roots of sweet potato in determining sink strength. The activity of SuSy altered the treatment modifying the partitioning by heat stress in potato tubers (Lafta and Lorenzen, 1995). The consistent activity of SuSy higher than the rate of invertase was observed during the development of wheat endosperm. Another work studied during the development of bananas reported that the enzyme activity was high and was constant during the entire phase of starch synthesis (Beatriz *et al.*, 1995).

Sucrose synthase activity was studied in tomato fruit and endosperm of rice grains (Nakamura *et al.*, 1989; Schaffer and Petreikov, 1997) and investigated the accumulation of transient starch in developing tomato. In this study, specific activities of UGPase, PGI and invertase were studied.

d) Sucrose phosphate synthase (EC 2.4.1.14)

Sucrose is the main form of translocated sugar in most plants. Its synthesis is catalyzed by sucrose phosphate synthase (SPS) and regulates the division of photosynthate intermediates between sucrose and starch in the leaves. The reaction catalyzed by SPS in sucrose biosynthesis is as follows:

UDP-G + Fruc-6-P ------> Sucrose phosphate + UDP

Geigenberg *et al.* (1999) have reported the inhibition of sucrose synthesis under water stressed condition in transgenic potato expressing low SPS activity. The reduction in SPS was found to have no major role in metabolism in non-

stressed tubers. The dominant role of SPS in *de novo* synthesis of sucrose was observed in sorbitol shocked Sweet potato (Wang *et al.*, 2000).

In mature leaves of plants, decreased SPS activity was observed under water deficitenvironment which accounted for the inhibition of photosynthetic mechanism (Vassey *et al.*, 1991). Change in sucrose partitioning between tubers and shoots in potato and decline in the net crop yield was reported as a result of heat stress. The increase in the enzyme activity occurred as a result of increase in the amount of SPS (Wolf *et al.*, 1990).

The modification in the covalent nature of SPS through reversible phosphorylation in response to light stress has been identified in some of the plant species (Huber *et al.*, 1989). The change in the kinetic properties of SPS was reported by Hubbard *et al.* (1990) in banana plant at the time of ripening. Oparka *et al.* (1987) reported that the tubers having high sink strength and high SPS activity initiates photosynthesis and biomass accumulation.

Studies by Schaffer and Petreikov (1997) revealed that the SPS activity was higher in potato tubers at high temperatures. In soya bean, SPS activity positively correlated with total dry matter accumulation and plant growth rate (Kerr *et al.*, 1984).

e) Invertase (EC 3.2.1.26)

Invertase (IT) is an enzyme belonging to hydrolases family which catalyzes an irreversible reaction of sucrose breakdown. The reaction occurs at an optimum temperature of 60°C and an optimum pH of 4.5. Based on the pH value, invertase appears as acidic and alkaline forms. In plants, the IT is localized in three sites such as cytoplasm, apoplast and vacuole. Instead of UDP-G as end product, IT produces glucose and form twice the number of hexoses. These two features provide greater capacity for IT to stimulate sugar sensors.

The transgenic potato tubers expressing cytosolic or apoplastic yeast invertase was developed to increase the sink capacity in potatowhere only the apoplastic invertase had a direct correlation with tuber yield (Hajirezaei *et al.*, 2000).In tomato, sucrose is used by the leaf which plays the role as transported

carbon.The IT in presence of UDP catalyzes the conversion of sucrose into UDP-G and fructose. The channeling of sucrose into apoplast and hexose to vacuolarsites through the plasmalemma and tonoplast is assisted by extracellular and vacuolar invertases.

Isolation, purification and characterization of various extracellular and vacuolar acid IT iso-enzymes were studied in tomato with highly diversified sink expression patterns (Nguyen and Foyer, 2001). Contrasting evidence was obtained regarding the role of acid IT in tomato fruit in sink establishment and maintenance. For this purpose, IT activity was measured in tomato fruit in relation to starch accumulation (Wang *et al.*, 1993). High invertase activity in potato tubers reduced sucrose content over 95% and significantly increased glucose content. Increase in glycolytic intermediates, catalytic activity of enzymes in respiratory pathway, CO_2 production and a reduction in starch biosynthesis proved the role of IT in channeling sucrose towards glycolytic pathway (Trethway *et al.*, 1998).

Acid invertase enzyme and was highly correlated with dry weight accumulation in the flowering organs of lily (Ramwala and Miller 1998).Sung *et al.* (1994) compared the role of IT and SuSy in pod elongation of beans and found that IT was associated with pod elongation and SuSy was positively linked to dry matter gain.

Elevated rate of IT activities also correlates with decreased content of stored sucrose in sugar beets while onset of sucrose storage is accompanied by a decrease in IT activity (Silvius and Snyder, 1979). The same was true for carrot roots (Ricardo and ap Rees, 1970), melon (McCollum *et al.*, 1988), citrus (Lowell, 1986)andwild tomato (Miron and Schaffer, 1991). In these plants, invertase was very active prior to sucrose gain and dropped significantly upon maturity. Invertase activity is high in plant tissues that are at a rapid stage of growth and development. Jain *et al.* (2008) understood the role of IT in sucrose transport and its utilization in developing grains such as sorghum (*Sorghum bicolor*).

The rapid increase in IT activity with simultaneous decrease in sucrose content of root tissue in response to wounding was studied in sweet potato. Acid

and alkaline IT were purified and studied from wounded and fresh tissues. The acid IT was 1,3-fructofuranosidase-specific and was unaffected by substrate or any cations or metabolites whereas the alkaline IT was sucrose-specific, inhibited by glucose and glucose 6-P and followed non-Michaelis-Menten kinetics (Matsushita and Uritani, 1974).

MATERIALS AND METHODS

Chapter 3
MATERIALS AND METHODS

The study entitled "Photosynthesis and Enzyme activities regulating starch biosynthesis in different varieties of sweet potato (*Ipomoea batatas* (L.) Lam.)" was conducted at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala during October 2015-August 2016. Details regarding the experimental materials and methodology adopted for various experiments are presented in this chapter.

3.1. Cultivation of Sweet potato

Four varieties of Sweet potato *viz.*, Sree Kanaka, Sree Arun, ST-13 and Kanhangad were cultivated in the field with three replications consisting of 25 plants each (Plate 1 and Plate 2). The crop was planted and grown under irrigated field conditions in Block I of ICAR-Central Tuber Crops Research Institute (CTCRI) during January 2016.

3.2. Sample Collection

Sweet potato leaves of different varieties were collected and kept for drying in a hot air oven at 62°C for 2-3 days. Tubers were collected, washed in tap water, sliced into small cubes and then kept for drying in a hot air oven at temperature 62°C for 3-4 days. After drying, the leaf and tuber samples were powdered using a homogenizer. These powdered samples were used for the quantification of starch and sucrose content. Fresh leaves and tubers were harvested for studying the activity of enzymes involved in regulating the starch biosynthesis.

3.3. Morphological parameters

3.3.1. Number of leaves per plant

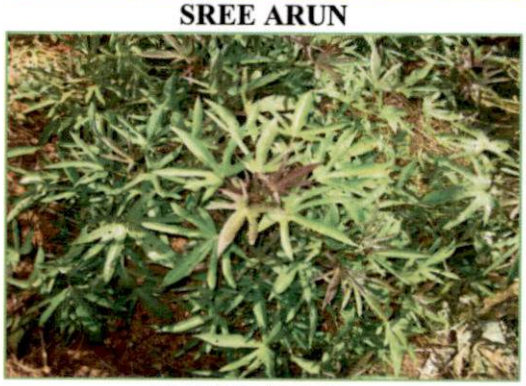
The total number of leaves per plant of sweet potato varieties/genotypes *viz.*, Sree Kanaka, Sree Arun, ST-13 and Kanhangad were counted in every month from February to May during the crop growth period.

Plate 1:Field plot of sweet potato cultivation

SREE KANAKA

SREE ARUN

ST-13



KANHANGAD

Plate 2: Four varieties of Sweet potato selected for the study (Sree Kanaka, Sree Arun, ST-13 and Kanhangad)

3.3.2. Leaf Area (cm^2) per plant

The leaf area per plant was calculated in four varieties in each month from February to May. Three varieties viz., Sree Kanaka, Sree Arun and ST-13 had non-lobed leaf structure. So the leaf area was calculated as follows:

Leaf area =l×b×0.45×total no. of leaves per plant

where, l is the length of the leaf and b is the breadth of the leaf

The variety Kanhangad had multi-lobed leaf and leaf area was calculated as follows:

LA = l ×b× no. of lobes×1.24×total no. of leaves per plant

Leaf area index (LAI) was calculated as:

$$LAI = \text{Leaf area in } cm^2/60 \times 20 \text{ cm}$$

3.3.3. Tuber yield

The tuber yield was recorded after the harvest of four Sweet potato varieties. The yield was expressed as kg per plant.

3.4. Biochemical parameters

3.4.1. Quantitative estimation of sucrose

The sucrose content in leaf and tuber samples was estimated by DNS(Di-nitro salicylic acid) colorimetric method (Wang*et al.*, 1993), Sadasivam and Manickam, 1992).

a) Extraction of sugar

- Ten ml of 80% acetone was added to 250mg powdered leaf sample taken in a beakerand the chlorophyll was removed by keeping in boiling water bath for 15min.
- The green coloured supernatant was decanted and the above step was repeated twice.
- Then 10ml 80% ethanol was added to the remaining residue and kept in a boiling water bath until the alcohol is evaporated.

- The sugar solution was collected and the ethanol extraction was repeated thrice.
- The sugar solution were pooled and made up to 25ml with distilled water in a standard flask.
- The tuber sample sugar extraction was same as the procedure stated above except the acetone treatment.

b) Sucrose content estimation by colorimetric method

- To 1ml of the extracted sugar taken in a test tube, 20µl of 11.9N HCl was added.
- Then incubation was carried out at 90°C for 5min.
- 50µl 5N KOH was added to stop the reaction.
- Then the sample was made up to 3ml with distilled water and 3ml DNS reagent (Appendix I)was added to it.
- The sample was incubated at 90°C for 5 min.
- After colour development, 1ml of 40% Rochelle salt solution was pipetted out and added to it (when the contents were still warm).
- The contents were mixed well.
- The tubes were cooled under running tap water and the absorbance was measured at 510nm using UV spectrophotometer (Thermo-scientific Model Evolution 201 UV-Visible Spectrophotometer). In each sample three replicates were taken for reading.
- The absorbance of the sample without adding the HCl also noted.
- The sucrose content was calculated by the difference in absorbance of HCl treated to untreated sample using glucose standard curve.

3.4.2. Quantitative Estimation of Starch

The starch content was estimated according to the method of Yoshida *et al.* (1971). The residue left in the tubes after sugar estimation was used for determining the starch content. The residue was treated with perchloric acid and

the starch was converted into simple sugars. This sugar was estimated by the phenol sulphuric acid method. The estimated sugar was then converted into starch content by multiplying with the factor 0.9.

a) Glucose Standard curve

100mg glucose was dissolved in 100ml distilled water. From this, 100ml was diluted to 100 ml with distilled water and this was used as a working standard. 0.2, 0.4, 0.6, 0.8 and 1ml of working standard were pipetted into a series of test tubes. The volume was made upto 1ml with distilled water. Set a blank with 1ml of distilled water. Then 1ml of 5% phenol was added to each tube. After that 5ml of 96% sulphuric acid was added gently to the sample and shaken well. The tubes were incubated at room temperature for 20min for cooling. The orange colour developed was read at 490nm using UV-visible spectrophotometer (Fig 2).

b) Starch estimation

- Two ml distilled water was added to the residue left in the centrifuge tubes after sugar extraction and kept in boiling water bath for 15min.
- Then 2ml 9.2N Perchloric acid (Appendix II) was added to each sample and incubated for 15min.
- Sample was diluted to 10ml with distilled water.
- Centrifuged the suspension at 10,000 rpm for 10 min.
- Collected supernatant to 25 ml standard flask.
- Then 2ml 4.6N Perchloric acid (Appendix II) was added to the pellet and kept for 15min and stirring constantly.
- Suspension was diluted to 10 ml with distilled water and again centrifuged at 10,000rpm for 10min.
- The supernatants were combined and the volume made up to 25ml with distilled water in a standard flask.
- The sugar content was analyzed by the method according to Dubois *et al.* (1956). In each sample three replicates were taken for reading.

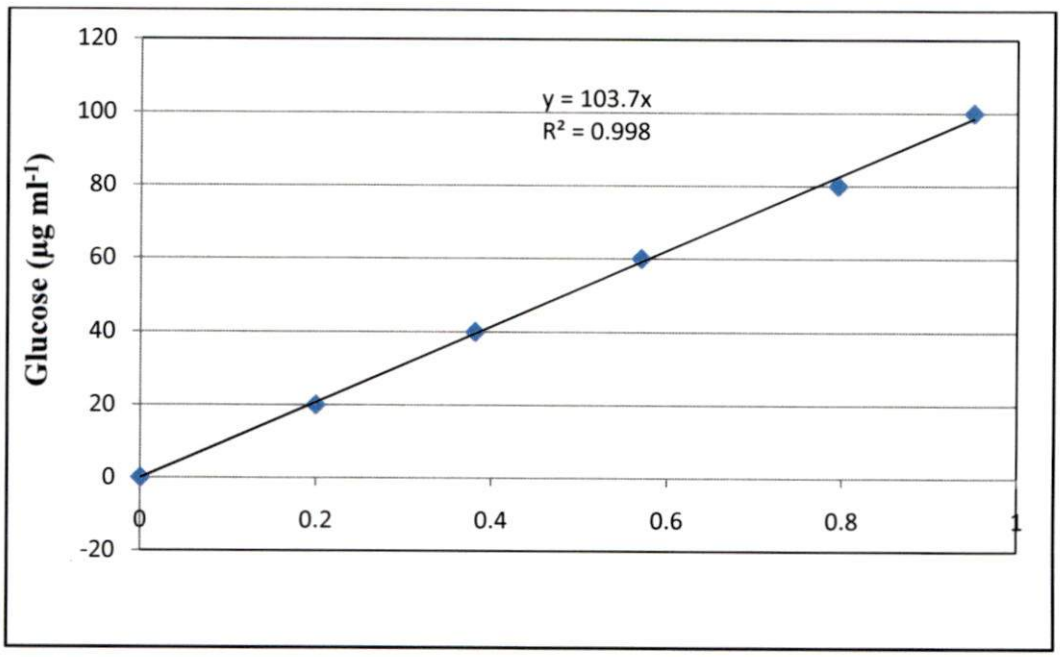


Fig 2. Glucose standardisation by Phenol sulphuric acid method

3.5. Rate of photosynthesis

Photosynthetic CO_2 uptake rate was measured using portable photosynthetic analyzer (LICOR, USA). Rate was measured in leaves in every month.

3.6. Assay of Enzymes involved in Starch Biosynthesis

3.6.1. Invertase Enzyme

The invertase assay was carried out by the method described by Tsai *et al.* (1970), Sadasivam and Manickam, (1992) and Wang *et al.* (1993).

a) Standard curve

25mg BSA was dissolved in 0.15M NaCl and the volume made up to 25ml with distilled water (1mg/ml). 0.01, 0.02, and 0.04 to 0.1ml of standard protein solution pipetted out into a series of test tubes. The volume in each tube was made up to 0.1ml with 0.1M phosphate buffer (pH-7.4). 0.1ml buffer alone serves as the blank. 5ml of 0.01% protein reagent (Appendix III) was added and mixed thoroughly by inversion. The absorbance was measured at 595nm using UV-visible spectrophotometer (Fig 3).

b) Enzyme extraction

- 500mg fresh leaf and tubers of four varieties of sweet potato were homogenized in a mortar and pestle with pre-cooled 10ml 20% glycerol.
- The homogenate was centrifuged at 10,000rpm for 10min at 4°C.
- The supernatant was collected and made up to 25ml with 20% glycerol and it was used for enzyme assay.
- 1-2ml of toluene was added to preserve the enzyme extract.
- The enzyme extracts were stored at 0-4°C when not in use.

c) Enzyme assay

- For the assay of invertase activity, the reaction mixture containing 10ml of 1M sodium acetate buffer (pH-5) (Appendix III), 5ml of 2.5% sucrose and 5ml of

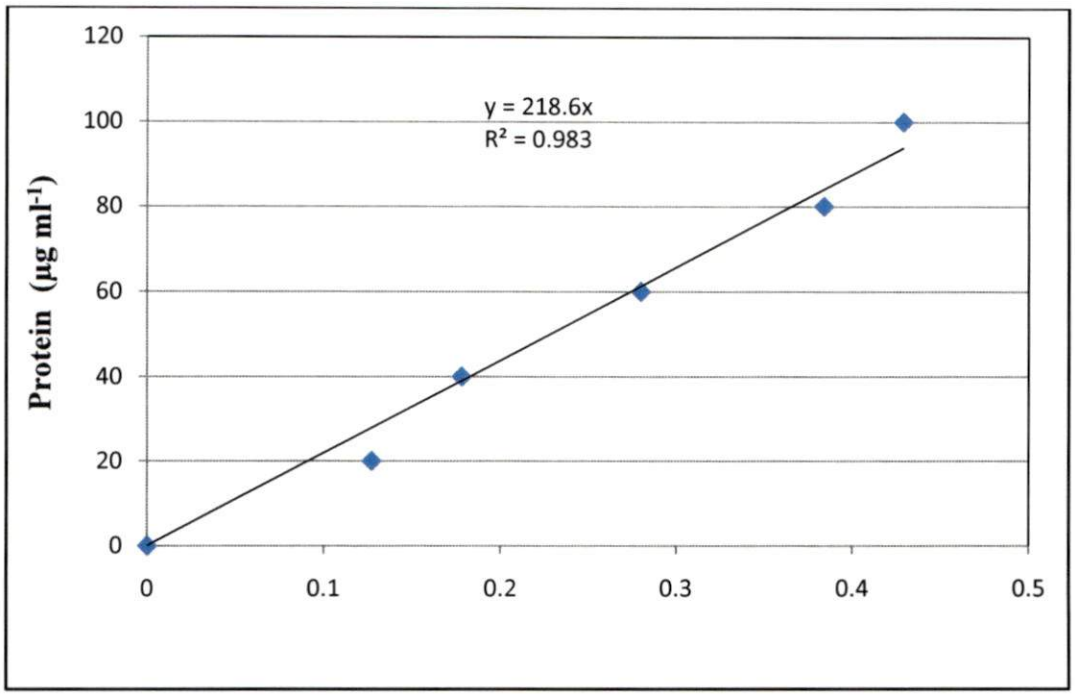


Fig 3. Protein standardisation by Bradford's method

enzyme preparation in a total reaction volume of 20ml was incubated at 37°C for 24 hrs.

- After incubation, 1ml of reaction mixture was pipetted out and the reaction terminated by adding 1ml DNS reagent (Appendix II).
- Then reducing sugar present in the reaction mixture was estimated by Dubois *et al.* (1956).
- Protein content in the enzyme extract was determined by protein standard curve (Fig 2) from the method of Bradford (1976).
- The enzyme activity was expressed as mg glucose released hr^{-1} mg $protein^{-1}$.

3.6.2. Sucrose Synthase (SuSy) Enzyme

SuSy activity was determined according to the method of Wardlaw *et al.* (1988).

a) Enzyme extraction

- One gram fresh leaf and tubers were weighed and homogenized with 8 ml extraction buffer (Appendix III).
- Centrifuged the sample at 10,000rpm for 10min at 4°C.
- Supernatant was collected and used for measuring SuSy activity.

b) Enzyme assay

- 90µl of enzyme preparation was taken in test tube.
- 50µl of 200mM HEPES-NaOH (pH-7.5), 20µl 5mM $MgCl_2$, 20µl 100mM UDP-G and 20µl 50mM fructose were added.
- The contents were mixed well.
- Incubated for 30 min at 30°C.
- After incubation, the reaction was terminated by adding 200µl of 1N NaOH.
- Heating the contents at 100°C for 10min.
- To this 0.5ml 1% resorcinol and 1.5ml 30% HCl was added.
- Then incubated the contents at 80°C for 8min.
- At last, reaction was stopped by keeping on ice pack.

- The absorbance was measured at 520nm using UV-Visible Spectrophotometer. In each sample three replicates were taken for reading.
- The enzyme activity was expressed as mg of sucrose synthesized/mg fresh weight of leaf or tuber using pre-established standards (Fig 3).

3.6.3. Sucrose Phosphate Synthase (SPS)

Activity of SPS was measured according to the method of Wardlaw *et al.* (1994). The enzyme extraction was done using the procedure stated above for SuSy.

a) Enzyme assay

- For SPS assay 100 mM fructose-6-phosphate was used instead of fructose in SuSy.
- 90µl of enzyme preparation was added to 50µl of 200mM HEPES-NaOH (pH-7.5), 20µl 5mM $MgCl_2$, 20µl 100mM UDP-G and 20µl 100mM fructose-6-phosphate.
- The contents were mixed well and incubated for at 30°C for 30min.
- After incubation, the reaction was terminated by adding 200µl of 1N NaOH.
- The contents were heated at 100°C for 10min.
- To this 0.5ml 1% resorcinol and 1.5ml 30% HCl was added and then incubated the contents at 80°C for 8min.
- At last, reaction was stopped by keeping on ice pack.
- The absorbance was measured at 520nm using UV-visible spectrophotometer. Three aliquots from each sample were used for reading.
- The enzyme activity was expressed as mg of sucrose synthesized/mg fresh weight of leaf or tuber using pre-established standards.

3.6.4. ADP Glucose Pyrophophorylase (AGPase) Enzyme

AGPase enzyme was assayed using the method of Nakamura *et al.* (1989).

a) Enzyme extraction

- 0.5g fresh leaf and tuber was used for extracting the AGPase enzyme.

- Fresh tissue was ground in a pestle and mortar with 2ml AGPase extraction buffer (Appendix III).
- Centrifugation was done at 10,000rpm for 10min at 4°C.
- The supernatant was collected and used as the enzyme preparation.

b) Enzyme assay

- For AGPase assay, 200µl of enzyme preparation was mixed with 850µl of reaction buffer taken in an eppendorf tube.
- Incubated the mixture at 30°C for 30min.
- After incubation, inactivate the enzymatic activity by keeping in boiling water for 30sec.
- Then centrifuged the mixture at 10,000rpm for 5min and transferred the supernatant to eppendorf tube.
- In another eppendorf tube, 500µl of supernatant and 15µl 10mM NADP were added and mixed thoroughly.
- 1µl of phosphoglucomutase (0.4U) and 1µl of Glucose-6-phosphodehydrogenase (0.35U) were added.
- Glucose-1-phosphate production was measured by the increasing A340 nm using UV-Visible spectrophotometer. Three aliquots from each sample were used for reading.
- The enzyme activity was expressed as Units min^{-1} 100 g fresh $weight^{-1}$.
- (1 unit = change in absorbance at 340nm by 100g fresh $tissue^{-1}$ min).

3.6.5. Starch Synthase (SS)

Measurement of SS activity was done by the method of Nakamura *et al.* (1989). The enzyme extraction was done using the procedure stated above for AGPase enzyme.

a) Enzyme assay

- For enzyme assay, 100µl enzyme extract was mixed with 180µl of reaction buffer I (Appendix III) taken in an eppendorf tube.

- Total volume of 280µl reaction mixture was incubated for 20min at 30°C for start of the reaction.
- Mixture was placed in a boiling water bath for 30sec.
- Then mixture was added with 100µl of reaction buffer II (Appendix III).
- Incubated the contents at 30° for 30min and heated in boiling water bath for 30sec.
- Then subjected to centrifugation at 10,000rpm for 5min at 4°C.
- From this, 300µl supernatant was mixed with 200µl of another reaction buffer III (Appendix III).
- Then 1µl of Hexokinase (1.4U) and 1µl of Glucose-6-phosphodehydrogenase (0.35U) were added to the above mixture.
- The enzymatic activity was measured as the increasing absorbance at 340nm using UV-Visible spectrophotometer. In each sample three replicates were taken for reading.

RESULTS

Chapter 4
RESULTS

4.1. Measurement of morphological parameters

The morphological parameters such as number of leaves per plant, leaf area in cm^2 and tuber yield were observed and changes were recorded at monthly intervals.

4.1.1. Number of leaves per plant

Leaf production in four varieties of Sweet potato (*Ipomoea batatas*) *viz.*, Sree Kanaka, Sree Arun, ST-13and Kanhangad was recorded and the leaf number was compared among different varieties (Fig 4). During the growth period, Sree Arun was observed to have the highest number of leaves (60±17) and the lowest number was observed in Kanhangad variety (45±15) (Table 1).

Table 1. The changes in the number of leaves per plant

Variety/Genotype	No. of leaves
Sree Kanaka	53±20
Sree Arun	60±17
ST-13	51±30
Kanhangad	45±15

4.1.2. Leaf area per plant

Leaf area of four varieties of sweet potato was calculated (Fig 5) and the highest mean value for leaf was observed in Kanhangad variety (5818.39±3232.73 cm^2) and the lowest leaf area was found in the variety ST-13(1533.46±1518.78 cm^2).

Leaf area index of four varieties was calculated (Fig 6) and the maximum LAI was observed in Kanhangad variety (4.85±2.69 cm) and lowest mean value for LAI in the variety ST-13 (1.27±1.26 cm) (Table 2).

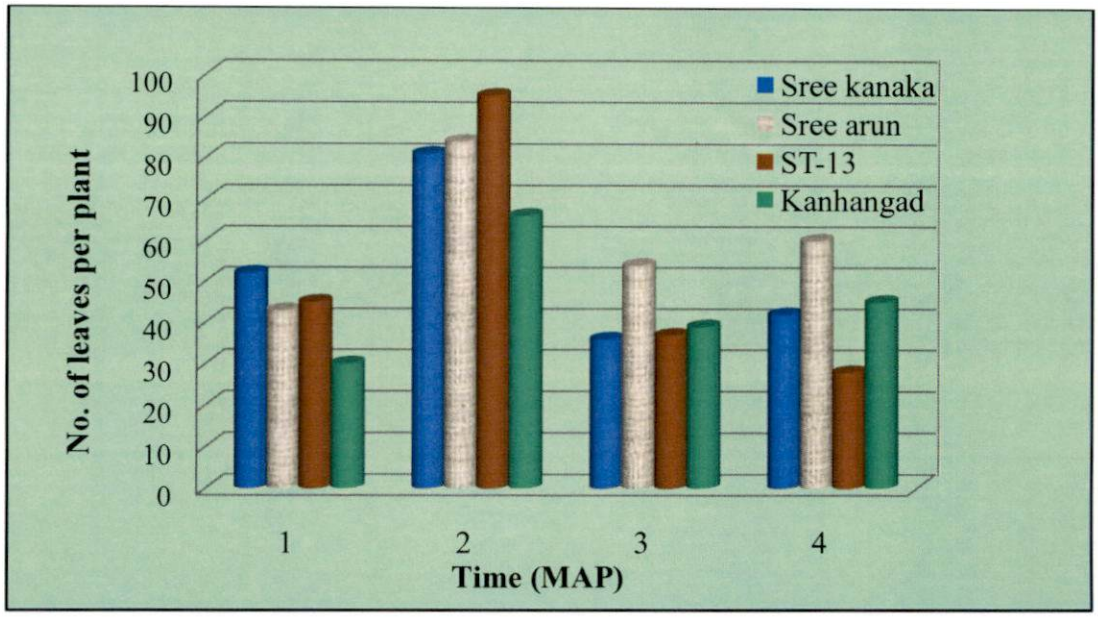


Fig 4: Changes in the number of leaves per plant in four Sweet potato genotypes during the growth period

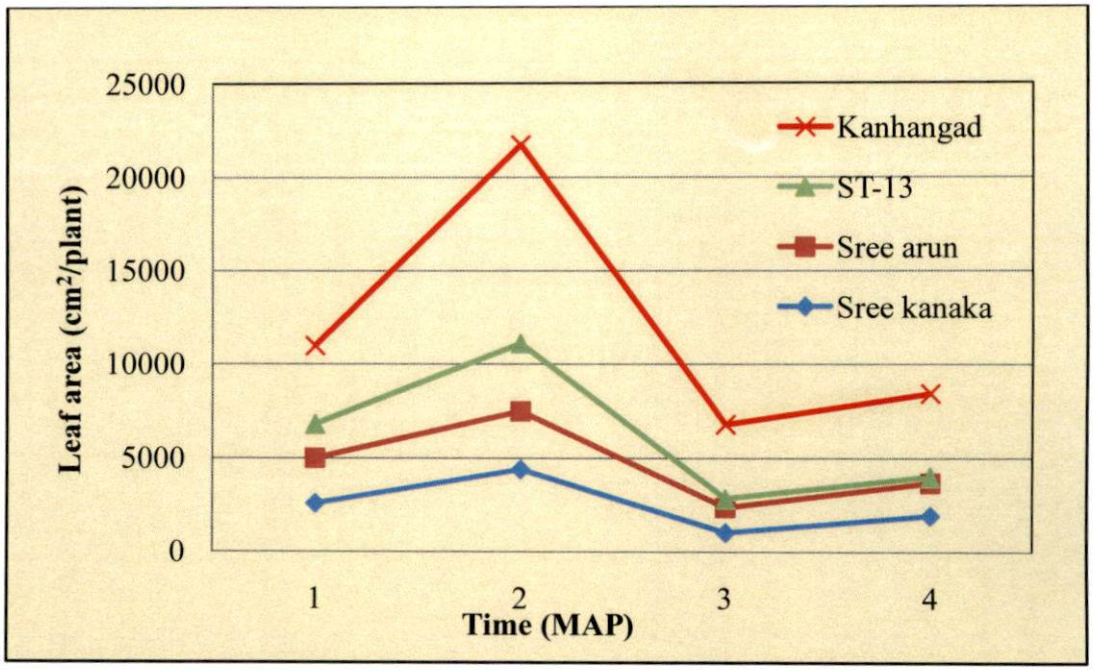


Fig 5: Variation in LA per plant in selected genotypes

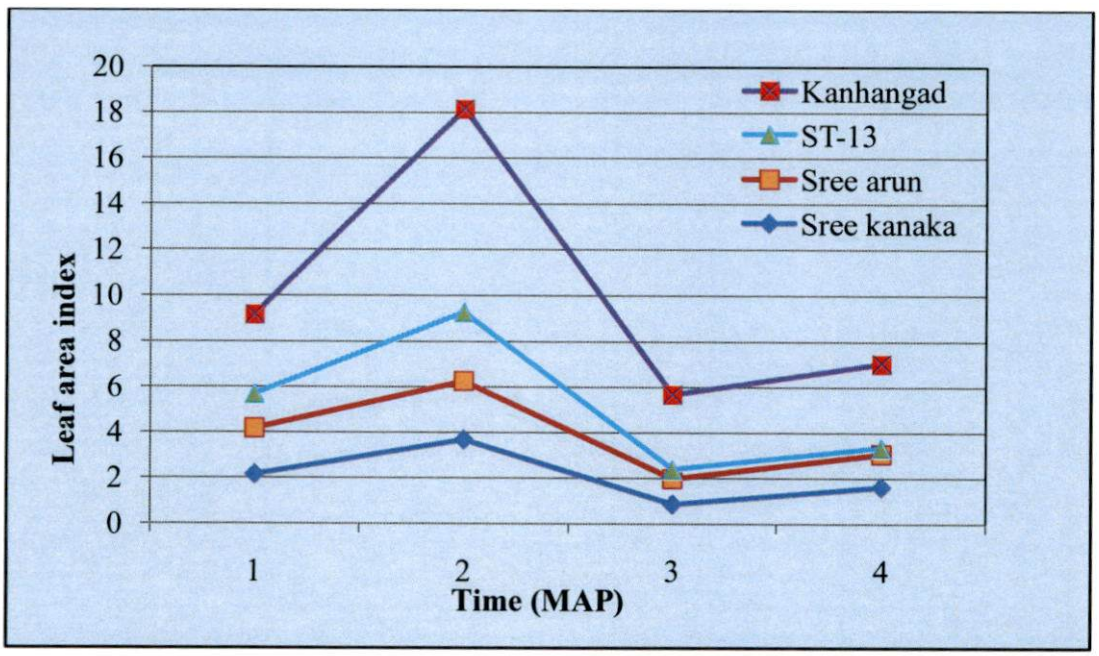


Fig 6: Variation in LAI in selected Sweet potato genotypes

Table 2. The mean value of LA and LAI of selected genotypes

Genotype	LA (cm^2)	LAI (cm)
Sree Kanaka	2487.24±1427.2	2.07±1.19
Sree Arun	2173.11±760.62	1.80±0.63
ST-13	1533.46± 1518.78	1.27±1.26
Kanhangad	5818.39±3232.73	4.85±2.69

4.1.3. Tuber yield per plant

Four varieties of Sweet potato 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 Sree Arun with 0.60±0.02 kg per plant and lowest yield in ST-13 with 0.35±0.02 kg per plant.

Table 3. The yield of selected Sweet potato tubers

Genotype	Tuber yield/ plant (kg)
Sree Kanaka	0.45±0.02
Sree Arun	0.60±0.01
ST-13	0.35±0.02
Kanhangad	0.58±0.04

4.2. Measurement of Biochemical parameters

4.2.1. Changes of Sucrose content

The Sucrose content in the tubers of Sweet potato varieties was recorded at monthly intervals. The sucrose content in leaves and tubers are presented in Fig 7 and Fig 8 respectively. The highest sucrose content was found in the leaves of Kanhangad (1.73±0.33) and the lowest in ST-13 leaves (1.27±0.28).

In tuber samples, ST-13 variety was observed as having the highest sucrose content (3.19±1.65) and Kanhangad with lowest sucrose (2.19±0.7) (Table 4).

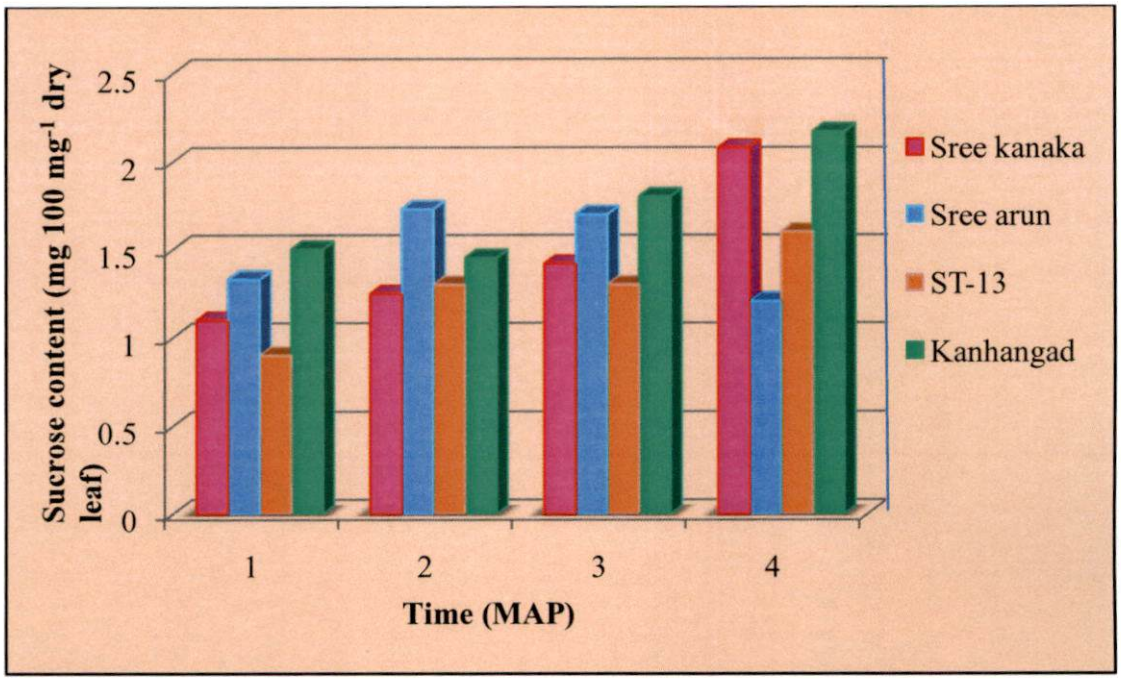


Fig 7: Distribution of sucrose content in the dry leaves of selected genotypes

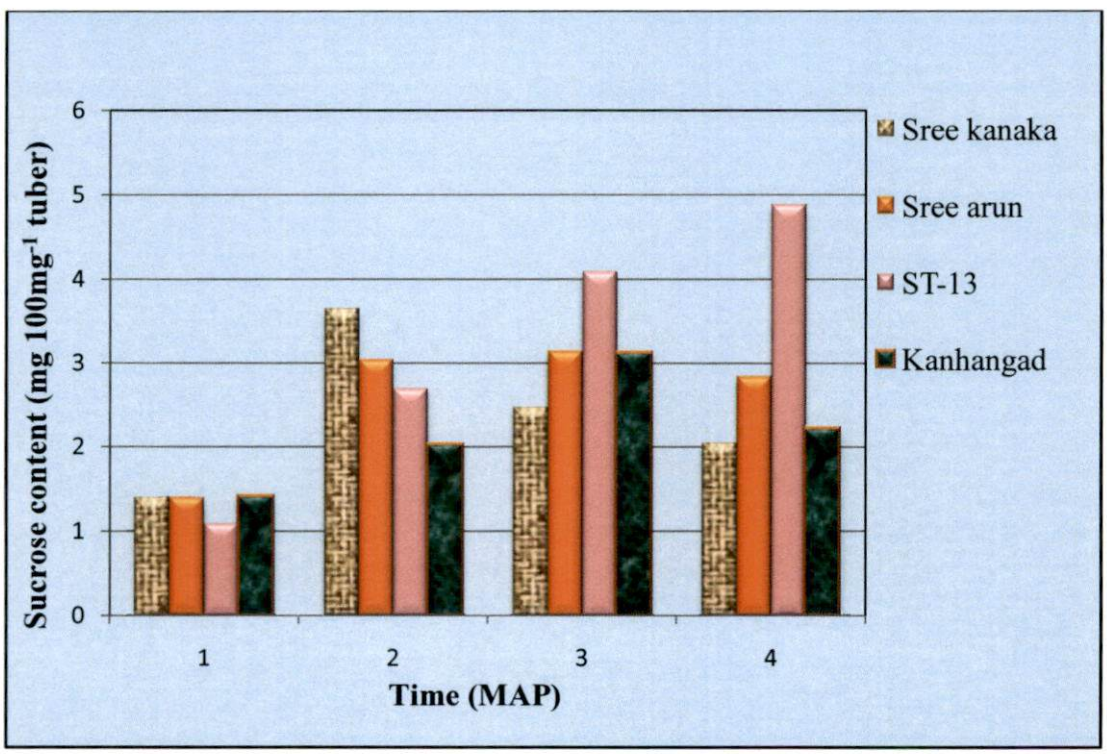


Fig 8: Distribution of sucrose content in the dry tubers of selected genotypes

Table 4. The mean Sucrose content of Sweet potato genotypes

Variety	Sucrose content (mg 100 mg^{-1} dry weight)	
	Leaf	Tuber
Sree Kanaka	1.46±0.43	2.56±0.94
Sree Arun	1.49±0.26	2.6±0.81
ST-13	1.27±0.28	3.19±1.65
Kanhangad	1.73±0.33	2.19±0.7

4.2.2. Changes of Starch content

The Starch content in the leaves (Fig 9) and in the tubers (Fig 10) of all the four varieties were recorded at monthly intervals. The highest starch content was found in Sree Arun (5.9±1.11) and the lowest in ST-13 variety (5.46±1.5) (Table 5).

Table 5. The mean Starch content of Sweet potato genotypes

Variety	Starch content (mg100 mg^{-1} dry weight)	
	Leaf	Tuber
Sree Kanaka	5.88± 1.18	13.49±3.04
Sree Arun	5.90±1.11	14.32±3.86
ST-13	5.46±1.5	11.60±2.1
Kanhangad	5.85±1.18	11.26±1.49

In tuber samples, the Starch content was higher in Sree Arun with 14.32±3.86 and lowest in the variety Kanhangad with 11.26±1.49. When comparing with leaves, the tubers were observed with the maximum Starch content.

4.3. Measurement of Photosynthetic Rate

Photosynthetic rate in leaves was measured using portable photosynthetic analyser. The highest P_N rate was observed (Fig 11) in Sree Arun variety with 25.3±3.86 $CO_2 \mu molm^{2-1}s^{-1}$ and the lowest rate in the Kanhangad variety with 23.2±3.75 CO_2 $\mu molm^{2-1}s^{-1}$(Table 6).

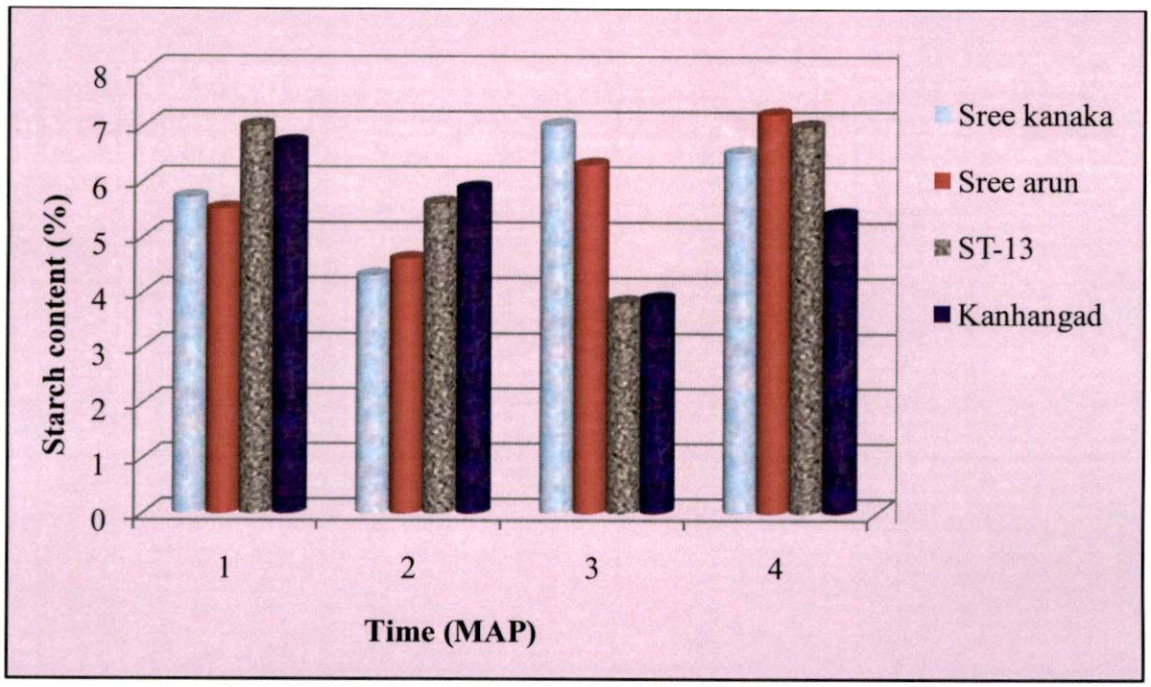


Fig 9: Distribution of starch content in dry leaves of Sweet potato varieties

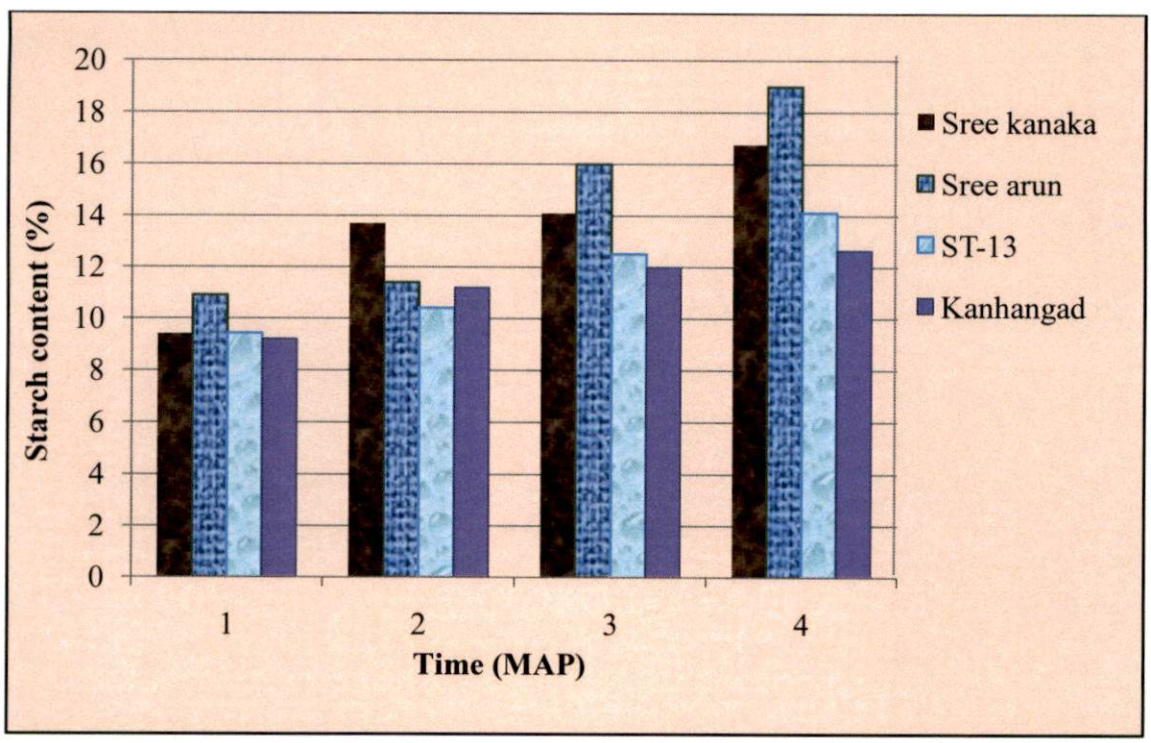


Fig 10: Distribution of starch content in dry Sweet potato tubers

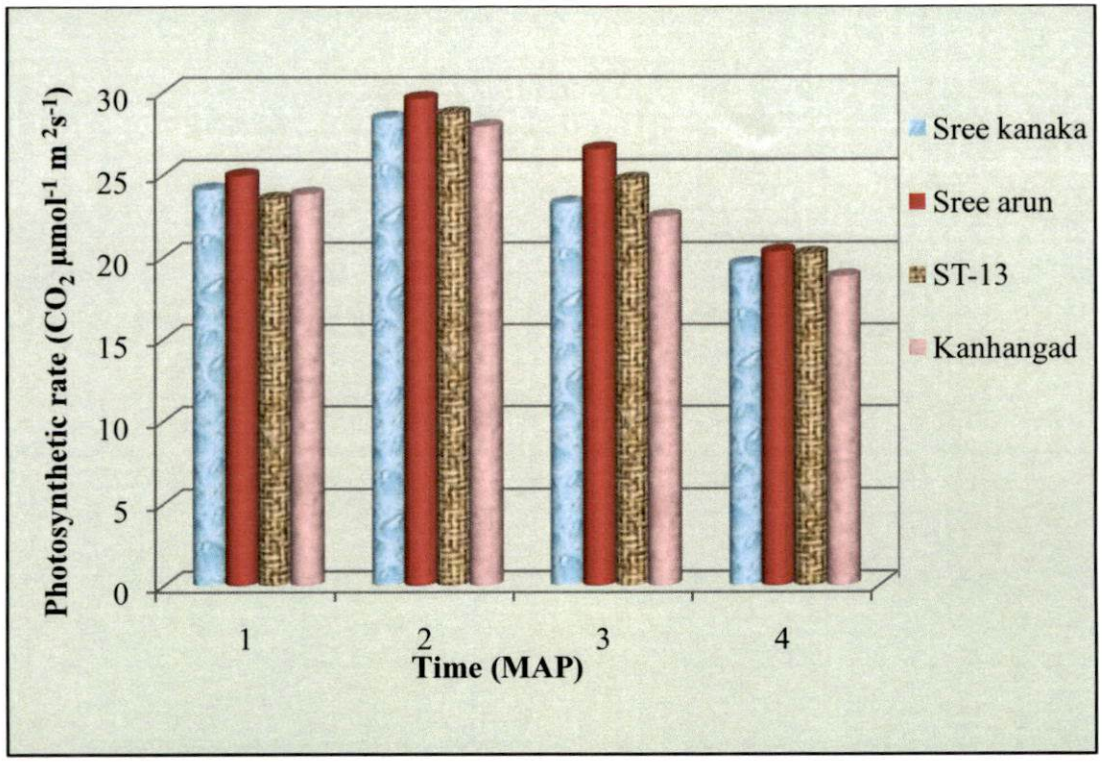


Fig 11. Effect of P_N rate in Sweet potato genotypes during the growth period

Table 6. The mean P_N rate in selected Sweet potato genotypes

Variety	P_N rate (CO_2 µmolm$^{2-1}s^{-1}$)
Sree Kanaka	23.79±3.62
Sree Arun	25.3±3.86
ST-13	24.22±3.5
Kanhangad	23.20±3.75

4.4. Measurement of Enzyme activity

a) Changes of the activity of Invertase

The invertase activity was assayed in both leaves (Fig 12) and in tubers (Fig 13) of Sweet potato. Changes in the activity were recorded every month. From this, invertase activity was found higher in leaf tissues (Table 7).

Table 7. Changes in Invertase activity in leafs and tubers of Sweet potato genotypes

Variety	Invertase activity (mg glucose releasedhr^{-1} mg $protein^{-1}$)	
	Leaf	Tuber
Sree Kanaka	1.83±0.72	1.67±0.43
Sree Arun	1.76±1.11	1.81±0.69
ST-13	1.56±0.68	1.89±0.52
Kanhangad	2.06±1.2	1.34±0.26

In leaves, Kanhangad variety was found to have highest invertase activity (2.06±1.2mg glucose released hr^{-1} mg $protein^{-1}$) and ST-13 had lowest (1.56±0.68mg glucose released hr^{-1} mg $protein^{-1}$).

Activity of invertase was found higher in ST-13 tubers with 1.89±0.52mg glucose released hr^{-1} mg $protein^{-1}$ while lowest activity was observed in Kanhangad tubers with 1.34±0.26mg glucose released hr^{-1} mg $protein^{-1}$.

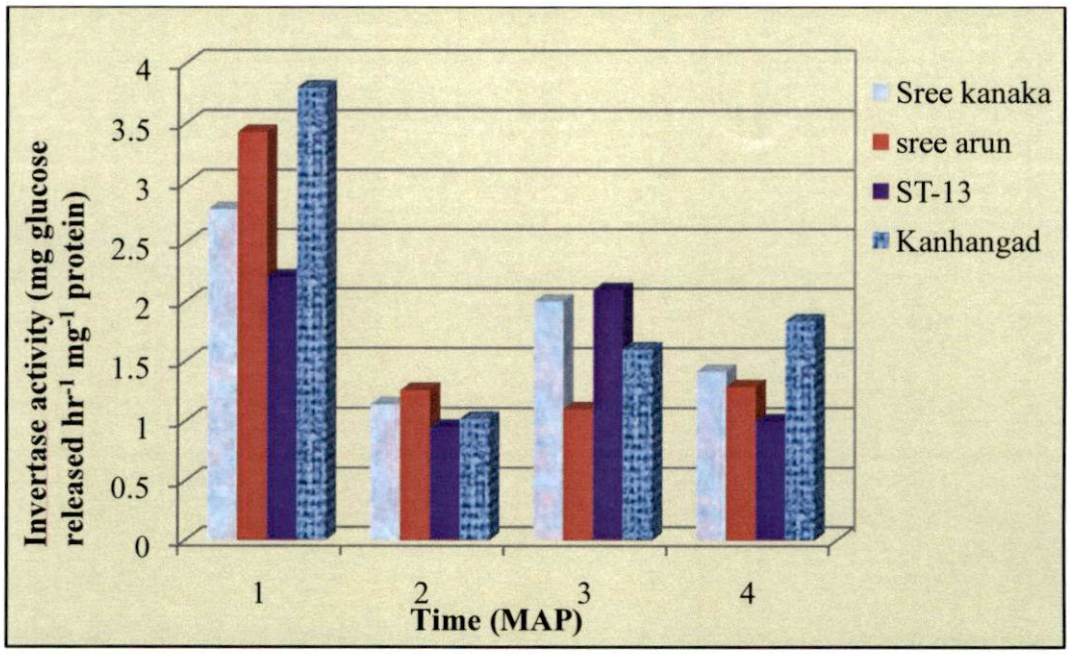


Fig 12.Time course of activities of Invertase in fresh leaves of selected genotypes

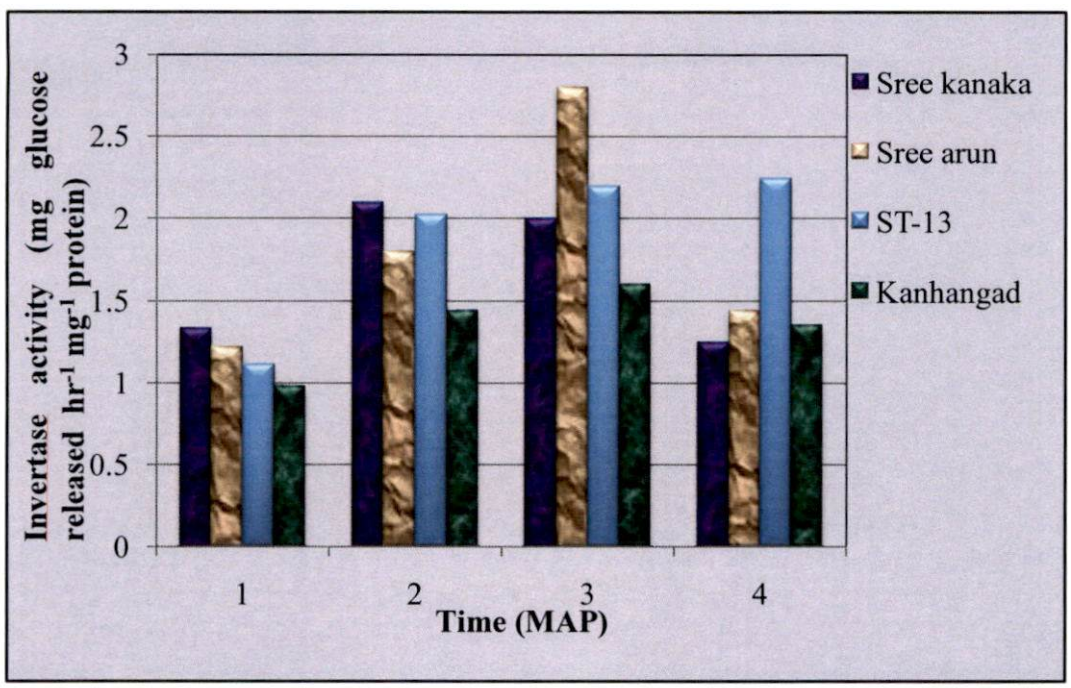


Fig 13: Time course of activities of Invertase in fresh Sweet potato tubers

b) Changes of activity of Sucrose Synthase

SuSy enzymatic activity was studied in leaves and tubers of four varieties of sweet potato. SuSy activity was found higher in leaf tissues than in tubers. Changes in leaves (Fig 14) and in tubers (Fig 15) were recorded at monthly intervals.

SuSy was highly active in the leaves of Sree Kanaka variety with 4.1±2.49mg sucrose synthesized 100mg fresh weight^{-1} while less activity was observed in Sree Arun leaves with 2.95±0.21 mg sucrose synthesized 100mg fresh weight^{-1}.

Higher SuSy activity was observed in the tubers of Kanhangad with 4.34±0.79mg sucrose synthesized 100mg fresh weight^{-1} while Sree Kanaka tuber was found to have the least enzyme activity with 1.51±0.37 mg sucrose synthesized 100mg fresh weight^{-1} (Table 8).

Table 8. Changes in SuSy activity in leafs and tubers of Sweet potato genotypes

	Sucrose synthase activity (mg sucrose synthesizedmg FW^{-1})	
Variety	Leaf	Tuber
Sree Kanaka	4.1±2.49	1.51±0.37
Sree Arun	2.95±0.21	2.59±0.31
ST-13	3.4±1.25	3.49±0.51
Kanhangad	3.85±2.56	4.34±0.79

c) Changes of activity of Sucrose Phosphate Synthase

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 16) and tuber (Fig 17) were recorded monthly.

In leaves, the SPS activity was higher in Sree Kanaka(3.69±2.46mg sucrose synthesized100mg fresh weight^{-1}) while lower in ST-13 (2.14±0.54mg sucrose synthesized 100mg fresh weight^{-1})

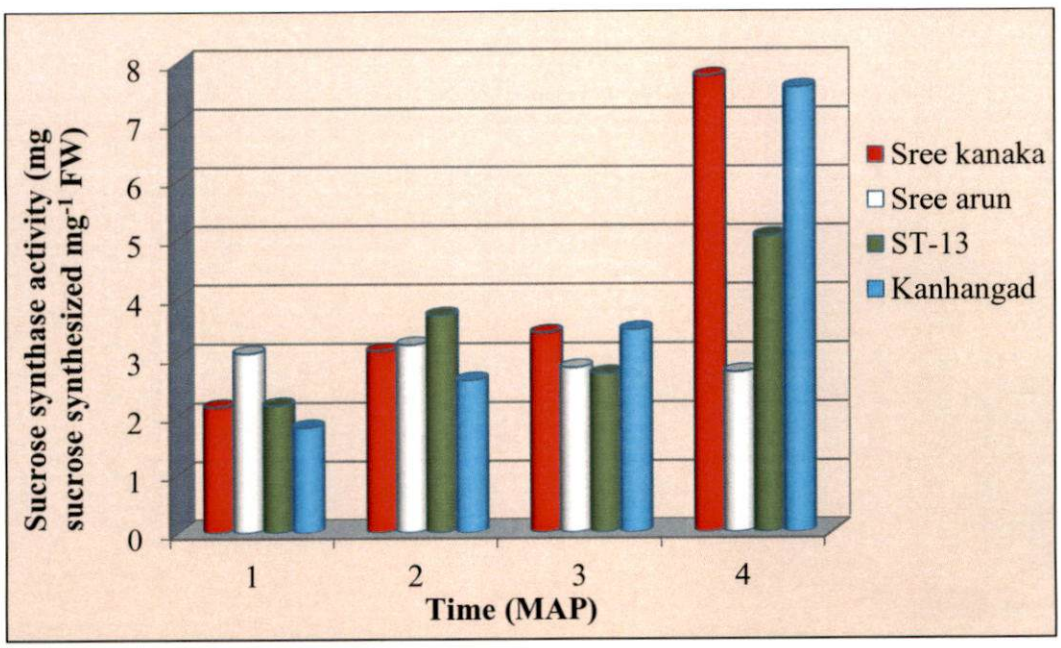


Fig 14: Time course of SuSy activity in fresh leaves of Sweet potato genotypes

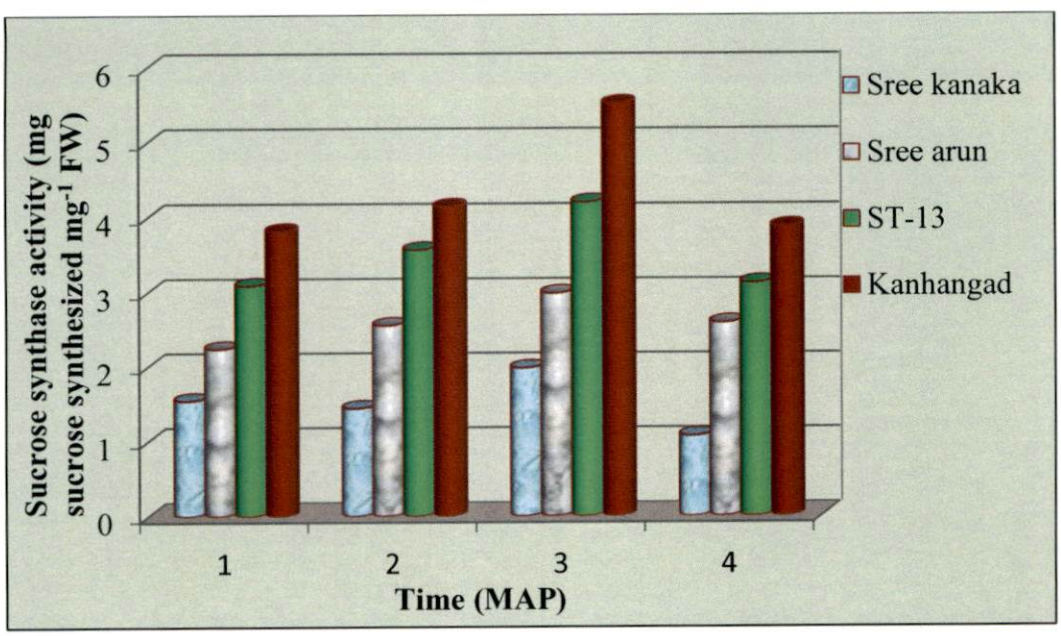


Fig 15: Time course of activities of SuSy in fresh Sweet potato tubers

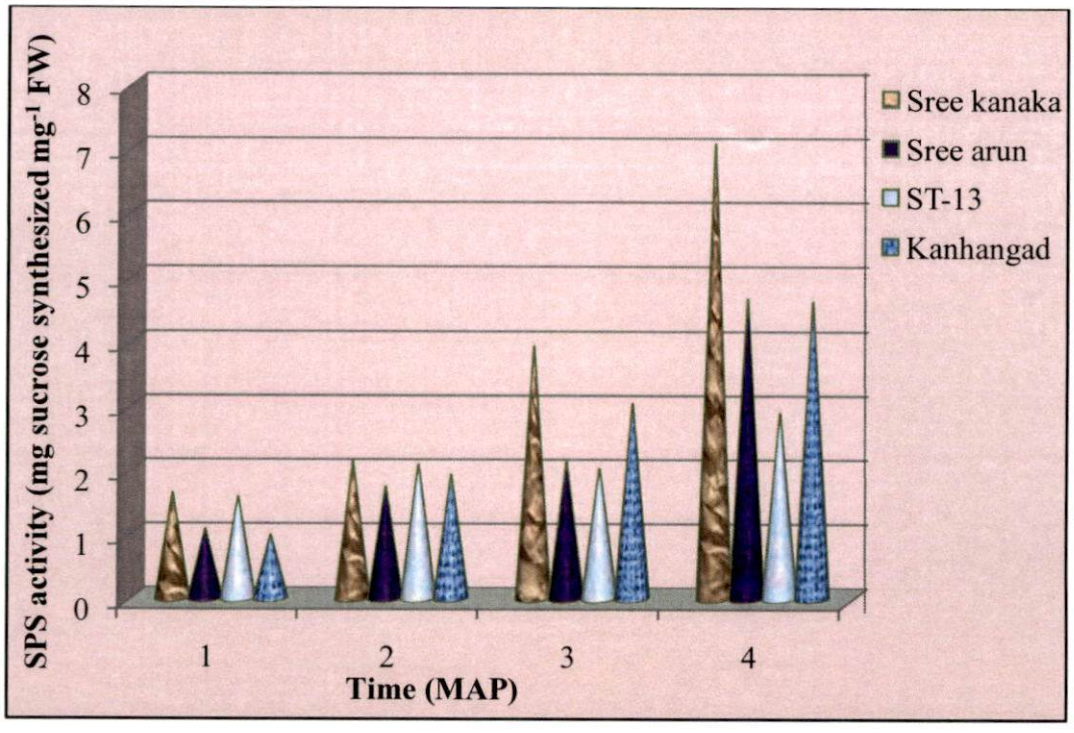


Fig 16: Time course of SPS activity in fresh leaves of selected genotypes

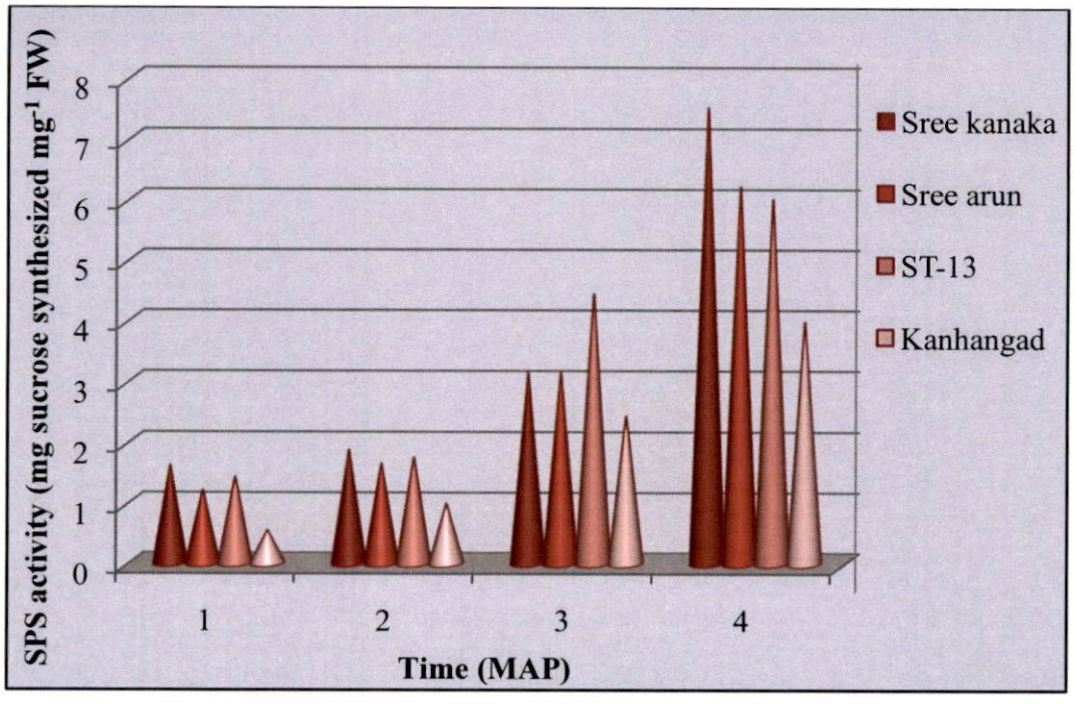


Fig 17: Time course of SPS activity in fresh Sweet potato tubers

In tubers, the SPS activity was found higher in Sree Kanaka with 3.54±2.73 mg sucrose synthesized 100mg fresh weight^{-1} and the lowest activity was found in Kanhangad with 1.98±1.55 mg sucrose synthesized 100mg fresh weight^{-1} (Table 9).

Table 9. Changes in SPS activity in leafs and tubers of Sweet potato genotypes

	Sucrose phosphate synthase activity (mg sucrose synthesizedmg FW^{-1})	
Variety	Leaf	Tuber
Sree Kanaka	3.69±2.46	3.54±2.73
Sree Arun	2.40±1.57	3.05±2.26
ST-13	2.14±0.54	3.40±2.2
Kanhangad	2.64±1.56	1.98±1.55

d) Changes of activity of ADP Glucose pyrophosphorylase

AGPase activity was assayed using leaf and tubers. Changes occurred during the growth of Sweet potato were recorded at monthly intervals and compared (Table 10). Changes in leaf and tuber are represented in Fig 18 and Fig 19 respectively.

Table 10. Changes in AGPase activity in leafs and tubers of Sweet potato genotypes

	AGPase activity (Units minute^{-1}mg fresh weight^{-1})	
Variety	Leaf	Tuber
Sree Kanaka	2827.4±860.79	3911±944.48
Sree Arun	3113.2±1336.22	8459.6±1268.74
ST-13	2569.2±1127.62	2643.4±1382.97
Kanhangad	2420.6±769.02	6802.2±1152.89

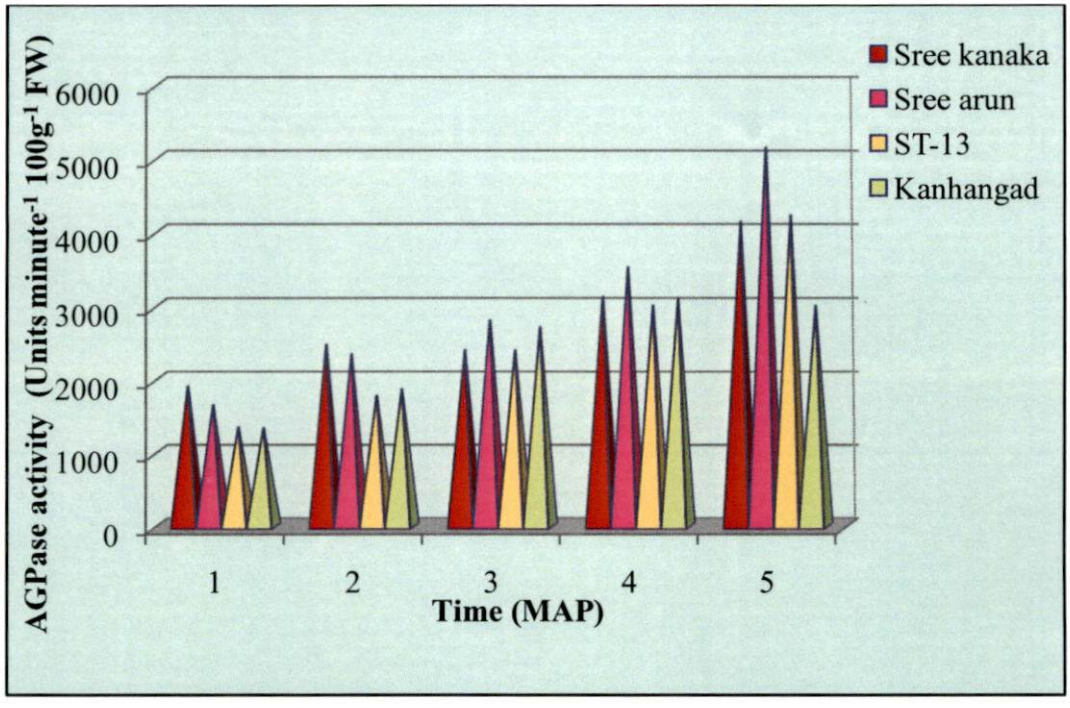


Fig 18: Time course of activities of AGPase in fresh leaves of selected genotypes

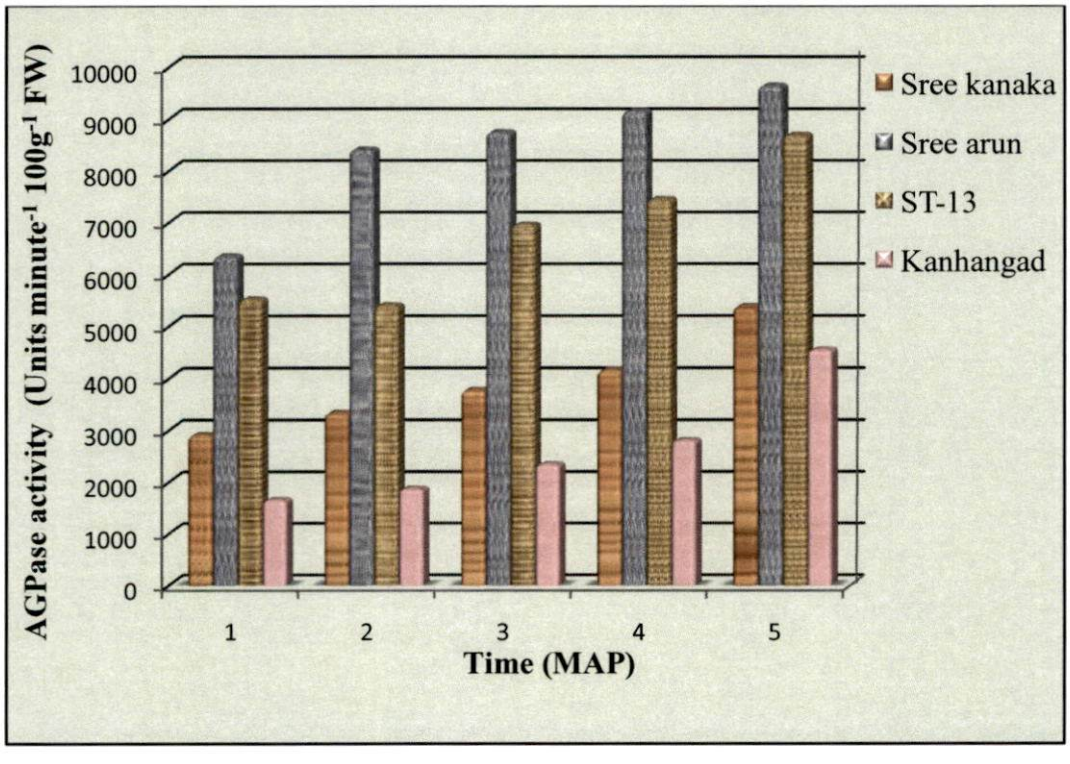


Fig 19: Time course of AGPase activity in fresh Sweet potato tubers

In leaves, Sree Arun (3113.2±1336.2 Units minute^{-1}) showed the highest activity while lowest activity was measured in Kanhangad (2420.6±769.02 Units minute^{-1}).

In tubers, Sree Arun has the highest AGPase activity (8459.6±1268.7 Units minute^{-1}) and ST-13has the lowest (2643.4±1382.97 Units minute^{-1}).

e) Changes of activity of Starch Synthase

Changes in the activity of SS were recorded monthly in leaves (Fig 20) and tubers (Fig 21) of four sweet potato varieties. In leaves, the SS activity was higher in the variety Sree Arun (9883±4356.76 Units minute^{-1}) while least activity was found in Kanhangad variety (2709±1099.75 Units minute^{-1}).

In tubers, the highest activity of SS was observed in Sree Arun variety (11887.18±8190.15 Units minute^{-1}) and lowest activity in Kanhangad with 3659±1316.73 Units minute^{-1} (Table 11).

Table 11. Changes in SS activity in leafs and tubers of Sweet potato genotypes

	Starch synthase activity (Units minute^{-1} mg FW^{-1})	
Variety	Leaf	Tuber
Sree Kanaka	5087.4±1886.79	4396.89±1941.3
Sree Arun	9883±4356.76	11887.18±8190.15
ST-13	7053.4±2959.14	5695.84±3332.93
Kanhangad	2709.8±1099.75	3659.06±1316.73

4.5. Correlation of P_N rate, Leaf area, sucrose content and starch content

The correlation between P_N rate and starch content in selected varieties of sweet potato were analyzed. From that, the P_N rate was positively and significantly correlated in the varieties such as Sree Kanaka and ST-13 were shown in the Fig 22. Sree Arun and Kanhangad variety were negatively correlated with the starch

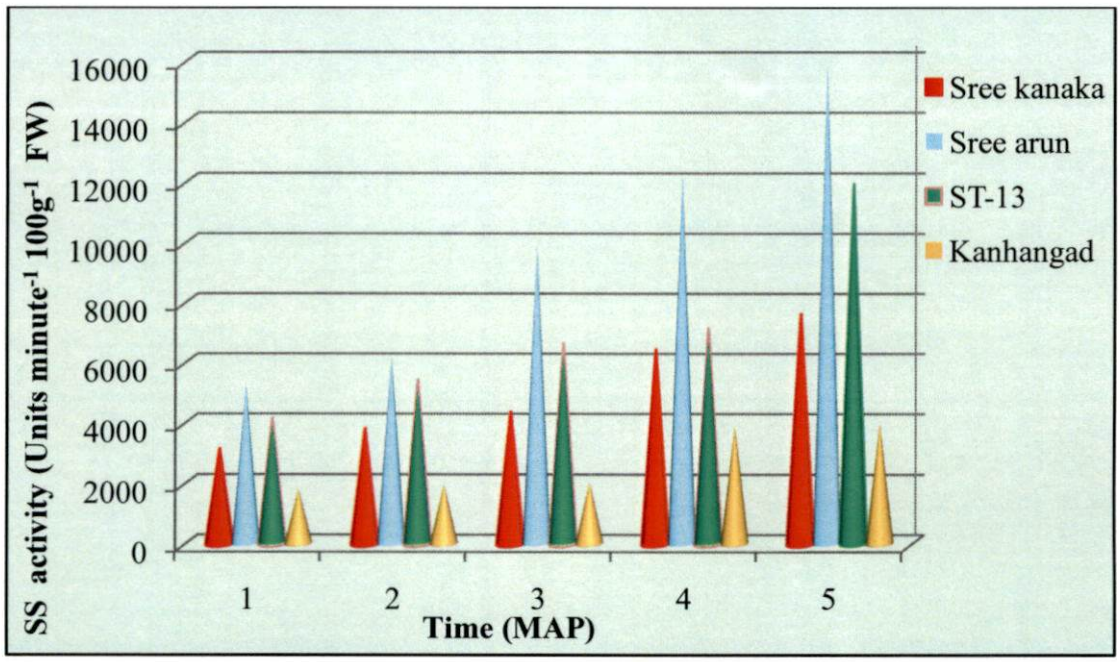


Fig 20: Time course of SS activity in fresh Sweet potato leaves

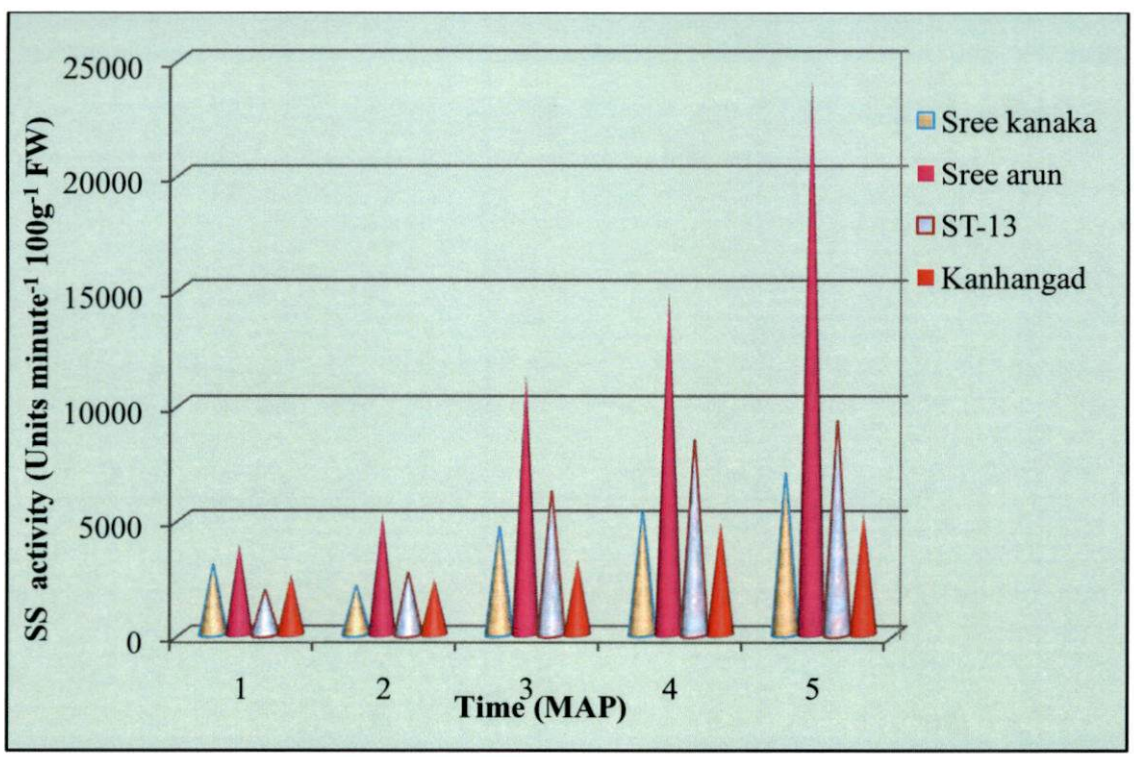


Fig 21: Time course of SS activity in fresh Sweet potato tubers

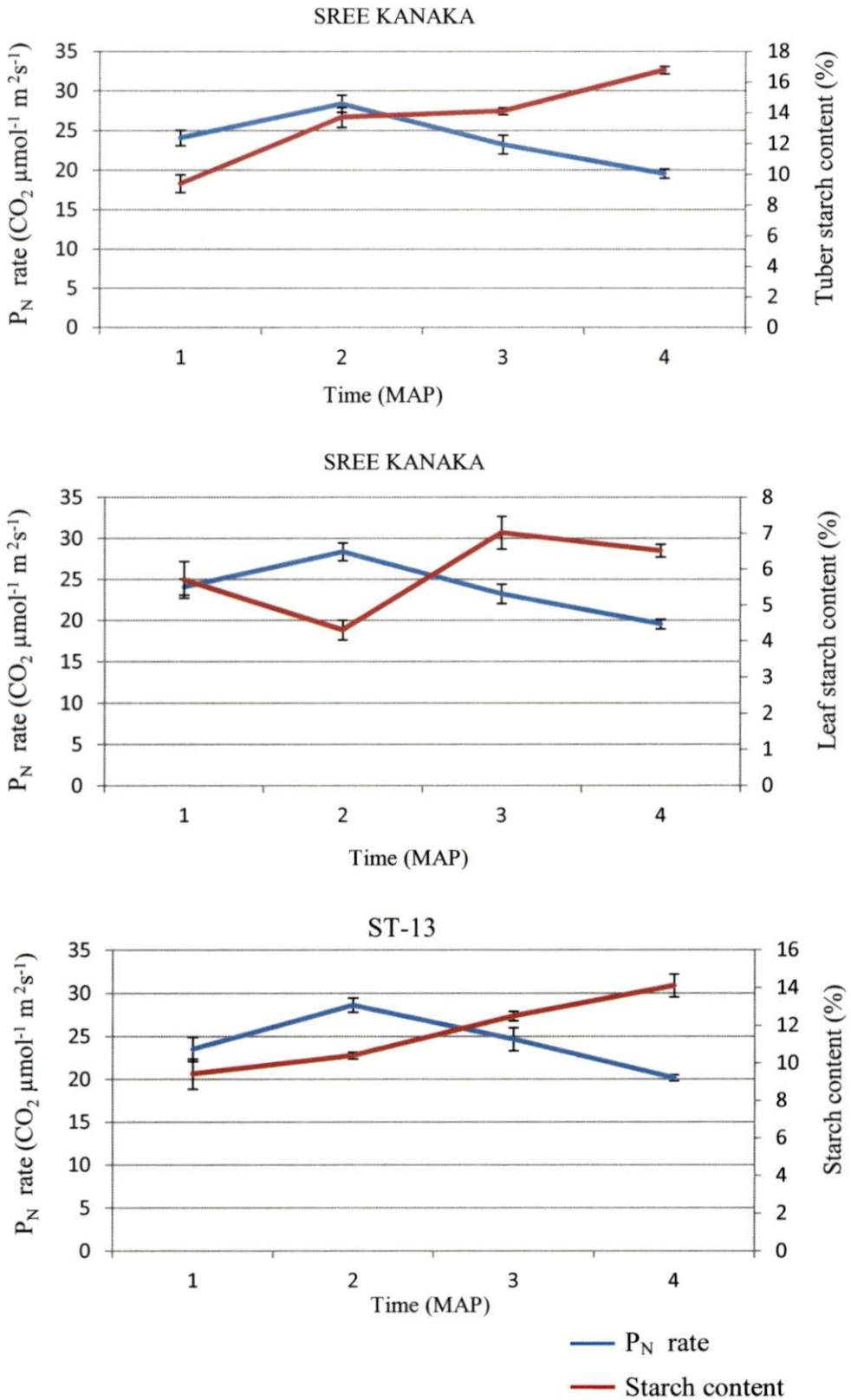


Fig 22: Correlation between P_N rate and starch content

content. As shown in Fig 23, the LA of Sree Kanaka and Sree Arun varieties were positively and significantly correlated with starch content. The LA of ST-13 and Kanhangad varieties were not significantly associated with starch content.

The correlation between sucrose content and starch content in selected varieties of sweet potato were analyzed. The Sree Kanaka and ST-13 varieties had a significant correlation between the tuber starch accumulations (Fig 24). Sree Arun and Kanhangad variety showed significantly negative correlation.

4.6. Correlation of the activities of enzymes involved in regulating starch biosynthesis and starch content

The correlations between Invertase, SuSy, SPS, AGPase, SS and tuber starch content were analyzed in selected varieties. From this correlation study, the activities of AGPase and SS in four varieties were positively and significantly correlated with starch content as shown in Fig 25 and Fig 26 respectively. The activities of Invertase and SuSy were not significantly associated with starch content. The correlation between the activity of SPS and starch content varied with different varieties. ST-13 and Kanhangad showed significantly positive correlation and Sree Kanaka and Sree Arun were not.

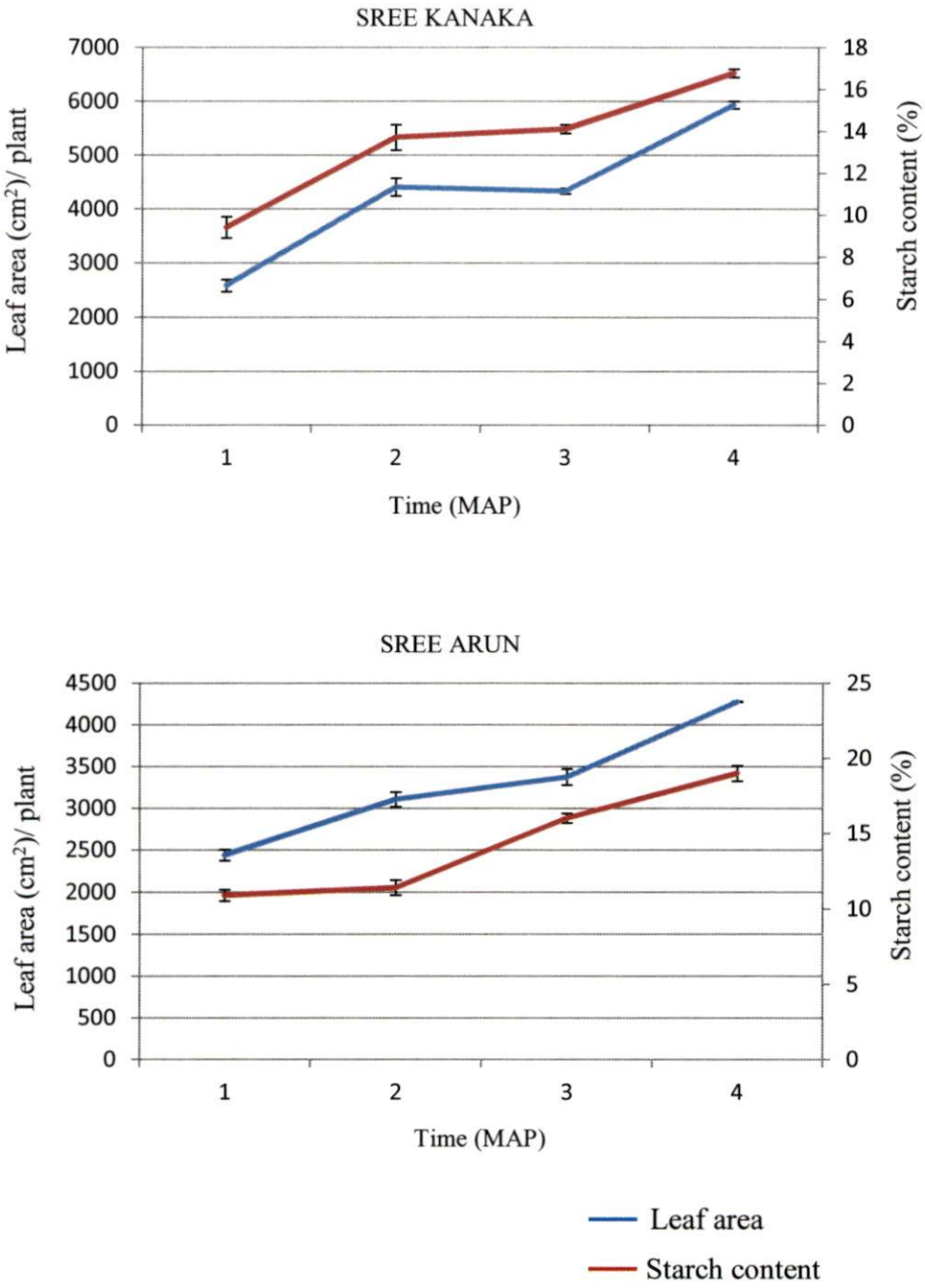


Fig 23: Correlation between Leaf Area and starch content

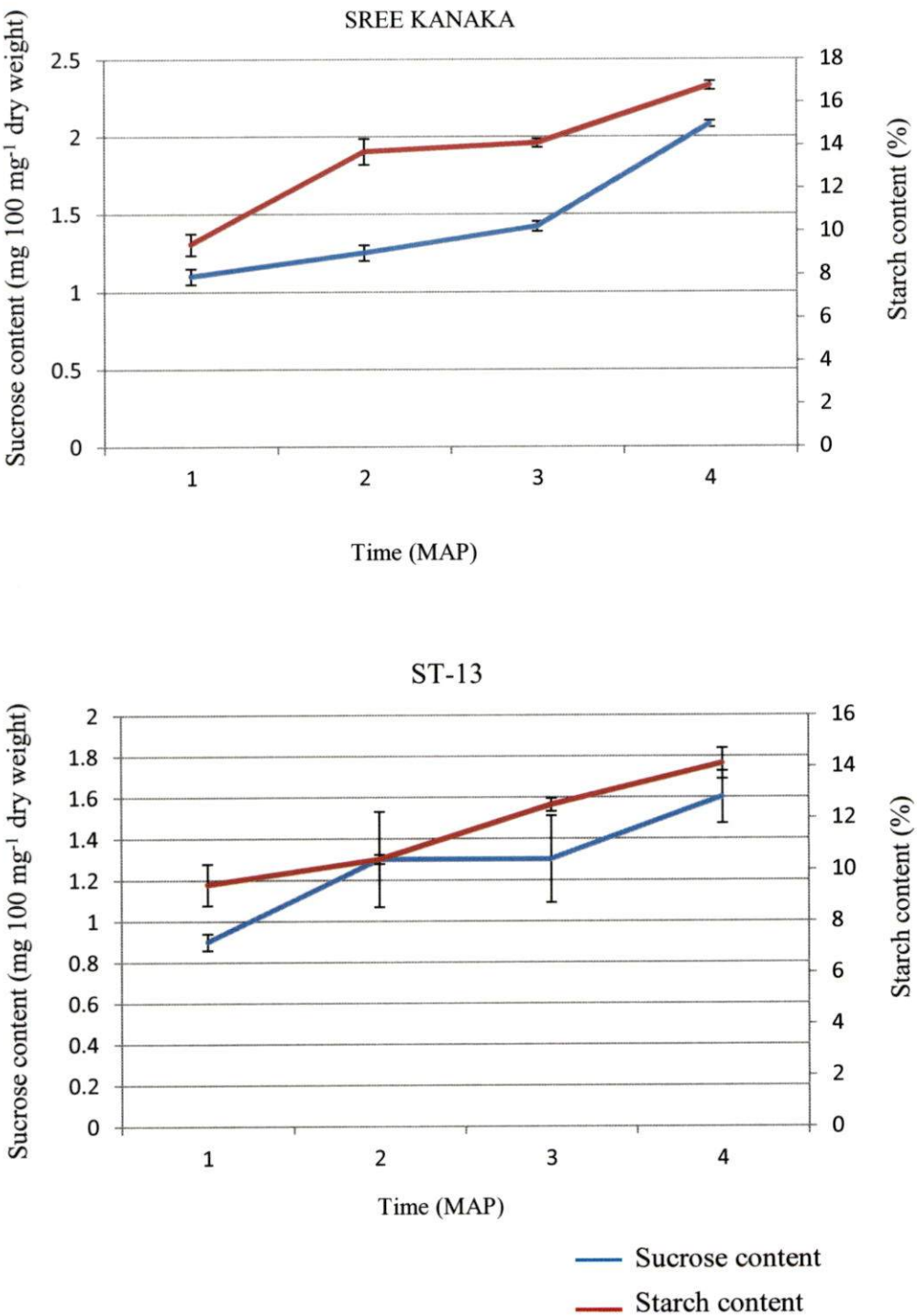
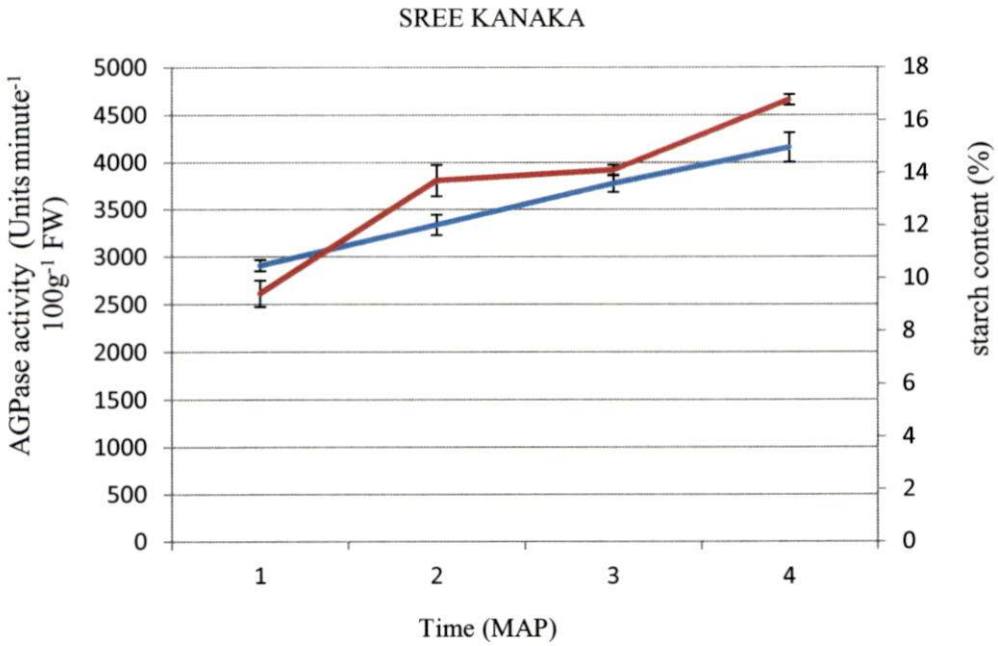
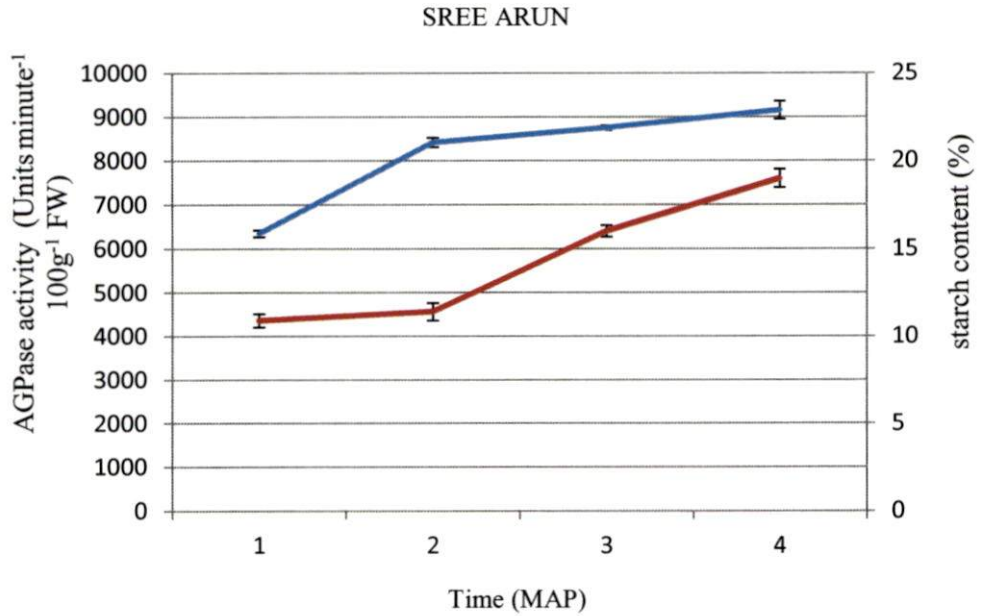


Fig 24: Correlation between sucrose content and starch content


SREE KANAKA
AGPase activity (Units minute-1 100g-1 FW)
starch content (%)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
18
16
14
12
10
8
6
4
2
0
1
2
3
4
Time (MAP)


SREE ARUN
AGPase activity (Units minute-1 100g-1 FW)
starch content (%)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
25
20
15
10
5
0
1
2
3
4
Time (MAP)

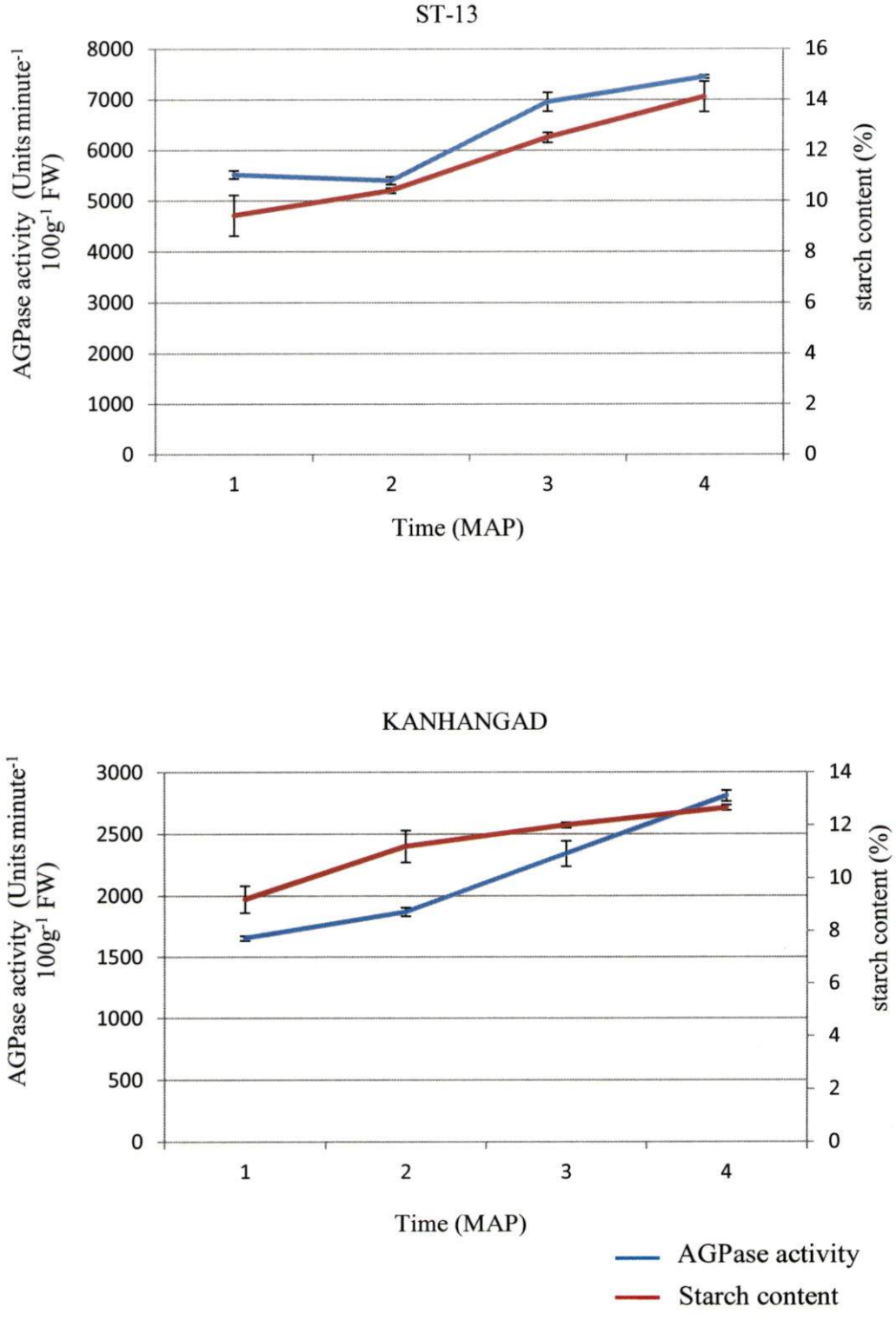
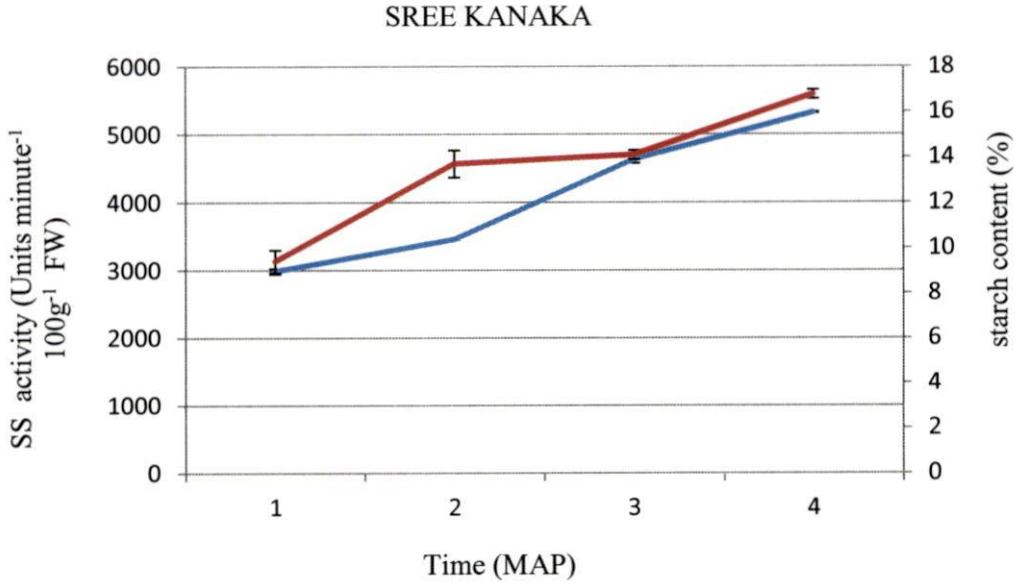
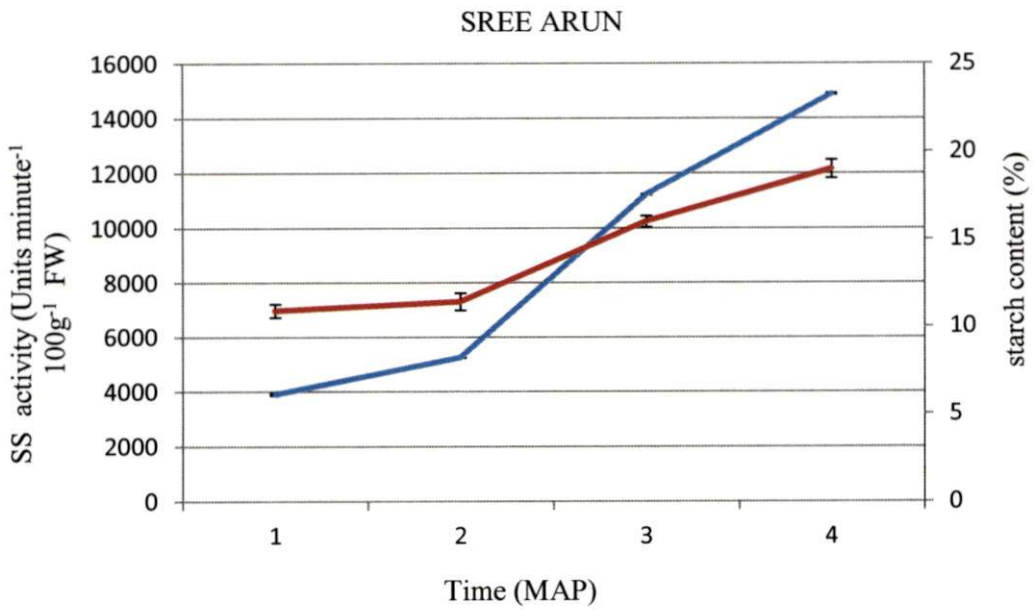


Fig 25: Correlation between AGPase activity and starch content


SREE KANAKA
SS activity (Units minute-1 100g-1 FW)
starch content (%)
6000
5000
4000
3000
2000
1000
0
18
16
14
12
10
8
6
4
2
0
1
2
3
4
Time (MAP)


SREE ARUN
SS activity (Units minute-1 100g-1 FW)
starch content (%)
16000
14000
12000
10000
8000
6000
4000
2000
0
25
20
15
10
5
0
1
2
3
4
Time (MAP)

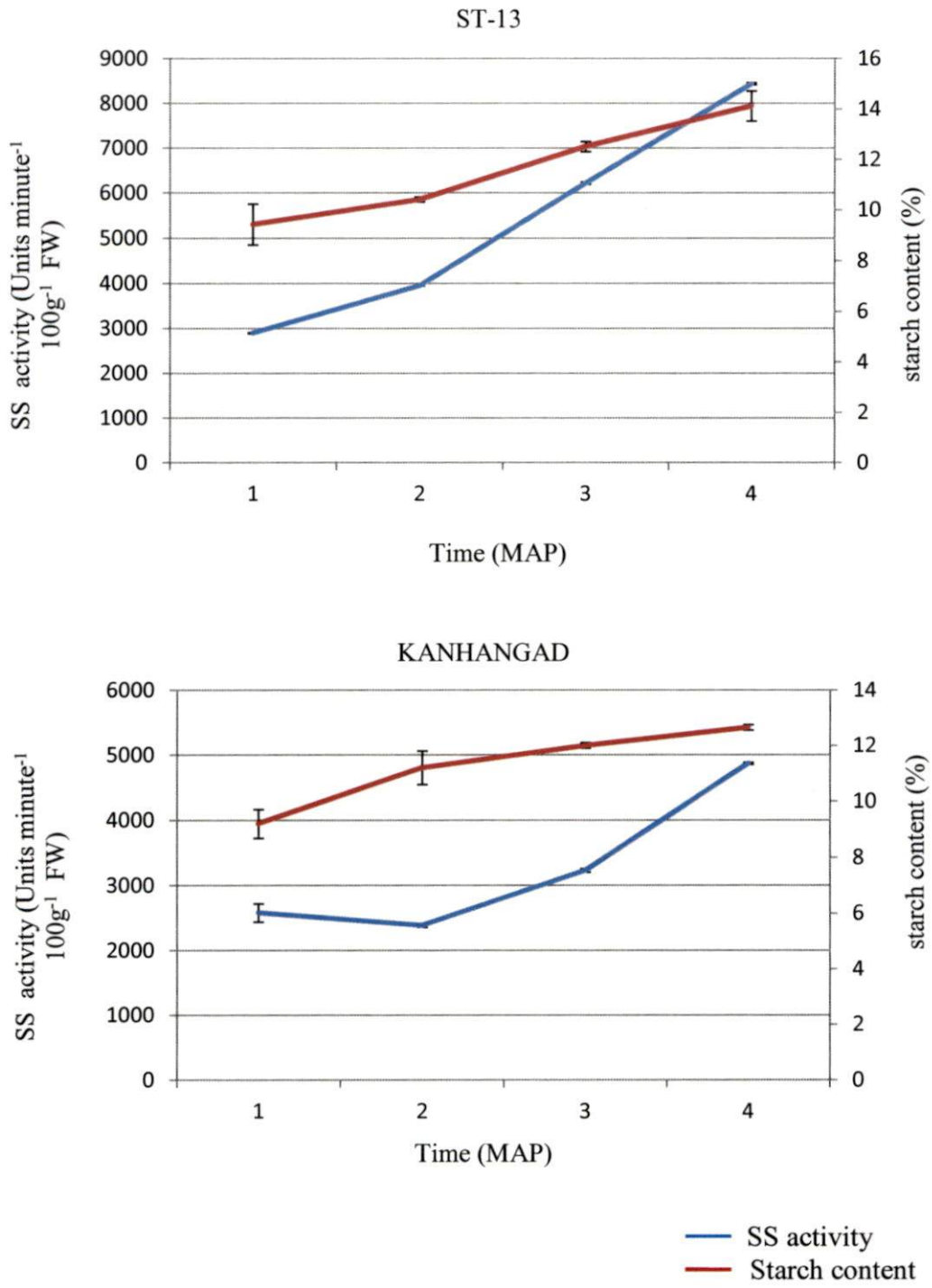


Fig 26: Correlation between SS activity and starch content

DISCUSSION

Chapter 5
DISCUSSION

Starch is the major storage carbohydrate stored in higher plants with many important functions. In photosynthesizing leaves, starch accumulates during the day and is remobilized at night to support continued respiration and growth in the dark (Geiger and Servaites, 1994). It is the second most abundant biopolymer on earth and the most important carbohydrate used for food and feed purposes. Therefore it has major role in our diet, feedstock and for many industrial applications such as bioethanol production (Smith, 2008). Understanding starch biosynthesis in plants could give the way to new strategies to improve starch yield.

The present study was conducted to find the activity of enzymes involved in the starch biosynthesis, sucrose and starch accumulation and photosynthetic rate and relation with starch content of four different varieties of Sweet potato *viz.*, Sree Kanaka, Sree Arun, ST-13 and Kanhangad grown under field conditions. Morphological parameters such as total number of leaves, leaf area and tuber yield and the activity of enzymes involved in sugar and starch accumulation were studied.

5.1. Effect of Morphological parameters on tuber yield

Observations on the total number of leaves, leaf area (cm^2) and tuber yield per plant were recorded at monthly interval during 1 to 4 months after planting. The influence of morphological parameters on starch content is discussed in the following section.

The total number of leaves in four varieties of sweet potato significantly influenced the sucrose and starch accumulation. The maximum number of leaves was recorded in the variety Sree Arun whereas the variety Kanhangad had the least number of leaves per plant.

Leaf area per plant was estimated in four varieties of sweet potato during 1 to 4 months after planting at monthly intervals. There was no definite relation between leaf area and tuber yield. Maximum leaf area was recorded in the variety Kanhangad. Some studies revealed that leaf area is an important parameter in determining plant productivity (Koester *et al.*, 2014). Leaf area index was also observed to be higher in Kanhangad variety. Kaur and Singh (2013) reported a negative relation among the LAI to dry matter and yield. A decreasing trend in leaf area was observed in the varietyST-13.

The influence of leaf area and leaf area Index (LAI) and the total no. of leaves on tuber yield are discussed here. The difference in tuber yield was observed among the varieties. Among different sweet potato varieties, Sree Arun had maximum tuber yield.

5.2. Effect of photosynthesis on tuber yield/ starch content

The relation between photosynthetic rate and tuber yield of four varieties of sweet potato were studied. Photosynthesis increased the starch accumulation and increased the maximum tuber yield. Maximum P_N rate was recorded in the variety Sree Arun which had higher starch accumulation in tubers. The tuber yield is strongly related to the net photosynthesis and dark respiration in potatoes (Tekalign and Hammes, 2004). The total dry matter yield of crops depends on the size of leaf canopy, longevity and the length of time the canopy persists (duration). The production of photosynthates by the leaves and the extent to which they can be accumulated in the sink representing the organs that are harvested significantly influences crop yield (Katoh *et al.*, 2015).

5.3. Effect of Biochemical parameters on tuber yield

Sucrose accumulation in leaf was found to be lower when compared with the sucrose content in tuber. It was reported that the sucrose content was greater in potato tubers (Clegg and Chapman, 1962) and in sugarcane (Waclawovsky, 2010). Sucrose content in tubers was higher in ST-13 and Kanhangad found to be the lowest in leaves. Sree Arun showed the higher starch content. Enhancement of

starch biosynthesis may stimulate photosynthesis in plants (Choi *et al.*, 1998). The proposed mechanism is that increasing seed sink strength may stimulate photosynthesis by pulling greater levels of sugars into developing seeds thereby reducing feedback inhibition of leaf sugars on photosynthesis (Sun *et al.*, 1999).

5.4. Effect of enzymatic activities on tuber yield

The activities of various enzymes such as ADP-Glucose pyrophosphorylase (AGPase), starch synthase (SS), sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and invertase (IT) were studied in leaves and tubers of four varieties of sweet potato.

Among four different varieties of sweet potato, Sree Arun had the maximum AGPase activity in leaf and tuber and had the maximum starch content. Joseph *et al.* (2001) identified that the activities of AGPase, SPS and IT paralleled net increases in starch and sucrose contents respectively in soybean. Increase in AGP activity in sink tissues of economically important plants have been reported to enhance seed size in maize (Giroux *et al.*, 1996) and starch content in potato (Hendriks *et al.*, 2003). The recently discovered redox regulation of AGPase provides a second and complementary way to regulate starch synthesis in response to light and the accumulation of sugars (Kolbe *et al.*, 2005).

SS activity of four varieties of sweet potato plants varied in leaves and tubers. Maximum SS activity was found in Sree Arun leaf and tuber and SS directs increased starch content in tubers. SS activity increased because of high rate of photosynthesis that may lead to the increased accumulation of starch content. Findings of the present study were in agreement with the findings of Zeeman *et al.* (2007). The data were consistent with the results of Tsai and Nelson (1966) where low starch content in the endosperm of maize mutant occurred due to the deficiency of AGPase and SS.

The SuSy activities significantly by varied among four varieties. The maximum SuSy activity with the variety Sree Kanaka is attributed to highest photosynthetic rate. This is because the SuSy catalyzed sucrose metabolism is greater in leaves of than in tubers. Therefore, enhancement of Susy activity

represents a potentially very useful strategy for increasing starch content and total yield in heterotrophic plant organs (Fernandez *et al.*, 2009). According to Ross and Davies (1992), the decrease in Susy activity was lower in developing potato (*Solanum tuberosum*) and considerable increases in hexose content and invertase activity.

In starch biosynthesis, SPS has an important role in increasing the tuber yield in relation to leaf area and photosynthetic rate. SPS activity was higher in Sree Kanaka in relation with the morphological parameters. In 1995, Lafta and Lorenzen reported that the SPS was highly active sink tissue and less in leaf of potato.

Invertase activity was not significant among four varieties of sweet potato. During tuber development, IT activity was high and declined during tuber maturation. The highest IT activity was found in ST-13 and Kanhangad had the lowest. The decrease in IT activity is accompanied by a decrease in the hexose to sucrose ratio and an increase in SuSy activity. This will leads to the accumulation of starch and sugars in leaf and sink tissues of the plant (Hajirezaei *et al.*, 2000).

The enzymes activities involved in starch biosynthesis assayed in the present study *viz.*, AGPase, SPS, SS, SuSy have been assayed in potato (Geigenberger *et al.*, 1998), rice (Nakamura *et al.*, 1989), wheat (Mahajan and Singh, 1990), maize (Vidra and Loerch, 1968), pea (Turner, 1969a,b) and sorghum (Jain *et al.*, 2008).

In this study, the variety Sree Arun had the maximum number of leaves per plant, tuber yield, starch content in both the leaves and tubers and greater activity of AGPase and SS in leaves and tubers. Manipulation of these parameters will improve the starch content or yield in low starch varieties of sweet potato.

SUMMARY

Chapter 6

SUMMARY

A study on "Photosynthesis and Enzyme activities regulating starch biosynthesis in different varieties of Sweet potato (*Ipomoea batatas* (L.) Lam.)"was conducted at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2015-2016.

The present study was focused on four varieties of Sweet potato *viz.*, Sree Kanaka, Sree Arun, ST-13 and Kanhangad in a field trail with three replications. Observation was made for morphological parameter study and photosynthetic rate.

Morphological parameters such as total number of leaves, leaf area and tuber yield were recorded at monthly intervals. The higher yield was obtained in Sree Arun variety with increased rate of photosynthesis. Biochemical parameters such as sucrose and starch content were recorded and showed gradual increase in each interval of time. Sucrose and starch content was higher in the variety ST-13 and Sree Arun variety respectively.

In this study, it was observed that four varieties showed variation in the morphological and biochemical parameters. Sree Kanaka was observed with relatively higher photosynthetic rate and starch content (13.49±3.04) with a total yield of 0.45 kg/ plant.

Enzymes involved in the starch biosynthesis (AGPase, SS, SuSy, SPS and IT) were studied in leaves and tubers of four sweet potato varieties. AGPase and SS were more active in tubers and promote tuberization. AGPase, SS and SPS activities were higher and least activity of SuSy and IT were studied. Sree Arun was observed that AGPase and SS activities were active simultaneously increasing the yield. ST-13 showed the lowest yield due to decrease in leaf area and lowest activities of IT, SPS and AGPase. Invertases were highly active in leaves when compared to tubers.

Activities of AGPase were found to be higher in leaves and tubers of Sree Arun variety and it increased the starch content or tuber yield in this variety. Lowest tuber yield and starch content was observed in the variety ST-13 due to the decreased action of AGPase, SS and SPS. SS activity was also higher in the leaves and tubers of Sree Arun variety. The SPS activity was higher in the tubers of sweet potato varieties especially Sree Kanaka variety.

From these findings, it was concluded that higher sucrose and starch accumulation was due to higher photosynthetic rate. It directly influenced the total tuber yield of sweet potato. The efficiency of enzymes involved in the starch biosynthesis will be engineered using molecular tools and thereby improving the starch content in low yielding varieties of sweet potato.

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Chapter 7

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APPENDICES

Chapter 8

APPENDICES

APPENDIX I: Reagents for Sucrose estimation

1. Dinitrosalicylic Acid Reagent Solution (1%) or DNS reagent
   - Dinitrosalicylic acid: 10 g
   - Phenol: 2 g
   - Sodium sulfite: 0.5 g
   - Sodium hydroxide: 10 g
   - Make upto 1 litre with distilled water
1. Sodium Potassium tartrate solution (40%) or Rochelle salt
2. Concentrate HCl (37.3%, 11.9 N) solution
3. 5N KOH
4. 80% Acetone
5. 80% Ethanol
6. 2% PVP (w/v)

APPENDIX II: Reagents for Starch estimation

1. 9.2N Perchloric acid

   Dilute 793ml of 70% Perchloric acid to one litre

2. 4.6N Perchloric acid

   Dilute 397ml of 70% Perchloric acid to one litre

3. 5% Phenol (w/v)
4. 96% H_2SO_4
5. Standard glucose (1 mg/ ml)

APPENDIX III: Reagents for Enzyme assay

1. 0.01% Protein reagent

   Dissolve 100mg of Coomassie brilliant blue G-250 in 50ml of 95% alcohol and 100 ml of 85%phosphoric acid and dilute to one litre with distilled water. Prepare fresh before use.

2. 0.1M phosphate buffer (pH-7.5)

   (A) K_2HPO_4 : 1.74 g in 100 ml of distilled water

   (B) KH_2PO_4 : 1.36 g in 100 ml of distilled water

   Mix 61.5 ml (A) and 38.5 ml of (B). Then made upto 100 ml buffer solution

3. Standard protein solution

   Dissolve 25 mg of BSA in 0.15 M NaCl and make up the volume to 25 ml with distilled water.

4. 2.5% sucrose solution (w/v)
5. 20% Glycerol (v/v)
6. 1 M sodium acetate buffer (pH-5)

   (A) Glacial acetic acid : 0.25 ml in 50 ml of distilled water

   (B) Sodium acetate: 4.1g in 50 ml of distilled water

   Mix 17.85 ml (A) and 32.15 ml of (B). Then made upto 50 ml buffer solution

7. Extraction buffer (For Susy and SPS)
   - 50 mM HEPES buffer (pH-7.5)
   - 7.5mm $MgCl_2$
   - 2mm EGTA
   - 5mM DTT
   - 2% PEG (w/v)
   - 2% PVP
8. 1N NaOH
9. 1% Resorcinol (v/v)
10. 30% HCl

11. Extraction buffer (For AGPase and SS)
    - 100mM HEPES-NaOH (pH-7.4)
    - 8mM $MgCl_2$
    - 2mM EDTA
    - 12.5% Glycerol
    - 5% PVP
    - 50mm β-mercaptoethanol
12. AGPase reaction buffer
    - 100mM HEPES-NaOH (pH-7.4)
    - 1.2 mM ADP Glucose
    - 3mM PPi
    - 5mM $MgCl_2$
    - 4mM DTT
13. Reaction buffer I (for SS)
    - 50mM HEPES-NaOH (pH-7.4)
    - 1.6 mM ADP Glucose
    - 0.7 mg Amylopectin
    - 15 mM DTT
14. Reaction buffer II (for SS)
    - 50mM HEPES-NaOH (pH-7.4)
    - 4mM PEP
    - 200mM KCl
    - 10mM $MgCl_2$
    - Pyruvate kinase (1.2 unit)
15. Reaction buffer III (for SS)
    - 50mM HEPES-NaOH (pH-7.4)
    - 10 mM glucose
    - 20 mM $MgCl_2$
    - 2mM NADP

ABSTRACT

PHOTOSYNTHESIS AND ENZYME ACTIVITIES REGULATING STARCH BIOSYNTHESIS IN DIFFERENT VARIETIES OF SWEET POTATO (*Ipomoea batatas* Lam.)

by

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(2011-09-116)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

requirement for the degree of

MASTER OF SCIENCE IN (INTEGRATED) BIOTECHNOLOGY

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2016

ABSTRACT

The present study entitled "Photosynthesis and Enzyme activities regulating starch biosynthesis in different varieties of Sweet potato (*Ipomoea batatas* Lam.)" was conducted at ICAR-CTCRI, Trivandrum during the period 2015-2016. The objective of the work was to the study the relation between photosynthesis, leaf area and enzyme activities involved in regulating starch biosynthesis in different genotypes of sweet potato and to identify the limiting factor(s) in low starch varieties of Sweet potato.

The rate of photosynthesis, morphological and biochemical parameters and enzymes activities involved in the starch biosynthesis were recorded at monthly intervals in selected varieties. Morphological parameters such as total number of leaves per plant, leaf area and tuber yield per plant were recorded during 1 to 4 MAP. Rate of photosynthesis was measured using IRGA (infra-red gas analyzer). The leaf area and photosynthetic rate were directly increasing the productivity which was found to be higher in the Sree Arun and Kanhangad variety. Biochemical parameters such as sucrose and starch content were estimated in leaves and tubers of sweet potato varieties. The sucrose content was relatively higher in leaves of Kanhangad variety and in tubers of ST-13 variety. Starch content was higher in the variety Sree Arun which had a direct relation with the photosynthetic rate and tuber yield. Activities of enzymes such as AGPase, starch synthase (SS), sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and invertase were measured in both the leaves and tubers of sweet potato. AGPase and SS were directly involved in the tuberization throughout the development which was observed in Sree Arun variety. SPS was highly active in leaves than tubers. SuSy and invertase are mainly involved in the sucrose metabolism in leaves and highly active during the day time. The increased activity of AGPase and SS in sweet potato tubers will open a new strategy for bioengineering the efficiency of enzymes to improve the starch content in low yielding sweet potato varieties. A detailed molecular study on enzymes involved in starch biosynthesis will be necessary for increasing or improving the starch content in tuber crops.