DEVELOPMENT OF GENE TAGGED SNP MARKERS FOR FUNCTIONAL ANALYSIS OF STARCH BRANCHING ENZYME GENES IN *ORYZA SATIVA* INDICA SUBSP.

Under the supervision of

Dr. HARVINDER SINGH

By

Vishakha Jain (111812) BTDD

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CERTIFICATE

This is to certify that the work entitled, "**Development of gene tagged molecular markers (SNP) for the functional analysis of starch branching enzyme genes in *Oryza Sativa* Indica Subsp.**" Submitted by Vishakha Jain in partial fulfilment for the award of degree of bachelor of Technology in Biotechnology of Jaypee University of Information Technology, Waknaghat (Solan) has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of the Supervisor:

Dr. Harvinder Singh

Date:

ACKNOWLEDGEMENT

Any assignment puts to litmus test an individual's knowledge, credibility and experience and thus, sole efforts of an individual are not sufficient to accomplish the desired work. Successful completion of a project involves interest and efforts of many people and so this becomes obligatory on my part to record thanks to them.

Therefore, first of all I would like to thank my guide and mentor Dr. Harvinder Singh for his guidance, help and constant encouragement throughout this project. Working under him was an enriching experience.

I express my heartfelt thanks to the Head of Department Dr. R.S. Chauhan for providing me the opportunity of doing this project.

Vishakha Jain (111812)

DATE:

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ABSTRACT

The starch branching enzyme genes and other genes like starch synthase genes, granule bound starch synthase gene play a crucial role in starch biosynthesis. These genes are involved in various physicochemical properties like amylose content and gelatinization temperature which are very important in determining the rice quality. The functional properties of these genes in 11 Indian rice genotypes were analysed. We focused on the Indica varieties which are consumed more in India and their starch properties affect the insulin levels of diabetic consumers. We tried to develop the SNPs to study variation in SBE gene sequences. The rice seeds were germinated at optimum temperature and genomic DNA was isolated from its seedlings. Amplification of SBE gene 1and 3 was carried on various genotypes. The physicochemical studies were also done to relate the glycemic levels and resistant starch with rice properties. In this study, we also estimated the amylose content of 11 rice varieties which showed that s5 variety (RR seed) has intermediate amylose levels which is preferable for consumption and grain elongation test was also performed to study the rice eating and cooking quality. Rice consumption has a significant effect on diabetic population so discovering such varieties with optimum levels of glycemic index is required which can be achieved by studying the genes involved in starch biosynthesis. Such candidate genes can be utilized for planning genetic manipulation studies to produce desirable rice varieties.

CHAPTER 1

Introduction

Rice is second most consumed crop in the world with its varieties in varies regions. It is of two types Indica varieties and japonica varieties. It is mostly consumed more in Asian and African countries. It is basically the seed of grass species *Oryza Sativa* with a genome size of 430Mb. It is also an important grain with regard to human consumption for nutrition and calorie intake providing more than one fifth of the calories consumed worldwide (Smith et al.). It is a predominant energy source for various countries in Asia and the Pacific, and other countries in North, South America and Africa. Rice is a monocot which is grown as an annual plant but in tropical areas of the world it can survive has a perennial plant with height about 1-1.8m. The cultivation is optimal for countries with low labour cost and high rainfall as it is labour intensive and requires a lot of water. The most approachable method of cultivating the rice is flooding the fields after the growing of young seedlings. The varieties of rice are classified as long, medium and short grain rice. The grain of the long grain rice tends to remain intact after cooking while the medium grain rice becomes sticky (Juliano et al.).

The two main varieties of rice i.e. Japonica and Indica also have lot of variation in characteristics and the area where it is cultivated.

Japonica varieties are mainly grown in japan and also called japan rice. The rice here is usually brown in colour which is further polished and sold as ready- made polished white rice but people also prefer to consume brown rice as unpolished due to its health benefits.

Indica varieties are the non- sticky, long- grained rice which is grown mostly submerged throughout Asia. Rice has been cultivated since ancient times also *Oryza* is the classical Latin word for Rice and *Sativa* means Cultivated. So in our study, we have done work on Indica varieties since it is grown in India and can be available readily for research purposes.

1.1 Background

Starch is primary form in which carbon is stored in plants and it makes up 50% or more of dry weight of many storage organs. Starch occurs as partially crystalline granules in plastids and is composed of two types of glucan polymer, amylose and amylopectin. Amylose is generally 25% and consists of predominantly linear chains α 1, 4 linked glucose residues. Amylopectin is generally 75 % consisting of shorter α 1, 4 chains connected by α 1, 6 linkages (Brown et al.).

Rice mainly contains two types of starch: amylose and amylopectin. Amylose is a long straight starch molecule that does not gelatinize while cooking and is non- sticky and fluffy after cooking. It also hardens while cooking and melts when the rice is re heated. Resistant starch commonly known as dietary starch is achieved from the amylose content. In case of diabetes which is a condition of disordered glucose metabolism, many metabolic studies now suggest that food sources of a carbohydrate vary greatly in their rate of absorption and effects on blood glucose and insulin concentration. One way of quantifying this variation in response to dietary carbohydrate is the glycemic index. Glycemic index is the incremental area under the curve of blood glucose produced by a standard amount of carbohydrates in a meal. The amylose content is inversely related to glycemic index. It has been observed that rice which has high amylose content usually have low glycemic index because amylose is harder to break down than simple sugars like glucose etc. and ensures a sustained release of sugar into blood without spiking immediately after a meal. It is less readily digested than amylopectin because it is linear and takes up less space. As a result, it is more preferred starch for storage in plants (Kay M Behall et al.).

Amylose

Amylose is a linear polymer made up of D- glucose units. It is synthesised in plastids from ADP glucose by starch synthase which adds glucose residue to the non- reducing ends of α 1,4 linked polymers. The carbon atoms on glucose are numbered, starting at the aldehyde (C=O) carbon, so in amylose, the 1- carbon on one glucose molecule is linked to the 4 carbon on the next glucose molecule (Nelson et al.).

It is also considered as an important form of resistant starch since it is in packed structure and takes time to digest (Cohen et al.).

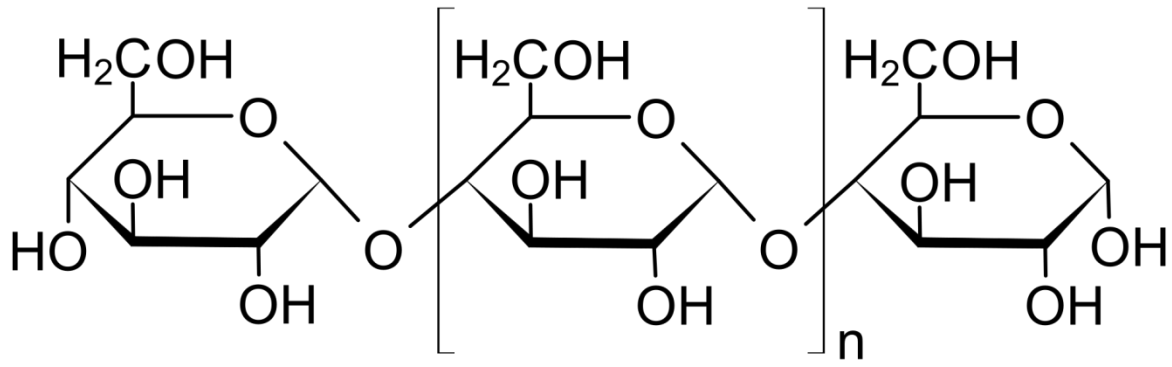


FIG1. Structure of amylose in starch granules

Amylopectin

It comprises about 75-80% of the starch in rice grains and is the crystalline form of starch which has some chain length along its structure. It consists of shorter α 1, 4 linked chains connected by α 1, 6 linkages. The structure of amylopectin can be generalized in terms of its types of chain (A, B and C) which differ in length. The A- chains (unbranched) are linked to B-chains and do not carry any other chains, the B-chains carry one or more A-chains or B-chains and the C-chains has the reducing end of the molecule. The chain length distribution data of various rice- Japonica and Indica rice suggests that Indica rice has more Amylopectin content as compared to Japonica rice when tested for its various rice varieties. Japonica rice has short chains of amylopectin (Ball, S. et al.).

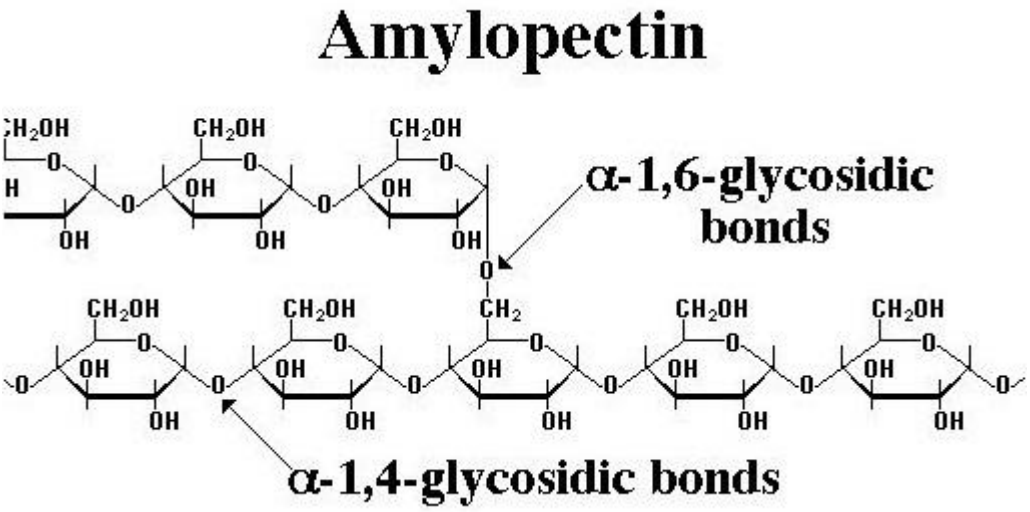


FIG2. Amylopectin structure in starch molecules

Starch Metabolism Pathway

There are various genes and their isoforms which are involved in the starch biosynthesis or metabolism. The various genes involved in starch biosynthesis are AGPase, Starch synthase, branching enzyme, debranching enzyme, and granule bound starch synthase (GBSS) and its isoforms which affect the physicochemical properties of the rice. Out of these genes I am potentially working on **Starch Branching Enzyme gene** which basically affects the amylopectin synthesis and its isoforms affects the properties like amylose content and gelatinization temperature (A M Smith et al.).

Here, amylose content basically has positive correlation with elongation ratio and volume expansion. It correlates positively with volume expansion of cooked rice. Rice having high amylose content shows high volume expansion (Nguyen Thi Lang and Bui Chi Buu et al., 2004).

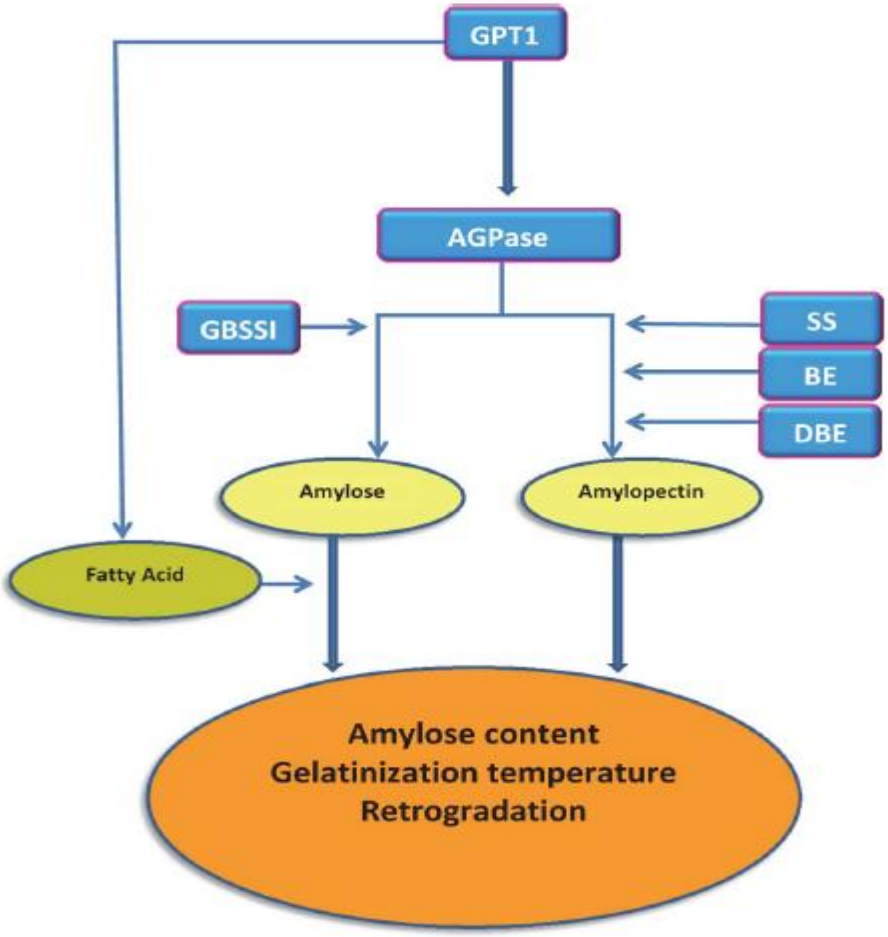


FIG3. Starch biosynthesis pathway

1.2 Physicochemical Properties of Rice

i. **Pasting Temperature:** The temperature at which irreversible swelling of the starch granules occur.

ii. **Chalkiness:** It is determined by the clarity of the endosperm. If part of the milled rice kernel is not transparent, it is often characterised as chalky. While chalkiness disappears upon cooking and has no effect on taste or aroma, it downgrades the quality of milled rice.

iii. **Gelatinization temperature:** Gelatinization is the irreversible melting process of starch. It is an endothermic process, and the peak temperature at which starch absorbs heat is called gelatinization temperature (GT). There are several ways to measure GT. The most common method is alkali spreading value (ASV), for which the degree of disintegration of milled rice is graded after immersion in potassium hydroxide for 24 hours. A more modern assay is by differential scanning calorimetry (DSC). This detects the peak temperature absorbed by the flour water mixture during gelatinization and is thus more precise than ASV (Jenkins et al.).

iv. **Peak viscosity:** The peak viscosity is defined as the maximum viscosity that occurs prior to the initiation of sample cooling. The highest viscosity reached during gelatinization of starch usually corresponding to the point where all the granules are swollen to occupy a high proportion of the available volume with each in contact with its immediate neighbours. On cooling the starch paste, the viscosity may rise above this level, but only the initial shoulder or true peak values determined on the hot paste are termed peak viscosity (Hans-dieter Belitz, Werner Grosch et al., 2004).

v. **Retrogradation:** Retrogradation is a reaction that takes place in gelatinized starch when the amylose and amylopectin chains realign themselves, causing the liquid to gel. In this study I am trying to investigate the role of starch- related gene i.e. SBE gene and its SNPs by assessing its contribution to variation in starch properties in a rice breeding population.

1.3 Research gap

With the advancement in research and technology more process has been made in this field of plants to improve the rice quality. *Oryza Sativa* was the cereal selected to be sequenced as a priority and has gained the status of model organism. Various works on the SNP detection has been done on the Japonica and Indica varieties but much of the work was focused on Japonica varieties. Numerous SNPs have been detected in Japonica varieties by various researchers. They have also developed other molecular markers like SSRs and CAP for this rice variety. But, the same has not been done for Indica varieties and people are still trying to study other aspects like abiotic stress tolerance and no significant work has been reported in Indica varieties in case of SNPs. This calls for the fact the need to study and develop SNPs in Indica varieties since it is widely consumed in India and no development in seen in this area. So studying the starch branching enzyme genes for functional analysis in Indica varieties makes it a novel work and will led to the contribution for improvisation of rice quality.

1.2 Objective

i. Detection of the putative SNPs in rice genotypes
ii. Association studies with properties like Amylose contentment and Gelatinization temperature

CHAPTER 2

Review of literature

Oryza Sativa, commonly known as Asian rice, is the plant species most commonly referred in English as rice. Rice is the second most consumed crop in the world. *Oryza sativa* is the cereal with smallest genome, of size 430Mb. It has been selected for studying the sequences and is considered as a 'Model Organism'. It is a monocot plant which basically belongs to the grass family. It requires lot of water to grow and is generally considered labour intensive to cultivate. It has been subject of studies on yield, hybrid vigour, genetic resistance to disease and adaptive responses, scientists try to explore the multitude of varieties that have adopted to a wide variety range of environmental conditions. There are various rice varieties in various regions of the world (Cecap, PhilRice and IIRR. 2000). The two known subspecies of rice are

An Indica variety which is long grain, less sticky rice which is mostly grown submerged in tropical Asia.

Japonica variety which is short grain, sticky rice which is grown in dry fields in temperate Asia, upland areas of Southeast Asia and high elevations in South Asia. This rice is mostly grown in Japan and consumed as unpolished rice which is brown in colour which is beneficial from health view. Generally rice is known to have various colours including white, brown, black, purple and red in some areas. Rice is known as the most common energy source of the world especially in countries with more labour intensive work and low economy. Due to this reason there is an increased demand to improve the rice quality (Glaszmann et al.)

The rice property widely affecting health is the amylose content because of its relation to the glycemic index. Glycemic index is known as the incremental area under the blood glucose curve which increases immediately after a meal. The amylose content is inversely related to the glycemic index suggesting that rice with high amylose content usually have a low glycemic index. So generally that is preferred as with low glycemic levels there will be less insulin demand with low release of sugar in the blood after a meal which is facilitated by resistant starch. Resistant starch commonly known as dietary starch is achieved from the amylose content. In a diabetic patient, due to the low insulin concentration in the body, the release of sugar into blood is less controllable than normal, leading to spike in blood sugar levels immediately after meals for them. High blood sugar, if left untreated can cause dehydration, electrolyte imbalance for short time and serious problems over long time. Hence it is necessary to maintain these levels in diabetic patient (Krezowski PA et al.).

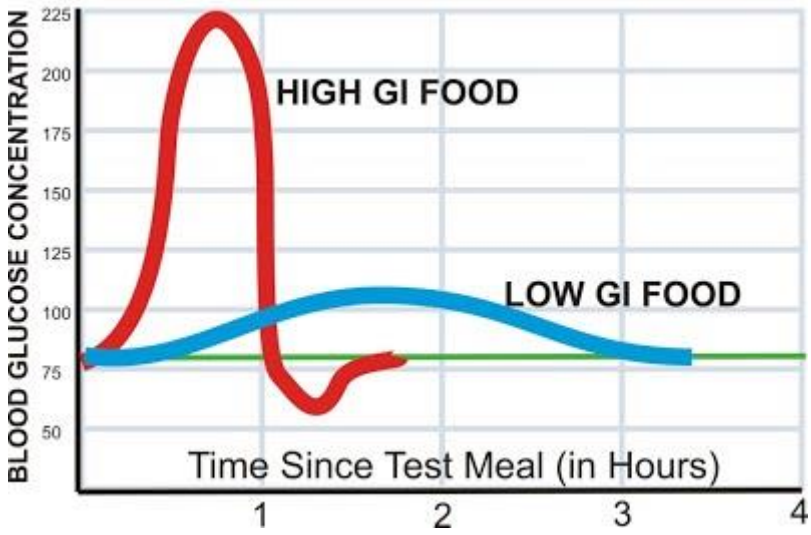


FIG4. Blood glucose concentration in relation to low glycemic index food and high glycemic index food Foods with high amylose content generally have a low glycemic index which is preferred (www.hollandclinic.com/Doctor-Holland-Book/chapter-8)

The natural history of population of pre domesticated ancestors along with the breeding systems and complexity of breeding practices is seen to influence the population structure of domesticated species. Among the rice species Orzya Sativa there is a well -known divergence between the two major subspecies Indica and Japonica. Among researchers it is considered of interest to develop a population based framework for analysis of diversity in Orzya sativa species due to the availability of rice genome sequence along with large collection of genetically available resources (Amanda J. Garris et al.).

The various starch properties are known to affect the cooking quality of rice and influence the human health by changing the glycemic index levels as seen in the figure and the levels of resistant starch. The incomplete digestion- absorption of resistant starch in small intestine leads to non- digestible starch fractions with physiological functions similar to dietary fibre with significant beneficial impacts.

Starch is produced in plants by converting the glucose 1- phosphate to ADP glucose via the enzyme glucose 1- phosphate adenylyltransferase. The enzyme starch synthase then adds the ADP- glucose through a 1, 4 glycosidic bonds to a growing chain of glucose residues which liberate ADP and create Amylose. Basically the starch branching enzyme introduces α 1, 6 glycosidic bonds between these chains creating the branched amylopectin (Smith, Alison M et al., 2001). These starch molecules seem to arrange themselves in plant in semi- crystalline forms. The starch is generally observed to become soluble in water when heated. The process of gelatinization occurs on heating where the granules swell and burst, the semi- crystalline amylopectin chains are open and the amylose starts leaching out of granule holding the water and increasing the mixture's viscosity. While cooking the starch granules forms a past and while cooling the semi- crystalline structure realigns itself becoming thick and losing water which is known as retrogradation of starch (Chun You et al., 2013). Generally the higher the amylose content of rice, the firmer the cooked grain of rice. The amylose is classified as

waxy 0-2%, very low 2-10%, low 10-20%, intermediate 20-25% and high 25-35%. The rice with the intermediate amylose content cooks softer and the grains stick together more easily.

Effect of Cooking on Amylose Content of Rice

Since rice contains two types of starch basically amylose and amylopectin. The effect of various physical treatments also varies according to the amount of amylopectin and amylose present in a particular rice subspecies. It is observed that out of the two, amylose is the long straight starch molecule that does not gelatinize on microwave cooking and hence rice varieties with high amylose content tend to cook fluffy with separated grains. The hardness of the cooked rice is correlated positively with the amylose content of rice but can provide more spread of values at intermediate and high amylose rice varieties. This amylose is analysed in rice by a means of colorimetric iodine method. As we know that diabetic patient have impaired insulin functions so meals which are high in carbohydrates can result in increase of sugar levels in blood soon after eating a meal. To decipher the most suitable method of cooking for diabetic patients we tried to find out its effect on amylose levels which can be related with glycemic index for diabetic patients. There is a vast variation in the amylose content of the rice depending on the way it is cooked. The methods involved are microwave, traditional and steam cooking (Ashish jain et al.) Other parameters tested while cooking with different methods were the resistant starch and gelatinization temperature. The onset gelatinization temperature was observed to be higher for Indica varieties than Japonica varieties due to the presence of long amylopectin chains. The resistant starch for steamed Indica and Japonica rice was observed to be 6.6% and 0.7% respectively (Reed MO et al., 2013). Among the three cooking methods it was concluded that microwave cooking was the preferred method as the amylose content was highest and can be used for diabetic patients since it will have less glycemic index with this method of cooking.

Another parameter studied was Retrogradation which is basically the re- crystallization of starch molecules on cooling after a heating process. This property can be correlated with levels of resistant starch also. Rice with high amylose content generally is considered highly retrograded rice with low glycemic index and hydrolysis index and more resistant starch. While the rice with low amylose content gets hydrolysed quickly showing higher hydrolysis index (Peisong Hu et al., 2004).

Genes Involved

The various genes involved in the starch biosynthesis are

ADP Glucose Pyrophosphorylase (AGPase), Granule Bound Starch Synthases (GBSS1 and GBSS2), Starch Synthases (SS1, SS2a, SS3b, SS4a, SS4b), Starch Branching Enzymes (SBE1, SBE2a, SBE2b), Debranching Enzymes (ISA1, ISA2, Pullulanase), Starch Phosphorylase (SPHOL), Glucose 6- Phosphate Translocator (GPT1)

Amylose is synthesised in plastids from ADP- glucose by Starch Synthase which adds glucose residue to the non- reducing ends of α 1,4 linked polymers. Amylopectin is synthesised from linear chain by starch branching enzymes which hydrolyses an α 1,4 linkage within chain by an α 1,6 linkage. Only SBE in amylopectin can introduce α 1, 6 glucosidic linkages into α polyglucans in plants. SBE is considered an important enzyme in case of amylopectin synthesis and in Australian varieties it contributes to amylose synthesis and various other properties. GBSS1 and SS2a genes are most correlated with starch properties whereas SS2b and SPHOL genes do not explain variation in starch properties. So far it is seen that GBSS1 influences amylose content and retrogradation. GPT1, SS1 and BE1 and SS2a are the other genes contributing to the retrogradation.

The amylose synthesis is widely characterised by GBSS1 enzyme gene mainly although in some Australian varieties SBE isoforms also played a crucial role (Kharabian-Masouleh et al.). The starch of mutant rice lacking GBSS1 and of transgenic plants in which GBSS1 has been reduced by expression of antisense RNA contains little or no amylose. GBSS1 is bound to starch granule. Isoforms of starch synthase other than GBSS1 are also found to be tightly bound to the granule. They become incorporated into the matrix of the granule as their amylopectin product crystallizes around them. When supplied with ADP glucose, the isolated granules incorporate the glucose unit into the starch. A soluble factor is needed for the amylose synthesis, soluble malto oligosaccharides such as maltose and maltotriose to the isolated granules allowed GBSS1 but not SS11 to synthesis amylose. GBSS1 can use these malto- oligosaccharides as initial substrates for synthesis of considerably longer chain which are unable to diffuse out. SS2 cannot use malto-oligosaccharides as substrates. This isoform can indeed use short malto- oligosaccharides as substrates adding only a single glucose unit before dissociating. The gene expression pattern is also different between SS1 and GBSS1 because the SS1 gene is expressed at the same level in both leaves and developing seeds (Baba et al., 1993) while the GBSS1 gene is present only in the developing seeds (Hirana and Sano., 1991).The fundamental difference in reaction mechanisms between SSII and GBSS1 is probably central to the unique ability of GBSSI to synthesize amylose.

Amylopectin synthesis depends on four enzymes i.e. AGPase, SS, SBE and DBE. The structure of amylopectin can be classified as L type or the S type. The L type amylopectin produced in many Indica type varieties can be distinguished from the S type amylopectin found in most Japonica type varieties in that the proportion of short chains of degree of

polymerization which is less than or equal to 10 in the former is specifically lower as compared to the latter. It is also observed that enzyme SSIIa is lower in Japonica rice varieties which is responsible for the elongation of Chain A suggesting that these varieties have low Amylopectin. When the lengths of chains reach degree of polymerization of 12 or longer, SBEIIb acts specifically on these chains (Alex chi wu et al.).

Single Nucleotide Polymorphisms (SNP) are the most abundant type of genetic variation found within all species and many important traits in rice and in human diseases are attributed to such sequence variations. Identifying SNP and associating them with starch properties brings the advancement in research regarding the functional analysis of rice enzyme genes. This highlights the improvement in crop quality which impact food quality and human nutrition and health. Kharabian- Masouleh et al. (2011) discovered more than 501 SNPs and 113 In/dels in 17 starch synthesis genes in an Australian rice breeding population using a combination of a target pooled long range PCR and Massively parallel sequencing (MPS) technology. Since allelic differences occur in more abundance in the rice genome it is easier to detect as compared to other markers for rice genome. Since these genes greatly influence the amylose and amylopectin synthesis in the rice varieties it is of concern to study the variation caused by these genes by one nucleotide changes in the genome affecting the eating and cooking quality of the rice (Chen H et al.).

The amylose content and the gelatinization temperature and gel consistency seems have most of the effect on the eating and cooking quality of rice. GBSS1 (waxy gene) which is responsible for the synthesis of amylose have encountered SNPs in the waxy locus which accounts for the subsequent amylose content in the rice varieties. The gene encoding the starch synthase studied on its effect on cooking quality and rice texture is exclusively expressed in the rice endosperm. Two SNPs within exon 8 [A/G] and [GC/TT] are associated with rice disintegration, eating quality and gelatinization temperature of starch (Umemoto et al., 2004). Various allele specific primers have been identified for both the properties to associate it with the rice quality. Out of the available mechanisms, the splicing of the Wx gene plays a key role for the reduced GBSS levels and low amylose content. The G to T transition at the 5' splice site of first intron blocks the splicing of pre-mRNA transcripts in the Wx gene. Another fact observed was that the G/T polymorphism in waxy locus in various rice varieties had the same AGTTATA sequence irrespective of the fact that it was Japonica or Indica subspecies. Apart from the G/T polymorphism the CTn repeats were also present in the waxy locus (Jixun Luo et al.).

The SNPs reported in SBE genes:

According to some research group who provided their research on Australian varieties some SNPs were reported in SBE genes also apart from the GBSSI and SSI genes. The SNPs were reported here in the three isoforms of the SBE gene i.e. SBEI, SBEIIa and SBEIIb.

SBEI: A C/T SNP at position 1588 was detected in this gene with 9 associated physiochemical traits. This SNP had high statistical significant values for amylose content and retrogradation and minor association with peak viscosity was also noted.

SBEIIa: this gene is involved in the amylopectin synthesis and amylopectin has its effects on gelatinization temperature and starch viscosity in some rice cultivars. The reported SNP was T/G at position 3266 but it had no significant correlation to the starch physiochemical properties in the Australian rice varieties.

SBEIIb: this enzyme gene is also known as the amylose extender in other cereals. Two SNPs were reported in this gene but there was no correlation to properties in these rice varieties. However, it is not necessary that the SNPs will not have an effect on physiochemical properties in other rice varieties (Ardashir et al).

Other markers in the SBE genes:

The CAPS markers were designed from the sequences and were further correlated with the starch properties and the STS marker was developed from the promoter region in the SBE1 gene. The CAPs marker was designed from the last exon of the SBE1 gene which detected T/C polymorphisms in both Indica and Japonica varieties. Together the information from the CAPS and STS markers revealed variations with the starch viscosity parameters i.e. hot viscosity, cool viscosity, consistency and peak viscosity among the rice cultivars (Yeupeng Han et al.).

Amylopectin role in Gelatinization temperature and specific allelic markers

The amylopectin structure can determine the gelatinization behaviour in the rice varieties which consists of α 1,4 D glucosyl units chains branched with α 1,6 bonds (Bulean et al., 1988). As we noted, the amylopectin structure has chain length of 19-23.(Takeda et al.,1987) The chain length in Indica varieties are higher i.e. 21-22 as compared to Japonica varieties stating that short chains are negatively correlated while the longer chains are positively correlated to gelatinization temperature (Nakumura et at., 2002). The underlying mechanism of amylopectin synthesis which affects the gelatinization temperature is very complex but visibly three classes of enzymes are involved in the synthesis of the amylopectin i.e. starch synthase, starch branching enzymes, starch debranching enzymes with their respective functions. These enzymes play a crucial role in the amylopectin synthesis, structure and its

distribution in rice. These structural changes in the amylopectin lead to the increase and decrease of the gelatinization temperature of the rice starch. Molecular markers tagged to genes SSs, SBEs, and two starch debranching enzyme (pullunase and isoamylase) have been evaluated for the effect of these genes on starch physiochemical properties. The SNPs analysed in these region had an effect on enzyme activity, amylopectin structure and gelatinization temperature. Also, the nucleotide diversity in the SSIIa gene was studied to decipher the relationships between the SNPs and the GT values. Researchers confirmed the role of GC/TT polymorphism in SSIIa gene helped to differentiate the rice from as high or medium Gelatinization temperature from low gelatinization temperature successively. In another study, 3 different alleles for SSIIa gene were reported which were present in Japonica and Indica varieties. These polymorphisms have leads to variation or changes in the gelatinization temperature depending on the rice varieties. However, still research is required to quantify the effects of individual or many enzymes in combinations to study properly the functional properties and their interdependence by applying the advanced functional genomics approach (Manish k. Pandey et al., 2012).

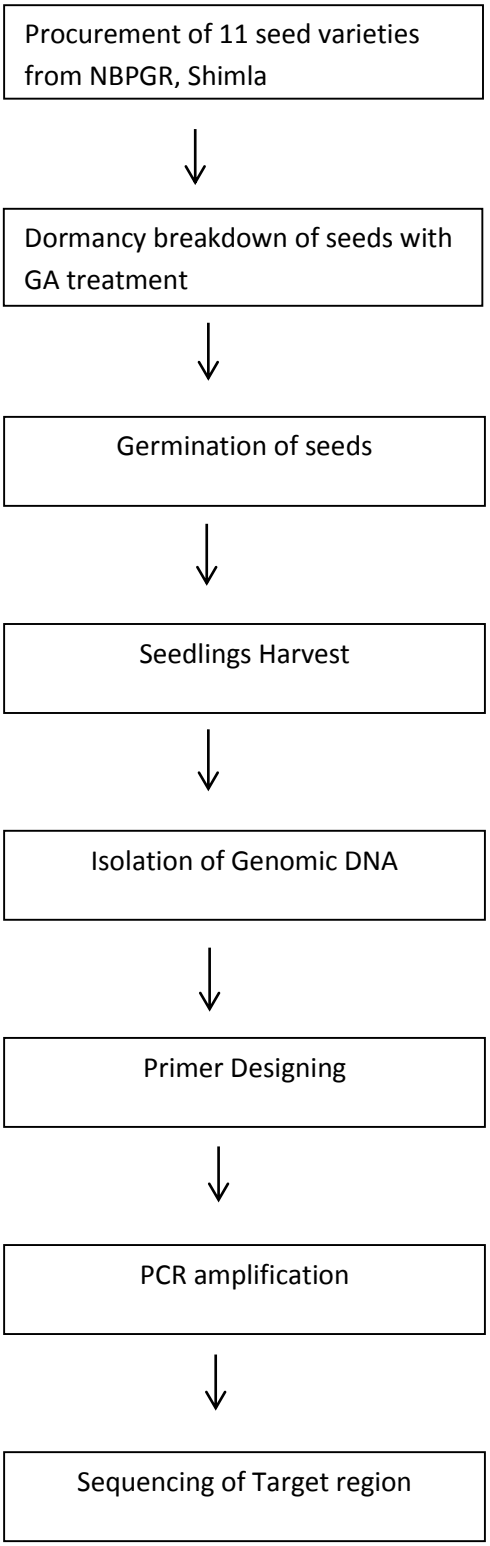
TABLE1. SNP present in the various starch related genes in 233 Australian breeding lines (Ardashir Kharabian- Masouleh et al. 2012)

No	Gene	SNP ID*	Coordinates on gDNA	Expected SNP	SNP Assayed†	Association with Physiochemical traits	Status
1	AGPS2b	TBGU388647	233	G/T	G/G	N/A	No polymorphism
2	AGPS2b	TBGI050742	1507	T/C	T/T	N/A	No polymorphism
3	SPHOL	TBGU168031	2501	G/T	G/G	N/A	No polymorphism
4	SPHOL	TBGU168032	2920	C/T	C/C	N/A	No polymorphism
5	SPHOL	TBGU168027	1001	C/A	C/C	N/A	No polymorphism
6	SPHOL	TBGU168024	176	G/T	G/G	N/A	No polymorphism
7	SPHOL	TBGU168039	5514	G/T	G/G	N/A	No polymorphism
8	GBSSI	WAXYEXIN1	246	T/G	T/G	P1,BD,FV,SB,MT,AC,PN	Highly associated
9	GBSSI	WAXYEX6	2494	A/C	A/C	SB,BD,MT,AC	Highly associated
10	GBSSI	WAXYEX10	3486	C/T	C/T	T1,FV,SB,MT,AC,PN	Highly associated
11	GBSSII	GBSSII_GA_1638	1638	G/A	G/A	PT, GT	Low-Medium association
12	SSI	TBGU272768	5153	T/C	T/C	FV,SB,MT	Low-Medium association
13	SSIIa	SSIIa_GA_Ref631	631	G/T	G/T	N/A	No association
14	SSIIa	ALKSSIIA4	4827–4828	GC/TT	GC/TT	BDV,SB,PKT,PT,GT,CHK	Highly associated
15	SSIIb	TBGU116115	3416	A/G	A/A	N/A	No polymorphism
16	SSIIb	TBGU116120	3948	G/C	G/G	N/A	No polymorphism
17	SSIIb	TBGU116121	3979	T/C	T/T	N/A	No polymorphism
18	SSIIb	TBGU116109	330	G/A	A/A	N/A	No polymorphism
19	SSIIb	TBGU116119	3946	C/T	C/C	N/A	No polymorphism
20	SSIIb	TBGU116116	3487	T/G	T/T	N/A	No polymorphism
21	SSIIIa	GA_Ref1058	1058	T/A	T/A	PT,MT,	Low-Medium association
22	SSIIIa	GA_Ref1680	1680	G/A	G/A	SB,PT,MT,AC,PN,GT	Low associated
23	SSIIIa	GA_Ref3136	3136	G/A	G/A	N/A	No association
24	SSIIIa	GA_Ref3391	3391	T/A	T/A	N/A	No association
25	SSIIIa	GA_Ref3559	3559	T/A	T/A	CHK	Low association
26	SSIIIa	GA_Ref4384	4384	G/A	G/A	N/A	No association
27	SSIIIa	GA_Ref1379	1379	A/C	A/C	FV,SB,PT,MT,AC,PN	Low-Medium association
28	SSIIIa	GA_Ref1708	1708	G/A	G/A	MT,AC,PN,GT	Low-Medium association
29	SSIIIa	GA_Ref3274	3274	G/A	G/A	N/A	No association
30	SSIIIa	GA_Ref6242	6242	T/C	T/C	N/A	No association
31	SSIIIa	GA_Ref1457	1457	A/C	A/C	N/A	No association
32	SSIIIa	GA_Ref1615	1615	C/T	C/T	N/A	No association
33	SSIIIa	GA_Ref1834	1834	C/T	C/T	N/A	No association
34	SSIIIa	GA_Ref2758	2758	G/A	G/A	N/A	No association
35	SSIIIa	GA_Ref1722ER	1722	G/A	G/A	FV,SB,PT,MT,AC,PN,GT	Low-Medium association
36	SSIIIa	GA_Ref2488	2488	C/T	C/T	N/A	No association
37	SSIIIa	GA_Ref3073	3073	G/A	G/A	N/A	No association
38	SSIIIa	GA_Ref1357	1357	G/A	G/A	MT	No association
39	SSIIIa	GA_Ref2080	2080	C/T	C/T	N/A	No association
40	SSIIIa	GA_Ref3481	3481	G/A	G/A	N/A	No association
41	SSIIIa	GA_Ref5466	5466	G/A	G/A	FV,SB,PT,MT,AC,PN,	Low-Medium association
42	SSIIIa	GA_Ref10761	10761	C/T	C/T	PT	Low association
43	SSIIIb	GA_Ref1315	1315	T/C	T/C	PT	Medium association
44	SSIIIb	GA_Ref4543	4543	C/A	C/A	PT	Medium association
45	SSIIIb	GA_Ref5451	5451	T/C	T/C	PT	Medium association
46	SSIIIb	GA_Ref7232	3232	T/G	T/G	PT	Medium-High association
47	SSIIIb	GA_Ref7255ER	7255	C/A	C/A	PKV	Medium association
48	SSIIIb	GA_Ref7437	7437	A/C	A/C	PT	Low-Medium association
49	SSIVa	GA_Ref4048	4048	C/T	C/T	PT,GT	Low-Medium association
50	SSIVa	GA_Ref7160	7160	A/G	A/G	PKT,PT,AC,PN,GT	Low-Medium association
51	SSIVa	GA_Ref7506	7506	A/T	A/T	PT,GT	Low-Medium association
52	SSIVa	GA_Ref7823	7823	T/C	T/C	PT,GT	Low-Medium association
53	SSIVa	GA_Ref8383	8383	C/A	C/A	PT,GT	Medium association
54	SSIVb	TBGU260749	5090	G/C	G/G	N/A	No polymorphism
55	SSIVb	TBGU260765	9525	G/A	G/G	N/A	No polymorphism
56	BEI	GA_Ref1558	1558	C/T	C/T	PV,BDV,FV,SB,PT,MT,AC,PN	Low-Medium association
57	BEIIa	GA_Ref3266	3266	T/G	T/G	N/A	No association
58	BEIIb	GA_Ref9035	9035	C/T	C/T	N/A	No association
59	BEIIb	GA_Ref10068	10068	C/A	C/A	N/A	No association

CHAPTER 3

MATERIALS AND METHODS

Work Plan:



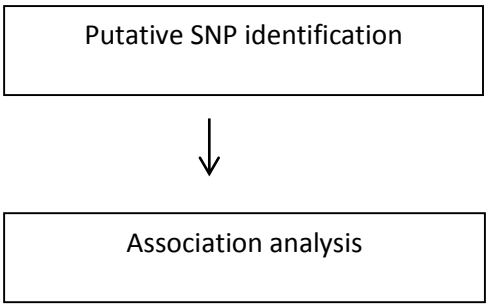


Table2. List of genotypes used in the study

S/No.	Variety Name	
1	S5 RR seed	NBPGR Shimla
2	S2 Sugandh seed	NBPGR Shimla
3	S3 Basmati seed	NBPGR Shimla
4	S4 Duplicate Basmati seed	NBPGR Shimla
5	Navrinut Lal	NBPGR Shimla
6	Navrinut White	NBPGR Shimla
7	Lal Dhan	NBPGR Shimla
8	Kard Dhan	NBPGR Shimla
9	Phul patash	NBPGR Shimla
10	Rangoli	NBPGR Shimla
11	Sukara Dhan	NBPGR Shimla

These 11 rice genotypes were procured from NBPGR Shimla. For dormancy breakdown Gibberellic acid treatment of 500ppm for 36 hours at 36 Celsius was given.

3.1 PRIMER DESIGNING

A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. They are required for DNA replication because the enzymes that catalyse this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3'- end of the primer, and copies the opposite strand. The primers were designed using the Primer 3 software.

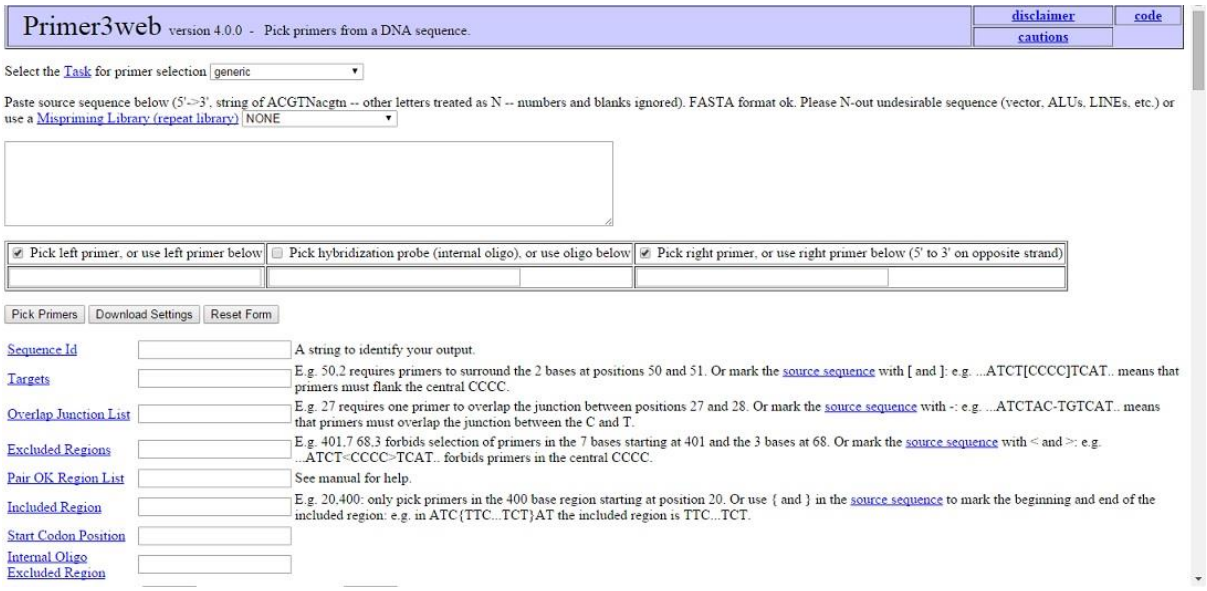


Fig5: screenshot of the Primer 3 software used for designing the primers.

3.2 DNA Isolation (CTAB METHOD)

Chemical Preparation:

1M Tris (pH 8.0)

Dissolve 12.11g of Tris base in 80ml of distilled water. Adjust pH to 8.0 by adding concentrated HCL or NaOH pellets. Allow the solution to cool to room temperature before making the final adjustments to the pH. Adjust the volume to 100ml with adding distilled water. Sterilize using an autoclave.

0.5 M EDTA (pH 8.0)

Dissolve 29.224 gm of EDTA in 180 ml of distilled water. Adjust pH to 8.0 by adding NaOH pellets. Allow the solution to cool to room temperature before making the final adjustments to the pH. Adjust the volume to 200ml with adding distilled water. Sterilize using an autoclave.

5M NaCl

Dissolve 58.44gm of NaCl in 180ml of distilled water. Raise the volume to 200ml and sterilize using an autoclave.

TE buffer

Dissolve 2ml of EDTA in 10ml of Tris Buffer. Sterilize using an autoclave.

50x TAE Buffer (pH 8.0)

Dissolve 121gm of Tris Cl in 450ml of distilled water. Adjust pH 8.0.

Add 50ml of 0.5 EDTA (pH 8.0)

28.55ml of glacial acetic acid

0.8 % Agarose Gel

0.8gm of Agarose dissolved in 100ml TAE.

Table3. CTAB Buffer (preparation)

Buffer component	Working Conc.	Stock	Volume
Tris Cl	100 mM	1M	10 ml
Nacl	1.4 M	5M	28 ml
EDTA	20mM	0.5M	4 ml
CTAB	2%		2g
PVP dH20	1%		1g Raise volume to 100ml

After autoclaving the buffer, store it in a cool, dry place. Add 500 µl of Beta mercaptoethanol freshly whenever needed to use.

Protocol

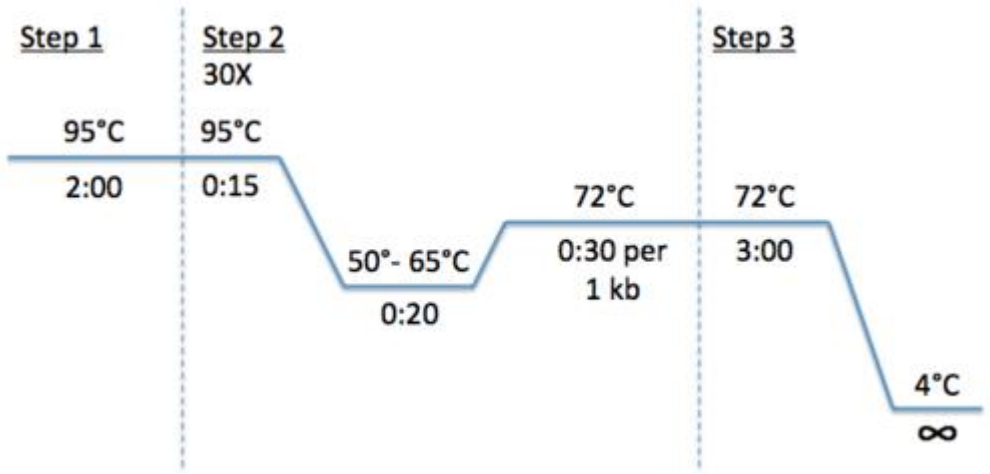
i. Grind 200mg of plant tissue to a fine powder in liquid nitrogen.
ii. Take the sample in microfuge tubes. (2ml)
iii. Add 500µl prewarmed cTAB buffer/ extraction buffer in microfuge tubes.
iv. Incubate the cTAB/ plant extract mixture for about 1-2 hours at 60 Celsius in a recirculating water bath.
v. After incubation add 0.6 volume of Chloroform: Isoamyl Alcohol (24:1) and mix the solution by inversion.
vi. Spin/centrifuge the CTAB/plant extract at 15000 rpm for 10 mins.
vii. Transfer the supernatant to clean microfuge tubes (1.5ml)
viii. Add 0.6 volume of chilled isopropanol (-20 Celsius). Mix well.
ix. Incubate it at -20 Celsius for 1-2 hours.
x. Centrifuge @ 15000 rpm for 10 mins.
xi. Discard the supernatant.
xii. Wash the pellet with 200µl of 70% ethanol (chilled).
xiii. Centrifuge at 15000 rpm for 10 minutes.
xiv. Discard the supernatant.
xv. Air dry the pellet. There should not be any smell of ethanol left.
xvi. Dissolve the pellet in 40-50 µl of autoclaved distilled water or TE buffer.
xvii. Keep the tubes at 4 Celsius overnight.

DNA quantification: Agarose Gel Electrophoresis

i. Prepare 0.8% solution of Agarose by melting 0.8g of Agarose in 100ml of 1X TAE Buffer in a microwave for 1 minute.
ii. Allow to cool for a couple of minutes then add 5µl ethidium bromide, stir to mix.
iii. Cast a gel using a supplied tray and comb. Allow the gel to set for a minimum of 20 minutes at room temperature on a flat surface.
iv. After proper solidification of gel remove the comb and keep gel in gel running tank. Pour running buffer gently.
v. Load 5 µl of DNA samples with loading dye in wells.
vi. Run the gel for 30 minutes to 1 hour at 100 Volts.
vii. Visualize the gel in the Gel Doc system through UV light chemiluniscence and photograph.
viii. Confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degradation.

3.3 PCR (Polymerase Chain Reaction)

FIG6. PCR Programme Conditions



Number of cycles- 35 cycles

Annealing temperature is specific for each set of primer.

TABLE4. Reaction Ingredients

DNA Template	1µl
Taq. Polymerase	.125µl
Primer (Forward)	.5µl
Autoclaved dH2O	8.875µl
10X Buffer	1.25µl
Primer (Reverse)	0.5µl
dNTPs	.25µl
	12.5µl

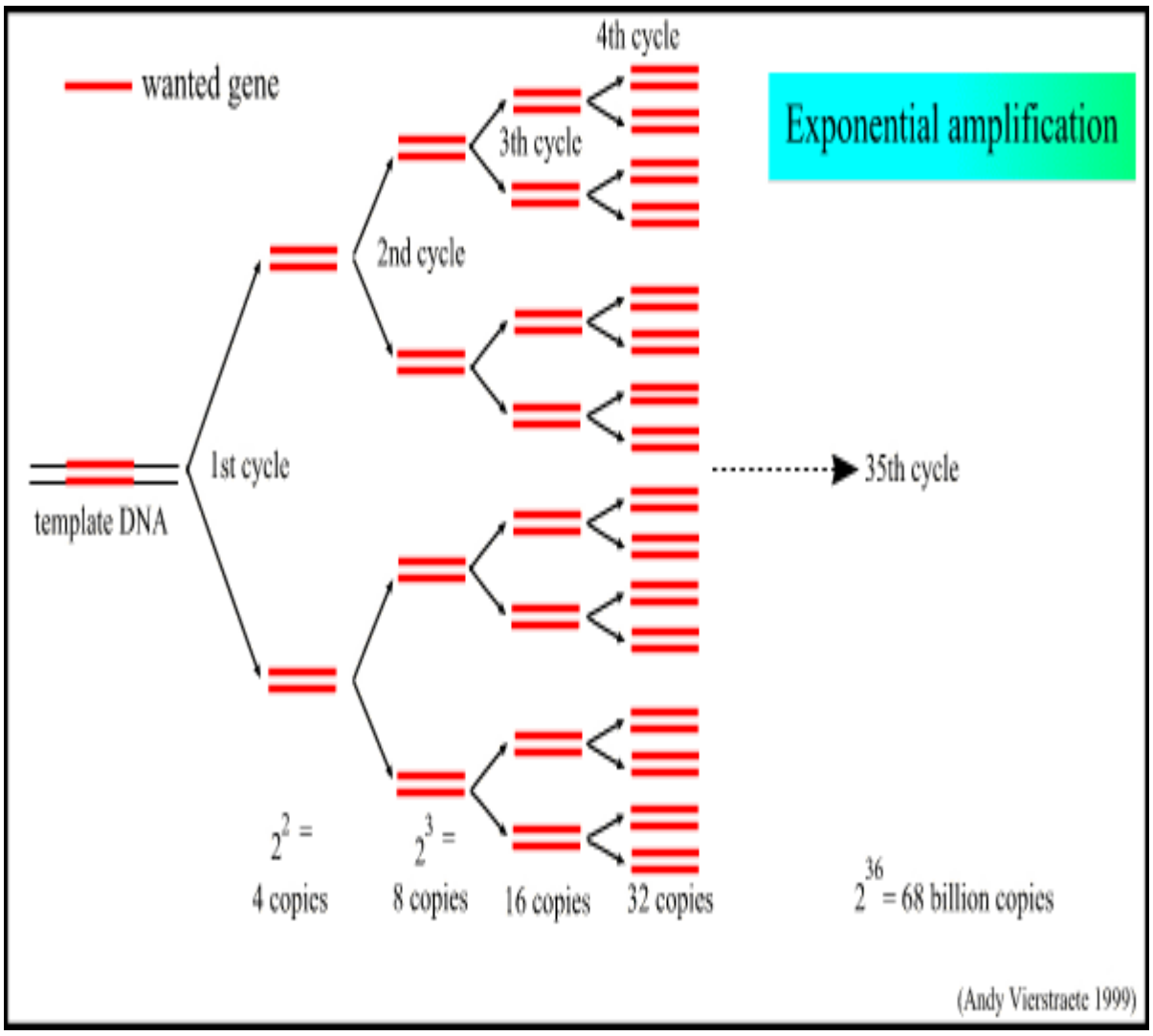


Fig7. Typical PCR amplification process of target region in the gene

3.4 Amylose estimation (Potassium Iodide Method)

The following were used for estimation of amylose content and for preparation of standard:

95% Ethanol: Prepared by adding 95ml of Ethanol and 5 ml of dH2O.

1N NaOH, Iodine: Potassium iodide solution

Standard amylose: Obtained from HIMEDIA

IN Acetic Acid: From NICE Chemicals

Glassware: Borosil

Water Bath: for incubation

Spectrophotometer: for measuring OD

Cuvette: for measuring OD of samples.

1N NaOH solution: Dissolve 40g of NaOH in 1000ml distilled water.

1N Acetic Acid solution: Dilute 57.5ml glacial acetic acid to 1000ml using distilled water.

Iodine: Potassium Iodine solution: Dissolve 0.26g of Iodine in 10ml of Potassium Iodine solution containing 2.6g of KI.

Standard Amylose Solution: Take 40mg of pure starch (amylose) in a 100ml volumetric flask and add 1ml of 95% ethanol and 9.0 ml of 1N NaOH. Shake well and boil over water bath for 10 minutes and make up the solution to 100ml using distilled water.

Protocol for Amylose Estimation

i. Weigh 100mg well powdered milled rice into 100ml volumetric flask.

ii. Add 1ml 95% ethanol and 9ml 1N NaOH.

iii. Heat the sample for 10 minutes in boiling water bath, cool it and make up the volume to 100ml.

iv. Pipette 5ml from the 100ml into another 100ml volumetric flask.

v. Add 1ml 1N acetic acid and then 2ml Iodine solution and make up the volume to 100ml.

vi. Shake, stand for 20 minutes and determine the percent Transmittance at 620nm using a colorimeter.

vii. Prepare a series of standard starch solution containing 0, 20, 40, 60, 80 and 100% amylose as in steps mentioned above.

viii. Read the transmittance of the standards at 620nm and plot a standard graph.

ix. Amylose content of the sample is determined in reference to the standard curve and expressed on percent basis.

x. Making of amylose standards:
    a. Pipette out 1, 2, 3, 4, and 5ml of the standard amylose into 100ml volumetric flasks in three replicates.
    b. Keep one flask as blank without adding anything.
    c. Add 1 ml 1N acetic acid and 2 ml I-KI solution to all flasks including blank.
    d. Make up all the flasks to 100ml using distilled water and cover all the flasks with a black cloth or aluminium foil to prevent direct light exposure. (I-KI disintegrates in light)
    e. Keep for 20 mins and take reading at 620nm.
    f. The standard includes blank, corresponding to 0%, 4%, 8%, 12%, 16% and 20% of amylose. Draw a standard curve using the absorbance reading.

3.5 Grain Elongation Test

The traits of elongation, volume expansion and water absorption are very important in determining the quality of cooked rice grains. Some varieties expand more in size than others upon cooking. Lengthwise expansion without increase in girth is considered a highly desirable trait in some high quality rice. Grain elongation appears to be a quantitative trait.
Amylose content showed significant positive association with elongation ratio and volume expansion. It correlates positively with volume expansion of cooked rice. Rice having high amylose content shows high volume expansion. (Nayak et al., 2003)

Protocol

i. The elongation test consists of measuring 25 whole milled kernels which are soaked in 20ml of distilled water for 30 minutes.

ii. The samples are placed in a water bath and the temperature is maintained at 98 Celsius for 10 minutes.

iii. The cooked rice is transferred to a petri dish covered with filter paper. 10 cooked whole grains are selected and measured in a photographic enlarger.

iv. The proportionate elongation is the ratio of the average length of cooked rice grains to the average length of raw rice grains.

CHAPTER 4

RESULTS

4.1 Seed germination

Seeds were given GA treatment for 36 hours and then maintained for 30 days in moist conditions invitro and optimum temperature for germination.

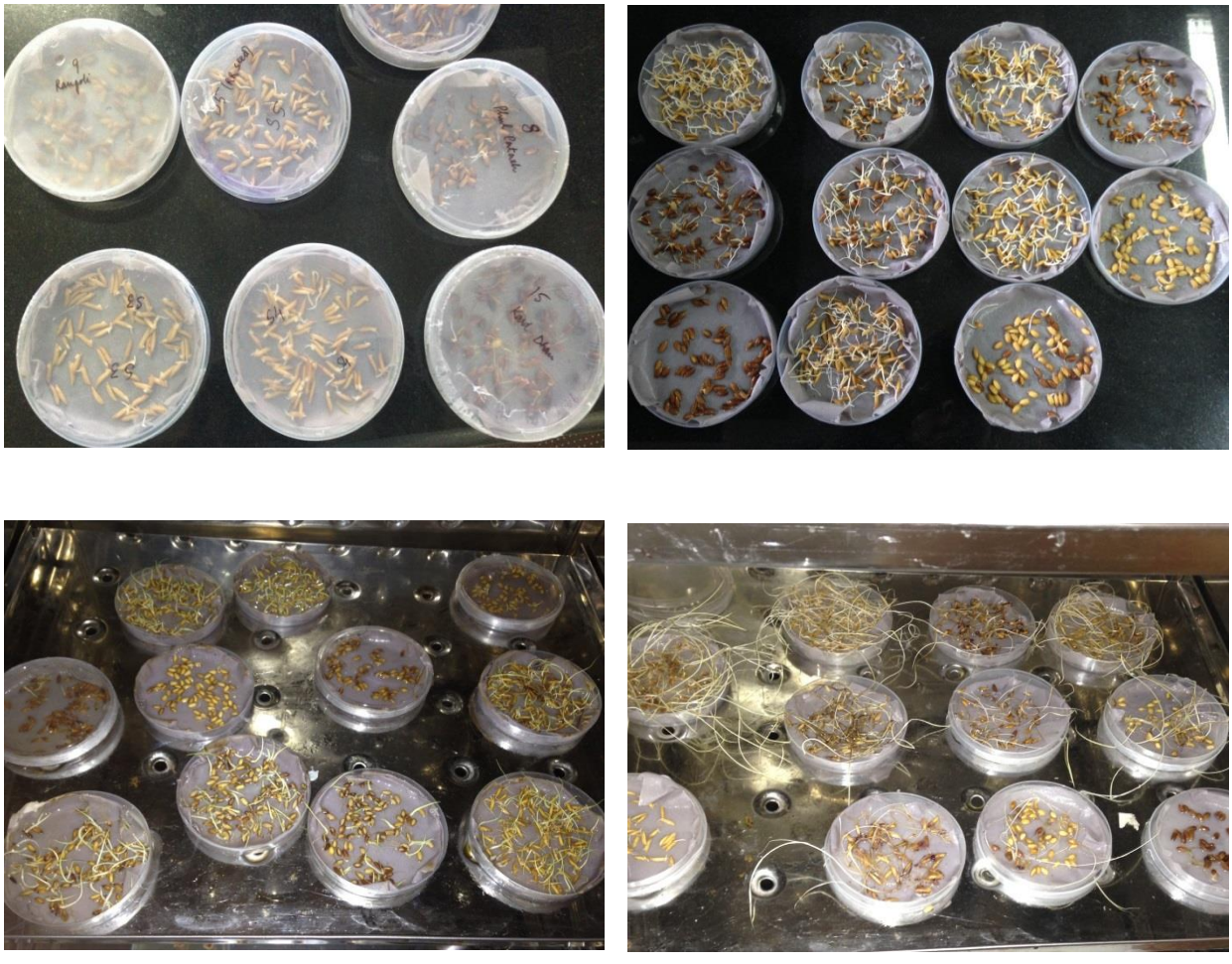


Fig 8-11. Seedling of day 1, day 5, day 10 and day 18

4.2 Primer 3 Results

Primers for gene sequence Starch Branching Enzyme1 and Starch Branching Enzyme 2 were designed using Software Primer 3 and providing other specifications.

TABLE5. List of Primers for SBE1 gene with GC content (%) and Annealing temperature (Tm)

Primer List	Sequence of primers	GC%	Tm (Celsius)	LENGTH (bp)
SBE1_1F	GACGTGAGAGTAAGCCCTGT	55.00	59.11	20
SBE1_1R	GTGCAGCAGTGGAGTGAAAA	50	58.98	20
SBE1_2F	TTTTCACTCCACTGCTGCAC	50	58.98	20
SBE1_2R	ACCCTAAAACCATCCTCGCT	50	58.71	20
SBE1_3F	AGCGAGGATGGTTTTAGGGT	50	58.71	20
SBE1_3R	GTGAAATCCTACCGGCAAGT	50	57.89	20
SBE1_4F	GTGTCTTGAATACTTGCCGGT	47.62	58.58	21
SBE1_4R	GACCTCCTCCACAACAGTCA	55	58.95	20
SBE1_5F	TACCTCGACCAGAAATGCCT	50	58.43	20
SBE1_5R	GGAGGATCCCAGTGTACACC	60	59.17	20
SBE1_6F	TGGTGTACACTGGGATCCTC	55	58.43	20
SBE1_6R	CTCCACCTTCATCAACTGGC	55	58.54	20
SBE1_7F	ACAAAGAGGACCGCAAATGG	50	58.75	20
SBE1_7R	TGCCTCACTTTACCCACACA	50	59.16	20
SBE1_8F	TGTGTGGGTAAAGTGAGGCA	50	59.16	20
SBE1_8R	TTCCTCCTCGAGTGCATTCA	50	58.73	20
SBE1_9F	TAGTATGGGCCCTTGTTGCT	50	58.70	20
SBE1_9R	TCTTGCCACAATCGTCCTTC	50	58.19	20
SBE1_10F	AAGCAACAAGTGGGGAGACT	50	59.15	20
SBE1_10R	GGACCAGTTCTTTGATGGGC	55	58.82	20

TABLE6. List of Primers with GC% and annealing Temperature (Tm)

Primer List	Sequences of Primers	GC%	Tm (Celsius)	LENGTH (bp)
SBE3_1F	GTCACCGCTACGTAGGATGA	55	58.98	20
SBE3_1R	CATCATCCTTTCTCGCGGTG	55	59.07	20
SBE3_2F	CACCGCGAGAAAGGATGATG	55	59.07	20
SBE3_2R	GCTTCCACCTAAACCCTCCT	55	59.01	20
SBE3_3F	AGGAGGGTTTAGGTGGAAGC	55	59.01	20
SBE3_3R	ACGTCACTTGCTCCAGATGA	55	59.03	20
SBE3_4F	CATTGTTGTAGGTGCCAGCC	55	59.47	20
SBE3_4R	GACTTCATCTATGCTCCCTCCA	50	59.03	22
SBE3_5F	CGCGGTTATGAGAAGTTTGGA	47.62	58.66	21
SBE3_5R	CACTACAGTAGCATCAAAACCGA	43.48	58.76	23
SBE3_6F	GGGGTGGGGTTCTCAACTTA	55	58.93	20
SBE3_6R	CCGTGCTACTCATTCCAACA	50	57.91	20
SBE3_7F	TGTTGGAATGAGTAGCACGG	50	57.91	20
SBE3_7R	AAATCTGCATTGCTCACCCA	45	58.07	20
SBE3_8F	TGGGTGAGCAATGCAGATT	45	58.07	20
SBE3_8R	AGATAAACTCGAATGCTAGCCA	40.91	57.33	22
SBE3_9F	TGGCTAGCATTCGAGTTTATCT	40.91	57.33	22
SBE3_9R	CGATGGTTATGGCCTCAGGA	55	59.24	20
SBE3_10F	TCCTGAGGCCATAACCATCG	55	59.24	20
SBE3_10R	ACCGGGTTTTCAATGGTCAA	45	57.93	20

4.3 DNA ISOLATION RESULTS

Fig12. DNA bands of sample s2, s5 and 9 on 0.8% Agarose gel

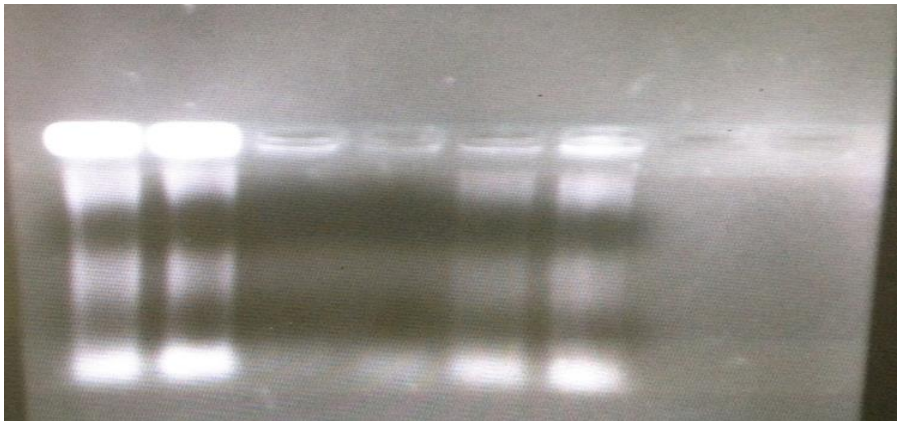


Fig13. DNA bands of samples s3, 15 and s4 on 0.8 % Agarose gel

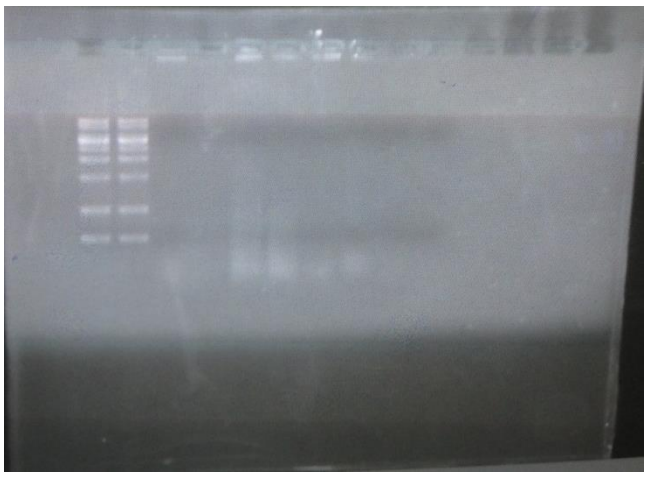


Fig14. DNA band of sample 8, s2 and 9 on 0.8% Agarose gel

DNA ISOLATION FIGURE:

DNA Bands obtained by running gel containing samples S2, S5 and 9.

DNA Bands obtained by running gel containing samples S3, S4 and 15.

DNA Bands obtained by running gel containing sample 1.

Table7. DNA quantification results (NANODROP)

Sample (name)	**Concentration (ng/µl)**
Blank (1)	7.52
Blank (2)	7.52
S3 (1)- Basmati seed	415
S3 (2)- Basmati seed	512
S4 (1)- Duplicate basmati seed	222
S4 (2)- Duplicate basmati seed	347
S5 (1)- RR seed	49.1
S5 (2)- RR seed	53.9
S2 (1)- Sugandh seed	370
S2 (2)- Sugandh seed	199
15 (1)- Kard Dhan	523
15 (2)- Kard Dhan	176
9 (1)- Rangoli	191
9 (2)- Rangoli	126
8 (1)- Phul patash	298

4.4 PCR amplification results:

The primers were designed by Primer 3 tool and used for amplification

Table8. List of primers with annealing temperature (Ta)

Primer	Ta (Celsius)
SBE1-(1f/1r)	52
SBE1-(2f/2r)	48
SBE3-(1f/1r)	54
SBE3-(2f/2r)	56

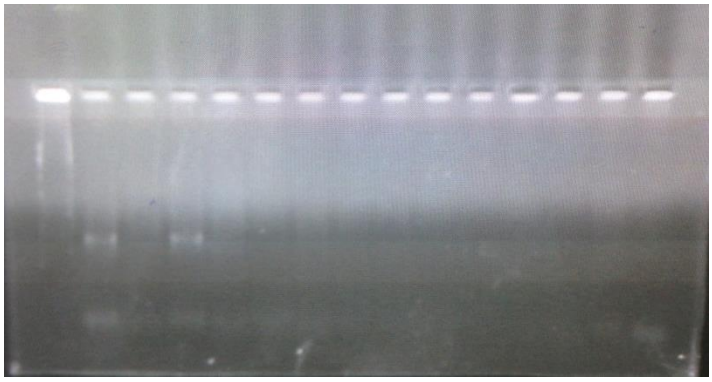


Fig15. PCR product from primers SBE1 (2f/2r), (1f/1r)

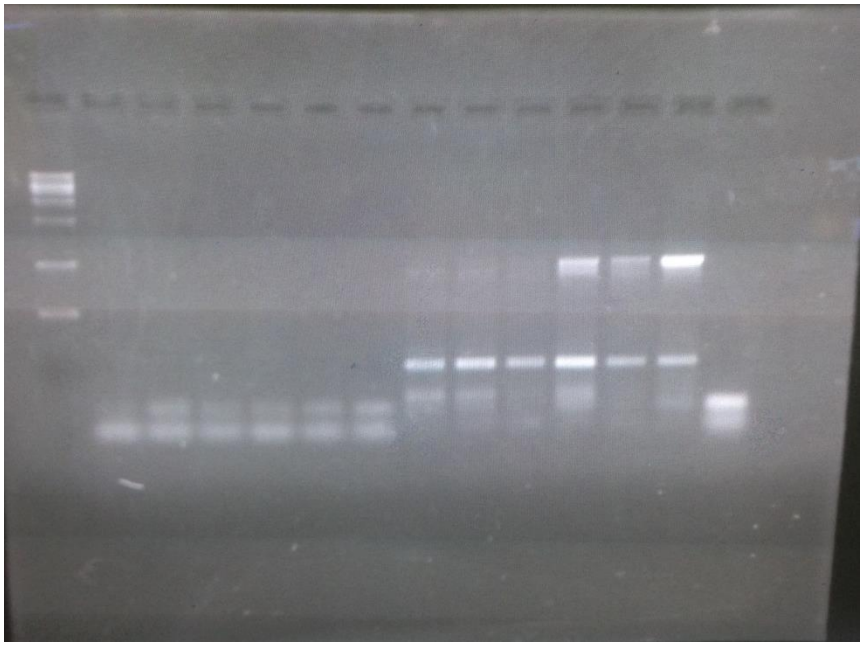


FIG16. PCR product from primers SBE3 (1f/1r), (2f/1r)

4.5 Amylose Estimation Results

Table9. Quantification of Amylose

Standard	A1 (Abs 620nm)	A2 (Abs 620nm)	A3 (Abs 620nm)	Average(Abs)
Blank	0	0	0	0
1	0.0245	0.0265	0.0293	0.02676667
2	0.05	0.046	0.0499	0.048633333
3	0.0776	0.0691	0.0693	0.072
4	0.0989	0.0989	0.0996	0.099133333
5	0.1239	0.1288	0.1176	0.123433333

The graph showing the values in the above table with abs on x axis and concentration on y axis

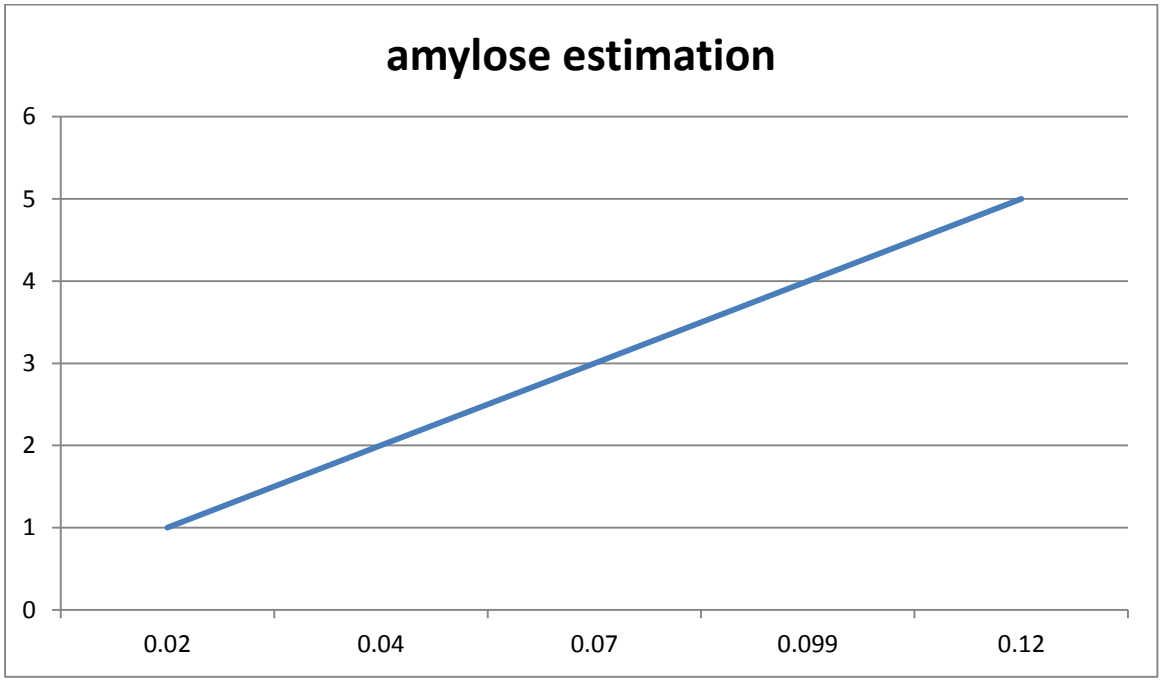


Fig17. Graph obtained by taking concentration on the y axis and absorbance at 620nm on the x axis

Table10. Amylose Quantification on 11 Indica Varieties

Sample	Absorbance (Abs 620nm)	Value of Y (mg/ml)	Charaterization
S5	0.5479	22.406505	Intermediate
S2	0.216	8.8152	Very low
S3	0.1671	6.812745	Very low
S4	0.2696	11.01012	Very low
7	0.189	7.70955	Very low
1	0.2427	9.908565	Very low
11	0.3886	15.88317	Low
15	0.3756	15.35082	Low
16	0.3384	13.82748	Low
8	0.3444	14.07318	Low
9	0.3384	13.82748	Low

The table enlists the amylose content of 20 varieties of rice. The values are obtained from extrapolating from the standard amylose curve and based on which characterization is done.

Table11. Co-relation between amylose content and Glycemic Index of rice (amylose is inversely proportional to glycemic index)

If	Then
Amylose content is high	Low Glycemic Index and the rice grains will show high volume expansion (not necessarily elongation) and a high degree of flakiness. The rice grains cook dry, are less tender, and become hard upon cooling.
Amylose content is low	High glycemic index and the rice grains will cook moist and sticky

4.6 Grain Elongation Test Results

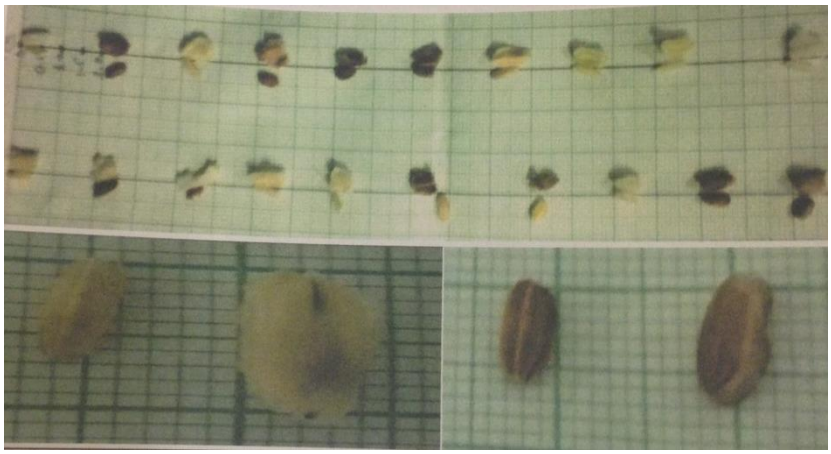


Fig18. Comparison of uncooked rice grain with cooked rice grain

Table12. Comparison of uncooked rice grain with cooked rice grain

S. No.	Variety Name	Uncooked rice Length (cm)	Cooked rice Length (cm)	Difference
1	Navri Nut	0.5	0.8	0.3
2	Kard Dhan	0.5	0.7	0.2
3	Lal Dhan	0.6	0.7	0.1
4	Navri Nut Lal	0.5	0.7	0.2
5	Phul Patash	0.6	0.7	0.1
6	Rangoli	0.6	0.8	0.2
7	Sukara Dhan	0.5	0.6	0.1
8	Sugandh Seed	0.7	1.0	0.3
9	Basmati seed	0.8	0.9	0.1
10	Duplicate basmati seed	0.7	0.8	0.1
11	RR seed	0.7	0.9	0.2

Inference: Amylose content significantly showed positive association with elongation ratio and volume expansion. It correlates positively with volume expansion of cooked rice. Rice having high amylose content shows high volume expansion.

CHAPTER 5

Conclusion

The research on rice varieties specially the Indica varieties is far below as compared to other rice varieties like Japonica varieties. As discussed earlier the research status is not beneficiary when it comes to helping the general population from the desired outcomes of the research. Researchers in various government institutes across India are trying to develop desired results with work associated like development of various markers for the analysis of functions in rice genome mostly regional land races, resistance to abiotic stress and other research institutes are trying to study the plant genetics and also the qualitative trait loci analysis for various crop plants including the rice. But globally we see that much of work is still being carried out on japonica varieties. This gap brings us the need to work on Indica varieties and develop the SNPs that are related to functional analysis of Starch branching enzyme genes. The SBE genes are mostly correlated to properties like gelatinization temperature, amylose content which in turn affects the grain elongation ability of rice on cooking.. These characteristics determine the quality of rice such as eating, cooking and processing qualities. We tried to correlate these genes with these properties by performing the DNA isolation further the PCR amplification with the gene specific primers for SBE1 and SBE3 gene respectively. It is believed that SNPs in these genes are highly inducing the characteristics properties and also the control of amylopectin chain length in the starch which is present in about 75%.Tests were performed for amylose estimation and further correlate it with glycemic index which is basically the incremental area under the blood glucose levels. The other test performed to study the physicochemical properties of rice was grain elongation test. The grain elongation test also seems to have a relation with amylose content of rice varieties and it was clearly noted that rice having higher amylose content have more volume expansion. Studying these SNPs and basically further performing physicochemical tests on rice grains of 11 varieties is of benefit to the farmers as they can cultivate the desired variety of rice among these land races once the land races which show most significant results are commercialised. The lowest amylose content was recorded in S3 variety with 6.812745mg/ml and the intermediate amylose content was recorded in S5 variety with 22.41mg/ml. This result was brought into light further in relation with glycemic index which haves an inverse relation with the amylose content. Usually the food which produces low glycemic index in our body is desirable especially for the diabetic patients who can't afford with immediate high insulin demand in the body which occurs in case of high glycemic index since diabetic patients have insulin resistance. Rice is the second most consumed crop in the world and the rice consumed in India generally has low amylose levels so it is desirable to find out the varieties which significantly have intermediate or high amylose levels. In my study the variety with intermediate amylose content is the S5 variety which is certainly more appropriate to consume than other 10 Indica varieties. The study is thus trying to lay emphasis on varieties of rice which are healthy to consume for people with diabetic problems. There are many research works going in India persuaded to study the various crop properties like maize, rice, wheat to basically enhance the agricultural industry in India and to ease out the job of a farmer for the production of more desirable crops which are good for consumption and can withstand abiotic and biotic stress in unfavourable environmental conditions. All such research work and the work including the studies on functional properties are booming the agriculture in India.

CHAPTER 6

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