MARKER ASSISTED BACKCROSS BREEDING IN RICE FOR DROUGHT TOLERANCE

By

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(2016-11-108)

THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Marker assisted backcross breeding in rice for drought tolerance"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me any degree, diploma, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"Marker assisted backcross breeding in rice for drought tolerance"** is a bonafide record of research work done independently by **Ms. Athulya S. Nair (2016-11-108)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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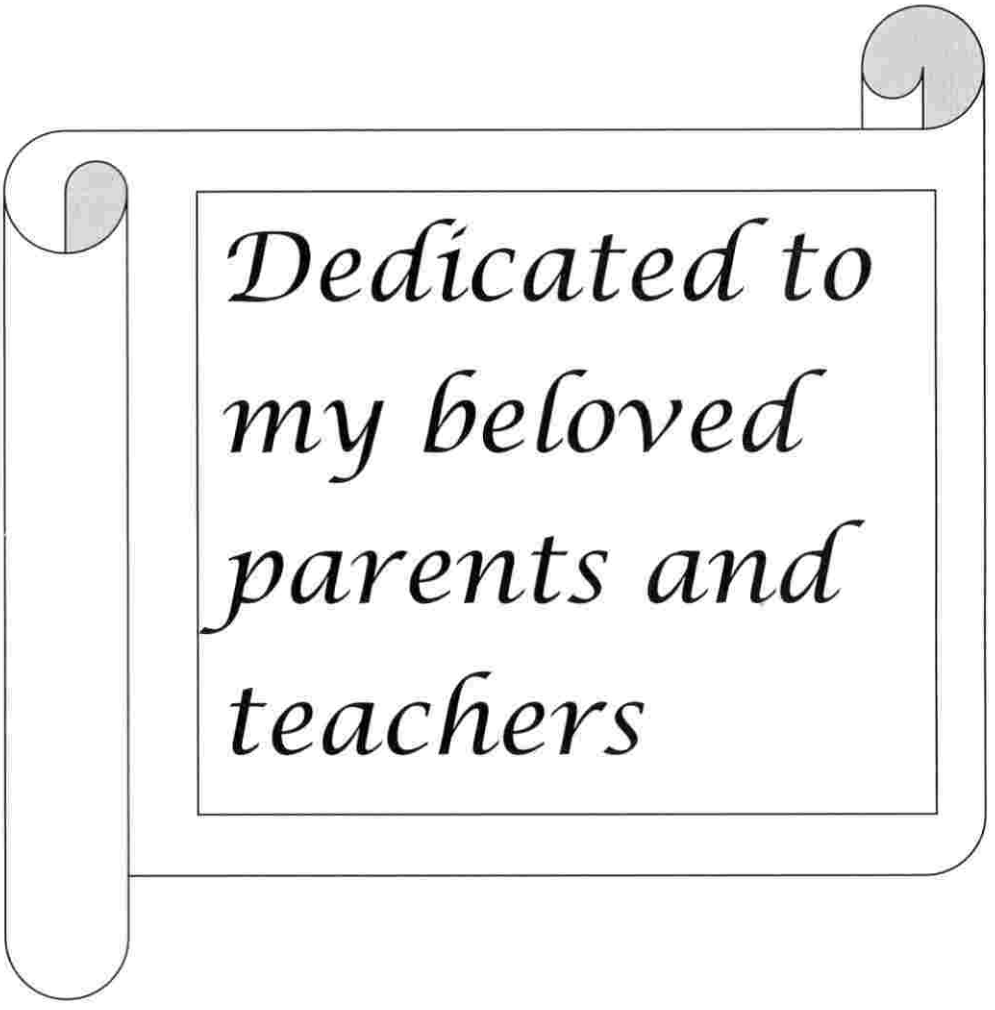
Dedicated to
my beloved
parents and
teachers

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ABBREVIATIONS

CPBMB	Centre for Plant Biotechnology and Molecular Biology
RARS	Regional Agricultural Research Station
KAU	Kerala Agricultural University
Ptb	Pattambi
CTAB	Cetyl Trimethyl Ammonium Bromide
TAE	Tris acetate EDTA
EDTA	Ethylenediaminetetraacetic acid
QTL	Quantitative Trait Loci
ABA	Abscisic acid
^0C	Degree Celsius
pH	Hydrogen ion concentrartion
cm	Centimeter
g	Gram
ng	Nanogram
ml	Millilitre
µl	Microlitre
M	Molar
min	Minute
Sec	Second
DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
PCR	Polymerase Chain Reaction
dNTP	Di- Nucleotide Triphosphate
SSR	Simple Sequence Repeats
HSP	Heat shock protein
ROS	Reactive Oxygen Species
H_2O_2	Hydrogen peroxide
Kb	Kilo base
Bp	base pair

EC	Electrical Conductivity
OD	Optical Density
UV	Ultra Violet
V	Volt
rpm	Revolution per minute
RIL	Recombinent Inbred Lines
MAS	Marker assisted selection

INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crop grown across the globe. It has shaped culture, diets and economics of millions of people across the world. Furthermore, rice cultivation is considered as the primary activity and a source of income for the households of developing countries in Asia and Africa (Nguyen, 2002). Rice production serves a major part of the national economy in India, the second largest producer of rice.

In Kerala, rice is the staple food and is an integral part in the life of its people. Although being a major food crop, the rice cultivation in the state is declining as there is a shift of cultivation to other crops (GOK, 2016). One of the reasons for this decline could be attributed to the unpredictability in climate. Palakkad, well known as the rice bowl of Kerala, was severely stricken by drought in the year 2016 (Kumar, 2016). In the present scenario of climate change and the dwindling water resources, the frequency of drought is more likely to increase in future.

The relatively less adaptability of rice to the water-limited conditions due to its semi-aquatic nature makes it vulnerable to losses from drought. Nearly 50 per cent of the world rice production is more or less affected by drought (Bouman, *et* al., 2005). Water stress adversely affects the rice production, in both upland as well as lowland ecosystems (Bimpong *et al.*, 2011a). In accordance with Srividhya *et al* (2011a), a yield reduction from 15–50 per cent can occur in rice, the yield loss being determined by the intensity of drought stress and crop growth period at which the stress occurs.

The occurrences of drought can be at any growth stage of rice plant and also can last for any time length. High sensitivity towards drought stress during seedling, vegetative, and reproductive stages are shown by modern rice varieties and a consistent yield reduction in rice can be resulted from even mild drought stress (Torres and Henry, 2016).

Seedling stage drought stress affects crop establishment and seedling survival rates. At vegetative stage, drought reduces leaf formation and tillering which in turn reduces the panicle number per plant; whereas, drought stress during reproductive stage effects the fertilization and grain filling leading to increased grain sterility, reduced number of grains per panicle and grain weight reduction (Pantuwan *et al.*, 2002a).

Traditional varieties which are adapted to the respective ecosystems were cultivated prior to the Green revolution. With the Green revolution, these traditional varieties were taken over by few high-yielding varieties that thrive best under the irrigated conditions. Even under mild stress conditions these high yielding varieties suffer from huge yield loss (Kumar *et al.*, 2008). Compared to other cereals, rice has an inherent variability in tolerance to drought as they are cultivated under diverse environments ranging from uplands to deep water ecosystems (Kumar *et al.*, 2014).

Breeding of new drought-tolerant rice cultivar helps in increasing and stabilizing the yield. Furthermore, it may also help in saving a large amount of water. The development of high yielding drought tolerant rice cultivars, with a significant yield advantage over the popular and widely adapted varieties under drought, should therefore be focused. The traditional breeding approach uses extensive phenotypic screening methods for crop improvement. Though these are effective they might delay production of climate-resilient germplasm. The speed and efficiency of plant breeding can be increased with the use of molecular breeding techniques (Whitford *et al.*, 2010).

For developing new drought-tolerant varieties, it is fundamental for breeders and molecular biologists to understand the genetic basis of drought tolerance in rice (Lang and Buu, 2008). Responses towards drought stress, at molecular level are multigenic traits. Various high-throughput molecular studies have helped in the identification of genes that get expressed under water deficit conditions (Shinozaki and Yamaguchi – Shinozaki, 2007).

Genes or QTLs controlling quantitative characters could be located by genome mapping using molecular markers (Manikavelu *et al.*, 2006). Once the QTLs are located it could be used in various breeding programmes for crop improvement. Marker-assisted backcross breeding helps in improving the targeted trait of a well-adapted, superior, elite breeding line by transferring of one or more genes or QTLs from a genetic source with the help of markers.

Jyothi (Ptb 39) is a most widely accepted high yielding rice variety released from Pattambi as well as from the state (Rosamma *et al.*, 2003). In studies conducted at Regional Agricultural Research Station, Pattambi, traditional and high yielding rice genotypes were phenotyped for drought-tolerant traits and Chuvannamodan has been identified as one of the varieties with drought tolerance (Beena *et al.*, 2018). Chuvannamodan (Ptb 30) an improved landrace, is recommended for *modan* / upland cultivation (Rosamma *et al.*, 2003). Fifteen traditional rice genotypes were phenotyped during reproductive stage for drought tolerance and further analysis of the proteome of the identified genotypes revealed that Chuvannamodan was tolerant to water stress (Babu, 2014).

With this background, the present endeavor was undertaken with an objective to improve the drought tolerance of rice variety Jyothi (Ptb 39) through marker assisted backcross breeding. The present study involves morphological characterization of Jyothi and Chuvannamodan and also the SSR polymorphism analysis between them. The current study was formulated till the production of BC_1F_1 seeds from the F_1s developed by crossing Jyothi (recurrent parent) and Chuvannamodan (donor parent).

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Rice

Rice, a semi-aquatic monocot annual grass, belonging to the family of Poaceae has a somatic chromosome number of 2n = 24. It comes under genus *Oryza* which encompass nearly 22 different species of which only two are cultivated and consumed by humans. The species *Oryza sativa* which has Asian origin is commonly cultivated all over the world. *Oryza sativa* also known as Asian rice can be categorized into three widely cultivated ecological varieties: *indica, japonica* and *javanica.* The species *Oryza glaberrima* also called as African rice is only cultivated in Africa (Muthayya *et al.*, 2014).

2.2. Varieties used for the current study

Chuvannamodan (Ptb30)

The variety was released during 1951. It was developed through mass selection from a local *kharif season* upland strain, Chuvannamodan. It is a short duration variety (105 days) having an average yield potential of 2200 kg ha^{-1}. The grain turns brownish red colour on maturity giving its panicle an attractive appearance. The kernel colour is red with a milling out-turn of 76.5 per cent. It shows satisfactory performance both under broadcasting and transplanting. Considering its drought tolerance character, it is particularly recommended for 'modan cultivation' (Rosamma *et al.,* 2003)

Fifteen traditional rice genotypes were phenotyped during reproductive stage for drought tolerance at CPBMB, Vellanikkara. In the study Chuvannamodan was found to exhibit better morphological traits as well as physiological traits like stomatal conductance, transpiration rate and membrane stability index under water deficit stress. Further, analysis of the proteome of the identified genotypes revealed that Chuvannamodan was tolerant to water stress (Babu, 2014).

In studies conducted at RARS, Pattambi, traditional and high yielding genotypes were phenotyped for drought-tolerant traits and Chuvannamodan was identified as one of the varieties with drought tolerance (Beena *et al.*, 2018).

Jyothi (Ptb39)

Jyothi was released during 1974. This is one of the most widely accepted high yielding rice variety released from Pattambi. This variety is developed from the cross between the short duration improved local strain *viz.*, Ptb 10 and the high yielding genotype *viz.*, IR8. Duration of this variety is 110 to 120 days. The variety has good coverage in Kerala as well as other states too. The average yield of the variety is 6 tons per hectare. The variety is red kernelled and photo-insensitive. The rice recovery is 72.9 per cent and the quality is very good. The variety can be cultivated by transplanting or direct sowing. Jyothi is moderately resistant to BPH and blast; but susceptible to sheath blight. (Rosamma *et al.*, 2003)

2.3. Drought

Drought, also mentioned as low moisture stress, is a type of abiotic stress that poses a serious problem in the growth and productivity of crops. In meteorological terms, drought refers to a reduction in rainfall compared to normal rainfall of a given region. Drought is mainly classified into four types. Meteorological drought occurs when a region receives a 25 per cent reduction in precipitation. Prolonged meteorological drought over a time period results in hydrological drought. Agricultural drought refers to a situation where soil moisture or annual rainfall fails to support the crop growth. Physiological drought occurs in a situation where physiological functions of plant are disrupted by water deficit (Rao, 2015).

Due to climate change, the exposure of crops to drought stress is likely to be increased in future. All the stages of the crop are prone to water stress. The impact of stress on the productivity of crop depends on its recurrence (over years),

the time of occurrence within the season, the rate of its outset, duration, intensity and some other minor factors (Shashidhar *et al.*, 2013).

Approximately 34 per cent of rice is cultivated in rainfed lowland, 9 per cent in rainfed upland, and 7 per cent in flood-prone areas, while irrigated ecosystem covers 50 per cent of total world rice area (Sandhu and Kumar, 2017). Rice, being a water-loving plant shows severe yield loss even under mild water stress. A comparison of data on the variability of rice production and the annual rainfall was done and it was found that drought is one of the most significant causes of year-to-year fluctuation in rice production in the six countries (Fukui, 1982).

2.4. Plant responses to drought

The plants exhibit various types of responses in order to endure the drought condition. These responses that can be at morphological, physiological, biochemical and molecular levels, are mainly shown to adapt to the adverse environmental stress.

2.4.1. Morphological responses

Effect on growth

As a survival technique, normally plants react to the drought stress by minimizing or pausing their growth (Zhu, 2002). The same applies to rice plants too, where the growth and development showed a significant reduction (Manikavelu *et al.*, 2006). The reduced turgor pressure caused as a result of drought stress disrupts cell growth (Taiz and Zeiger, 2006). The cell elongation and expansion can be equally affected by the drought stress (Shao *et al.*, 2008). Thus the overall cell enlargement is also arrested (Jaleel *et al.*, 2009).

Drought can be detrimental to rice seedling germination (Swain *et al.*, 2014). Drought also severely affects the tillering by reducing the tiller number (Mostajeran and Rahimi-Eichi, 2009). Water stress also influences height of plants (Ashfaq *et al.*, 2012). There occurs a substantial reduction in biomass

production (Farooq *et al.*, 2009). Reduction in fresh and dry weight of shoots (Centritto *et al.*, 2009) and roots (Ji *et al.*, 2012) due to drought were also observed.

Effect on leaf traits

In rice plants the flag leaf plays a major role in grain filling. The flag leaf characteristics are thus recognized to have a positive correlation with the yield under drought (Biswal and Kohli, 2013). Leaf rolling considered as an acclimation response in rice helps in the maintenance of internal water status in plants (Turner *et al.*, 1986) by reducing light inception, transpiration rate and dehydration of leaf (Kadioglu and Terzi, 2007).

The genetic basis of difference in the leaf rolling among various rice genotypes and the QTLs associated with it has been reported (Salunkhe *et al.*, 2011). When the internal water deficit occurs, leaf angle is a character usually associated with plasticity in leaf rolling (Chutia and Borah, 2012). Water deficit also has an impact on leaf traits like leaf number (Farooq *et al.*, 2010), leaf area and leaf area index of plants (Kumar *et al.*, 2014).

Effect on yield attributes

An apparent reduction in rice crop yield was brought about by drought stress (Bouman *et al.*, 2005). A reduction in grain size and weight has been observed as an outcome of drought stress (Castillo *et al.*, 2006). Decrease in the seed setting rate and 1000 grain weight was also reported by Ji *et al.* (2012). An increase in spikelet sterility is mostly observed as a consequence of drought stress (Raman *et al.*, 2012). Drought is found to reduce the grain yield by reducing the filling period of rice grains (Shahryari *et al.*, 2008)

Effect on root traits

The crop function under drought is largely determined by the root system of the rice. Root length and root biomass can predict the rice yield under drought

(Feng *et al.*, 2012). Jaleel *et al.* (2008) reported an increase in root growth of *Catharanthus roseus* resulted from water stress. In maize the root growth was not considerably inhibited during water stress (Sacks *et al.*, 1997) confirming the least sensitivity of roots towards low water potentials induced growth inhibition. Generally, under limited water availability, plants show an increase in the root: shoot ratio (Wu and Cosgrove, 2000).

2.4.2. Physiological responses

Effect on photosynthesis

The water deficit condition causes premature senescence in leaves, decreases the leaf expansion, oxidizes the chloroplast lipids and damages the proteins and pigments which in turn results in the impairment of photosynthetic machinery and gas exchange parameters (Menconi *et al.*, 1995). A comparison of maize plants under drought and well watered condition reported that the stomatal conductance, intercellular CO_2, net photosynthesis, water use efficiency and transpiration rate showed a decline under water deficit condition (Anjum *et al.*, 2011). Samarah *et al.* (2009) reported that the reduction in photosynthetic activity under drought stress could be attributed to both stomatal and non-stomatal mechanisms.

Effect on Chlorophyll contents

Manivannan *et al.* (2007) observed that different sunflower varieties showed a major drop in chlorophyll a, chlorophyll b and also the total chlorophyll content under drought stress. Drought stress causes excessive swelling and appearance of lipid droplets in chloroplast, damages the chloroplast membrane and the lamellae vesiculation resulting in reduced chlorophyll content (Kaiser *et al.*, 1981).

Effect on water relations

Most of the plants show considerable reduction in the relative water content (RWC) as response to the low moisture stress. Marked reduction in RWC and water potential were exhibited by leaves when subjected to water stress

(Nayyar and Gupta, 2006). Siddique *et al.* (2000) reported that plant's encounter with drought stress led to a substantial decrease in the leaf water potential, RWC and transpiration rate, with an associated rise in leaf temperature. The interaction of severity, span of drought and the species affected determines the RWC (Yang and Miao, 2010).

Effect on stress hormones

Apart from being a growth regulator, abscisic acid (ABA) during stress conditions also functions as stress hormone mediating plant's response by modifying various signal transduction pathways. Wang *et al.* (2007) studied the dynamic accumulation of ABA in response to drought stress in rice. ABA confers drought tolerance in the plant by inducing an increase in antioxidant enzymes (Latif, 2014) and improving expression of resistance proteins, its transport and carbon metabolism (Zhou *et al.*, 2014).

2.4.3. Biochemical responses

Osmolyte accumulation

Under drought, the maintenance of leaf turgor could be achieved by improving water uptake by the mechanism of osmotic adjustment which is done by to accumulation of solutes like proline, soluble carbohydrates and glycine betaine in the cytoplasm. The process of solute accumulation under drought stress strongly relies on the rate of plant water stress. The accumulation and mobilization of proline was observed in wheat to enhance its tolerance to drought condition (Nayyar and Walia, 2003).

Proline accumulation

Kemble and MacPherson (1954) first reported the accumulation of free proline during water stress in rye grasses. Accumulation of proline as an osmolyte contributes to drought tolerance and better performance of plants (Vajrabhaya *et al.*, 2001). Proline can also play the role of an antioxidative defence molecule, metal chelate, and signalling molecules (Hayat *et al.*, 2012). Considerable change

in proline concentration was observed when rice was exposed to drought stress (Mostajeran and Rahimi-Eichi, 2009).

Polyamine accumulation

Small positively charged Polyamine (PAs) molecules are also involved in the drought stress response (Calzadilla *et al.*, 2014). The suggested roles of polyamines include enhancing the DNA binding activity of transcription factors thus regulating gene expression (Panagiotidis *et al.*, 1995), maintaining ionic balance, preventing senescence, membrane stabilization, radical scavenging, (Bouchereau *et al.*, 1999). They are also believed to be involved in phosphorylation of proteins and the conformational transition in DNA (Martin-Tanguy, 2001).

When rice is exposed to drought stress, accumulation of polyamines occurs as an immediate response (Basu *et al.*, 2010). Study by Yang *et al.* (2007) suggested capacity of rice to enhance polyamines synthesis in leaves, mainly spermidine (Spd) and spermine (Spm) in the free form and putrescine (Put) in the insoluble-conjugated form, as early response to drought stress. Exogenous application of polyamines has improved net photosynthesis, water use efficiency, leaf water status. It was also found alleviate the oxidative damage on cellular membranes and produce of free proline, anthocyanins and soluble phenolics (Farooq *et al.*, 2009).

Antioxidants

Drought causes an imbalance in the generation and quenching of reactive oxygen species (ROS) like superoxide radical, hydrogen peroxide hydroxyl free radical, and singlet oxygen (Smirnoff, 1998). A complex antioxidant system involving non-enzymatic as well as enzymatic antioxidants protects plant cells from the detrimental effects of ROS. Ascorbate (AsA) and glutathione (GSH) comes under non-enzymatic antioxidants while the enzymatic antioxidants consist

of catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione cycle, monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) (Noctor and Foyer, 1998).

The expressions of antioxidants are crucial in the ROS scavenging system thus improving the drought tolerance in rice plants (Wang *et al.*, 2005). The activities of AsA, GSH, APX is reported to be increased during drought stress in rice (Selote and Khanna-Chopra, 2004). It was also found that the levels DHAR, GR, SOD, MDHAR (Sharma and Dubey, 2005), CAT and phenylalanine ammonia-lyase (Shehab *et al.*, 2010) were also increased. Li *et al.* (2011) proposed that antioxidant enzymes response in rice seedlings is altered by mild drought preconditioning, thus helping them to survive in the drought stress environment.

2.4.4. Effects of drought at the molecular level

The response towards the abiotic stresses like drought is shown by inducing several regulatory and functional genes in plants. The stress-inducible gene products are mainly of two groups (Harb *et al.*, 2010). The first group involves the enzymes required for the biosynthesis of various osmoprotectants, late embryogenesis abundant proteins, anti-freeze proteins, chaperones and detoxification enzymes that preserve the cells by protecting from desiccation. The second group involves transcriptional factors and protein kinases that regulate the gene expressions and signal transduction in stress response (Seki *et al.*, 2003). Over expression of transcriptional factors like AREB1 and DREB/CBF are reported to improve tolerance of rice to drought stress (Oh *et al.*, 2005).

2.5. Drought resistance in rice

Shallow root system, reduced photosynthetic rate and high leaf senescence associated with the rapid closure of stomata even under mild water stress makes rice a highly susceptible crop towards drought (Hirasawa, 1999). As other crops, rice resists drought stress using three different mechanisms: drought escape,

drought avoidance and drought tolerance. Drought escape strategy involves adjusting the plant life cycle, which facilitates the completion of the highly vulnerable developmental stages before serious soil water deficit occurs (Pantuwan *et al.,* 2002b). Drought avoidance mechanism allows plants in maintaining relatively higher tissue water potential in spite of a shortage of soil moisture which is achieved by either reducing the water loss or by maintaining the supply of water. Drought tolerance mechanisms are considered adaptative and are induced by drought stress, allowing plants to survive under low tissue water content (Haque *et al.,* 1992). When the timing of drought is unpredictable rice varieties exhibiting drought avoidance and tolerance mechanisms are required (Pantuwan *et al.,* 2002b). Choice of early maturing varieties that shows drought escape may be a wise choice when stress is terminal and predictable.

Drought escape using short duration varieties

In spite of limited water availability in drought-prone areas of southern US, the drought escape strategy allows rice to produce grains (Kumar *et al.*, 2008). Short-duration varieties from the Aus germplasm, which attains maturity in as little as 80 days, are being used for the upland cultivation in drought-prone upland areas of Bangladesh and eastern India. Though these varieties can escape from the terminal drought they cannot be drought-resistant (Pantuwan *et al.,* 2002b).

Drought avoidance through deeper root distribution

The upland rice varieties possessing deeper root system were able to access more water present in the deep soil layers, thus allowing it to maintain a better yield potential in drought condition (Mambani *et al.*, 1983). A higher deep-root weight to shoot weight ratio exhibited by upland rice complements its drought resistance (Fukai and Cooper, 1995) The crop improvement programmes for a deep root system in rice could be considered as a promising way to improve the water uptake of plants that ultimately results in better grain yield under drought stress (Mambani and Lal, 1983; Fukai and Cooper, 1995). Root

characteristics like increased root thickness and improved xylem vessel size can also result in better water absorbtion (Yambao *et al.*, 1992)

Samson *et al.* (2002) reported that rice genotypes having deep, coarse roots with a higher ability of penetration and branching and also a higher root to shoot ratio showed drought avoidance. Rice genotypes that avoid drought generally have a deep, coarse root with good ability for branching and soil penetration, higher root to shoot ratio, leaf rolling elasticity, improved cuticular resistance and early stomatal closure (Wang *et al.*, 2006).

Drought avoidance by stomatal control

Stomatal closure minimizes the impact of drought by reducing water loss. The associated reduction in CO_2 intake as a result of stomatal closure causes reduced photosynthesis (Sharkey and Seemann, 1989). Thus, this survival mechanism can cause associated yield reduction (Price, 2002). Under short frequent and relatively mild drought stress the early stomatal closure may not be desirable. Rice varieties were reported to show considerable genetic variation for stomatal sensitivity towards the leaf water status (Price *et al.*, 1997).

In C3 species, carbon isotope discrimination (CID) provides an integrated measurement of transpiration efficiency (Condon *et al.*, 1990). Selection for high CID in plants can result in improvement of yield under drought-prone environments as the stomata tends to close even at a relatively mild level of drought stress in rice (Bernier *et al.*, 2008).

The stomatal closure is more connected to soil water status than leaf water potential as it is mainly determined by chemical signals such as abscisic acid production in the dehydrating plant roots (Hadi *et al.*, 2016). Leaf rolling is also a drought avoidance mechanism that reduces the water loss (Turner *et al.*, 1986).

Rice genotypes that can maintain water status through ABA biosynthesis, other biochemical mechanisms or adapted root systems are able to minimize the yield losses caused by drought (Singh *et al.*, 2012).

Drought tolerance

Genetically, drought tolerance in rice is considered as a complex trait involving various morpho-physiological mechanisms including increased elasticity in the cells, maintenance of turgor pressure by osmotic adjustment, cell size reduction and dehydration tolerance by the protoplasmic resistance which is controlled by polygenes (Li and Xu, 2007). The level of drought tolerance in plants is determined by their response to tissue water potential (Mitra, 2001) and the traits linked with such phenomenas are generally considered as the secondary traits. The secondary traits such as osmotic adjustment, RWC, leaf rolling and stomatal conductivity have been used for the selection of drought tolerance (Kato *et al.*, 2006).

2.6. Breeding programmes in rice for drought resistance

The progress of breeding programs in rice for drought resistance has a slower pace. The reason for this may be due the fact that drought resistance trait is controlled by polygenes having differential effects, and unpredictability in timing and severity of drought (Bernier *et al.*, 2008). Sacrificing yield potential against higher yield during drought years is not an attractive option to farmers. Thus, plant breeders must select varieties capable of producing relatively high yields in both stress and non-stress environments (Rosielle and Hamblin, 1981).

Direct selection for yield is the most commonly used selection strategy by cereal breeders to improve yield in water-limited environments. Combining the secondary traits with the selection based on yield can improve the selective response, where the physiological processes constituting the grain yield under target environment is well known as well as when measuring of the secondary traits are feasible (Araus *et al.*, 2002)

The selection process may be undertaken for the rice lines showing ideal maturity period under well-watered conditions and least delay in flowering during drought stress. The lines maintaining a high spikelet fertility rate and/or showing a relatively low rate of leaf drying under drought stress can be selected. The traits

like delay in flowering and good spikelet fertility both are reported to possess moderate heritabilities and are found highly correlated with the grain yield under reproductive-stage drought stress. Carbon isotope discrimination (CID), an indirect but integrative measure stomatal conductance, is also suggested to be an effective criterion for selection of grain yield in drought stress (Lafitte *et al.*, 2003).

Leaf water potential (LWP) during the stress conditions is currently employed by the Thai rainfed lowland rice breeding program as a secondary trait for selection (Jongdee *et al.*, 2006). This measurement shows a strong correlation to spikelet sterility under drought stress and is less influenced by the timing of stress than spikelet sterility (Jongdee *et al.*, 2002).

As the root system is difficult to study, the root characteristics are not generally considered as secondary selection traits by breeders (O'Toole and Bland, 1987). Apart from contributing for the drought avoidance mechanisms, selecting for a better root system, could also enhance the weed competitiveness and the nutrient absorption potential of upland rice (Yadav *et al.*, 1997).

2.7. Use of molecular markers for improvement of drought resistance in rice

Quantitative trait locus (QTL) refers to a chromosomal region where one or more genes affect phenotypic values of a quantitatively inherited trait like grain yield or plant height (Thomson *et al.*, 2006). The QTLs are identified by correlation of the phenotypic values of lines with different markers at the given chromosomal loci (Salvi and Tuberosa, 2005).

The mapping studies are conducted to detect molecular marker tightly linked to a gene of interest, thus allowing the selection of the desirable genes on the basis of marker genotype rather than the plant's field phenotype (Jongdee *et al.*, 2002). The technique, namely marker-assisted selection (MAS), can be used as an effective tool to improve traits that are either controlled by a few genes or where performance of phenotypic evaluation is difficult. Considering the relative

difficulty associated with phenotyping for drought resistance, MAS in breeding for drought resistance will be greatly helpful (Bernardo, 2002)

Use of molecular markers for selection of hybrids

Yashitola *et al.* (2002) used 13 microsatellite and 5 STS markers for analysing of six hybrids and their parental lines. The analysis showed two alleles (one allele per parent) to be present in the hybrids where parental lines were detected as polymorphic. The study also suggested that a single, appropriately chosen microsatellite marker was significant enough for assessing the hybrid seed purity.

Latif *et al.* (2017), in a study to examine the performance of F_1 hybrids, used the simple sequence repeat (SSR) markers to identify true hybrids of F_1 developed from the cross of Gogo dryland rice (Situ Bagendit and Towuti) x Paddy field rice (Ciherang and Cibogo).

In a biofortification study on rice(Brar *et al.*, 2018), the F_1s obtained from the cross between high yielding genotypes and micronutrient (Iron and Zinc) rich genotypes were identified by using the microsatellite markers employing both by agarose and polyacrylamide gel electrophoresis techniques.

Microsatellite markers could be exploited for fingerprinting the hybrids, assessing variation within parental lines and testing the genetic purity of hybrid seed lot in rice. Nandakumar *et al.* (2004), employed ten sequence tagged microsatellite sites (STMS) markers were employed for fingerprinting 11 rice hybrids and their parental lines.

Molecular markers and QTLs related to drought resistance

Zhang *et al.* (2001) reported a comprehensive study of mapping the drought resistance components in a population of 154 lines of doubled-haploid

plants in rice (*Oryza sativa* L.). A genetic linkage map constituting 315 DNA markers was been constructed. Forty one quantitative trait loci (QTLs) for osmotic adjustment and root traits were determined and individually described 8–38 per cent of the plant's phenotypic variance. Rice marker RM263 was found linked to QTL for osmotic adjustment on chromosome number 2.

Quantitative trait loci (QTLs) associated with plant water desiccation indicators, phenology and yield traits under both irrigated and water deficit conditions were mapped in a population of 154 doubled-haploid (DH) lines of rice derived from the cross between CT9993-5-10-1-M/IR62266-42-6-2. A total of 47 QTLs were determined that individually justified 5 to 59 per cent of the phenotypic difference. A region containing major QTLs for grain yield, number of grains per panicle and plant height under water stress was identified on chromosome 4. Rice marker RM212 on chromosome 1 was found linked with QTL for relative water content, $rwc_{1.1}$ (Babu *et al.*, 2003).

Babu *et al.* (2003) also genetically dissected the nature of connection of root traits and capacity for osmotic adjustment with the rice production under water stress. Root traits displayed a positive correlation towards the yield and yield components under water deficit condition.

In the study by Kumar *et al.* (2005), bulked line analysis (BLA) was conducted to determine microsatellite markers linked to the drought tolerance in rice plants. Of seven polymorphic primers obtained between the bulks, two primers - RM223 and RM263 were found to co-segregate in all the individual rice accessions constituting the bulks. These SSR markers were expected to be linked to drought resistance.

Fine mapping of QTLs related to drought resistance traits was done through bulked segregant analysis and the region RM212–RM302–RM8085–RM3825 in chromosome 1, was identified to harbour major effect QTLs for drought-resistance traits across many genetic backgrounds in the rice (Salunkhe *et al.*, 2011).

A major QTL, $qDTY_{1.1}$, for grain yield under the drought stress was determined on rice chromosome 1 being flanked by markers RM11943 and RM431. This was the first reported QTL in rice with a major and consistent effect in multiple elite genetic backgrounds under both drought stress and non-stress situations. Markers RM263 linked to QTL $qDTY_{2.3}$ (Chromosome 2), RM216 linked to QTL $qDTY_{10.1}$ (Chromosome 10) and RM12091 linked to QTL $qDTY_{1.1}$ (Chromosome 1) were also reported (Vikram *et al.*, 2011).

A major drought grain yield QTL, $qDTY_{12.1}$, was identified on chromosome 12. It is the only QTL reported to have shown a large effect in multiple recipient genetic backgrounds as well as under highly varied upland and lowland rice ecosystems. $qDTY_{12.1}$, $qDTY_{2.3}$ and $qDTY_{3.2}$ are important regions for improving grain yield under drought of susceptible varieties of both lowland and upland ecosystems. RM28166 and RM22 was reported being linked to $qDTY_{12.1}$and $qDTY_{3.2}$ respectively (Mishra *et al.*, 2013)

Selective genotyping was combined with SSR genotyping to map the quantitative trait loci (QTLs) linked with the drought resistance in rice. A total of 229 BC_2F_2 lines developed from the cross between OM1490/WAB880-1-38-18-20-P1HB were assessed for root length (RL), root dry weight (RDW), and flowering (DRF) under drought condition. A microsatellite map was constructed using 232 markers to determine linkage to the target traits. The QTLs were determined for water stress tolerance giving an emphasis on 2 QTLs for root length, and 2 QTLs for root dry weight. The phenotypic differences were explained by each QTL ranging from 20.7 per cent to 30.8 per cent for dry root weight (DRW) and from 6.2 to 3.4 per cent for morphological characters related to drought at flowering. Single marker analysis identified rice markers RM 237, RM204, RM38, RM50 to be linked to grain yield under drought stress (Lang *et al.*, 2013).

Bimpong *et al.* (2011b) evaluated 513 BC_2F_3 progenies of alien introgression lines (AILs), developed from the crosses of *Oryza sativa* (IR64) ×

O. glaberrima. Thirty three of the AILs showed higher yields, thus demonstrating their scope of being used as genetic material for the transfer drought-related traits from *O. glaberrima* to *O. sativa*. The analysis identified thirty three quantitative trait loci (QTLs; including ten novel) for different traits. A QTL at RM208 on chromosome 2 had a positive affect for yield under stress, accounting for about 22 per cent of the genetic variation. Based on the single-point analysis the marker RM208 was reported to be linked to QTLs bm2.2 (biomass), hi2 (harvest index), ph2 (plant height), tn2.1 (tiller number), ps2.2 (panicle fertility), dth2.2 (days to heading), ypp2 (yield per plant).

In the drought mapping population of CR143-2-2/ Krishnahamsa, association study between phenotype and genotype was carried out to detect QTLs, using single marker analysis (SMA). The phenotypic trait, days to 50 per cent flowering (DFF) at reproductive stage stress exhibited an association with chromosome 1 and 6. A new QTL, $qDFF_{1.1}$ controlling flowering under drought stress was reported and RM3825 marker in chromosome 1 was detected to be linked with it. RM527 on chromosome 6 was reported to be linked to QTL that controls flowering under terminal drought stress. DNA marker RM3 on Chromosome 6, was reported to be associated with QTL, $qDTY_{6.2}$, for yield under drought stress (Barik *et al.*, 2018).

Physiological and molecular characterization of rice for drought tolerance reported a significant positive correlation between plot yield and physiological traits like stomatal conductance, photosynthetic rate, RWC and transpiration rate under severe water deficit condition. Single marker analysis identified 15 markers linked to at least one of the trait investigated. Markers RM17 and RM470 were found to be linked with photosynthetic rate, stomatal conductance, transpiration rate and RWC. RM20A was reported to be linked to transpiration rate, photosynthetic rate and RWC. RM264 was observed to be associated with photosynthetic rate and transpiration and RM315 was found linked to harvest index and plot yield (Ramchander *et al.*, 2016)

A mapping population consisting of 140 RILs, derived from the cross between IR64 and landrace INRC10192, were used in the evaluation of response of rice seedlings towards the water deficit condition induced by PEG and to map the associated genetic regions with seedling traits under stress. It was reported that two QTLs on chromosome 2 at the interval RM106-RM5897 were associated with the shoot dry weight under stress condition. The marker interval RM493- RM302, on chromosome1, was reported to harbor QTLs for root to shoot ratio under drought stress. The intervals RM493-RM302 and RM106-RM5897 were found to be water supply-specific regions having effects only during the stress conditions, suggesting the water deficit promoted the expression of QTLs located in these regions. The QTL qrs1.2 a stable QTL across control and stress treatments was found to be located between RM493- RM302 and the QTL qrs2.1 was found to be located between interval RM106- RM262 (Srividhya *et al.*, 2011b).

An introgressed population derived from inter-varietal backcross inbred lines (BILs) of Swarna x WAB 450 was screened for physiological and the yield component traits to determine QTL for drought tolerance. Ten QTLs for physiological, productivity and related traits were detected under water stress whereas 5 QTLs were detected under non stress condition. RM253 was found to be linked to qRWC 6-1, for relative water content under drought stress (Sangodele *et al.*, 2014). RM212 was reported to be associated with MQTL1.2 in chromosome 1(Swamy *et al.*, 2011).

RILs developed from the cross between japonica upland rice 'IRAT109' and the paddy rice 'Yuefu' were used for mapping QTLs of developmental root traits. Eighty four additive-effect QTLs and eighty six pairs of epistatic QTLs were identified for six root traits at five stages. The interval between RM525-RM263 was reported to be linked with QTLs brt2, rn2d, rdw2c, rfw2c associated with basal root thickness, root dry weight, root number and root fresh weight respectively (Qu *et al.*, 2008).

Marker RM242 in chromosome 9 was reported to be associated with maximum root length (Courtois *et al.*, 2000). Twenty three putative QTLs, 4 for root to shoot ratio, 4 for maximum root length, , 4 for deep root weight to shoot ratio, 2 for root thickness, 2 for root number, 5 for tiller number and 2 for plant height were detected in recombinant inbreed (RI) population derived from cross between Vietnamese upland rice accessions.. SSR markers RM221 RM242, RM250, RM263 and RM270 were found to be linked to QTL regions (Thanh *et al.*, 2006).

Fine-mapping studies on four QTLs for grain yield (GY) under drought, qDTY2.1, qDTY2.2, qDTY9.1 and qDTY12.1, were conducted using 4 different backcross-derived populations which were screened in 16 experiments. QTL, $qDTY_{9.1A}$, in chromosome 9 flanked by RM321 and RM566 was found to be associated with grain yield under drought in both lowland and upland conditions (Dixit *et al.*, 2012).

The chromosomal regions associated to the drought tolerance in rice were detected using SSR mapping system on F_2 mapping population derived from the cross between drought susceptible Taichung 189 (japonica type) and drought tolerant Milyang 23 (*indica* type). A total of 4 QTLs were detected, including lr4.1 (linked to RM518 on chromosome 4), lr8.1 (linked to RM72 on chromosome 8), lr12.1 (linked to RM20A on chromosome12) and lr10.1 (linked to RM228 on chromosome10). Five rice markers, RM5443 (y1.1, chromosome 1), (y4.1, chromosome 4), RM136 (y6.1, chromosome 6), RM3 (y6.2, chromosome 6) and RM537 RM3231 (y8.1, chromosome 8) were reported to be associated with yield (Lin *et al.*, 2007).

Swamy *et al.* (2017), characterized Malaysian rice genotypes for yield and yield-related traits and through structured association mapping, significant marker-trait associations were identified. RM262, RM26, RM249, RM25694, RM224, RM552, and RM28048 were found to be associated with grain yield under drought. In work done by Anupam *et al.* (2017) the marker RM518 was

used for evaluating the genotypes for QTL qDTY4.1, where the R87707-446-B-B was used as a drought resistant check.

In study Nguyen *et al.* (2013) on QTLs linked to the drought tolerance, found that the QTL for number of days to leaf rolling between the marker interval RM335–RM307 on chromosome 4 having a considerable effect on the tolerance trait. In another study by Zheng *et al.* (2006) for identifying QTLs for rice root growth under flooding and upland conditions marker RM 335 was found to be linked to root weight.

In a study on validation of SSR markers related to drought, four SSR markers viz., RM263 (qDTY2.3), RM3825 (MQTL1.1), RM212 (MQTL1.2) and RM22 (qDTY 3.2) were evaluated for their use in marker assisted selection (MAS) in BC_1F_1 plants derived from four different crosses. Marker RM263 (qDTY2.3) and RM3825 (MQTL1.1) were consistently associated with yield per plant in all cultivars and all the BC_1F_1 polpulation. Whereas, SSR marker RM212 (MQTL1.2) and RM22 (qDTY3.2) was reported to validate in two BC_1F_1 population each. Hence the markers could be utilized for MAS for improvement of drought tolerance in rice (Awasthi and Lal 2014a).

The marker RM225 was found putatively linked to a QTL on chromosome 6 for root length. Apart from this marker also showed linkage with relative growth rate (Subashri *et al.*, 2009). A similar result was obtained earlier in the study by Venuprasad *et al.* (2002), where the QTLs for root weight were mapped in this region on chromosome 6.

Marker-assisted backcross breeding for water stress

In a study conducted by Siangliw *et al.* (2007), three doubled haploid lines 1) IR68586-F2-CA-31 (DH103) 2) IR68586-F2-CA-54 (DH126) and 3) IR68586-F2-CA-143 (DH212) derived from a cross between CT9993 and IR62266 were employed as donors of the QTLs associated with drought tolerance. These doubled haploids were crossed to KDML105 which served as a recipient for QTLs of drought tolerance. The F_1s derived was again backcrossed with

KDML105. One hundred and three BC_3F_3 backcross introgression lines of KDML105 were obtained as a result of backcrossing and marker-assisted selection.

Dixit *et al.* (2017) developed and characterized high-yielding drought-tolerant NILs of Sabitri. Two QTLs ($qDTY_{3.2}$ and $qDTY_{12.1}$) identified to show large effects on grain yield under water stress, previously reported in the background of this variety in separate mapping studies, were introgressed and pyramided to develop the NILs. The marker-assisted backcross breeding combined with the phenotypic selection for the yield potential and grain type led to the development of early maturing NILs having similar yield potential with higher tolerance to drought.

A study (Shamsudin *et al.,* 2016) was conducted with an objective of enhancing the grain yield under reproductive stage water stress of the elite Malaysian rice cultivar MR219. Through marker-assisted breeding 3 major drought yield QTLs ($qDTY_{2.2}$, $qDTY_{3.1}$, and $qDTY_{12.1}$) which has a consistent effect on the grain yield under reproductive-stage water stress were introgressed. This resulted in the development of drought-tolerant MR219 lines showing a yield advantage of more than 1500 kg ha^{-1}. This is considered as the first report where qDTYs exhibited an increase in the yield under drought in genetic backgrounds apart from those in which the qDTYs were identified.

The study was undertaken to introgress root trait genes to a high yielding rice variety MRQ74 from an aerobic rice variety, AERON1, through marker-assisted backcross breeding (MABC). Two foreground markers, RM242 and RM263, related to root traits and 57 background markers were used throughout the breeding program. Eventually, six best rice lines were identified in BC_2F_1 population to carry root trait genes for drought tolerance. These lines were closely related to the recurrent parent by having a similar phenotypic appearance and the higher recovery percentage (Ab Jalil *et al.*, 2018).

Awasthi and Lal (2014b), on the basis of the presence of gene MQTL1.1, selected plants for drought tolerance from the cross between (Sarjoo- 52× Nagina-22) × Sarjoo- 52 of rice. Flanking markers RM 212- RM 3825 were identified from parental screening, to produce reproducible and polymorphic bands. These markers were used for foreground selection.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study on 'Marker assisted backcross breeding in rice for drought tolerance' was carried out at Regional Agricultural Research Station, Pattambi, Palakkad and Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Thrissur during the period 2016- 2018. The materials used and methodologies adopted for the present study are discussed in this chapter.

3.1. Materials

3.1.1. Plant material

High yielding rice variety Jyothi (Ptb 39) and traditional upland rice variety Chuvannamodan (Ptb 30) were used for the present study. The seed materials were obtained from Regional Agricultural Research Station, Pattambi. These varieties were crossed to produce the F_1 generation. The selected F_1 plants were then used for backcrossing with Jyothi.

3.1.2 Laboratory chemicals, glassware and plastic ware

The chemicals used in this study were procured from GeNei, HIMEDIA and SRL. The plastic wares from Tarsons India Ltd were used. The glasswares used were of borosil.

3.1.3 Equipment and machinery

The research work was carried out using facilities available at RARS, Pattambi and CPBMB, Vellanikkara. The autoclave used for sterilization was of EQUITRON. The waterbath of Rotek was used for the experiment. Centrifugation was done using high speed refrigerated centrifuge (REMI C-24BL). Estimation of quantity and quality of DNA was done using Eppendorf BioPhotometer plus spectrophotometer. Refrigerator (LG) was used for storing samples. The microwave oven of LG was used. Thermal cycler of Eppendorf was used for doing PCR. Horizontal gel electrophoresis system of BIO-RAD (Sub-Cell Model 192, USA) was used for agarose gel electrophoresis. Gel Doc (GELSTAN 4X

Advanced- Medicare) was used for imaging and documentation of gel. Plant samples were dried in Hotair oven (Rotek).

3.2 Methods

3.2.1 Cultural operations

The seeds were treated with *Pseudomonas fluorescens* overnight, and then sown in petriplates for germination. The germinated seeds were then planted in trays. After two weeks, the healthy seedlings were transplanted to pots or polythene bags. For hybridization purpose, the plants were raised in pots and three plants were maintained in a pot. For morphological studies, the plants were grown in polybags of 100 cm length and 60cm diameter filled with potting mixture. A total of twenty polybags each having a single plant was maintained for each variety. The potting mixture prepared by mixing soil and cow dung (2:1) was used for both pots and polybags.

3.2.2. Morphological Characterization

Plant height

The height of the plant was measured from the ground level to the tip of the plant and the mean value was expressed in centimetre.

Number of productive tillers

The number of productive tillers was recorded by counting the total number of grain-bearing tillers per plant at maturity.

Days to fifty per cent flowering

The actual number of days from sowing to ear emergence in fifty per cent of the plants was recorded.

Filled grains per panicle

Number of filled grains per panicle was recorded from randomly selected panicles of five representative plants at maturity and mean value was computed.

Per cent sterility

From each panicle, per cent sterility was calculated as below:

(No of unfilled grains per panicle/ Total no of grains per panicle)× 100

1000 grain weight

The weight of 100 grains was taken using electronic balance and the value was then multiplied by 10 to get 1000 grain weight and expressed in grams.

Grain density

The weight of 100 grains was taken using electronic balance. The volume of these 100 seeds was calculated by the water displacement method and the grain density was calculated by using the formula:

(100grain weight/volume of 100grains)

Measurement of water mining traits

a) **Root to shoot ratio**

The plant samples with intact shoot ant roots together were dried in hot air oven at 40°C for 5days. The dry weights were taken using electronic balance. After weighing the root and shoot dry weights separately the root to shoot ratio was calculated.

b) **Root volume**

The total root volume of plants were recorded by water displacement method and expressed in cc.

c) **Root dry weight**

The plant roots were dried in hot air oven. The dry weights were recorded by using electronic balance and the mean values were expressed in grams.

Plate 1. Chuvannamodan plants 55 days after sowing

Plate 2. Jyothi plants 55 days after sowing

d) Root length

Root length was measured from the crown of the root to tip of the root and expressed in centimetre.

3.2.3. Molecular analysis

Parental polymorphism study between varieties Jyothi (high yielding drought susceptible and Chuvannamodan (drought tolerant) was conducted using 120 SSR markers. The polymorphic markers was used for confirmation of hybridity of F_1 plants obtained after crossing of parents (Jyothi and Chuvannamodan)

3.2.3.1. DNA isolation

The leaves were collected from the plants in the morning and were brought to lab after wrapping in aluminum foil. The CTAB method (Murray and Thompson, 1980) was used for DNA extraction. The reagents used and the procedure are as follows.

Reagents

a) 4% CTAB buffer
b) Chloroform: isoamyl alcohol (24:1)
c) Chilled isopropanol
d) 70% ethanol
e) Sterile distilled water

Procedure for DNA isolation

a) From the leaf sample collected, 0.1g was weighed and transferred to pre-chilled mortar and pestle. It was then ground in liquid nitrogen and transferred to sterile tube (2ml).
b) Pre-warmed CTAB buffer (1ml) and 2.5µl β- mercapto ethanol was added to the above extraction tissue mix. The sample was mixed well and then incubated at 65°C in water bath for 30 minutes

c) After incubation, the sample was centrifuged at 6400rpm for 30 minutes and the supernatant was collected.
d) To this sample 1ml of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly by inversion.
e) The sample was then centrifuged at 15000 rpm for 15 minutes and the aqueous phase was collected in a sterile tube
f) To the aqueous phase, 2.5µl Rnase was added and incubated at 37°C for ½ hr after proper mixing
g) After incubation again 1ml of Chloroform: isoamyl alcohol (24:1) was added and the sample was mixed well for 15 minutes
h) The sample was then centrifuged at 10000 rpm for 2 minutes and the aqueous phase was collected
i) To this aqueous phase, 0.6^{th} volume of ice-cold isopropanol was added and mixed well the crude sample is then kept at 4°C for 2hrs for precipitation
j) The tube was then centrifuged at 15000 rpm for 20 minutes, the supernatant was discarded and the DNA pellet was washed with 70 per cent ethanol
k) After drying the pellet properly, the DNA was dissolved in100µl sterile distilled water and stored at -20°C

3.2.3.2. The quality and quantity assessment of DNA by spectrophotometer

The purity of DNA sample was assessed using Eppendorf Biophotometer plus spectrophotometer. The absorbance was recorded at 260nm and 280nm and the OD ratio was recorded to assess the purity of the sample. The DNA is considered pure if the ratio falls between 1.8 and 2.0. The concentration of DNA was indicated in ng/µl.

a) The device was switched on and the arrow key was used to select the method 'dsDNA'
b) Using the enter key, the selected method was opened
c) Then the cuvette shaft was opened by sliding the cover backward

d) The cuvette was filled with blank solution. Sterile distilled water was used for measuring blanks.
e) The filled cuvette was inserted into the cuvette shaft and the blank key was pressed
f) After taking blank reading, the cuvettes filled with sample solutions were kept in cuvette shaft and the sample key was pressed
g) The results displayed on the screen were recorded

3.2.3.3. The quality assessment of DNA by electrophoresis

The quality of isolated plant DNA was also evaluated using the Agarose Gel Electrophoresis method (Sambrook *et al.*, 1989). The reagents used for AGE are as follows

Reagents

a) TAE buffer
b) Agarose
c) Ethidium bromide
d) Gel loading dye
e) Molecular weight marker (1kb)

Procedure

Electrophoresis of DNA samples in 0.8% agarose gel was done for checking the quality of DNA samples.

a) The casting apparatus was properly sealed and the comb was placed.
b) To 100ml of 1X TAE buffer, 0.8g of agarose was added to prepare 0.8% agarose gel
c) The agarose was melted by boiling it in microwave oven and then 3µl of ethidium bromide was added to the solution
d) The solution was then mixed gently for uniform mixing of ethidium bromide.

e) The solution was then poured to the casting tray without formation of air bubbles and allowed it to solidify
f) Once the gel was solidified, the combs were removed and the gel was transferred to buffer tank of the electrophoresis unit
g) The buffer tank was filled with a sufficient amount of 1X TAE buffer to immerse the gel
h) Now 2µl of gel loading dye was mixed with 5µl of DNA sample and was then loaded into the wells
i) Apparatus was closed and about 80V power supply was given to run the gel until the dye reaches ¾ th of the gel
j) The gel was then taken from the electrophoresis unit and examined under gel documentation system.

3.2.3.4. Gel documentation system

The gel image was captured using gel documentation system. The gel pictures were observed for intactness of bands and RNA or protein contamination. The image of the gel was captured using controls in the imaging device window displayed on the computer screen.

3.2.3.5. Parental polymorphism study

The DNA samples of parents Jyothi and Chuvannamodan were genotyped using 120 SSR markers for identifying the polymorphic markers. The markers for screening were randomly selected from the *Grammene* database. The polymorphic markers obtained were used for the hybridity testing.

DNA normalization for PCR

In order to bring all DNA concentrations to a relatively equal level, normalization was done by proper dilutions. DNA was diluted to 10ng/µl concentration and the dilutions were done using sterile distilled water. For the PCR reaction 3 µl of the diluted samples were used.

Polymerase chain reaction (PCR)

The amplification was done in flat capped thin-walled PCR tubes of 0.2ml. Each PCR tube contains a total reaction mixture of 10µl.

The details of PCR reaction mix are given below in Table 1:

Table 1. Details of PCR reaction mix

Sr. No.	Components	Quantity (µL)
1.	Sterile distilled water	3.85
2.	10X Taq assay buffer	1.00
3.	dNTP mix(10Mm each)	0.50
4.	$MgCl_2$ (25mM)	0.25
5.	Taq DNA polymerase (3U)	0.40
6.	Forward primer (10µM)	0.50
7.	Reverse primer (10µM)	0.50
8.	DNA sample (10ng/µl)	3.00
Total		10.00

Amplification was carried out in PCR machine and the reaction was as follows

a) Initial denaturation at 95°C for 3 minutes
b) Denaturation at 94°C for 50 seconds
c) Primer annealing 55°C - 68°C for 50 seconds
d) Primer extension at 72°C for 1 minute
   Step b) to d) repeat 30 cycles
e) Complete primer extension at72°C for10 minutes
f) Final hold at 4°C, until removal

Separation of PCR products by gel electrophoresis

The amplified products are separated on 2 per cent agarose gel. Agarose (2g) was added to 100ml 1X TAE buffer and it was melted in the oven. Once

melted ethidium bromide was added and was poured to casting tray for solidification. When solidified the gel was transferred to gel tank of electrophoresis apparatus. PCR product (10µl) was mixed with gel loading dye (2µl) and added to the wells. A voltage of 80V was applied across the gel for 2hrs for separation of product. After electrophoresis, the banding pattern was observed in gel documentation system and the photograph was taken.

Polymorphism analysis

The banding pattern of the 120 SSR markers (selected from *Grammene* database for each variety (Jyothi and Chuvannamodan) was observed and the polymorphic markers were identified. The polymorphic markers between the varieties were then used to confirm hybridity of F_1 plants.

Table 2. SSR markers used for parental polymorphism study

Sr. No	Primer	Chromosome	Sequences
1.	RM1	1	Forward- GCGAAAACACAATGCAAAAA
			Reverse- GCGTTGGTTGGACCTGAC
2.	RM24	1	Forward- GAAGTGTGATCACTGTAACC
			Reverse- TACAGTGGACGGCGAAGTCG
3.	RM140	1	Forward- TGCCTCTTCCCTGGCTCCCCTG
			Reverse- GGCATGCCGAATGAAATGCATG
4.	RM212	1	Forward- CCACTTTCAGCTACTACCAG
			Reverse- CACCCATTTGTCTCTCATTATG
5.	RM237	1	Forward- CAAATCCCGACTGCTGTCC
			Reverse- TGGGAAGAGAGCACTACAGC
6.	RM243	1	Forward- GATCTGCAGACTGCAGTTGC
			Reverse- AGCTGCAACGATGTTGTCC
7.	RM272	1	Forward- AATTGGTAGAGAGGGGAGAG
			Reverse- ACATGCCATTAGAGTCAGGC

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
8.	RM297	1	Forward- TCTTTGGAGGCGAGCTGAG
			Reverse- CGAAGGGTACATCTGCTTAG
9.	RM302	1	Forward- TCATGTCATCTACCATCACAC
			Reverse- ATGGAGAAGATGGAATACTTGC
10.	RM315	1	Forward- GAGGTACTTCCTCCGTTTCAC
			Reverse- AGTCAGCTCACTGTGCAGTG
11.	RM319	1	Forward- ATCAAGGTACCTAGACCACCAC
			Reverse- TCCTGGTGCAGCTATGTCTG
12.	RM323	1	Forward- CAACGAGCAAATCAGGTCAG
			Reverse- GTTTTGATCCTAAGGCTGCTG
13.	RM431	1	Forward- TCCTGCGAACTGAAGAGTTG
			Reverse- AGAGCAAAACCCTGGTTCAC
14.	RM443	1	Forward- GATGGTTTTCATCGGCTACG
			Reverse- AGTCCCAGAATGTCGTTTCG
15.	RM472	1	Forward- CCATGGCCTGAGAGAGAGAG
			Reverse- AGCTAAATGGCCATACGGTG
16.	RM490	1	Forward- ATCTGCACACTGCAAACACC
			Reverse- AGCAAGCAGTGCTTTCAGAG
17.	RM493	1	Forward- TAGCTCCAACAGGATCGACC
			Reverse- GTACGTAAACGCGGAAGGTG
18.	RM1195	1	Forward- ATGGACCACAAACGACCTTC
			Reverse- CGACTCCCTTGTTCTTCTGG
19.	RM1287	1	Forward- GGAAGCATCATGCAATAGCC
			Reverse- GGCCGTAGTTTTGCTACTGC
20.	RM3412	1	Forward- AAAGCAGGTTTTCCTCCTCC
			Reverse- CCCATGTGCAATGTGTCTTC

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
21.	RM3825	1	Forward- AAAGCCCCCAAAAGCAGTAC
			Reverse- GTGAAACTCTGGGGTGTTCG
22.	RM8046	1	Forward- AGTACGATTTCTGTCAGCGTTGCTTAGT
			Reverse- GGATGAAAGTTGATGGATGATCTACTTGTT
23.	RM8094	1	Forward- AAGTTTGTACACATCGTATACA
			Reverse- CGCGACCAGTACTACTACTA
24.	RM10346	1	Forward- GCTTGATCTGCCCTTGTTTCTTGG
			Reverse- AACTCGAGCGGCCTTCTCAGC
25.	RM10702	1	Forward- ACGATAAATCAAGCGGCTACTCG
			Reverse- CTTTCAGCAGCACCTTCTCAGG
26.	RM10745	1	Forward- TGACGAATTGACACACCGAGTACG
			Reverse- ACTTCACCGTCGGCAACATGG
27.	RM10772	1	Forward- GCACACCATGCAAATCAATGC
			Reverse- CAGAAACCTCATCTCCACCTTCC
28.	RM10793	1	Forward- GACTTGCCAACTCCTTCAATTCG
			Reverse- TCGTCGAGTAGCTTCCCTCTCTACC
29.	RM12091	1	Forward- CTGCAAATGCACAGGAATCAGG
			Reverse- TCCTCTCGCCTTTCTTTCTCTCC
30.	RM10	2	Forward- TTGTCAAGAGGAGGCATCG
			Reverse- CAGAATGGGAAATGGGTCC
31.	RM106	2	Forward- CGTCTTCATCATCGTCGCCCCG
			Reverse- GGCCCATCCCGTCGTGGATCTC
32.	RM183	2	Forward- GGAGCGGGAGAGAGAGCCACG
			Reverse- TGCCGATGAAGGACTGCGACGC
33.	RM207	2	Forward- CCATTCGTGAGAAGATCTGA
			Reverse- CACCTCATCCTCGTAACGCC

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
34.	RM208	2	Forward- TCTGCAAGCCTTGTCTGATG
			Reverse- TAAGTCGATCATTGTGTGGACC
35.	RM211	2	Forward- CCGATCTCATCAACCAACTG
			Reverse- CTTCACGAGGATCTCAAAGG
36.	RM250	2	Forward- GGTTCAAACCAAGCTGATCA
			Reverse- GATGAAGGCCTTCCACGCAG
37.	RM263	2	Forward- CCCAGGCTAGCTCATGAACC
			Reverse- GCTACGTTTGAGCTACCACG
38.	RM266	2	Forward- TAGTTTAACCAAGACTCTC
			Reverse- GGTTGAACCCAAATCTGCA
39.	RM301	2	Forward- TTACTCTTTGTGTGTGTGTGAG
			Reverse- CTACGACACGTCATAGATGACC
40	RM406	2	Forward- GAGGGAGAAAGGTGGACATG
			Reverse- TGTGCTCCTTGGGAAGAAAG
41.	RM525	2	Forward- GGCCCGTCCAAGAAATATTG
			Reverse- CGGTGAGACAGAATCCTTACG
42.	RM561	2	Forward- GAGCTGTTTTGGACTACGGC
			Reverse- GAGTAGCTTTCTCCCACCCC
43.	RM16	3	Forward- CGCTAGGGCAGCATCTAAA
			Reverse- AACACAGCAGGTACGCGC
44.	RM22	3	Forward- GGTTTGGGAGCCCATAATCT
			Reverse- CTGGGCTTCTTTCACTCGTC
45.	RM157B	3	Forward- CCTCCTCCTCACGAATCCCGCC
			Reverse- GGGCTTCTTCTCCGCCGGCTTC
46.	RM282	3	Forward- CTGTGTCGAAAGGCTGCAC
			Reverse- CAGTCCTGTGTTGCAGCAAG

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
47.	RM570	3	Forward- GTTCTTCAACTCCCAGTGCG
			Reverse- TGACGATGTGGAAGAGCAAG
48.	RM19	4	Forward- CAAAAACAGAGCAGATGAC
			Reverse- CTCAAGATGGACGCCAAGA
49.	RM273	4	Forward- GAAGCCGTCGTGAAGTTACC
			Reverse- GTTTCCTACCTGATCGCGAC
50.	RM335	4	Forward- GTACACACCCACATCGAGAAG
			Reverse- GCTCTATGCGAGTATCCATGG
51.	RM401	4	Forward- TGGAACAGATAGGGTGTAAGGG
			Reverse- CCGTTCACAACACTATACAAGC
52.	RM451	4	Forward- GATCCCCTCCGTCAAACAC
			Reverse- CCCTTCTCCTTTCCTCAACC
53.	RM470	4	Forward- TCCTCATCGGCTTCTTCTTC
			Reverse- AGAACCCGTTCTACGTCACG
54.	RM471	4	Forward- ACGCACAAGCAGATGATGAG
			Reverse- GGGAGAAGACGAATGTTTGC
55.	RM518	4	Forward- CTCTTCACTCACTCACCATGG
			Reverse- ATCCATCTGGAGCAAGCAAC
56.	RM537	4	Forward- CCGTCCCTCTCTCTCCTTTC
			Reverse- ACAGGGAAACCATCCTCCTC
57.	RM5414	4	Forward- ACCATGGTTCAAGAGTGAAA
			Reverse- ACAGCTCAACCTGTTGAGTG
58.	RM5687	4	Forward- GATCGCTGGCGATTGATC
			Reverse- GACTTGTGGGGTGGTTTTTG
59.	RM39	5	Forward- GCCTCTCTCGTCTCCTTCCT
			Reverse- AATTCAAACTGCGGTGGC

Table 2. contd.

Sr. No	Primer	Chromosome	Sequences
60.	RM55	5	Forward- CCGTCGCCGTAGTAGAGAAG
			Reverse- TCCCGGTTATTTTAAGGCG
61.	RM159	5	Forward- GGGGCACTGGCAAGGGTGAAGG
			Reverse-GCTTGTGCTTCTCTCTCTCTCTCTCTC
62.	RM249	5	Forward-GGCGTAAAGGTTTTGCATGT
			Reverse-ATGATGCCATGAAGGTCAGC
63.	RM267	5	Forward- TGCAGACATAGAGAAGGAAGTG
			Reverse- AGCAACAGCACAACTTGATG
64.	RM274	5	Forward- CCTCGCTTATGAGAGCTTCG
			Reverse- CTTCTCCATCACTCCCATGG
65.	RM405	5	Forward- TCACACACTGACAGTCTGAC
			Reverse- AATGTGGCACGTGAGGTAAG
66.	RM440	5	Forward- CATGCAACAACGTCACCTTC
			Reverse- ATGGTTGGTAGGCACCAAAG
67.	RM3	6	Forward- ACACTGTAGCGGCCACTG
			Reverse- CCTCCACTGCTCCACATCTT
68.	RM30	6	Forward- GGTTAGGCATCGTCACGG
			Reverse- TCACCTCACCACACGACACG
69.	RM50	6	Forward- ACTGTACCGGTCGAAGACG
			Reverse- AAATTCCACGTCAGCCTCC
70.	RM190	6	Forward- CTTTGTCTATCTCAAGACAC
			Reverse- TTGCAGATGTTCTTCCTGATG
71.	RM204	6	Forward- GTGACTGACTTGGTCATAGGG
			Reverse- GCTAGCCATGCTCTCGTACC
72.	RM225	6	Forward- TGCCCATATGGTCTGGATG
			Reverse- GAAAGTGGATCAGGAAGGC

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
73.	RM253	6	Forward- TCCTTCAAGAGTGCAAAACC
			Reverse- GCATTGTCATGTCGAAGCC
74.	RM276	6	Forward- CTCAACGTTGACACCTCGTG
			Reverse- TCCTCCATCGAGCAGTATCA
75.	RM314	6	Forward- CTAGCAGGAACTCCTTTCAGG
			Reverse- AACATTCCACACACACACGC
76.	RM469	6	Forward- AGCTGAACAAGCCCTGAAAG
			Reverse- GACTTGGGCAGTGTGACATG
77.	RM527	6	Forward- GGCTCGATCTAGAAAATCCG
			Reverse- TTGCACAGGTTGCGATAGAG
78.	RM588	6	Forward- GTTGCTCTGCCTCACTCTTG
			Reverse- AACGAGCCAACGAAGCAG
79.	RM589	6	Forward- ATCATGGTCGGTGGCTTAAC
			Reverse- CAGGTTCCAACCAGACACTG
80.	RM3805	6	Forward- AGAGGAAGAAGCCAAGGAGG
			Reverse- CATCAACGTACCAACCATGG
81.	RM18	7	Forward- TTCCCTCTCATGAGCTCCAT
			Reverse- GAGTGCCTGGCGCTGTAC
82.	RM214	7	Forward- CTGATGATAGAAACCTCTTCTC
			Reverse- AAGAACAGCTGACTTCACAA
83.	RM234	7	Forward- TTCAGCCAAGAACAGAACAGTGG
			Reverse- CTTCTCTTCATCCTCCTCCTTGG
84.	RM320	7	Forward- CAACGTGATCGAGGATAGATC
			Reverse- GGATTTGCTTACCACAGCTC
85.	RM336	7	Forward- CTTACAGAGAAACGGCATCG
			Reverse- GCTGGTTTGTTTCAGGTTCG

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
86.	RM1209	7	Forward- AATGGAGCTCCTGACTCTAAAGC
			Reverse- TGCATCTCCTACAGAAACAAGG
87.	RM38	8	Forward- ACGAGCTCTCGATCAGCCTA
			Reverse- TCGGTCTCCATGTCCCAC
88.	RM195	8	Forward- AGAAAGAGAGGCCGTCGGCGGC
			Reverse- GGGCTCACCCCCAAACCTGCAG
89.	RM210	8	Forward- TCACATTCGGTGGCATTG
			Reverse- CGAGGATGGTTGTTCACTTG
90.	RM223	8	Forward- GAGTGAGCTTGGGCTGAAAC
			Reverse- GAAGGCAAGTCTTGGCACTG
91.	RM264	8	Forward-GTTGCGTCCTACTGCTACTTC
			Reverse- GATCCGTGTCGATGATTAGC
92.	RM544	8	Forward- TGTGAGCCTGAGCAATAACG
			Reverse- GAAGCGTGTGATATCGCATG
93.	RM108	9	Forward- TCTCTTGCGCGCACACTGGCAC
			Reverse- CGTGCACCACCACCACCACCAC
94.	RM201	9	Forward- CTCGTTTATTACCTACAGTACC
			Reverse- CTACCTCCTTTCTAGACCGATA
95.	RM205	9	Forward- CTGGTTCTGTATGGGAGCAG
			Reverse- CTGGCCCTTCACGTTTCAGTG
96.	RM215	9	Forward- CAAAATGGAGCAGCAAGAGC
			Reverse- TGAGCACCTCCTTCTCTGTAG
97.	RM219	9	Forward- CGTCGGATGATGTAAAGCCT
			Reverse- CGTCGGATGATGTAAAGCCT
98.	RM242	9	Forward- GGCCAACGTGTGTATGTCTC
			Reverse- TATATGCCAAGACGGATGGG

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
99.	RM566	9	Forward- ACCCAACTACGATCAGCTCG
			Reverse- CTCCAGGAACACGCTCTTTC
100.	RM147	10	Forward- TACGGCTTCGGCGGCTGATTCC
			Reverse- CCCCCGAATCCCATCGAAACCC
101.	RM171	10	Forward- AACGCGAGGACACGTACTTAC
			Reverse- ACGAGATACGTACGCCTTTG
102.	RM216	10	Forward- GCATGGCCGATGGTAAAG
			Reverse- TGTATAAAACCACACGGCCA
103.	RM228	10	Forward- TCTAACTCTGGCCATTAGTCCTTGG
			Reverse-AAGTAGACGAGGACGACGACAGG
104.	RM258	10	Forward- TGCTGTATGTAGCTCGCACC
			Reverse- TGGCCTTTAAAGCTGTCGC
105.	RM269	10	Forward- GAAAGCGATCGAACCAGC
			Reverse- GCAAATGCGCCTCGTGTC
106.	RM333	10	Forward- GTACGACTACGAGTGTCACCAA
			Reverse- GTCTTCGCGATCACTCGC
107.	RM6100	10	Forward- TCCTCTACCAGTACCGCACC
			Reverse- GCTGGATCACAGATCATTGC
108.	RM6132	10	Forward- CCGCCATCTCTCTTCAGTTC
			Reverse- CAGTGCATAGAGGAGAGGACG
109.	RM21	11	Forward- ACAGTATTCCGTAGGCACGG
			Reverse- GCTCCATGAGGGTGGTAGAG
110.	RM206	11	Forward- CCCATGCGTTTAACTATTCT
			Reverse- CGTTCCATCGATCCGTATGG
111.	RM209	11	Forward- ATATGAGTTGCTGTCGTGCG
			Reverse- CAACTTGCATCCTCCCCTCC

Table 2 contd.

Sr. No	Primer	Chromosome	Sequences
112.	RM224	11	Forward- ATCGATCGATCTTCACGAGG
			Reverse- TGCTATAAAAGGCATTCGGG
113.	RM229	11	Forward- CACTCACACGAACGACTGAC
			Reverse- CGCAGGTTCTTGTGAAATGT
114.	RM286	11	Forward- GGCTTCATCTTTGGCGAC
			Reverse- CCGGATTCACGAGATAAACTC
115.	RM17	12	Forward- TGCCCTGTTATTTTCTTCTCTC
			Reverse- GGTGATCCTTTCCCATTTCA
116.	RM20A	12	Forward- ATCTTGTCCCTGCAGGTCAT
			Reverse- GAAACAGAGGCACATTTCATTG
117.	RM235	12	Forward- AGAAGCTAGGGCTAACGAAC
			Reverse- TCACCTGGTCAGCCTCTTTC
118.	RM247	12	Forward- AAGGCGAACTGTCCTAGTGAAGC
			Reverse- CAGGATGTTCTTGCCAAGTTGC
119.	RM28048	12	Forward- TTCAGCCGATCCATTCAATTCC
			Reverse- GCTATTGGCCGGAAAGTAGTTAGC
120.	RM28166	12	Forward- TGCTTGCAAACATTGCTTCTGG
			Reverse- ACTGATGTACTGAACACGGGAAGG

3.2.4. Hybridization of plants

For the production of F_1 plants, staggered sowing of recurrent parent Jyothi and donor parent Chuvannamodan was done at weekly intervals from 16 April 2018 to 10 May 2018 for 5 weeks. For backcrossing the F_1 plants Jyothi was sown in a staggered manner with one-week interval from 27 July 2018 to 22 August 2018 for 5 weeks. The hybrid seeds were sown from 08 August 2018.

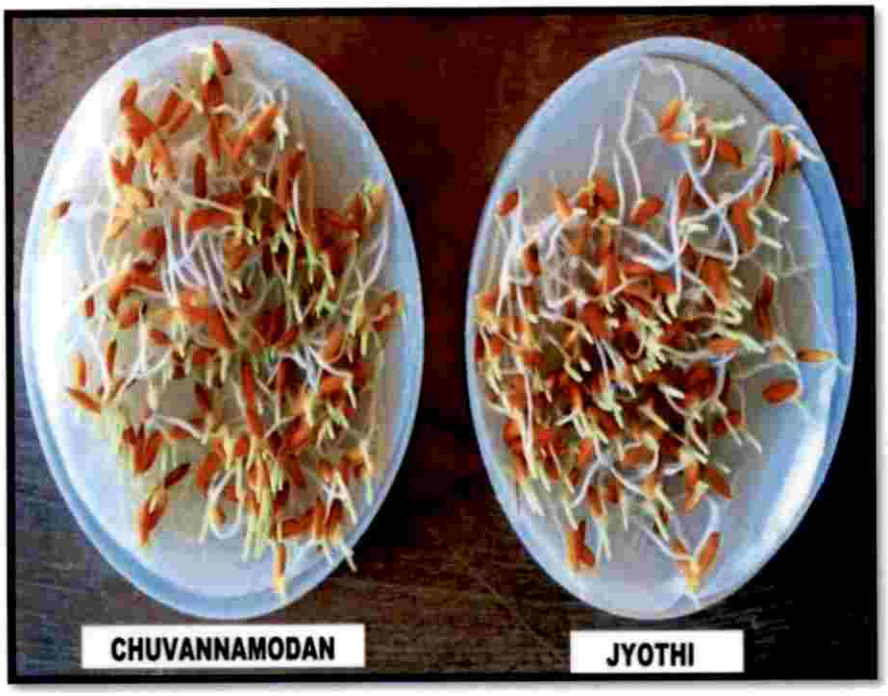


Plate 3. Seeds sown in petriplates for germination

Plate 4. Germinated seedlings transplanted to trays

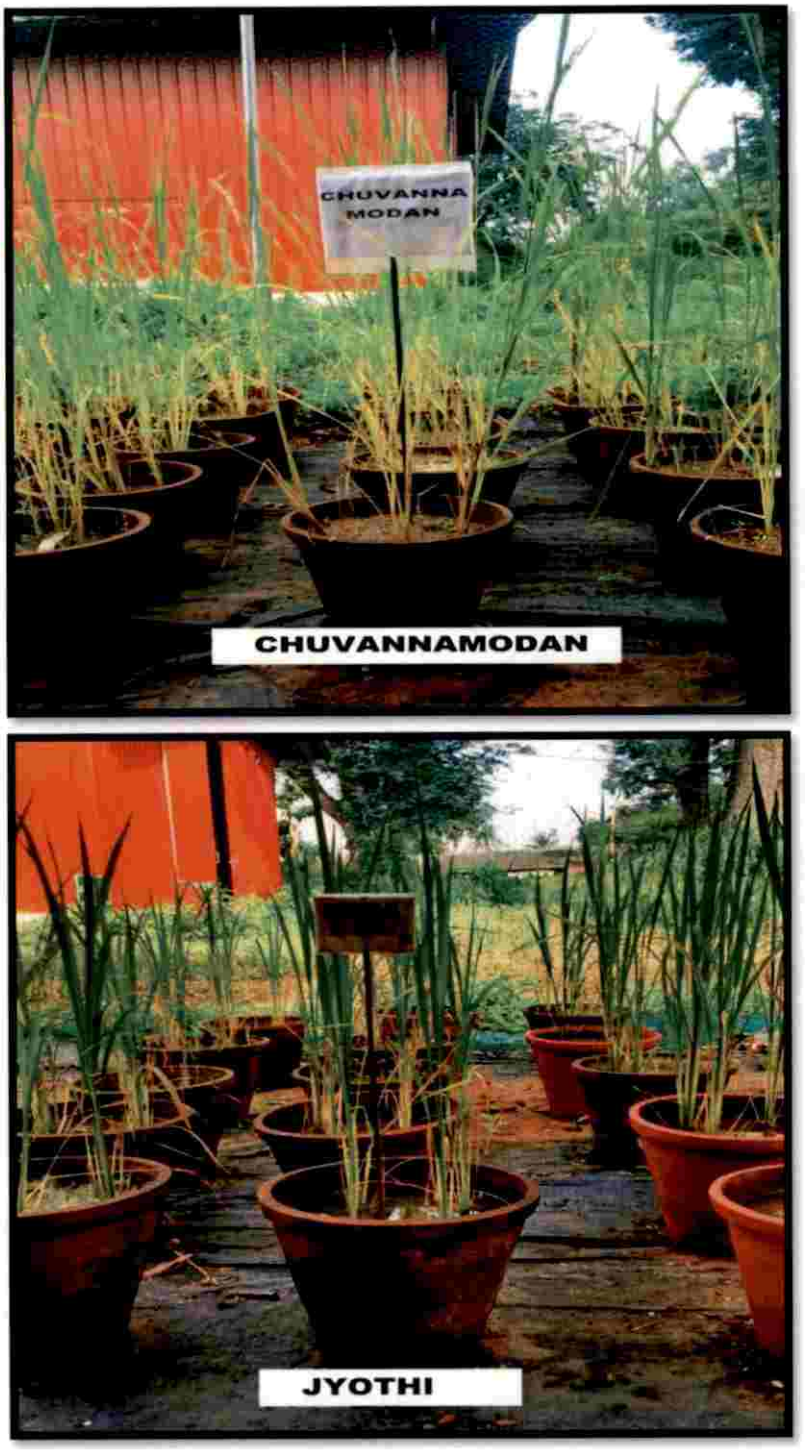


Plate 5. Plants of Chuvannamodan and Jyothi raised for hybridization

Emasculation

Clipping method was used for emasculating the panicles. For emasculation, the evening time period from 4 PM was preferred. The panicles that were half emerged from the flag leaf were selected for emasculation. The leaf sheath was opened up and the panicles were fully exposed. Immature florets located at the base of the panicle with height of anther less than half of florets were removed. Florets which are likely to open on the following day, with height of anthers equal to or more than half the florets, were selected for emasculation. Top one-third portion of each selected floret was clipped off with the help of scissors, to expose anthers. The anthers were then removed with the help of the forceps. Butter paper bags were used to cover the emasculated panicles, which was then tagged and labelled.

Pollination

Pollination was done in three subsequent days after emasculation. Pollination was done between 8.00 AM and 1.00 PM. For collecting pollen, panicles of the male parent, which were ready to dehisce were selected. Pollen grains were collected by gently tapping the full bloomed panicle in a petridish containing distilled water. The collected pollen grains were transferred to the stigma of the emasculated spikelets of the female parent with the help of a thin brush. To avoid contamination by foreign pollen, re-bagging was done after pollinating the panicles. Seed setting was checked a week after hybridization. At maturity, seeds were harvested, dried and stored.

Production of F_1s

Jyothi (Ptb 39) and Chuvannamodan (Ptb 30) were crossed to produce the F_1 seeds. For the crossing programme Jyothi (Ptb 39) was taken as the female parent.

Confirmation of F_1 hybridity

DNA isolation (Murray and Thompson, 1980) was done from all F_1 plants obtained after crossing Jyothi (Ptb 39) and Chuvannamodan (Ptb 30). Then, the PCR analysis of these F_1 progenies was carried out using polymorphic SSR markers obtained after polymorphism study. The progenies with double bands *i.e.* bands from both parents were confirmed as true hybrids.

Production of BC_1F_1 population

The true hybrid plants were further backcrossed with the recurrent parent Jyothi (Ptb 39) to produce BC_1F_1 seeds. The seeds were again sown to raise the BC_1F_1 population.

RESULTS

4. RESULTS

The study on 'Marker assisted backcross breeding in rice for drought tolerance' was carried out at Regional Agricultural Research Station, Pattambi, Palakkad and Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Thrissur during the period 2016- 2018. The results obtained are presented below.

4.1. Morphological characterization of parents

The details of the morphological characterization are presented in the Table 3

Table 3. Morphological Characterization of parents

Observations	Chuvannamodan (Ptb 30)			Jyothi (Ptb 39)		
	Max.	Min.	Mean	Max.	Min.	Mean
Plant height (cm)	154	114	141	100	80	91
No of productive tillers	43	25	33	25	12	17
Days to 50 per cent flowering	76			93		
No. of filled grains per panicle	76	64	71	134	84	104
Sterility (Percentage)	12.50	8	11.40	25	10.60	18.40
1000 grain weight (g)	27.80	26.20	27.00	29.50	27.50	28.30
Grain density (g/cm^3)	1.10	1.08	1.09	1.13	1.09	1.11
Root length (cm)	125	96	109	76	60	68
Root volume (cm^3)	120	80	96	40	25	32
Root dry weight (g)	36	28	31.6	20	9	14.4
Root to shoot ratio	0.32	0.29	0.31	0.29	0.20	0.24

4.1.1. Plant height

The plant height was measured from the ground level to the tip of the plant and the mean value was expressed in centimetre. The average height of Chuvannamodan (Ptb 30) was 141cm and that of Jyothi (Ptb 39) was 91 cm.

4.1.2. Number of productive tillers

The total number of grain-bearing tillers per plant was counted at maturity. The average number of tillers in Chuvannamodan (Ptb 30) was 33 while it was 17 in Jyothi (Ptb 39).

4.1.3. Days to fifty per cent flowering

The actual number of days from sowing to ear emergence in fifty per cent of the plants was recorded. Chuvannamodan (Ptb 30) took 76 days to reach 50 per cent flowering whereas Jyothi (Ptb 39) took 93 days to attain 50 per cent flowering.

4.1.4. Filled grains per panicle

The number of filled grains per panicle was recorded from randomly selected panicles from each of five representative plants at maturity and mean value was computed. The number of filled grains per panicle for Chuvannamodan (Ptb 30) was obtained as 71 and that in Jyothi (Ptb 39) was 104.

4.1.5. Per cent sterility

The per cent sterility recorded in varieties Chuvannamodan (Ptb 30) and Jyothi (Ptb 39) was 11.40 and 18.40 respectively.

4.1.6. 1000 grain weight

The weight of 1000 grains for Chuvannamodan (Ptb 30) and Jyothi (Ptb 39) was 27.00 g and 28.30 g respectively.

4.1.7. Grain density

The grain density of Chuvannamodan (Ptb 30) was obtained as 1.09 g/cm^3 and it was 1.11 g/cm^3 in Jyothi (Ptb 39).

4.1.8. Measurement of water mining traits

a) Root to shoot ratio

After weighing the root and shoot dry weights, the root to shoot ratio was calculated. The root to shoot ratio of Chuvannamodan (Ptb 30) was obtained as 0.31 and 0.24 in Jyothi (Ptb 39).

Plate 6. Comparison of Jyothi and Chuvannamodan at vegetative stage (65 days after sowing)

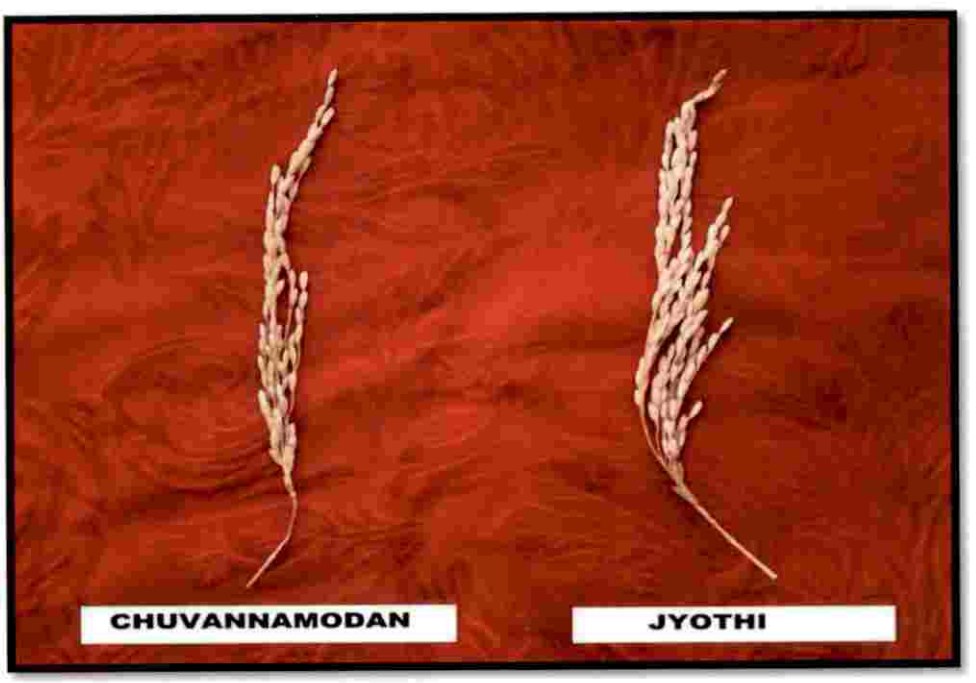


Plate 7. Comparison of Panicle characteristics of Chuvannamodan and Jyothi

b) Root volume

The total root volume of each genotype was taken by the water displacement method. The root volume of Chuvannamodan (Ptb 30) was 96 cm^3 and that of Jyothi (Ptb 39) was obtained as 32 cm^3.

c) Root dry weight

The root dry weight of Chuvannamodan (Ptb 30) and Jyothi (Ptb 39) was 31.6 g and 14.4 g respectively.

d) Root length

Root length was measured from the crown of the root to tip of the root and expressed in centimetre. The average root length of Chuvannamodan (Ptb 30) was found to be 109 cm and that of Jyothi (Ptb 39) was 68 cm.

4.2. Parental polymorphism study

4.2.1. DNA isolation

DNA of both parents Jyothi (Ptb 39) and Chuvannamodan (Ptb 30) were isolated using the CTAB method. The quality and quantity of DNA isolated were checked using spectrophotometer. The quality of DNA was also analyzed using agarose gel electrophoresis.

4.2.1.1. Quantity and quality analysis by spectrophotometer

The quality and quantity of isolated DNA from both the parental varieties Jyothi (Ptb 39) and Chuvannamoda (Ptb 30) were analyzed using the spectrophotometer. The result of the analysis is given in Table 4

Table 4. Quantity and quality analysis of parental DNA

Genotype	$A_{260/280}$	Quantity (ng/µl)
Chuvannamodan	1.93	1660
Jyothi	1.86	1720

Plate 8. Comparison of root traits of Chuvannamodan and Jyothi

Plate 9. Hybridization of Jyothi and Chuvannamodan

The quantity of DNA of Chuvannamodan (Ptb 30) and Jyothi (Ptb 39) was 1660ng/µl and 1720ng/µl respectively. The OD value of DNA of Chuvannamodan (Ptb 30) and Jyothi (Ptb 39) was 1.85and 1.86 respectively.

4.2.1.2. Quality analysis by agarose gel electrophoresis

The quality analysis of DNA was done by agarose gel electrophoresis. A single, good, intact band of DNA without shearing was observed. The DNA was free from RNA and protein contamination.

4.2.2. Primer screening for polymorphism study

Parental polymorphism study was done by genotyping the parents (Chuvannamodan and Jyothi) with SSR markers. The DNA of both the varieties was screened using 120 SSR markers which were distributed throughout the rice chromosomes. The result of the study is given in Table 5.

Table 5. Parental polymorphism analysis

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
1.	RM1	Monomorphic	1	1
2.	RM3	Polymorphic	1	1
3.	RM10	Monomorphic	1	1
4.	RM16	Monomorphic	1	1
5.	RM17	Polymorphic	1	1
6.	RM18	Monomorphic	1	1
7.	RM19	Polymorphic	1	1
8.	RM20A	Monomorphic	2	2
9.	RM21	Monomorphic	1	1
10.	RM22	Polymorphic	1	1

Table 5 contd.

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
11.	RM24	Monomorphic	1	1
12.	RM30	Monomorphic	1	1
13.	RM38	Monomorphic	1	1
14.	RM39	Monomorphic	1	1
15.	RM50	Monomorphic	1	1
16.	RM55	Monomorphic	1	1
17.	RM106	Polymorphic	1	1
18.	RM108	Monomorphic	1	1
19.	RM140	Polymorphic	1	1
20	RM147	Monomorphic	2	2
21.	RM157B	Monomorphic	2	2
22.	RM159	Monomorphic	1	1
23.	RM171	Monomorphic	1	1
24	RM183	Monomorphic	1	1
25.	RM190	Monomorphic	1	1
26.	RM195	Monomorphic	1	1
27.	RM201	Monomorphic	1	1
28.	RM204	Polymorphic	1	1
29.	RM205	Polymorphic	1	1
30.	RM206	Monomorphic	1	1
31.	RM207	Monomorphic	1	1
32.	RM208	Polymorphic	1	1

Table 5 contd.

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
33.	RM209	Polymorphic	1	1
34.	RM210	Monomorphic	2	2
35.	RM211	Monomorphic	1	1
36.	RM212	Polymorphic	1	1
37.	RM214	Monomorphic	1	1
38.	RM215	Monomorphic	1	1
39.	RM216	Polymorphic	1	1
40	RM219	Monomorphic	1	1
41.	RM223	Monomorphic	1	1
42.	RM224	Monomorphic	1	1
43.	RM225	Polymorphic	1	1
44.	RM228	Polymorphic	1	1
45.	RM229	Monomorphic	1	1
46.	RM234	Monomorphic	1	1
47.	RM235	Polymorphic	1	1
48.	RM237	Polymorphic	1	1
49.	RM242	Polymorphic	1	1
50.	RM243	Monomorphic	1	1
51.	RM247	Monomorphic	1	1
52.	RM249	Polymorphic	1	1
53.	RM250	Monomorphic	1	1
54.	RM253	Polymorphic	1	1

Table 5 contd.

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
55.	RM258	Monomorphic	1	1
56.	RM263	Polymorphic	1	1
57.	RM264	Polymorphic	1	1
58.	RM266	Monomorphic	1	1
59.	RM267	Monomorphic	1	1
60.	RM269	Monomorphic	1	1
61.	RM272	Monomorphic	1	1
62.	RM273	Monomorphic	1	1
63.	RM274	Polymorphic	1	1
64.	RM276	Monomorphic	1	1
65.	RM282	Monomorphic	1	1
66.	RM286	Monomorphic	1	1
67.	RM297	Polymorphic	1	1
68.	RM301	Monomorphic	1	1
69.	RM302	Monomorphic	1	1
70.	RM314	Monomorphic	1	1
71.	RM315	Monomorphic	1	1
72.	RM319	Monomorphic	1	1
73.	RM320	Polymorphic	1	1
74.	RM323	Monomorphic	1	1
75.	RM333	Polymorphic	1	1
76.	RM335	Polymorphic	2	1

Table 5 contd.

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
77.	RM336	Monomorphic	1	1
78.	RM401	Polymorphic	1	1
79.	RM405	Polymorphic	1	1
80.	RM406	Monomorphic	1	1
81.	RM431	Polymorphic	1	1
82.	RM440	Polymorphic	1	1
83.	RM443	Monomorphic	1	1
84.	RM451	Monomorphic	1	1
85.	RM469	Monomorphic	1	1
86.	RM470	Polymorphic	2	2
87.	RM471	Polymorphic	1	1
88.	RM472	Monomorphic	1	1
89.	RM490	Polymorphic	1	1
90.	RM493	Polymorphic	1	1
91.	RM518	Polymorphic	1	1
92.	RM525	Polymorphic	1	1
93.	RM527	Polymorphic	1	1
94.	RM537	Monomorphic	1	1
95.	RM544	Monomorphic	1	1
96.	RM561	Monomorphic	1	1
97.	RM566	Polymorphic	1	1
98.	RM570	Monomorphic	1	1

Table 5 contd.

Sr. No.	Primer	Nature of amplification	Number of amplicons	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
99.	RM588	Monomorphic	1	1
100.	RM589	Polymorphic	1	1
101.	RM1195	Monomorphic	1	1
102.	RM1209	Monomorphic	1	1
103.	RM1287	Polymorphic	1	1
104.	RM3412	Monomorphic	1	1
105.	RM3805	Monomorphic	1	1
106.	RM3825	Polymorphic	1	1
107	RM5414	Polymorphic	1	1
108.	RM5687	Monomorphic	2	2
109.	RM6100	Polymorphic	1	1
110.	RM6132	Monomorphic	1	1
111.	RM8046	Monomorphic	1	1
112	RM8094	Monomorphic	1	1
113.	RM10346	Polymorphic	1	1
114.	RM10702	Monomorphic	1	1
115.	RM10745	Polymorphic	1	1
116.	RM10772	Polymorphic	1	2
117.	RM10793	Monomorphic	1	1
118.	RM12091	Polymorphic	2	2
119.	RM28048	Monomorphic	1	1
120.	RM28166	Monomorphic	1	1

The results of screening using 120 primers showed that 47 primers were polymorphic among parents (Table 6). The polymorphic SSR primers were RM3, RM17, RM19, RM22, RM106, RM140, RM204, RM205, RM208, RM209, RM212, RM216, RM225, RM228, RM235, RM237, RM242, RM249, RM253, RM263, RM264, RM274, RM297, RM320, RM333, RM335, RM401, RM405, RM431, RM440, RM470, RM471, RM490, RM493, RM518, RM525, RM527, RM566, RM589, RM1287, RM3825, RM5414, RM6100, RM10346, RM10772, RM10745 and RM12091.

Table 6. List of polymorphic markers and their amplicon size

Sr. no.	Primer	Chromosome	Amplicon size	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
1.	RM140	1	287bp	263bp
2.	RM212	1	140bp	123bp
3.	RM237	1	156bp	166bp
4.	RM297	1	143bp	182bp
5.	RM431	1	288bp	273bp
6.	RM490	1	88bp	101bp
7.	RM493	1	230bp	217bp
8.	RM1287	1	164bp	181bp
9.	RM3825	1	154bp	131bp
10.	RM10346	1	339bp	296bp
11.	RM10772	1	477bp	387bp
12.	RM10745	1	181bp	196bp
13.	RM12091	1	168bp, 70bp	126bp, 62bp
14.	RM106	2	269bp	286bp

Table 6 contd.

Sr. no.	Primer	Chromosome	Amplicon size	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
15.	RM208	2	194bp	179bp
16.	RM263	2	200bp	156bp
17.	RM525	2	107bp	130bbp
18.	RM22	3	189bp	178bp
19.	RM19	4	214bp	234bp
20	RM401	4	266bp	217bp
21	RM471	4	119bp	101bp
22	RM518	4	159bp	180bp
23.	RM335	4	160bp, 108bp	110bp
24.	RM470	4	255bp, 133bp	244bp, 102bp
25.	RM5414	4	94bp	120bp
26.	RM249	5	128bp	146bp
27.	RM274	5	172bp	159bp
28.	RM405	5	88bp	93bp
29.	RM440	5	183bp	177bp
30.	RM3	6	152bp	140bp
31.	RM204	6	106bp	119bp
32.	RM225	6	142bp	123bp
33.	RM253	6	143bp	126bp
34.	RM527	6	252bp	265bp
35.	RM589	6	198bp	211bp
36.	RM320	7	230bp	221bp

Table 6 contd.

Sr. no.	Primer	Chromosome	Amplicon size	
			Jyothi (Ptb 39)	Chuvannamodan (Ptb 30)
37.	RM264	8	200bp	156bp
38.	RM205	9	129bp	158bp
39.	RM242	9	217bp	189bp
40.	RM566	9	240bp	221bp
41.	RM228	10	236bp	216bp
42.	RM333	10	160bp	176bp
43.	RM216	10	150bp	131bp
44.	RM6100	10	171bp	185bp
45.	RM209	11	180bp	207bp
46.	RM17	12	160bp	172bp
47.	RM235	12	128bp	142bp

Table 7. List of polymorphic markers reported to be linked to drought tolerance

Sr No.	Chromosome	Primer	Reference
1.	1	RM212	Swamy *et al.* (2011)
2.	1	RM431	Vikram *et al.* (2011)
3.	1	RM493	Srividhya *et al.* (2011b)
4.	1	RM3825	Barik *et al* (2018)
5.	1	RM12091	Vikram *et al.* (2011)
6.	2	RM106	Srividhya *et al.*(2011b)
7.	2	RM208	Bimpong *et al.* (2011b)
8.	2	RM263	Vikram *et al.* (2011)

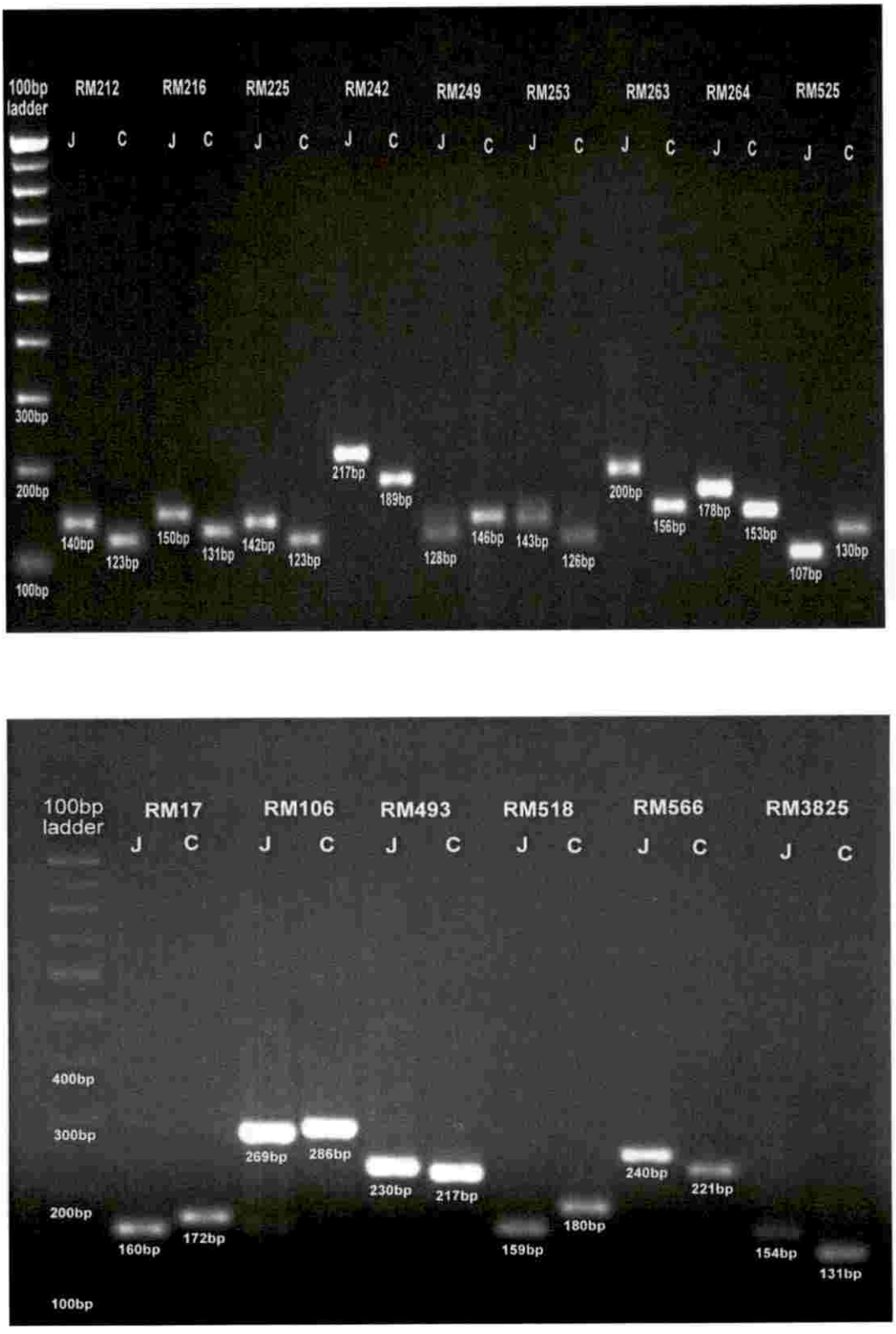
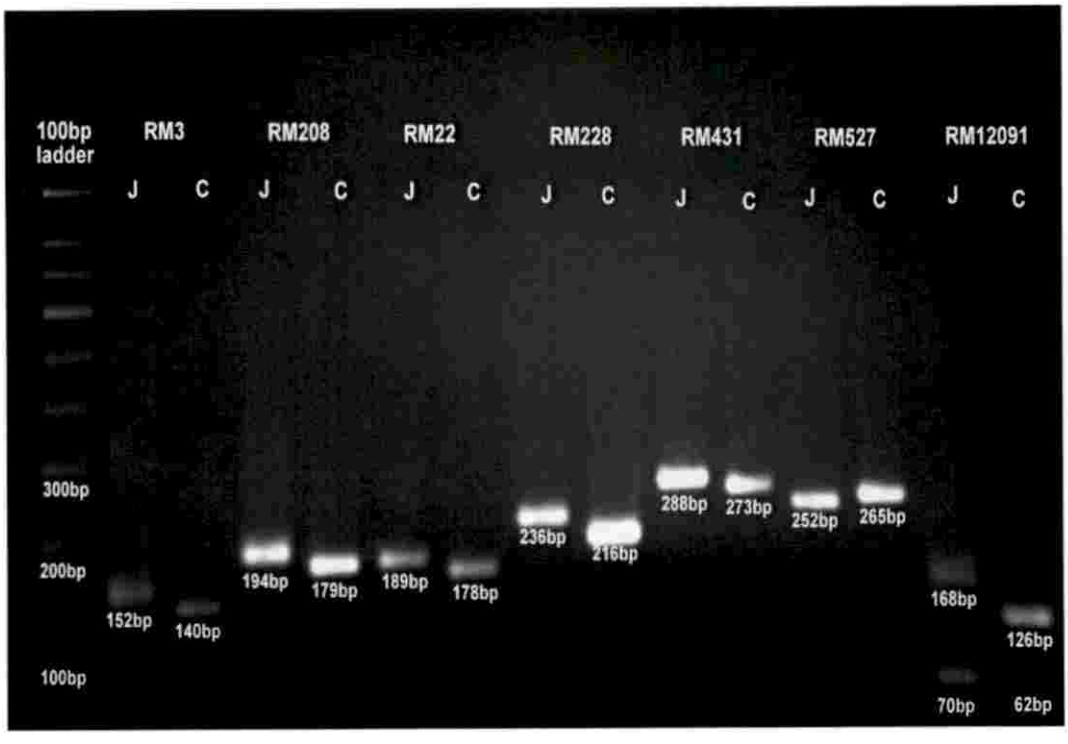


Figure 1. Amplification profile of polymorphic markers

J- Jyothi (Ptb 39), C- Chuvannamodan(Ptb 30)



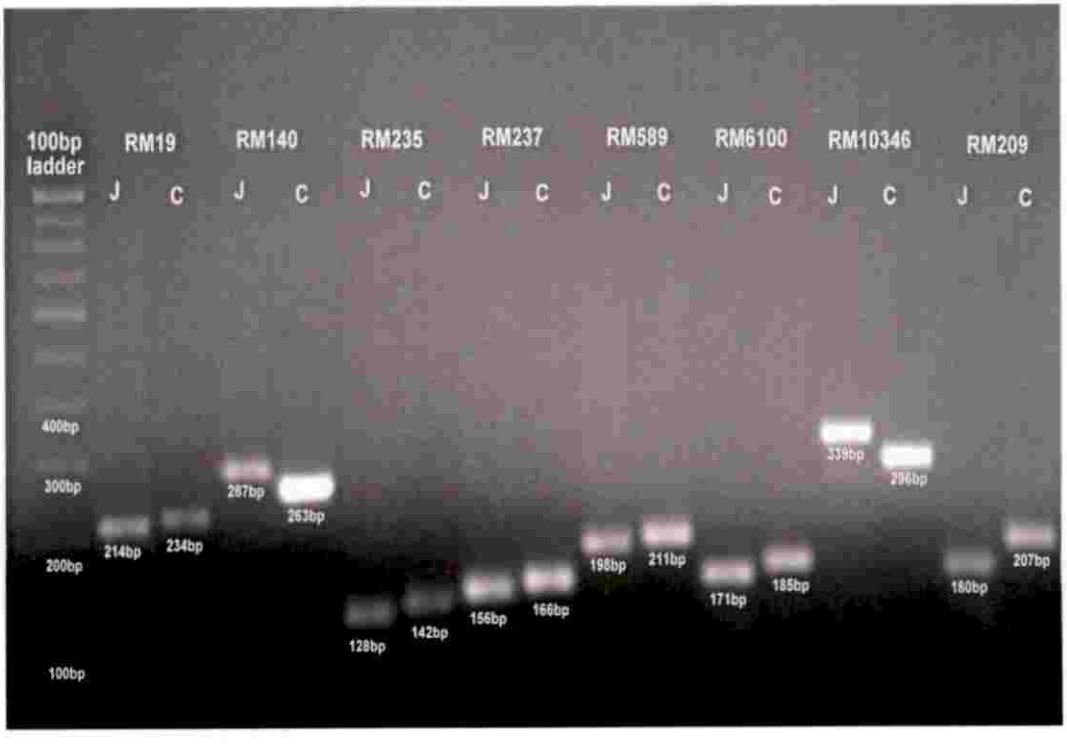
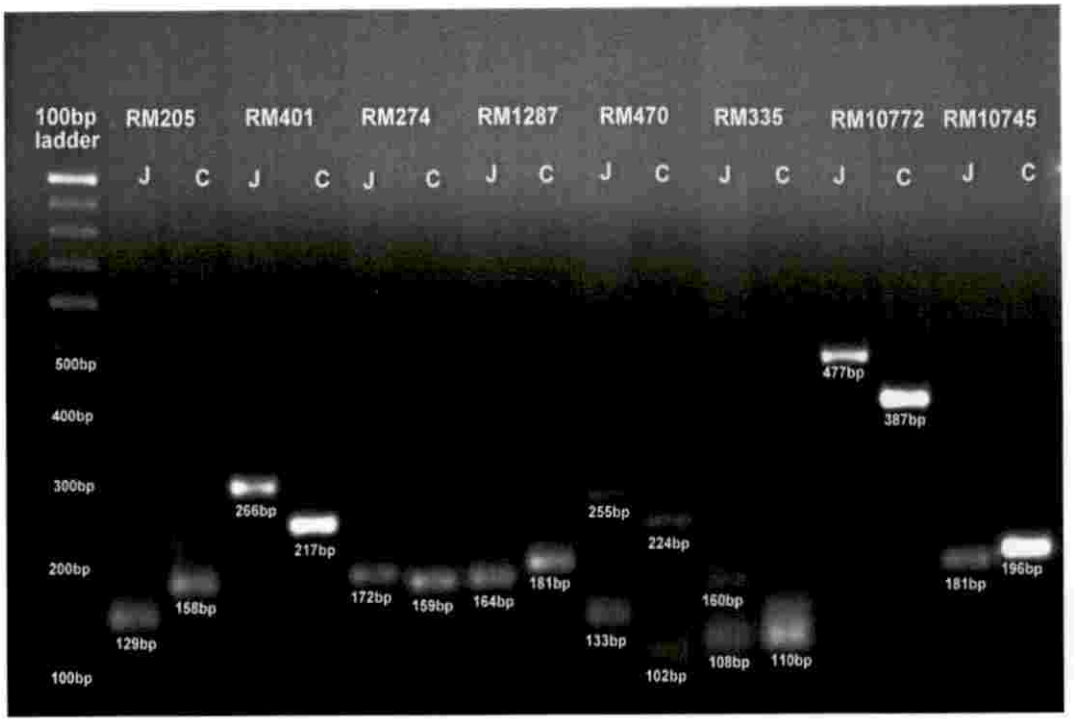


Figure 1. Amplification profile of polymorphic markers

J- Jyothi, (Ptb 39) C- Chuvannamodan(Ptb 30)



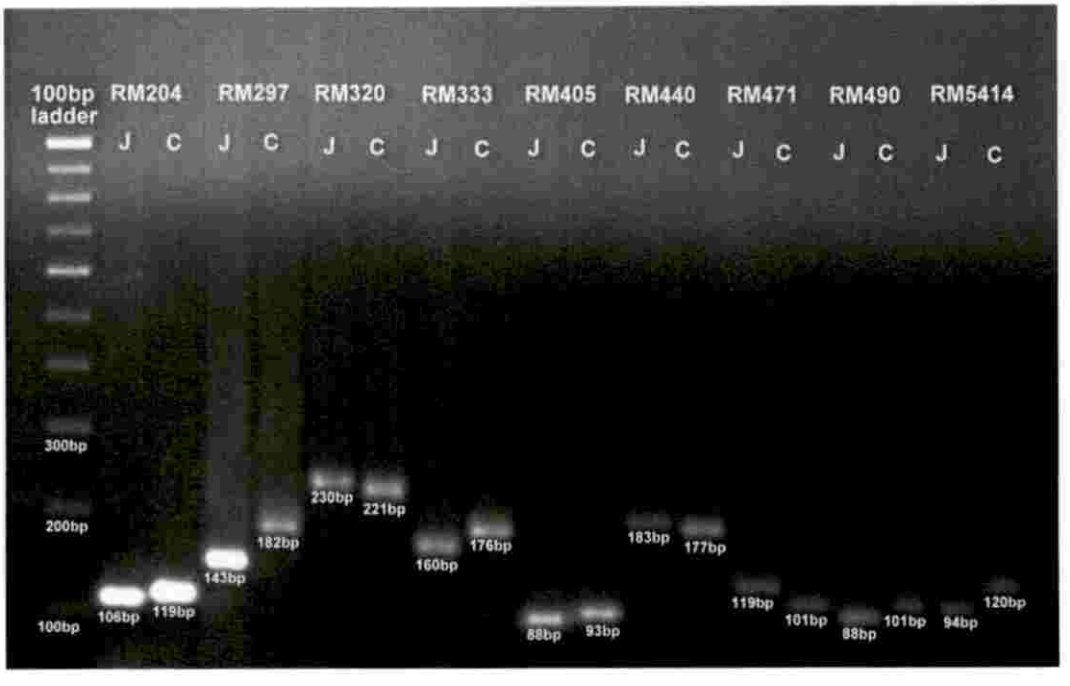


Figure 1. Amplification profile polymorphic markers

J- Jyothi(Ptb 39), C- Chuvannamodan(Ptb 30)

Table 7 contd.

9.	2	RM525	Qu *et al.* (2008)
10.	3	RM22	Vikram *et al.* (2011)
11.	4	RM518	Lin *et al.* (2007)
12.	4	RM470	Ramchander *et al.* (2016)
13.	4	RM335	Nguyen *et al.* (2013)
14.	5	RM249	Swamy *et al.* (2017)
15.	6	RM3	Barik *et al* (2018)
16.	6	RM225	Subashri *et al.* (2009)
17	6	RM253	Sangodele *et al.* (2014)
18.	6	RM527	Barik *et al* (2018)
19.	8	RM264	Ramchander *et al.* (2016)
20.	9	RM242	Courtois *et al.* (2000)
21.	9	RM566	Dixit *et al.* (2012)
22.	10	RM216	Vikram *et al.* (2011)
23.	10	RM228	Lin *et al.* (2007)
24.	12	RM17	Ramchander *et al.* (2016)

4.3. Production of F_1s

Jyothi (Ptb 39) was crossed with Chuvannamodan (Ptb 30) to produce the F_1 seeds. A total of 45 hybrid seeds were obtained after hybridization. These F_1 seeds were sown for raising the F_1 plants. Six seeds germinated and grew to maturity.

4.4. Confirmation of hybridity

The F_1 seeds were raised in pots. Only six seeds germinated and grew to maturity. DNA was isolated from these plants. The quality and quantity of DNA were checked with spectrophotometer and agarose gel electrophoresis. PCR analysis was done and the hybridity was confirmed using polymorphic SSR markers RM3825 and RM263 which are also linked to drought QTLs.

4.4.1. Quality and quantity analysis by spectrophotometer

The result of quality analysis by spectrophotometer is given in Table 8

Table 8. Quality and quantity analysis of F_1s by spectrophotometer

Genotype	$A_{260/280}$	Quantity (ng/µl)
P1	1.89	1660
P2	1.93	1650
P3	1.88	1650
P4	1.84	1670
P5	1.85	1720
P6	1.86	1750

The range of quantity of DNA isolated from the F_1s (P1- P6) were between 1650-1750 ng/µl and their ratio of UV absorbance ranged from 1.8 to 1.93.

4.4.2. Quality analysis by agarose gel electrophoresis

The quality analysis of DNA was done by agarose gel electrophoresis. On documentation, it showed a single, good, intact band of DNA without shearing. The DNA was also free from RNA and protein contamination

4.4.3. Genotyping with selected polymorphic SSR markers

The DNA of plants isolated were genotyped using the polymorphic SSR markers RM3825 and RM263. The true hybrids showed double bands specific to each parent. Among the 6 plants, P5 and P6 showed double bands.

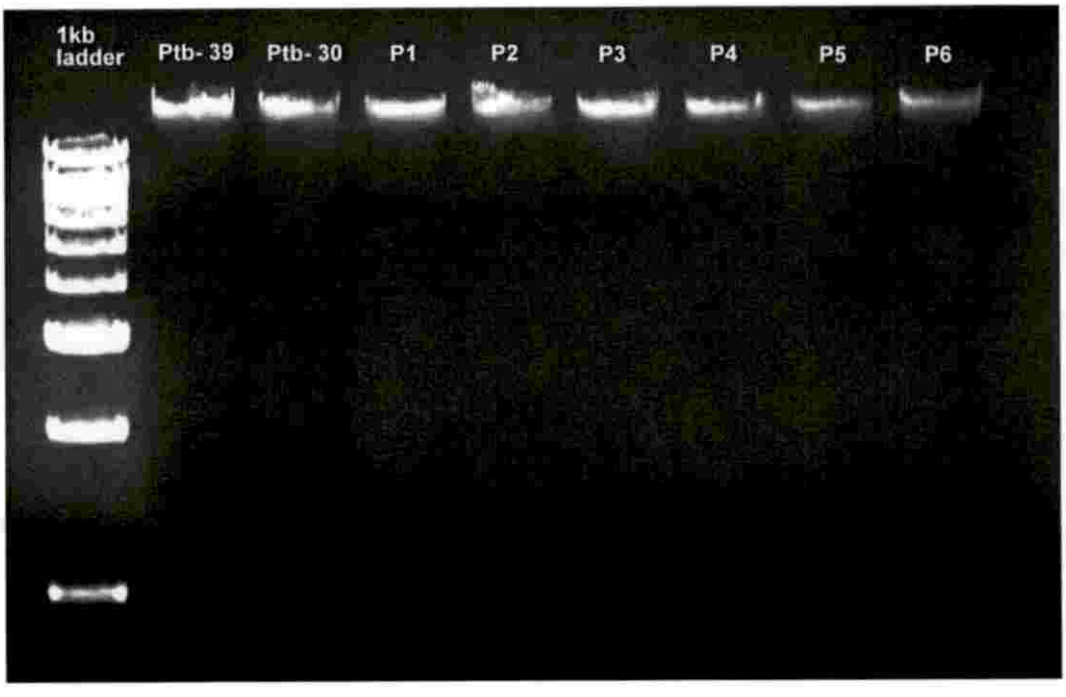


Figure 2 . DNA isolation from parental varieties and F_1plants (Ptb- 39: Jyothi, Ptb- 30: Chuvannamodan, P1-P6: Plant No.1 – Plant No. 6)

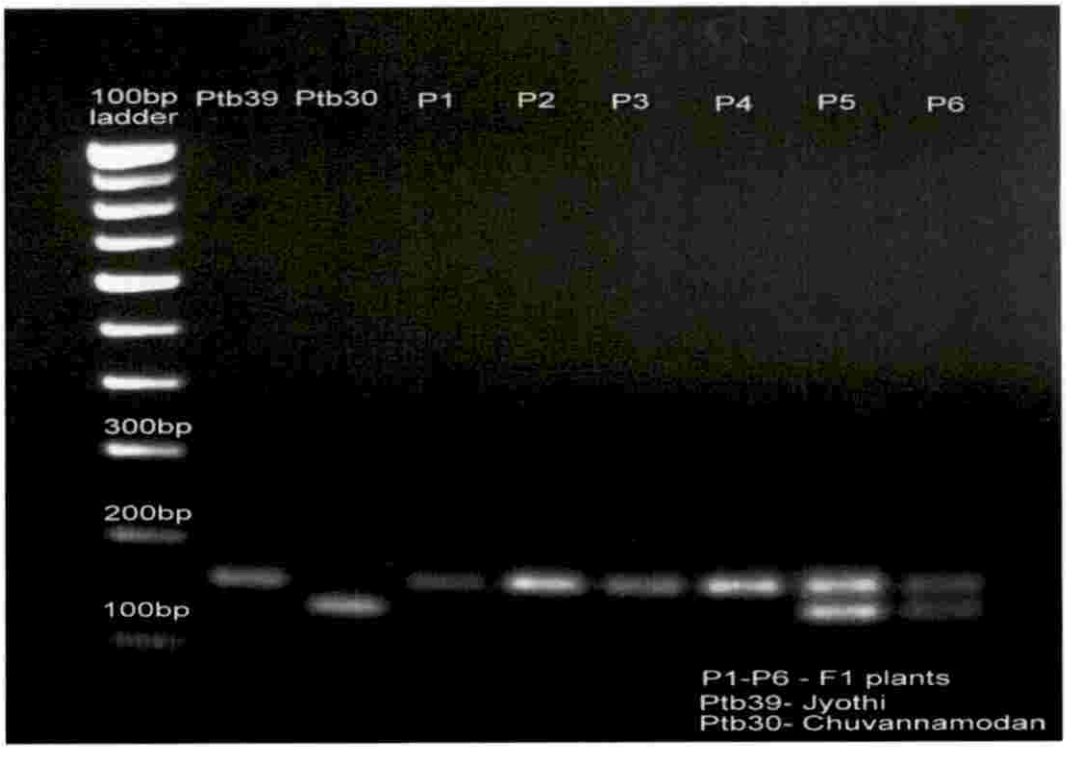


Figure 3. Hybridity confirmation using RM3825

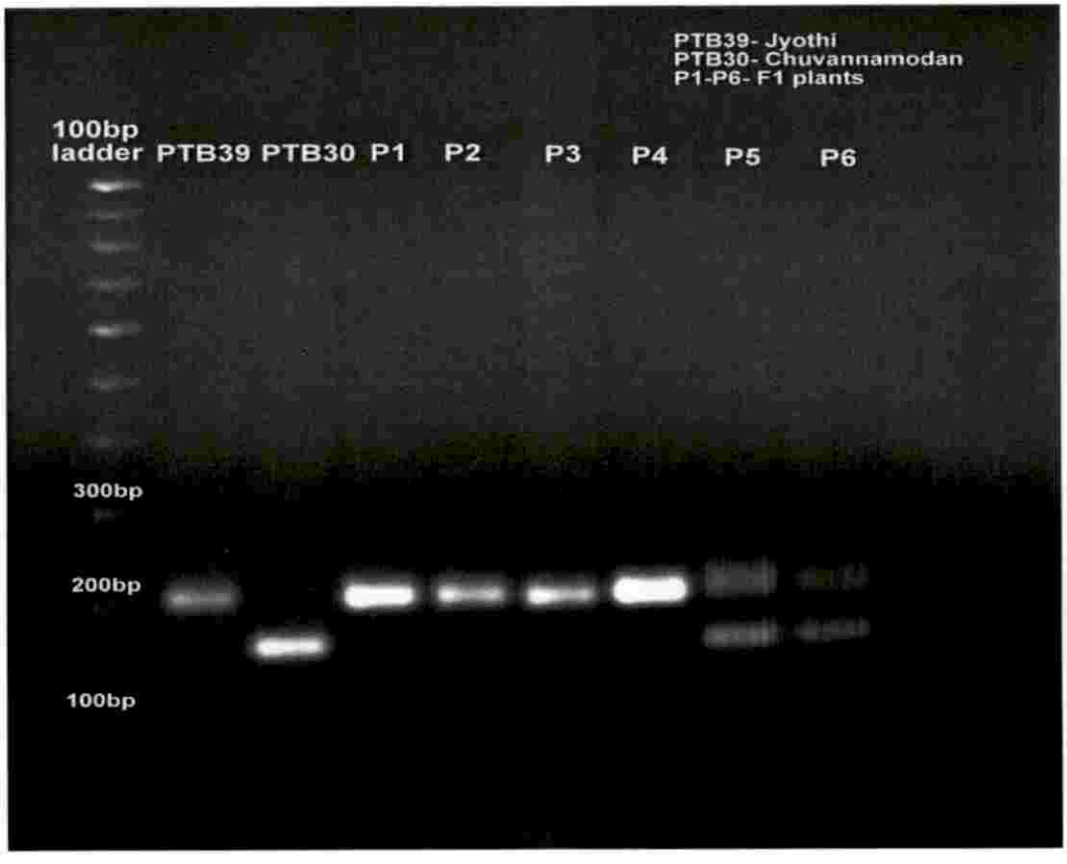


Figure 4. Hybridity confirmation using RM263

Plate 10. Plant No. 6 and Plant No. 5 confirmed as hybrids

Plate 11. BC_1F_1 plants 20 days after sowing

4.5. Production of BC_1F_1 population

The identified hybrids were backcrossed with Jyothi (Ptb 39) to produce BC_1F_1 seeds. Panicles were also allowed to self for the production of F_2 seeds. A total of 15 BC_1F_1 seeds were obtained as a result of backcrossing. The F_2 seeds will be used for developing recombinant inbred lines for genetic mapping.

4.6. Evaluation of BC_1F_1 population

Of the 15 BC_1F_1 seeds sown, 4 seeds germinated. The vigour of these 4 seedlings obtained were very poor and they eventually got dried by 20 days after sowing. Thus the further evaluation of the BC_1F_1 population couldn't be done.

DISCUSSION

5. DISCUSSION

The study on 'Marker assisted backcross breeding in rice for drought tolerance' was carried out at Regional Agricultural Research Station, Pattambi, Palakkad and Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Thrissur during the period 2016- 2018. The results obtained for the present study are discussed below.

5.1. Varieties used for the current study

Chuvannamodan (Ptb30)

The variety was released during 1951. It was developed through mass selection from a local *kharif season* upland strain, Chuvannamodan. It is a short duration variety (105 days) having an average yield potential of 2200 kg ha^{-1}. The grain turns brownish red colour on maturity giving its panicle an attractive appearance. The kernel colour is red with a milling out-turn of 76.5 per cent. It shows satisfactory performance both under broadcasting and transplanting. Considering its drought tolerance character, it is particularly recommended for 'modan cultivation' (Rosamma *et al.*, 2003)

Fifteen traditional rice genotypes were phenotyped during reproductive stage for drought tolerance at CPBMB, Vellanikkara. In the study Chuvannamodan was found to exhibit better morphological traits as well as physiological traits like stomatal conductance, transpiration rate and membrane stability index under water deficit stress. Further, analysis of the proteome of the identified genotypes revealed that Chuvannamodan was tolerant to water stress (Babu, 2014).

In studies conducted at RARS, Pattambi, traditional and high yielding genotypes were phenotyped for drought-tolerant traits and Chuvannamodan was identified as one of the varieties with drought tolerance (Beena *et al.*, 2018).

Jyothi (Ptb39)

Jyothi was released during 1974. This is one of the most widely accepted high yielding rice variety released from Pattambi. This variety is developed from the cross between the short duration improved local strain *viz.*, Ptb 10 and the high yielding genotype *viz.*, IR8. Duration of this variety is 110 to 120 days. The variety has good coverage in Kerala as well as other states too. The average yield of the variety is 6 tons per hectare. The variety is red kernelled and photo-insensitive. The rice recovery is 72.9 per cent and the quality is very good. The variety can be cultivated by transplanting or direct sowing. Jyothi is moderately resistant to BPH and blast; but susceptible to sheath blight. (Rosamma *et al.*, 2003)

5.2. Morphological characterization of parents

The morphological traits of parents (Jyothi and Chuvannamodan) grown in polybags under control condition were recorded.

Both the varieties, Chuvannamodan and Jyothi, exhibited a vigours growth. This may be due to the ideal growth condition and availability of surplus nutrients. Chuvannamodan grew taller than Jyothi consistent with earlier reports (Rosamma *et al.*, 2003).

Profuse tillering was observed in both the varieties which also could be attributed to the ideal growing conditions in polybags with abundant nutrient availability. The number of productive tillers was 33 and 17 for Chuvannamodan and Jyothi respectively. This was against earlier observation were the productive tiller number of 4 and 5 were reported under field condition for Chuvannamodan and Jyothi respectively (Rosamma *et al.*, 2003).

Chuvannamodan flowered earlier (76 days) than Jyothi (93 days). Early flowering could be regarded as a drought escape mechanism in late-stage drought stress. In spite of limited water availability in drought-prone areas of southern US the drought escape strategy allows rice to produce grains (Kumar *et al.*, 2008).

Yield attributes like filled grains per panicle, 1000 grain weight and grain densities were higher in Jyothi than Chuvannamodan. This is on par to the previous reports (Rosamma *et al.*, 2003) and Jyothi is a high yielding variety. Though Jyothi showed higher yield, per cent sterility was observed to be higher in higher in Jyothi.

The root length, root volume root dry weight and root to shoot ratio were higher in Chuvannamodan than Jyothi. Similar results were reported by Babu (2014) for root traits of Chuvannamodan and Jyothi for water mining traits (root traits). In a study on root traits of 80 genotypes, Chuvannamodan was identified as a deep-rooted variety with thick roots (Beena *et al.*, 2018).

Samson *et al.* (2002) reported that rice genotypes having deep, coarse roots with a higher ability of penetration and branching and also a higher root to shoot ratio showed drought avoidance. Rice genotypes that avoid drought generally have a deep, coarse root with good ability for branching and soil penetration, higher root to shoot ratio, leaf rolling elasticity, improved cuticular resistance and early stomatal closure (Wang *et al.*, 2006).

The upland rice varieties possessing deeper root system were able to access more water present in the deep soil layers, thus allowing it to maintain a better yield potential in drought condition (Mambani and Lal, 1983). A higher deep-root weight to shoot weight ratio exhibited by upland rice complements its drought resistance (Fukai and Cooper, 1995).

5.3. Parental polymorphism study

DNA of good quality was isolated from both the parents Jyothi and Chuvannamodan using CTAB method. The A260/A280 ratio of DNA extracts was 1.93 and 1.86. A value less than 1.8 or greater than 2.0 would have indicated a high degree of protein contamination and RNA respectively (Manchester, 1996).

Both the parental varieties were screened using 120 SSR markers which were distributed throughout the rice chromosomes. Out of 120 primers, 47

primers showed polymorphism between the parents. Among the 47, thirteen markers were from chromosome 1, four markers from chromosome 2, one marker from chromosome 3, seven markers from chromosome 4, four markers from chromosome 5, six markers from chromosome 6, one marker from chromosome 7, one marker from chromosome 8, three markers from chromosome 9, four markers from chromosome 10, one marker from chromosome 11 and two markers from chromosome 12.

The 47 polymorphic SSR primers amplified in parental population were RM3, RM17, RM19, RM106, RM140, RM204, RM205, RM208, RM209, RM212, RM216, RM22, RM225, RM228, RM235, RM237, RM242, RM249, RM253, RM263, RM264, RM274, RM297, RM320, RM333, RM401, RM405, RM431, RM440, RM471, RM490, RM493, RM518, RM525, RM527, RM566, RM589, RM1287, RM825, RM5414, RM6100, RM10346 and RM10745.

A total of 24 primers among the 47 polymorphic are reported to be linked to drought tolerance. Of these 24 five markers were from chromosome 1, four markers from chromosome 2, one marker from chromosome 3, three markers from chromosome 4, one marker from chromosome 5, four from chromosome 6, one marker from chromosome 8, two markers from chromosome 9, two markers from chromosome 10 and one marker from chromosome 12.

The SSR polymorphic markers amplified in parental population reported to be linked to drought resistance are RM3, RM17, RM22, RM106, RM208, RM212, RM216, RM225, RM228, RM242, RM249, RM253, RM263, RM264, RM335, RM431, RM470, RM493, RM518, RM525, RM527, RM566, RM3825 and RM12091.

Barik *et al.* (2018) reported a new QTL, $qDFF_{1.1}$ controlling flowering under drought stress and RM3825 marker in chromosome 1 was detected to be linked with it. In the same study, marker RM527 on chromosome 6 was found to be linked to QTL that controls flowering under terminal drought stress and DNA

marker. RM3 on Chromosome 6 was reported to be associated with QTL, $qDTY_{6.2}$, for yield under drought stress.

Rice markers RM212 and RM3825 on chromosome 1 were found to be associated with large effect QTLs for drought-resistance traits across several genetic backgrounds in rice (Salunkhe *et al.*, 2011). In a study Swamy *et al.* (2011) reported that the marker RM212 was associated with MQTL1.2 in chromosome 1. Rice marker RM212 on chromosome 1 was found linked with QTL for relative water content, $rwc_{1.1}$ (Babu *et al.*, 2003).

Vikram *et al.* (2011) identified a major QTL, $qDTY_{1.1}$, for grain yield under drought stress on rice chromosome 1 flanked by markers RM11943 and RM431. The study also reported molecular markers RM263 being linked to QTL $qDTY_{2.3}$ (Chromosome 2), RM216 being linked to QTL $qDTY_{10.1}$ (Chromosome 10) RM12091 being linked to QTL $qDTY_{1.1}$ (Chromosome 1) and RM 22 being linked to QTL $qDTY_{3.2}$.

In study by Srividhya *et al* (2011b), on chromosome 2 the marker interval RM106-RM5897 were reported to harbor two QTLs for shoot dry weight under stress condition and the marker interval RM493- RM302, on chromosome1, was reported to be associated with root to shoot ratio under drought stress. The intervals RM493-RM302 and RM106-RM5897 were found to be water supply-specific regions having effects only during the stress conditions, suggesting the water deficit promoted the expression of QTLs located in these regions.

The QTL qrs1.2 a stable QTL across control and stress treatments was found to be located between RM493- RM302 and the QTL qrs2.1 was found to be located between interval RM106- RM262 (Srividhya *et al.*, 2011b).

A QTL at RM208 on chromosome 2 was reported to positively affected yield under water deficit stress, accounting for 22 per cent of the genetic variation and based on the single-point analysis of IR64 × *O. glaberrima* (BC_2F_3) AILs the marker RM208 was reported to be linked to QTLs bm2.2 (biomass), hi2 (harvest

index), ph2 (plant height), tn2.1 (tiller number), ps2.2 (panicle fertility), dth2.2 (days to heading), ypp2 (yield per plant) (Bimpong *et al.*, 2011b).

A study done by Awasthi and Lal (2014a) validated four SSR markers viz., RM263 (qDTY2.3), RM3825 (MQTL1.1), RM212 (MQTL1.2) and RM22 (qDTY 3.2) to be associated with drought tolerance in rice and hence these markers could be used in MAS for crop improvement programmes. Rice marker RM263 was found linked to QTL for osmotic adjustment on chromosome number 2 (Zhang *et al.*, 2001).

Qu *et al.* (2008) reported that the marker interval between RM525-RM263 on chromosome 2 was reported to be linked with QTLs brt2, rn2d, rdw2c, rfw2c associated with basal root thickness, root number, root dry weight and root fresh weight respectively. The study by Mishra *et al* (2013) in IR74371-46-1-1×Sabitri inbred lines reported that QTL, $qDTY_{3.2}$ linked to marker RM22 on chromosome 3, was associated with grain yield under drought stress.

Lin *et al.* (2007) reported QTLs, lr4.1 (linked to RM518 on chromosome 4) and lr10.1 (linked to RM228 on chromosome10), related to leaf rolling under drought stress. In work done by Anupam *et al* (2017) the marker RM518 was used for evaluating the genotypes for QTL qDTY4.1, where the R87707-446-B-B was used as a drought resistant check.

Ramchander *et al.* (2016) from the study focused on the physiological and molecular characterization drought tolerance in rice reported that marker RM17 and RM470 were linked with photosynthetic rate, transpiration rate, stomatal conductance and RWC under drought stress. It also reported marker RM264 to be linked to photosynthetic rate and transpiration under water deficit stress.

In study Nguyen *et al.* (2013) on QTLs linked to the drought tolerance, found that the QTL for number of days to leaf rolling between the marker interval RM335–RM307 on chromosome 4 having a considerable effect on the tolerance trait. In another study by Zheng *et al.* (2006) for identifying QTLs for rice root

growth under flooding and upland conditions marker RM 335 was found to be linked to root weight.

The marker RM225 was found putatively linked to a QTL on chromosome 6 for root length. Apart from this marker also showed linkage with relative growth rate (Subashri *et al.*, 2009). A similar result was obtained earlier in the study by Venuprasad *et al.* (2002), where the QTLs for root weight were mapped in this region on chromosome 6.

Marker RM242 in chromosome 9 was reported to be associated with maximum root length (Courtois *et al.*, 2000).SSR marker RM242 were found to be linked to QTL regions associated with root traits related to drought resistance (Thanh *et al.*, 2006). RM253 was found to be linked to qRWC 6-1, for relative water content under drought stress (Sangodele *et al.*, 2014).

QTL, $qDTY_{9.1A}$, in chromosome9 flanked by RM321 and RM566 was found to be associated with grain yield under drought in both lowland and upland conditions (Dixit *et al.*, 2012). RM249 was reported to be associated with grain yield under drought stress (Swamy *et al.*, 2017).

In bulk line analysis, out of seven polymorphic primers obtained between the drought tolerant and drought susceptible bulks, two primers - RM223 and RM263 co-segregated in all the individual rice accessions constituting the bulks. These SSR markers were expected to be linked to drought resistance (Kumar *et al.*, 2005).

Two foreground markers, RM242 and RM263, related to root traits and 57 background markers were used throughout the breeding program to introgress root trait genes to a high yielding rice variety MRQ74 from an aerobic rice variety, AERON1, through marker-assisted backcross breeding (MABC) (Ab Jalil *et al.*, 2018).

In marker assisted breeding program drought specific markers RM 212-RM 3825 linked to drought tolerance QTL, MQTL1.1, was used for foreground selection (Awasthi and Lal, 2014b)

The drought specific markers can be further used for foreground selection in advanced stages of backcrossing and the background markers can be selected from the polymorphic markers.

5.4. Production of hybrids and confirmation of hybridity

Jyothi was crossed with Chuvannamodan to produce the F_1 seeds (45 Nos.). These were sown and the six F_1 plants obtained were genotyped using the SSR polymorphic markers RM3825 and RM263, which were also drought specific. Among the six plants two showed double bands specific to each parent confirming their hybridity. Thus two plants Plant No. 5 (P5) and Plant No.6 (P6) were confirmed as true hybrids

Yashitola *et al.* (2002) used 13 microsatellite and 5 STS markers for analysing of six hybrids and their parental lines. The analysis showed two alleles (one allele per parent) to be present in the hybrids where parental lines were detected as polymorphic. The study also suggested that a single, appropriately chosen microsatellite marker was significant enough for assessing the hybrid seed purity.

Latif *et al.* (2017), in a study to examine the performance of F_1 hybrids, used the simple sequence repeat (SSR) markers to identify true hybrids of F_1 developed from the cross of Gogo-dryland rice (Situ Bagendit and Towuti) x Paddy-field rice (Ciherang and Cibogo).

In a biofortification study on rice (Brar *et al.*, 2018), the F_1s obtained from the cross between high yielding genotypes and micronutrient (Iron and Zinc) rich genotypes were identified by using the microsatellite markers employing both by agarose and polyacrylamide gel electrophoresis techniques.

5.5. Production of BC_1 F_1

The confirmed true hybrids were then backcrossed with Jyothi to produce the BC_1F_1 seeds. These could be used for advancing further backcross generations. Few panicles allowed for selfing produced F_2 seeds which also could be advanced for the production of RILs.

SUMMARY

6. SUMMARY

The study entitled 'Marker assisted backcross breeding in rice for drought tolerance' was carried out at Regional Agricultural Research Station, Pattambi, Palakkad and Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Thrissur during the period 2016- 2018. The objective of the study was to improve drought tolerance of rice variety Ptb 39 (Jyothi) through marker assisted backcross breeding.

Rice variety Jyothi (Ptb 39) is a high yielding variety, popular in Kerala. Chuvannamodan (Ptb 30) is a traditional variety suitable for upland cultivation. Both the varieties were characterized for their morphological traits. The varieties Jyothi (Ptb 39) and Chuvannamodan (Ptb 30) showed good vegetative growth. The morphological characterization revealed existence of variability between Jyothi (Ptb 39) and Chuvannamodan (Ptb 30). Both the varieties showed considerable differences in root traits which gives an insight to their distinction drought tolerance.

Chuvannamodan (Ptb 30) exhibited better root traits than Jyothi (Ptb 39) thus indicating its better tolerance to water deficit stress. All water mining traits like root length, root volume, root dry weight and root to shoot ratio was higher for Chuvannamodan (Ptb 30). Also Chuvannamodan (Ptb 30) exhibited earlier flowering than Jyothi (Ptb 39). But yield attributes were good for Jyothi as it is a high yielding variety.

In the parental polymorphism study using 120 SSR markers, Jyothi and Chuvannamodan (Ptb 30) were found to be distinct from each other at 47 marker loci *i.e.* there are 47 polymorphic markers. Among 47 polymorphic markers obtained, 24 markers are reported to be linked to drought tolerance.

As an initial step for incorporation of drought tolerance from Chuvannamodan (Ptb 30) to Jyothi (Ptb 39) both the varieties were crossed to obtain the F_1 plants. During hybridization Jyothi (Ptb 39) is taken as the female

parent. A total of 45 F_1 seeds were obtained after hybridization. These seeds were sown and only six seeds germinated and grew to maturity.

These six F_1 plants were further checked for confirming their hybridity using the polymorphic markers and two plants among the six were confirmed as true hybrids. Plant No. 5 and Plant No.6 (P5 and P6) were confirmed to be true hybrids (Jyothi / Chuvannamodan) through the hybridity confirmation test using markers RM3825 and RM263, where both these plants showed double bands each band representing specific parent.

For further advancement of the backcross breeding programme the true hybrids were backcrossed with the recurrent parent Jyothi (Ptb 39) to produce the BC_1F_1 seeds (15 Nos.). Also few panicles were allowed to self to produce the F_2 seeds. Thus fifteen BC_1F_1s have been produced to advance the backcross breeding programme aimed at incorporating drought tolerance in popular HYV Jyothi (Ptb 39).

- The BC_1F_1s serve as material for further backcross breeding programme to generate Jyothi variety with drought tolerance
- F_2 population can be forwarded to develop RILs for mapping qualitative traits and quantitative traits related to drought tolerance
- F_2 can also serve as the base population for the development of breeding lines through pedigree selection method
- F_2 can also be used in BSA studies to identify markers linked to contrasting traits between parents.

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ANNEXURES

ANNEXURES

1. **CTAB Buffer**

   CTAB- 4g

   Tris HCl- 100 mM

   EDTA-20 mM,

   NaCl1.4 M

2. **Washing solution**

   70% ethanol

3. **TAE buffer50X (100ml)**

   Tris base: 24.2g

   Glacial acetic acid: 5.71

   0.5M EDTA: 10ml

4. **TAE Buffer for gel (100ml)**

   2ml 50X TAE buffer

   98ml distilled water

5. **Loading dye**

   0.25% bromophenol blue

   0.25% xylene cyanol

   30% glycerol in water

MARKER ASSISTED BACKCROSS BREEDING IN RICE FOR DROUGHT TOLERANCE

By

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ABSTRACT OF THESIS

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important food crop grown across the globe. The crop is cultivated in diverse environments ranging from uplands to deep water ecosystems. Drought is one of the major constraints for rice production in rainfed lowlands. In the present scenario of climate change, the frequency of drought is more likely to increase in the future, making drought resistance in rice varieties indispensable. During the era of post-green revolution, many locally adapted traditional rice varieties (TRVs) were replaced by high-yielding varieties (HYVs) that thrive best in the irrigated ecosystem. Most of these HYVs suffer heavy yield loss even under mild water deficit conditions. Hence, improving drought tolerance of high yielding varieties is imperative. Considering this, the study 'Marker assisted backcross breeding in rice for drought tolerance,' was executed with an objective to improve drought tolerance in high yielding rice variety Ptb 39 (Jyothi; J) using Ptb 30 (Chuvannamodan; Ch) as donor parent, through marker assisted backcross breeding.

Jyothi is a popular high yielding rice variety of Kerala derived from the cross between Ptb 10 and IR 8. Chuvannamodan is an improved landrace, recommended for '*Modan*' *i.e.,* upland cultivation owing to its drought tolerance ability. Morphological characterisation of both the varieties was done at Regional Agricultural Research Station, Pattambi, during 2018. Chuvannamodan registered a higher vegetative growth than variety Jyothi. The plant height and the number of productive tillers were 141 cm and 33 respectively in Chuvannamodan, while, in comparison, it was 91 cm and 17 in Jyothi. It was also observed that Chuvannamodan flowered earlier (Days to 50% flowering: 76 days) than variety Jyothi (Days to 50% flowering: 93 days). However, Jyothi out-performed Chuvannamodan with respect to the yield traits like number of filled grains per panicle (Ch: 71 and J: 104), 1000-grain weight (Ch: 27.00 g and J: 28.30 g), and grain density (Ch: 1.09 g/cm^3 and J: 1.11 g/cm^3). The sterility in Chuvannamodan and Jyothi was 11.40 per cent and 18.40 per cent respectively.

The water mining traits like root length, root volume, root dry weight and root to shoot ratio were higher in variety Chuvannamodan. The root length, root volume, root dry weight and root to shoot ratio of Chuvannamodan was respectively 109cm, 96cm^3, 31.60g and 0.31, while, it was 68cm. 32cm^3,14.40g and 0.24 respectively in variety Jyothi.

The genetic polymorphism study between Jyothi and Chuvannamodan was studied using 120 SSR markers. Forty-seven markers were found to be polymorphic between the two genotypes. Among these polymorphic markers, 24 are reported to be linked to drought tolerance traits.

Forty five F_1 seeds were obtained by hybridizing variety Jyothi (as female parent) and Chuvannamodan (as male parent). Staggered sowing of the two varieties was done at weekly intervals for this purpose. Only six F_1 seeds germinated. The test for confirmation of hybridity was conducted in these plants along with the parents. The polymorphic markers RM3825 and RM263, which are reported to be linked to drought traits were used for hybridity testing. Two plants, P5 (Plant No.5) and P6 (Plant No.6), were confirmed to be true hybrids as they were found to be heterozygous for the parental alleles. The hybrids (P5 and P6), were then backcrossed to the recurrent parent Jyothi to produce BC_1F_1 seeds (15 Nos.). Simultaneously, selfing of the F_1s to generate F_2s (300 Nos.) was also done.

In order to advance further the marker assisted backcross breeding programme aimed at imparting drought tolerance to Jyothi, the BC_1F_1s produced need to be genotyped further to identify progenies with resistant alleles for drought tolerance. The F_2 population can be forwarded to develop RILs (Recombinant inbred lines) that would enable mapping of qualitative traits and quantitative trait loci related to drought tolerance. The F_2s can also serve as the base population for the development of advanced breeding lines through pedigree selection.