ANALYSIS OF INTERACTION AMONG TRAITS IN QTL-NIL AND THE ASSESSMENT OF MOLECULAR NETWORKS IN MARKER ASSISTED BACK CROSS PROGENIES OF RICE (*Oryza sativa* L.)

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DEPARTMENT OF CROP PHYSIOLOGY

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BENGALURU **DECEMBER, 2020**

Affectionately Dedicated

To

My Beloved Family

and

Chairperson

DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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CERTIFICATE

This is to certify that the thesis entitled "**ANALYSIS OF INTERACTION AMONG TRAITS IN QTL-NIL AND THE ASSESSMENT OF MOLECULAR NETWORKS IN MARKER ASSISTED BACK CROSS PROGENIES OF RICE (*Oryza sativa* L.)**" submitted in partial fulfilment of the requirement for the award of **DOCTOR OF PHILOSOPHY** in **CROP PHYSIOLOGY** to the University of Agricultural Sciences, Bangalore, is a record of bonafide research work carried out by **POOJA BHARTI** bearing **ID No. PALB 4040** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

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December, 2020 ***Pooja Bharti***

ANALYSIS OF INTERACTION AMONG TRAITS IN QTL-NIL AND THE ASSESSMENT OF MOLECULAR NETWORKS IN MARKER ASSISTED BACK CROSS PROGENIES OF RICE (*Oryza sativa* L.)

POOJA BHARTI

THESIS ABSTRACT

A set of 260 $DCBC_3F_4$ lines developed by introgressing root traits and WUE through marker assisted back cross breeding were phenotyped for target traits and yield along with the parents under semi-irrigated aerobic conditions. Based on the phenotyping, 25 transgressive near isogenic lines (NIL) differing in root and WUE viz., HD+HR, HD+LR, LD+HR and LD+LR were selected for further characterization. The performance of these selected NILs was significantly better than the recurrent parent (IR64) across various seasons and locations. The consistency of traits across seasons demonstrated the stability of the QTLs and NILs. Based on Marker class analysis the 25 lines were classified into phenotypic classes differing in root traits with low $\Delta^{13}C$ and higher yield. All low root types possessed alleles of RM 2584, RM 1388 and RM 16 from AC-39020. On the other hand, high root types had RM 2584 and RM 1388 alleles from AC-39020 and RM 16 alleles from non-donor parent. Further, from the 25 NILs, six lines were selected based on marker/QTL combination and an additional seven lines from among 260 $DCBC_3F_4$. These lines were characterized for physiological traits in the MLM phenomics facility. All the 13 TILs identified as superior transgressive seggregants, performed better than the recurrent parent. Further, the NILs with donor marker alleles RM 2584 and RM 1388 allele and non-donor allele of RM 16 had significantly higher root traits. The marker RM 16 seemed to be associated with genomic regions that act like negative regulator of root growth. Scientific investigations reported in literature also suggests RM 16 as negative regulator. Bioinformatics and co-localization study suggested that gene *Os03g43400* present within RM 16 region was the candidate gene responsible in inhibiting lateral root growth.

December, 2020

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ಗುರುತುಕಾರಕದ ಸಹಾಯದಿಂದ ಅಭಿವೃದ್ಧಿ ಪಡಿಸಿದ ಭತ್ತ(*Oryza sativa* L.)ದ ಸಂತತಿಗಳ ಗುಣಸ್ಥಾನ-NILದ ಸಂವಹನ ಮತ್ತು ಅನುವಂಶಿಕ ಜಾಲದ ವಿಶ್ಲೇಷಣೆ

ಪೂಜ ಭಾರತಿ

ಪ್ರಬಂಧ ಶೀರ್ಷಿಕೆ

ಬರನಿರೋಧಕ ಗುಣಗಳಾದ ಅಧಿಕ ಬೇರು ಮತ್ತು ನೀರು ಬಳಕೆಯ ಸಾಮರ್ಥ್ಯತೆಗಳನ್ನು DNA ಗುರುತುಕಾರಕಗಳ ಸಹಾಯದಿಂದ ಸಂಯೋಜಿಸಲಾದ 260 ತಳಿಗಳನ್ನು ಪ್ರಸ್ತುತ ಸಂಶೋಧನೆಯಲ್ಲಿ ಬಳಸಲಾಯಿತು. ಸಂಯೋಜಿಸಿದ ಗುಣಗಳ ವೈವಿಧ್ಯತೆಯ ಆಧಾರದ ಮೇಲೆ ಈ ತಳಿಗಳನ್ನು ನಾಲ್ಕು ಭಾಗಗಳಾಗಿ ವಿಂಗಡಿಸಿ ಅವುಗಳ ಬೆಳವಣಿಗೆ, ಇಳುವರಿ ಮತ್ತಿತರ ಗುಣಗಳನ್ನು ಹಲವಾರು ಋತು ಮತ್ತು ಸ್ಥಳಗಳಲ್ಲಿ ಸುಧೀರ್ಘವಾಗಿ ವಿಶ್ಲೇಷಿಸಲಾಯಿತು. ಬರ ನಿರೋಧಕ ಗುಣಗಳನ್ನು IR64 ಎಂಬ ಪ್ರಸಿದ್ಧ ತಳಿಯ ಅನುವಂಶಿಯ ಹಿನ್ನೆಲೆಯಲ್ಲಿ ಅಭಿವೃದ್ಧಿಪಡಿಸಿದ್ದರಿಂದ ಈ ಗುಣ ಸಂಯೋಜಿಸಿದ ತಳಿಗಳ ಕ್ಷೇತ್ರ ಕ್ಷಮತೆಯನ್ನು IR-64 ತಳಿಗೆ ಹೋಲಿಸಲಾಯಿತು. ಗುಣ ಸಂಯೋಜಿಸಿದ ತಳಿಗಳು IR-64 ಗಿಂತ ಸರಾಸರಿ ಶೇಕಡಾ 30 ರಿಂದ 35 ರಷ್ಟು ಹೆಚ್ಚಿನ ಇಳುವರಿ ಹೊಂದಿರುವುದು ಕಂಡು ಬಂದಿತು. ಬೇರು ಗುಣದಾನಿ ತಳಿಯಾದ AC-39020 ಇಂದ RM 2584, RM 1388 ಮತ್ತು RM 16 ಗುರುತುಕಾರಕಗಳ ಸಹಾಯದಿಂದ ಹೆಚ್ಚಿನ ಬೇರಿನ ಗುಣಗಳನ್ನು ಹಾಗೂ RM 16 ಗುರುತುಕಾರಕದಿಂದ ಬೇರಿನ ಗುಣವನ್ನು ಕುಗ್ಗಿಸುವ ಸಾಮರ್ಥ್ಯವಿರುವುದು ಕಂಡುಬಂದಿತು. ಈ ಸಂಶೋಧನೆ ಗುರುತುಕಾರಕಗಳ ದಕ್ಷತೆಯನ್ನು ನಿರ್ಧರಿಸಲು ಉಪಯುಕ್ತವಾಯಿತು. ಬಹುತೇಕ ಗುಣ ಸಂಯೋಜಿಸಿದ ತಳಿಗಳು ಶೇಕಡಾ 98 ರಷ್ಟು IR-64ದ ಅನುವಂಶಿಯ ಹಿನ್ನೆಲೆಯನ್ನು ಹೊಂದಿದ್ದರೂ ಅವುಗಳ ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿ ಗಮನಾರ್ಹವಾಗಿ ಹೆಚ್ಚಿನಮಟ್ಟದಲ್ಲಿ ಇರುವುದನ್ನು ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಪ್ರಪ್ರಥಮವಾಗಿ ವರದಿಯಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯಿಂದ ಹೊರಬಂದ ಗುರುತುಕಾರಕಗಳು ಮತ್ತು ಗುಣದಾನಿ ತಳಿಗಳನ್ನು ಬರ ನಿರೋಧಕ ಗುಣಗಳ ಅಭಿವೃದ್ಧಿಗೆ ಬಳಸಬಹುದೆಂದು ಕಂಡುಬಂದಿದೆ. ಈ ತರಹದ ಗುಣ ಸಂಯೋಜಿತ ತಳಿಗಳ ಅಭಿವೃದ್ಧಿಯಿಂದ ಭತ್ತದ ವ್ಯವಸಾಯದಲ್ಲಿ ಹೆಚ್ಚಾಗಿ ವ್ಯಯವಾಗುವ ನೀರನ್ನು ಉಳಿಸಲು ಸಹಾಯಕವಾಗುತ್ತದೆ.

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LIST OF ABBREVIATIONS

AC	-	Aerobic condition
RL	-	Root Length
RV	-	Root volume
RW	-	Root weight
SL	-	Shoot length
SW	-	Shoot weight
NT	-	No. of Tillers
SLA	-	Specific Leaf Area
SCMR	-	SPAD chlorophyll meter reading
TLA	-	Total Leaf Area
TDM	-	Total dry matter
$\Delta^{13}C$	-	Discrimination against 13 C
WUE	-	Water Use Efficiency
CID	-	Carbon isotope discrimination
IRMS	-	Isotopic Ratio Mass Spectrometer
DFF	-	Days to fifty percent flowering
TW	-	Test weight
SF	-	Spikelet fertility
PL	-	Panicle length
HI	-	Harvest index
MTA	-	Marker trait association
LM	-	Linkage mapping
LD	-	Linkage disequilibrium

TIL	-	Trait Introgressed Lines
SSR	-	Simple Sequence Repeats
QTL	-	Quantitative Trait Loci
NIL	-	Near Isogenic Line
MLM	-	Mini Lysimeter
MAS	-	Marker Assisted Selection

I INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple foods in Asia. It belongs to the Poaceae family with 2n=24. Out of the 25 species of genus *Oryza*, only 2 species are being cultivated *i.e., Oryza sativa* and *Oryza glaberrima.* Because of the importance as one of the staple foods, its enhanced production is needed to feed the growing population. Rice consumes more than 80 per cent of the water supplied for agriculture (Bouman, 2007). With urbanization and industrialization, fresh water is becoming limiting. Water unavailability/ deficiency is one of the primary causes of the reduction in crop yield (Boyer, 1982). Various methedologies have been develped to investigate the possibility of growing rice that reduces the requirement of water. The usage for water can be reduced by decreasing seepage loss, percolation and evaporation, adopting promising techniques such as intermittent irrigation, saturated soil culture and the system of rice intensification (SRI). These techniques still use extended flooding periods so that water loss stays high. Therefore, essentially the distinct strategy would be to grow rice on non-flooded aerobic soil similar to an upland crop to save considerable amount of water. A new strategy known as aerobic approach has shown excellent potential to save water but with a serious yield compromise (Bouman *et al.,* 2000). Yield loss while saving water needs to be avoided. Therefore, tackling drought can provide better yield even under water shortage conditions (Arvind *et al.,* 2018). Thus, it is imminent that rice cultivars must be improved genetically to suit aerobic method for stabilizing productivity.

Breeding to enhance productivity for water limited conditions has been attempted over many years, where conventional breeders used yield as a trait under water limited conditions. But, due to the high G X E interaction for yield and a narrow variability among the already improved lines, further selection for yield is not effective (Araus *et al*., 2008, Reynolds and Tuberosa, 2008). Dissection of yield into component traits led to a great deal of success (Araus *et al.,* 2008). Similarly, attempts have been made to identify specific morpho-physiological traits associated with plant growth under water limited conditions. It is being emphasized that for a comprehensive improvement in drought adaptation, several important traits need to be introgressed in an elite background. Genetic enhancement in rice through such component trait based breeding is one of the

most appropriate strategies to tackle drought. Among the numerous traits, water acquisition by roots, water use efficiency (WUE), water conservation though epicuticular wax load and cellular level tolerance (CLT) found to have higher relevance during water limited conditions. Introgression of root and WUE traits in elite gentic background appears to improve the yield under aerobic conditions.

A conventional breeding approach to combine root traits and WUE led to the development of a cultivar namely KMP-175 which is significantly higher yielding and now released as a variety for Karnataka region under the name Daksha (Sheshshayee *el al*., 2018).

Breeding for the appropriate physiological traits is complex, as these traits have polygenic inheritance and are difficult to quantify. In this direction, much progress is made in understanding the genetic basis of complex traits through Quantitative Trait Loci (QTL) mapping strategy. Through traditional bi parental linkage and association mapping approaches, there has been phenomenal success in defining QTLs for several such traits. Successful quantitative trait identification of QTLs relies on the choice of contrasting parents and the level of complex trait segregation in the recombinants. To achieve the desirable phenotypic and yield levels pyramiding QTLs may be an effective approach.

The effect of genetic loci in a pyramiding program may not always be as expected because of the complexity in gene and gene networks. Pyramiding QTL will not only help to understand interactions among genetic loci but it also improves the efficiency of marker-assisted selection for desirable loci in breeding programs.

From this context, the major emphasis of the study is to identify the best trait introgressed lines and interactions among QTL-NILs for root and water use efficiency traits.

The specific objectives are as follows:

1. To study the interactions of water use efficiency and root traits in the QTL-NILs
2. Identification and physiological characterization of the best trait introgressed line
3. To study the molecular network responsible for variation in traits.

II REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) has been cultivated for centuries and played a major role in supplying the food and also in shaping the socio-economic progress of those regions where it is cultivated. Continuous upsurge in world population, increase in deterioration of arable land, lack of fresh water and global climate changes all highlights the need for developing stress tolerant plants. Drought is the foremost abiotic stress factor affecting plant growth and development & thereby reducing crop yield. It is estimated that 70% of the crop yield loss is owing to abiotic stresses, particularly drought stress (Bray *et al*., 2000). Rice is considered to be inimitably greatest drought-susceptible crop due to its small root system, low cuticular wax, and swift stomatal closure. Nearly 23 million hectares of rain-fed rice face drought stress (Sahebi *et al.,* 2018).

2.1 Drought and productivity

As a sessile organism, plant encounters a wide range of adverse situations in the environment. Abiotic and biotic stresses normally have an undesirable impact on growth and yield of crop plants (Passioura, 1986). Water deficit/ drought is a widespread challenge to sustainable agriculture. Plant growth and development is affected at all the stages due to water deficit but the degree and nature of damage and recovery capacity and the impact on the productivity depends on the developmental phase at which crop encounters stress. So, sustaining crop productivity below water deficit needs to be addressed instantly.

The total cultivated rice area in India accounts to 42.75 million ha, but only 25.12 m ha is irrigated i.e., about 60% of total area is under irrigation (Mandal *et al.,* 2019). In the semi-arid tropics alike India, rain-fed agriculture is progressively becoming unproductive due to lengthy rain free months. The traditional answer to water shortage in rain fed area is irrigation. Nevertheless, its use in numerous cropping area is limited because either it is not available or the large capital costs of equipment and pumping exclude its wide spread use. Although the susceptibility of cereals to water deficit during reproductive stage and its direct impact on yield have been well known over the last century, the development towards overcoming this problem has been slow.

2.2 Resistance of rice crop to water stress

About 80% of the agricultural area globally is rain fed and water constraint is known to cause substantial reduction in the yield of crop plants, which is a major risk to food security. Rice crop is more sensitive to drought at tillering and reproductive stage. The major influence of drought is on spikelet fertility (Raju *et al.,* 2016). Approximately 50% of Asia's rice is rain fed and water stress is the most important aspect limiting rice productivity in the rain fed habitats (Widawsky and O'Toole, 1990; Fischer *et al*., 2003).

Crop plant selected for economic yield needs to survive drought stress through mechanisms that maintain crop yield (Basu *et al.,* 2016). These mechanisms are linked to plant processes at various level of organization (Blum, 1982).

Plant mechanisms for drought tolerance are

1) The ability of a plant to escape phases of drought, especially during the most sensitive periods of development (drought escape).
2) The ability of a plant to recover from the water limited period by producing new leaves from the buds or to maintain greenness of leaves that makes plant able to survive the dry spell (drought avoidance).
3) The ability of a plant to endure or withstand a dry period by maintaining a favorable internal water balance under drought (drought tolerance).

Drought stress during tillering stage of rice reduces height, tiller number, panicle number, leaf area and grain yield (Bhattacharjee *et al.,* 1973). In addition to these leaf rolling, drought stress at late vegetative and reproductive stage leads to reduction in number of panicles per plant, spikelet fertility and 1000 grain weight (De-Datta and Seshu, 1973). The reduction in yield is higher when plants are exposed to moisture stress at panicle initiation phase, the milk ripe or dough ripe stage.

2.3 Aerobic rice as a strategy to save water

The main challenge in sustainable agriculture is to produce more food grains using less water. Hence, plant system performance should be evaluated in terms of water

use efficiency and water productivity along with the land productivity. Rice plant is mainly grown in submerged condition; however, there is a necessity to develop strategies for growing rice under semi-irrigated aerobic conditions to decrease water use in rice production. Irrigated rice cultivation in asia is typically transplanted into puddled paddy fields. Land preparation consists of soaking with water followed by ploughing and harrowing of saturated soil. After crop establishment, the fields are kept submerged with 5-10 cm of water. Wetland preparation coupled with huge losses of water by seepage, percolation and evaporation when the soil is submerged results in requiring more water for the production of lowland rice (Bouman and Tuong, 2001). Saving of irrigation water and increase water use efficiency is possible if rice could be grown under aerobic conditions. However, new varieties need to be developed to make the concept of "Aerobic rice" successful. Upland rice also exists but it suffers at unfavorable environments without access to irrigation with low external inputs and low yield potential. Aerobic rice must be responsive to high inputs (water, nutrients) to reach high yields under non-flooded conditions.

2.3.1 Drought tolerant mechanisms

Plants respond, acclimatize and survive under drought stress by initiation of various morphological, biochemical, physiological and molecular responses (Farooq *et al*, 2009). Drought tolerance is well-defined as the capability to grow, perpetuates and display economic yield under suboptimal water supply. Drought affects the water relations of plants at cellular, tissue, organ and whole plant levels, causing specific as well as unspecific reactions, damage and adaptation reactions (Beck *et al.*, 2007). To cope with drought, tolerant plant initiate defense mechanisms under water deficit condition (Chaves and Oliveira, 2004), Though drought adaptive mechanisms *viz.*, Drought escape (DE), Drought avoidance (DA) and Drought tolerance (DT) possess various implications (Table 2.1), they are typically involved together with the plant functions to insure survival (Turner *et al.,* 2001). However, due to its complicated nature, little effort was produced to dissect the genetic factors of drought resistance.

Table 2.1: List of important mechanisms and traits associated with drought adaptation in rice

Sl. No.	Mechanisms		Specific traits	References
1	**Drought escape**		Rapid Phenology/ plasticity	Chaves *et al*., 2003; Barrnabs *et al.,* 2008
			Photoperiod sensitivity	Kumar *et al.,* 2007
2	**Dehydration avoidance**	**Minimizing water loss/ Pessimists**	Stomatal conductance	Ludlow and Muchow, 1990
			Leaf rolling	Courtois *et al,* 2000
			Stay greenness	Ishimaru *et al.,* 2001
			Canopy temperature	Garrity and O'Toole, 1995
			Water Use efficiency	Dingkuhnu *et al.,* 1991
			Epicuticular wax	Baenziger *et al.,* 1983
		Maximizing water uptake/ Optimists	Root traits	O'Toole and Bland 1987; Ludlow, 1990
			Hydraulic conductivity	Henry *et al.,* 2011
3	**Dehydration tolerance**		Osmotic adjustment	Lilley *et al.,* 1996
			Cell-membrane stability	Tripathy *et al.,* 2000
			Antioxidant capacity and chaperons	Moran *et al.,* 1994, Mittler, 2002
			Desiccation tolerance	Lilley *et al.,* 1996
			Cellular level tolerance	Raju *et al.,* 2012
			Relative water content	Courtois *et al.,* 2000

In this framework, a better consideration of the genetic make-up and molecular basis of features (main and secondary) underlying the adaptive response of plants across a wide spectrum of soil moisture is a noteworthy prerequisite for more efficient and targeted breeding operations (Araus *et al.,* 2002; Salekdeh *et al.,* 2009). Drought mitigation characteristics were classified as primary, secondary and integrative characteristics (Table 2.2). These drought responses traits have been studied widely and their significance was validated (Paterson, 1995; Sheshshsyee *et al.,* 2003; Lafitte *et al.,* 2004; Passioura, 2007; Kamoshita *et al.,* 2008; Serraj *et al.,* 2009; Songsri *et al.,* 2008; Passioura, 2010; Passioura, 2012; Tuberosa, 2012). However, reassuring heritability of physiological or secondary characters that are highly connected with yield gives a better chance for plant breeding in drought-prone regions (Stiller *et al.,* 2005). Therefore, the major focus has been to device novel approaches (analytical, trait-based breeding) for achieving the job of trait pyramiding to improve drought stress tolerance of rice crop to withstand productivity under water limited conditions.

It is proved that pyramidizing several appropriate features will considerably increase rice productivity (Sheshshayee *et al.,* 2012). Thus, a breeding strategy based on "trait" is most suitable. Li *et al.,* 2005, Sheshshayee *et al.,* 2012, Bheemanahalli *et al.,* 2014 showed the significance of several adaptive drought characteristics in breeding for stress tolerance and enhanced productivity. Of the many features, water acquisition connected with better root systems, greater water effectiveness, better water preservation strategies connected with epicuticular waxes and elevated inherent stress tolerance at cellular level are often regarded to be the most appropriate physiological features connected with rice productivity under water circumstances. To achieve an extensive increase in drought tolerance and productivity, introducing several of these characteristics to an agronomically superior background is crucial. However, this trait introgression is best accomplished by adapting breeding protocols aided by molecular markers.

Table 2.2: List of drought adaptive traits and QTLs reported in rice

Sl. No	Traits	References
	Morphological traits	
1	Biomass	Lian *et al.,* 2005
2	Flowering time	Brondani *et al.,* 2002
3	Leaf area	Yan *et al*., 2003
4	Leaf & shoot dry weight	Yan *et al*., 2003; Lian *et al.,* 2005
5	Leaf rolling	Venuprasad *et al.,* 2007b
6	Panicle exertion	Hittalmani *et al.,* 2003
7	Plant height	Yan *et al.,* 1998b
8	Root traits	Nguyen *et al.,* 1997, Kamoshita *et al.,* 2008
9	Tiller number	Yan *et al.,* 1998a
10	Panicle associated traits	Hittalmani *et al.,* 2003
11	Yield	Hittalmani *et al.,* 2003; Bernier *et al.,* 2007
	Physiological traits	
12	Abscisic acid content	Quarrie *et al.,* 1997
13	Canopy temperature	Garrity and O'Toole, 1995; Yue *et al.,* 2006
14	Cell viability	Miura *et al.,* 2002
15	Gas exchange parameters	Centritto *et al.,*2009
16	Leaf senescence	Ishimaru *et al.,* 2001
17	Membrane integrity	Tripathy *et al.,* 2000
18	Osmotic adjustment	Lilley and Ludlow, 1996
19	SCMR	Yue *et al.,* 2006, Mohankumar *et al.,* 2012
20	Stomata conductance	Dingkuhn *et al.,* 1991, Price *et al.,* 1997, Sinclair *et al.,* 2011
21	Leaf water status/relations	Courtois *et al.,* 2000
22	Water Use Efficiency by $D^{13}C$	Dingkuhn *et al.,* 1991; Condon *et al.,* 2004; Impa *et al.,* 2005
	Other traits	
23	Protein content	Ye *et al.,* 2010
24	Harvest index	Hittalmani *et al.,* 2003
25	Drought index score	Bernier *et al.,* 2009
26	Spikelet sterility	Hittalmani *et al.,* 2003

2.4 Traits linked to drought tolerance

Plants have evolved various morpho-physiological mechanisms that facilitate their growth and survival under stress. These morpho-physiological traits that support plants under drought conditions can be categorized as constitutive traits (also expressed under well-watered condition) and drought-responsive or induced traits (expressed under pronounced water shortage condition).

2.4.1 Root traits

Several drought resistance mechanisms have been adopted by rice plants to overcome drought impacts, ranging from cellular to whole plant level. Rice plants retain growth and productivity by preserving the association between tissue water and beneficial carbon gain from deeper soil profiles by mining water (Fukai and Copper, 1995).

Roots provide vital functions including water and nutrient uptake for plant growth, serve as storage organs, anchor plants in the soil, and are the site of interactions in the rhizosphere with pathogenic and useful bacteria. The plasticity of root growth and development in reaction to altering soil moisture and nutrient status offers possibilities for exploring natural variation to recognize useful root features to improve agricultural system efficiency (Kano *et al.,* 2011, Grossman *et al.,* 2012, Lynch *et al.,* 1995). The root system architecture (RSA) is jointly referred to as the spatial distribution of all root components in a specific development setting. RSA is dynamic and influenced by the internal setting (humidity of the soil, temperature, nutrients and pH) and the surrounding microbial communities that affect the manner a plant detects and reacts to its environment (Bao *et al.,* 2014, Robbins *et al.,* 2015). Various root features allow crops in varying settings to react adapt and flourish. Strategies for implementing "root breeding" involve identifying the subterranean root characteristics that allow a plant to use water and nutrients in distinct settings more effectively. Various studies recognized connections between root characteristics and plant productivity (Kell *et al.,* 2011, Hufnagel *et al.,* 2014). Understanding the root traits resulting in greater yields and enhanced stress tolerance would provide concrete objectives for breeders to select people with the

faultless root phenotypes to be used as parents and create breeding lines to progress through the method of crop improvement. The success of RSA modification breeding programs depends on the particular feature being designated in distinct plants, the trait heritability, and the capacity to correctly and effectively phenotype various genotypes origins (Meister *et al.,* 2014). The root system also adds to soil health, referring to the soil's ongoing ability to operate as a living ecosystem that supports crops, livestock and eventually people (Doran *et al.,* 2000, Saikia *et al.,* 2015).

Despite the importance of root systems to improve the acquisition of water and nutrients, breeding efforts that select and modify specific root characteristics are limited (Zhu *et al,* 2005). Genetic gains in the manufacturing of forage and grain are significant purposes for plant breeding programs and these gains could be enhanced by knowing the root features that add to improved plant efficiency. The task is to create technologies to correctly represent and capture the RSA for non-destructive root phenotyping. These methods should enable ongoing monitoring of root development and its response to various growing conditions as well as relatively high-throughput systems as part of the breeding program to effectively evaluate a large number of genotypes. A number of methods are available for the assessment of root traits in the soil profile (Table 2.3). As an alternative method to root phenotyping in field experiments, a numeral of studies have measured roots (root length, root diameter, root depth root pulling force, deep root to shoot ratio, root number, root growth plasticity (Table 2.4), root penetration ability and root length density) in plants full-grown under controlled situations. It allows more rapid and accurate analysis of root features. A rational compromise to evade both the unusual conditions present in hydroponics and aeroponics and the trouble of studying roots in the field is circumvented by rising plants in pots, columns (PVC pipes), root structures, monolith, minirhizotrons, and/or observation chambers filled with soil (Smit *et al.,* 2000; Azhiri-Sigari *et al.,* 2000; Wade *et al.,* 2000; Sheshshayee *et al.,* 2011a, b). The most preferably accepted methods for root studies are outlined in the Table 2.3.

Table 2.3: List of root phenotyping approaches reported in the literature

Sl. No.	Phenotyping Methods for root traits	References
1	Basket method	Uga *et al.,* 2009, 2011
2	Hydroponic system	Henry *et al.,* 2011
3	PVC pipes	Hemamalini *et al*., 2000
4	PVC tubes	Asch *et al*., 2005
5	Root boxes	Wang *et al*., 2009
6	Root structure	Sheshshayee *et al*., 2011a,b
7	Semi-automated 3D Root Imaging	Christopher *et al.,* 2013
8	Soil-filled glass rhizotrons for visualizing roots	Price *et al.,* 2002
9	Three-Dimensional Root Phenotyping	Clark *et al.,* 2011
10	2D root system platform	Clark *et al.,* 2012
11	Wax-petrolatum layer system	Yu *et al*., 1995
12	Semi-automated 3D Root Imaging	Christopher *et al.,* 2013
13	Leaf temperature (as surrogate for root traits)	Dharmappa *et al.,* 2019

Table 2.4: List of root related traits and QTLs reported in rice

Sl. No.	Root characters	Proposed function	References
1	Maximum root depth	Potential for absorption of moisture and nutrients in deeper soil layer	Nicou *et al.,* 1970; Kato *et al.,* 2006
2	Root to shoot ratio	Assimilate allocation	Asch *et al.,* 2005
3	Root volume	The ability to permeate large volume of soil	Mohankumar *et al.,* 2012
4	Root diameter	Potential for penetration ability, branching, hydraulic conductivity	Armenta-Soto *et al.,* 1983
5	Root length density	Rate of water and nutrient uptake	Mohankumar, 2010
6	Hardpan penetration ability	Ability to penetrate subsurface hardpans	Babu *et al.,* 2001; Clark *et al.,* 2000, 2008
7	Hydraulic conductivity	Rate of water uptake	Henry *et al.,* 2011
8	Deep root to shoot ratio	Potential for absorption of soil moisture and nutrient in deeper soil layers	Yoshida and Hasegawa, 1982
9	Root pulling force	For root penetration into deeper soil layers	O'Toole and Bland, 1987
10	Root branching	Power of soil exploration	Fitter, 1991; Ingram *et al.,* 1994
11	Root length	Potential for absorption of moisture and nutrients in deeper soil layer	Nicou *et al.,* 1970; Kato *et al.,* 2006,
12	Root number	Physical strength, potential for root system architecture	Armenta-Soto *et al.,*1983
13	Root dry weight	To explore a greater soil volume	Yadav *et al.,* 1997

To choose superior varieties, the identification of QTLs connected with drought is relevant (Bernier *et al.,* 2007). QTLs linked to several associated characteristics of root architecture have therefore been known in rice (Kamoshita *et al.,* 2008; McCouch and Jung, 2013). Most QTL mapping trials were performed using progenies (F2, home inbred lines, doubled haploid lines besides mostly recombinant inbred lines) extracted from distinct subspecies (japonica x indica) instead of the same species (Fischer *et al.,* 2012; McCouch and Jung 2013). These parental lines often show slight morphological distinctions, but their off springs show significant genetic variability for numerous root characteristics (transgressive seggregation). The exploitation of these transgressive seggregants has been accomplished by QTL discovery. The number of rows used was between 56 (Hemamalini *et al.,* 2000) and 220 (Kamoshita *et al.,* 2002). Variations in root structure connected features among the progenies caused in the identification of QTLs ranging from 1 to 19 and the phenotypic variation described by each QTL ranged from about 4% to 66.6% (Gowda *et al.,* 2011). More lately, variations in characteristics between naturally variable accessions to germplasms are being analysed in order to define the genomic region governing complicated traits (McCouch and Jung 2013).

Our conceptual framework emphasizes that maintaining leaf water relationships through effective root water mining and efficient use of water to produce biomass is most important to achieve an extensive increase in both drought tolerance and efficiency (Sheshshayee *et al.,* 2011a, & b).

2.4.2 WUE a major trait to enhance drought adaptation

WUE is described in various aspects. Instantaneous WUE (WUEis) which is also known as transpiration effectiveness (TE), is usually evaluated at the single leaf level as the total quantity of carbon assimilated (A) per mole of water transpired (T) (Farquhar & Richards 1984; Farquhar, *et al.,* 1989; Condon *et al.,* 2002; Bacon, 2004). Intrinsic WUE (WUE intrinsic or WUEic) is defined as the proportion between photosynthesis (A) and stomatal conductance (gs) (Choi *et al.,* 2007), which is believed to be closer to autonomous physiological reactions of gas exchange characteristics to particular environmental circumstances. WUE is typically calculated for agronomists and plant

breeders as the collected dry matter divided by the amount of water spent by the crop throughout the growth cycle (WUE integrative or WUEie) (Tuberosa *et al.*, 2007) (Table 2.5). Crop biomass measurements and precise water budget needed for estimating WUE are labour-intensive, time-consuming and costly, and therefore unappealing to plant breeders, particularly under field circumstances.

Efforts were formed to comprehend the genetic variability of TE in various plant species by finding surrogates strongly related to characteristics. Reports proposed a favourable connection between TE and the content of chlorophyll, SCMR and leaf nitrogen (Nageswararao *et al.,* 2001; Bindumadhava *et al.,* 2003). The existence of genotypic difference in rice for gas exchange parameters and WUEic (A/gs) was demonstrated by worldwide studies. It was low throughput because of its instantaneous nature of the leaf level measurement and is the significant restriction. During 1980s innovation of hopeful Carbon Isotope Discrimination (CID, $\Delta^{13}C$) technique was introduced to field has a time averaged surrogate for gauging WUE at whole plant level (O'Leary, 1981; Farquhar *et al.,* 1982; Udayakumar and Prasad, 1994; Sheshshayee *et al.,* 2003) and it faster the crop breeding activities.

Carbon isotope discrimination (CID) gives the proportion of stable carbon isotopes ($^{13}C/^{12}C$) in dry matter to atmospheric CO_2 (O'Leary, 1981; Condon *et al.*, 1990). The linkage between the average ^{13}C and the observed WUE variants is well established (Farquhar *et al.,* 1982) and commonly accepted (Sheshshayee *et al.,* 2003 and reference therein). Over the era of accumulation of biomass (Condon *et al.,* 1990, 2004; Araus *et al.,* 2002; Rebetzke *et al.,* 2002; Chen *et al.,* 2011), commonly but not always (Turner *et al.,* 2007). It is also a good forecaster of stomatal behaviour (Condon *et al.,* 2002) and WUE in plants under drought pressure (Tambussi *et al.,* 2007). It is also used as a WUE surrogate in various plant species including rice (Dingkuhn *et al.,* 1991; Peng *et al.,* 1998; Impa *et al*., 2005; Mohankumar *et al.,* 2012).

On the source of leaf gas exchange rates under irrigated circumstances, these methods stayed used to study WUE in tropical japonica and indica cultivars (Peng *et al.,* 1998).

Table 2.5: Some of the common explanations of water-use efficiency (WUE) and the corresponding phenotyping methods reported in literature

Sl. No.	Level/Time scale	Equation/ Formula	Methods	Reference
1	Leaf (Minutes or hours)	$WUE_{Instantaneous}$ = Photosynthesis (A, μ mol m^{-2} s^{-1})/ Transpiration (T, m mol water m^{-2} s^{-1})	Infra-red gas analyser (IRGA)	Peng *et al.*, 1998 Impa *et al.*, 2005
		$WUE_{Intrinsic}$ = Photosynthesis (A, μ mol m^{-2} s^{-1})/ Stomatal conductance (gs, mol water m^{-2} s^{-1})	Infra-red gas analyser (IRGA)	Hall *et al.*, 1992 Choi *et al.*, 2007
		WUE= SCMR vs. SLA (negative relation)	SPAD chlorophyll meter reading (SCMR), Specific leaf area (SLA)	Nageswararao *et al.*,2001 Sheshshayee *et al.*, 2006
2	Crop (Weeks to months)	$WUE_{integrative}$ = Aboveground biomass /Seasonal evapotranspiration	Gravimetric method	Impa *et al.*, 2005 Tuberosa *et al.*, 2007
		$WUE_{economic}$ = Grain yield/Seasonal evapotranspiration	Gravimetric method	Chen *et al.*, 2011
3	Seedling/ whole plant (Time averaged)	$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_p)/(1+\delta^{13}C_p/1000)$	Isotope Ratio Mass Spectrometer (Carbon isotope discrimination)	Farquhar *et al.*, 1982, 1989 Udayakumar and Prasad, 1994 Sheshshayee *et al.*, 2003

Most of the indica cultivars had greater T than tropical japonica lines, and the A / T ratio for tropical japonica was 25–30 percent greater than for indica. And the observed differences in A/T were confirmed by lower values in the tropical japonica associated to indica. Numerous rice reports displayed the association between WUEic and the complete WUE plant respectively by gas exchange and stable isotope (Sheshshayee *et al.,* 2003). Additionally, Impa *et al.,* 2005 also verified the connection between the rice germplasm gravimetrically determined WUE and $\Delta^{13}C$ and demonstrated the use of WUE genetic variability as a feature in the breeding programme.

Despite determining important genetic variability using a solid, high-performance evaluation strategy, breeders were not passionate about exploiting WUE variability. The discrepancy of the WUE-biomass relationship, which showed helpful, negative and even natural correlations (Richards *et al.,* 2002; Sheshshayee *et al.,* 2003; Condon *et al.,* 2004), was the main concern of large-scale breeding programs (Sheshshayee *et al.,* 2012). The hidden secrets of WUE dependent gs (conductance / stomata mediated) and gm dependent (capacity / mesophyll mediated) were critically clarified (Udayakumar *et al.,* 1998). Sheshshayee *et al.,* 2012) and reviewed the WUE sub-components and debated how WUE could still be regarded as a prospective feature for crop improvement. The authors proved with experimental information that, after optimizing water use (through root) and/or light capture characters through canopy cover, growing WUE has tremendous importance. Improved WUE could be achieved by two feasible methods (Flexas *et al.,* 2010):

- Increasing CO_2 diffusion to the carboxylation locations by keeping *g*s, which could be accomplished by cumulative mesophyll conductance to CO_2 (*g*m)
- Improving the Rubisco carboxylation efficiency, which might be realized by presenting carboxylase enzyme by other species

Rice genotypes with intrinsically higher gm have been able to withstand greater circumstances of water deficit (Centritto *et al.,* 2009). This understanding opens up an alternative to use molecular breeding along with transgenic approach to study genetic variety, choice and utilization of elevated WUE in crop improvement.

Genetic variation of these characteristics is usually controlled by QTL under significant environmental influence. Understanding the genetic foundation of WUE in water-limited environments is essential for crop improvement. Martin & Nienhuis (1989) recorded the first QTL recognized for ubiquitous $\Delta^{13}C$ in tomatoes (*Lycopersicon esculentum* and *L. pennellii*), followed by QTL for ubiquitous $\Delta^{13}C$ in other species including rice (Impa *et al.,* 2005; Nadarajan *et al.,* 2005; Laza *et al.,* 2006; Takai *et al.,* 2009; Xu *et al.,* 2009; This *et al*., 2010). Masle *et al.,* 2005 produced the first report to identify a gene from QTL in Arabidopsis for water use efficiency. Cloned a gene called ERECTA, a Leucine-rich receptor (LRR-RLK) responsible for the pattering of the leaf surface. Takai *et al.,* (2009) discovered that g_s were associated with a QTL controlling flower at $\Delta^{13}C$ on the long side of chromosome 3 in rice. Diab *et al.,* (2008) reported QTL on the same locus (gwm389) for $\Delta^{13}C$ and transpiration. However, no single QTL has been recognized in cereals including rice with large $\Delta^{13}C$ with a big impact, and the majority of QTL identified for $\Delta^{13}C$ have minor impacts. Two years of dry-season field testing at IRRI lately verified the connection between grain yield and CID among rice lines segregating for a significant drought yield QTL (Source: IRRI website). A few groups have found QTLs and candidate genes for WUE, which Chen *et al.,* 2011 has widely evaluated.

Gravimetric measurements provide reliable estimates of WUE, as they let accurate measurement of transpiration and dry matter making including roots (Rao and Wright., 1994, Udayakumar *et al*., 1998a). Boominathan (2001) showed considerable variability in WUE ranging from 2.49 – 5.41g/litre among rice genotypes adopting gravimetric approach. Other studies also showed genetic variability in WUE by means of this approach (Impa, 2002) where WUE is valued as the ratio of dry matter formed to the amount of water lost through transpiration over a period of time. The measurement of WUE is tedious and exhaustive because of the real-world problems associated with the measurement of transpiration and dry matter of root. A Mini-Lysimeter based phenomics platform has relevance for the precise maintenance of soil and plant moisture status across large number of accessions. The approach involves the real time measurement of transpiration and soil moisture status in container grown plants exposed to natural field

environment conditions. The evapotranspiration (ET) interfaced replenishment of water provides an option to maintain the desired soil moisture status across the genotypes irrespective of variation in transpiration. Non-invasive phenotyping tools like Fieldspec (Spectral reflectance characteristics), Planteye (imaging growth), IR Camara (thermal imaging) are also added to the facility to make it high through put.

Physiological WUE is evaluated by measuring CO_2 fixation and transpiration rate. The measurements are typically done on a single leaf over a partial period of time. The biomass produced is a purpose of photosynthetic rate. Therefore, WUE at a single leaf level is the ratio of carbon assimilation rate (A) to transpiration (T). the transpiration rate is determined by the intrinsic stomatal conductance and the current leaf to air vapour pressure difference (V). The plants studied are full-grown under a very similar environmental state, it can be anticipated that the leaf to air vapour pressure will be comparable and hence, the major factor that determines transpiration would be the intrinsic stomatal conductance (gs). Though not sturdy and statistically significant, a positive suggestion between WUE measured by gravimetry and A/gs was observed which recommends that A/gs could be a useful gauge of variation in whole plant WUE (Boominathan, 2001). Poor relationship between intrinsic WUE (A/gs) and WUE measured gravimetrically was found in Cowpea signifying that instantaneous measure of WUE, however dynamic but not constant, hence do not relay with season long WUE (Bindumadhava, 2000).

2.5 Strategies to discover QTLs and for pyramid drought adaptive traits

The genome databases have been accumulating since the first rice genome mapping and sequence data of various rice varieties. High through put and high-performance genotyping availability has also helped to increase the amount of rice linkage maps and genetic stocks (McCouch *et al.,* 2002; Fischer *et al.,* 2012). Drought study in rice is getting more attention because of these data heaves that open the door to the identification of QTLs and hence drought tolerance genes.

Identifying QTLs based solely upon traditional phenotypic assessment is inspiring. Further, identification of agronomically important QTLs and their use in crop

improvement involves mapping these QTLs with molecular markers in a crop species genome. It is helpful to identify QTLs with genetically related DNA markers to incorporate genes into enhanced cultivars through marker-assisted selection (MAS), map-based cloning of tagged genes, and to improved understand the genetics of complicated characteristics (Asins, 2002). The two most commonly used methods for QTL mapping are linkage analysis and association mapping. The QTL assessment is based on the concept of identifying a marker genotype connection. Markers are used to split the mapping population into distinct genotypical groups based on the existence or lack of a specific marker locus and to determine whether there are important variations in the measured characteristic between groups (Tanksley, 1993; Young, 1996). A significant difference between phenotypic means of the groups, depending on the marker system and type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait.

Quantitative traits such as roots and WUE that have been hard to enhance in rice because they are regulated by many genes (Feldman *et al.,* 2018). Each gene with a tiny impact and are extremely affected by the environment. In such cases, in another sector or background, the lines that do well in one year or in one context may fail. These limitations, however difficult, make the prospect of marker-assisted selection (MAS) very appealing for these traits. The mixture of association mapping and linkage mapping can therefore provide the authority and resolution to detect stable QTL for interest characteristics (Krill *et al.,* 2010; Manenti *et al.,* 2009). Ultimately, the efficacy of any MAS operation will rely on a thorough strategy designed to develop superior varieties of rice with increased drought tolerance (Figure 2.2).

Over the years, progress in the methodology of molecular genetics has led to widespread use of codominant molecular markers, in particular Simple Sequence Repeats (SSRs). Over the previous 20 years, SSRs have been the most commonly used markers for genotyping crops because they are extremely informative, codominant, multi-allele genetic markers that can be experimentally reproduced and transferred between associated species (Mason, 2015).

In specific, SSRs are helpful for wildlife species (i) in genetic distance-based diversity research; (ii) gene flow estimation and rate crossing; and (iii) in evolutionary studies, primarily to infer intraspecific genetic relationships. On the other side, SSRs are frequently used for (i) building linkage maps for cultivated crops; (ii) mapping loci involving quantitative characteristics (QTL); (iii) estimating the degree of kinship; (iv) using marker assisted selection (v) defining DNA fingerprints for cultivars (Jonah *et al.,* 2011; Kalia *et al.,* 2011). SSRs have been useful for creating integrated maps for plant species in which full-sub families are used for constructing linkage maps (Garcia *et al.,* 2006; Souza *et al.,* 2013; Pereira *et al.,* 2013), and for merging genetic, physical, and sequence-based maps (Temnykh, 2001), providing breeders and geneticists with a tool to link phenotypic and genotypic difference (Mammadov *et al.,* 2012; Hayward *et al.,* 2015).

The chief limiting issue in QTL mapping is the precision localization of QTL n which depends on the number of progenies in the study. After identifying QTLs, projected effects of the identified QTLs are often varying with different genetic backgrounds. An over estimate of the QTL effects in limited progenies leads to wrong guesses, a phenomenon called the 'Beavis effect' (Bernardo, 2008; Xu, 2003). Hence, the challenge for molecular breeders is to discover stable heritably and major QTLs that function independently of genetic background, and further to develop an effective breeding method for the utilization of such QTLs under drought condition. To provide a most stable and reliable forecast of QTL effects, reasonable population size, replicated field trials from multi-sites and across seasons are typically required. Consistent molecular markers and better statistical systems are also vital to address the problem (Kearsey and Farquhar, 1998).

Complex traits have been the goal for slow but steady breeding gain over the past century. Association mapping (AM) has arisen as a promising method for complex trait dissection (McCouch and Jung, 2013) and it emphases on association within populations of different individuals, which inspects a collection of diverse accessions viz., varieties, landraces and breeding lines without generating mapping populations. These accessions represent historical recombination events thus most of the alleles whole represents either

strong linkage or linkage disequilibrium (LD). Further, such populations also disclose significant allele diversity and hence, the population genetics-based LD mapping would surge the resolution of QTL (Yu & Buckler, 2006; Abdurakhmonov & Abdukarimov, 2008; Rafalski, 2010). In this approach, QTL discovery for varied traits can be attained simultaneously with the identification of specific trait donor genotypes. This led to the identification of robust markers associated with the traits. Markers and trait donor genotype identification exemplified the advantages of AM over linkage mapping in discovering markers for adaptive traits. Introgressions of several diverse drought-adaptive traits on to elite popular genetic background using stable multi trait QTL donors could help to boost the efficiency of molecular breeding and also to sustain yield under resource limited conditions.

Although diverse groups of distinct individuals are considered in association mapping, a subtle kinship among individuals is inevitable. This kinship results in false discovery of marker-traits association. A better worry is the possibility of neglecting these QTLs. These potentially hazardous errors are often mentioned to as type I and type II errors, respectively (Rafalski 2010). Type I error refers to detection of false marker-trait association and type II error is probability of missing genuine causal association. Edward Buckler and his co-workers advanced a novel statistical method to circumvent these faults. They confirmed that evaluating the kinship between individuals can meaningfully overcome false positive association among markers and traits. They referred this strategy as association in structured population. Germplasm assemblies of diverse genetic families and with different collection histories, likely differ in their alleles (Bernardo 2008) and the same QTL would be probable to be present in different populations, assuming that the particular QTL is consistent. By applying all the historic recombination events from germplasm development, association mapping can deliver a high-resolution genetic map (fine mapping) and deliver more precise sites of individual QTL (Oraguzie *et. al*., 2007; Maccaferri *et. al*., 2011), or a step near to positional cloning (Rafalski, 2010), which is more interesting but more gratifying refining quantitative traits (Sorkheh *et. al*., 2008). Association study in species with low rates of noticeable recombination (inbreeding species and those with low levels of DNA level diversity) can

be carried out with a reasonable number of markers evenly dispersed across the genome via genome scanning. Self-pollinated species (rice) normally have fewer levels resolution and experience low levels of recombination and thus a high linkage disequilibrium (late breakdown of linked genomic regions).

2.6 Backcross breeding strategy for improving drought resistance

2.6.1 Conventional backcross breeding

The backcross breeding method was proposed by Harlan and Pope. Now backcrossing has develop an extensively us ed plant breeding method in various crop species (Allard, 1999). This method is most commonly used for incorporating one or few traits into an adapted or elite variety. Crop varieties with better drought resistance and WUE have been developed through conventional backcross breeding method. For example, in rice, the International Rice Research Institute made a total of 322 crosses between three elite recurrent parents (IR64, Teqing, and IR68552-55-3-2) and 163 donor varieties of diverse origins (Yu *et al*., 2003) to develop varieties. The backcross progenies derived from these crosses that showed much higher yields compared to the recurrent parents in severe-drought lowland or upland nurseries (Laffitte *et al.*, 2006). Likewise, to develop new rice varieties with improved drought resistance and high yield, significant crossing and backcrossing using elite paddy rice varieties as recurrent parents and upland drought-resistant rice as donors. In recent years, a sequence of water-saving and drought-resistant rice varieties have been advanced and released, such as Huhan3, Huhan2B, and Hanyou3 (Luo, 2010). Because of the polygenic nature, low heritability, and high levels of genotype–environment interface of drought resistance linked traits, the advancement of conventional breeding in crops is still quite slow (Luo, 2010).

2.6.2 Marker Assisted Backcross (MABC) breeding

With the accomplishment in Marker Associated Selection (MAS) in crop breeding for simple traits, namely *R* genes for drought resistance, the Mas technology has also been used in breeding programs to advance crop drought resistance. Molecular markers that are strongly linked with economically vital traits have been used for MABC in rice including resistance of bacterial blight, blast, brown plant hopper, green plant hopper,

gall midge, virus infections and tolerance to salinity, submergence, drought, cold, semi-dwarf, grain quality and used in MAS.

The usual approach of MABC is to introgress QTLs from drought-resistant donor genotype to high-yielding but drought-sensitive recipient line. There are three major types of selection in MABC i.e. foreground, recombinant and background selection. Foreground selection, is first step in which the breeder chooses plants having the marker allele of the donor parent at the target locus. The aim is to sustain the target locus in a heterozygous form (one donor allele and one RP allele) till the final backcross is finished. The selected plants are self-pollinated and progeny plants recognized that are homozygous for the donor allele. Those markers which have already been developed and they are strongly linked to the target gene or QTL should be used to select the target locus of donor parent in early back crossed (BC) progenies for the selection of plants that are having the target gene. This is known as 'foreground selection'.

The second level includes selecting backcross progeny with the target gene and recombination events between the locus and linked flanking markers is called as 'recombinant selection' (Collard, 2007). The main drive of recombinant selection is to decrease the size of the donor chromosome segment covering the target locus. This selection is significant because the rate of reduction of the donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent (Hospital, 2005). By use of conventional breeding methods, the donor section can remain very large even with many backcross generations (e.g. >10 cM; (Salina *et al.*, 2003). Using markers that flank a target gene (e.g. <5 cM) on either side), linkage drag can be diminished.

A CSSL (Chromosome Segment Substitution Line) population, advanced mapping population, is generally developed through advanced backcrossing, selfing, and then marker-assisted selection (MAS) (Qiao *et al.,* 2016). Each line consists of a single or a few chromosomal segments from the donor parent in the background of the recurrent parent. These populations can be used to estimate individual QTLs precisely owing to negligible genetic background noise. Thus, they are adequate for fine mapping and

characterization of target QTLs and further analyses. Further, phenotypic effects of QTLs are always influenced by genetic backgrounds and environmental factors. Therefore, the development of advanced mapping populations such as introgression lines (ILs) and chromosome segment substitution lines (CSSLs) to analyze QTLs has received great attention (Qiao *et al.,* 2016).

Chromosome Segment Subsitution Line from interspecific hybridization represents a powerful genetic resource for genomic research. The first complete set of substitution lines were constructed in tomato by Eshed and Zamir, 1994. These consisted of near isogenic lines (NILs) carrying single *Lycopersicon pennellii* chromosomal segments in an otherwise homogeneous *L. esculentum* background which represented the entire genome of wild tomato. Hirabayashi *et al.,* 2010 developed introgressed lines (ILs) from *O. rufipogon* and *Oryza glumaepatula* in a japonica cultivated rice background firstly.

2.7 QTLs mining to identify novel genes

Genetic studies of many traits in rice have generated data suggesting that specific regions of rice chromosomes contain sites that influence expressions of quantitative traits or quantitative trait loci (QTL). QTL mapping has found more than 9100 genomic regions in rice for various traits. While GRAMENE is a endless resource for significant plant genomes including rice, it alone does not deliver an easy way for QTL-based biological mining information. Selection of candidate genes depends on a wealth of information obtained through traditional genetical and molecular approaches and this biological info on rice are deposited on the public domain databases (Table 2.6). Scientists uses these databases to apply bioinformatics methods and data integration systems to find the maximum promising candidate genes for the trait in question and to elucidate functions of such genes. In QTL and under the molecular regulations, several public resources were created for gene mining (Table 2.6). It offers biologically supported evidence vital for targeting gene groups or networks engaged in regulating QTL-based characteristics (Thongjuea *et al.,* 2009; Nagamura *et al.,* 2011).

Table 2.6: List of rice databases available for crop improvement

Sl. No.	Database name	Database Link
1	Rice genome sequence data	*www.msu.edu, www.bgi.org*
2	Full-length cDNA clones	*http://cdna01.dna.affrc.go.jp/cDNA/*
3	Rice biological and molecular data	*www.gramene.org, www.graingenes.org*
		http://www.ncgr.ac.cn/ricd
		http://cdna01.dna.affrc.go.jp/PIPE
4	Rice annotation	*http://rice.plantbiology.msu.edu/*
		http://rapdb.dna.affrc.go.jp/
		http://ricegaas.dna.affrc.go.jp/
		http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp
5	Transcription factor	*http://drtf.cbi.pku.edu.cn/*
		http://ricetfdb.bio.uni-potsdam.de/
6	Rice expression	*http://cdna02.dna.affrc.go.jp/RED/*
		http://mpss.udel.edu/
		http://www.ricearray.org/index.shtml
		http://ricexpro.dna.affrc.go.jp/
7	Rice co-expression	*http://ricefrend.dna.affrc.go.jp/*
8	Comparative genomics	*http://greenphyl.cirad.fr/*
9	Mining genes from QTLs	*http://rice.kps.ku.ac.th:8080/RiceGeneThresher*
		http://agri-trait.dna.affrc.go.jp/
10	Rice mutants	*http://rmd.ncpgr.cn/*
		http://www.ricefgchina.org/mutant
11	Small RNA	*http://sundarlab.ucdavis.edu/smrnas/*
		http://retroryza.fr/
12	Rice proteome	*http://gene64.dna.affrc.go.jp/RPD/main_en.html*
13	BAC/EST resources	*www.genome.arizona.edu*
14	Phenotype of the mutants	*http://tos.nias.affrc.go.jp/*
15	SNP resources	*http://www.plantgenome.uga.edu/snp*
		http://www.ricesnp.org/
		http://shenghuan.shnu.edu.cn/ricemarker
16	Rice diversity	*http://www.ricediversity.org/*
17	RiceVarMap	http://ricevarmap.ncpgr.cn/v2/
18	Rice TE database (RiTE-db)	https://omictools.com/rite-db-tool
19	DroughtDB	http://pgsb.helmholtz-muenchen.de/droughtdb

The MSU Rice Genome Annotation Project Database and Resource is a National Science Foundation project that provides sequence and annotation data intended for the rice genome. This database provides rice genome sequence from the Nipponbare subspecies and annotation of the 12 rice chromosomes. These data are available through search pages and our Genome Browser that provides an integrated display of annotation data.

Several such rice databases have been intended and created to incorporate catalogs from public domain rice databases involving genetic information, genome annotation, expressed sequence tags (ESTs), protein data like protein domains, gene ontology (GO), metabolic pathway, protein-protein interaction prediction, and stress-responsive genes. Table 2.6 tabulates the list of rice databases cited in the literature. RiceGeneThresher is also one of the system's embedded information sources and offers strong web-based apps and versatile instruments to deliver tailored rice biological information (Thongjuea *et al.*, 2009). This scheme promotes full-genome gene mining for QTLs by enquiring marker intervals and biologically endorsed evidence that is critical to targeting clusters or networks of genes engaged in regulating QTL characteristics.

Anybody may navigate from QTL to candidate genes and from candidate gene to SNP discovery by using these accessible database resources (Table 2.6). *Insilico* assessment showed the significance of genomic data to identify and isolate novel and superior alleles of agronomically significant genes from plant gene pools in order to be appropriately deployed to develop enhanced varieties. Allele mining is a hopeful approach to dissecting naturally occurring allelic variation in candidate genes that control important agronomic characteristics that have potential uses in crop improvement programs. It enables to track allele evolution, identify fresh haplotypes, and develop allele-specific markers aimed at use in marker-assisted selection. So, the mixture of connection and linkage mapping would lead to the detection of markers from genic region and/or candidate genes, which would lead to the documentation of the most efficient marker allele contribution. Introgressing QTLs superior allelic variety (haplotype) would considerably improve MAS's achievement in introgressing the tolerance of drought.

III MATERIAL AND METHODS

Exponentially growing population, water scarcity with increasing demand, climate change and biotic & abiotic stresses are major hazards to food security. Rice, which is the major staple food crop, is also a water intensive crop. Several efforts have been taken to develop water saving strategies in rice plants. Drought is climatically induced calamity that slows down rice yield production in semi-arid, arid and humid areas. Tackling drought by plants can provide different way to rice research and development. An effective breeding approach that develops drought-tolerant rice varieties can lead to food security under situations of higher food demand, depleting resources and predicted climate variations. Though a huge number of published articles are accessible for drought associated QTLs and candidate genes, a few QTLs have been used in Marker assisted selection (MAS) for development in drought traits. In a previous study a successful effort was made to introgress two drought adaptive traits (root along with water use efficiency) into the elite cultivar, IR-64 (Dharmappa *et al.*, 2019). However, the effect of selected genetic loci in a pyramiding program is not always as expected, due to the complexity of gene networks among genetic regions (Kumar *et al*., 2018). Apart from this, quantitative traits such as roots and WUE are highly influenced by the environment. In such conditions, in another field or background, lines that do well in one environment or in one genetic background may fail. These constraints led to future line of work to check the stability of performance of TILs/NILs across seasons and to identify the interaction among traits. This chapter describes various experiments carried out to study interaction among traits, identification of best trait introgressed lines and molecular network responsible for variation in trait.

A set of 173 diverse rice (*Oryza sativa* L.) germplasm accessions were obtained from National Rice Research Institute (NRRI), Cuttack. Panel has been extensively phenotyped for root, water use efficiency, yield and other associated morpho-physiological traits in previous studies (Mohankumar, 2010). From the germplasm accessions, trait donor lines for drought adaptive traits were identified. Among the various trait donor lines, AC-39020 and IET-16348 was selected as root and water use efficiency donor, respectively. Using the selected donors, focused multi-parent marker

assisted backcrossing strategy was developed to pyramid root and WUE traits into IR-64 background. Sixteen markers were used in foreground selection and 120 neutral markers were used for background selection. Selected donor parents (AC-39020 and IET-16348) were hybridized separately with IR-64. The resulting F_1s were hybridized to get DCF_1s. True F_1s were identified using foreground markers and the selected plants that were heterozygous were backcrossed to IR-64 to get the $DCBC_1F_1$s. Phenotypic and molecular characterization of all $DCBC_1F_1$ and consequently backcrossing with recipient parent and selfing followed by selection leads to development of 260 $DCBC_3F_4$ progenies (Dharmappa *et al.,* 2019) (Fig. 3.1).

3.1 Plant Material

A set of 260 $DCBC_3F_4$ (TILs) lines along with the three parents was the plant material used in the study.

3.1.1 Phenotyping for drought adaptive traits

All the 260 $DCBC_3F_4$ lines and the parents (recurrent and donor) were evaluated for various drought adaptive traits at Dept. of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore, India. Climatic conditions prevailed during the cropping season are given in Table 3.1.

Table 3.1: Details of the experimental location

Experiment	Details
Place	Bengaluru
Year and season	2016 (Kharif)
Latitude and longitude	130 05’N;770 34’E
Altitude (mean sea level)	930
Site/conditions	Aerobic condition
Soil type	Red loamy

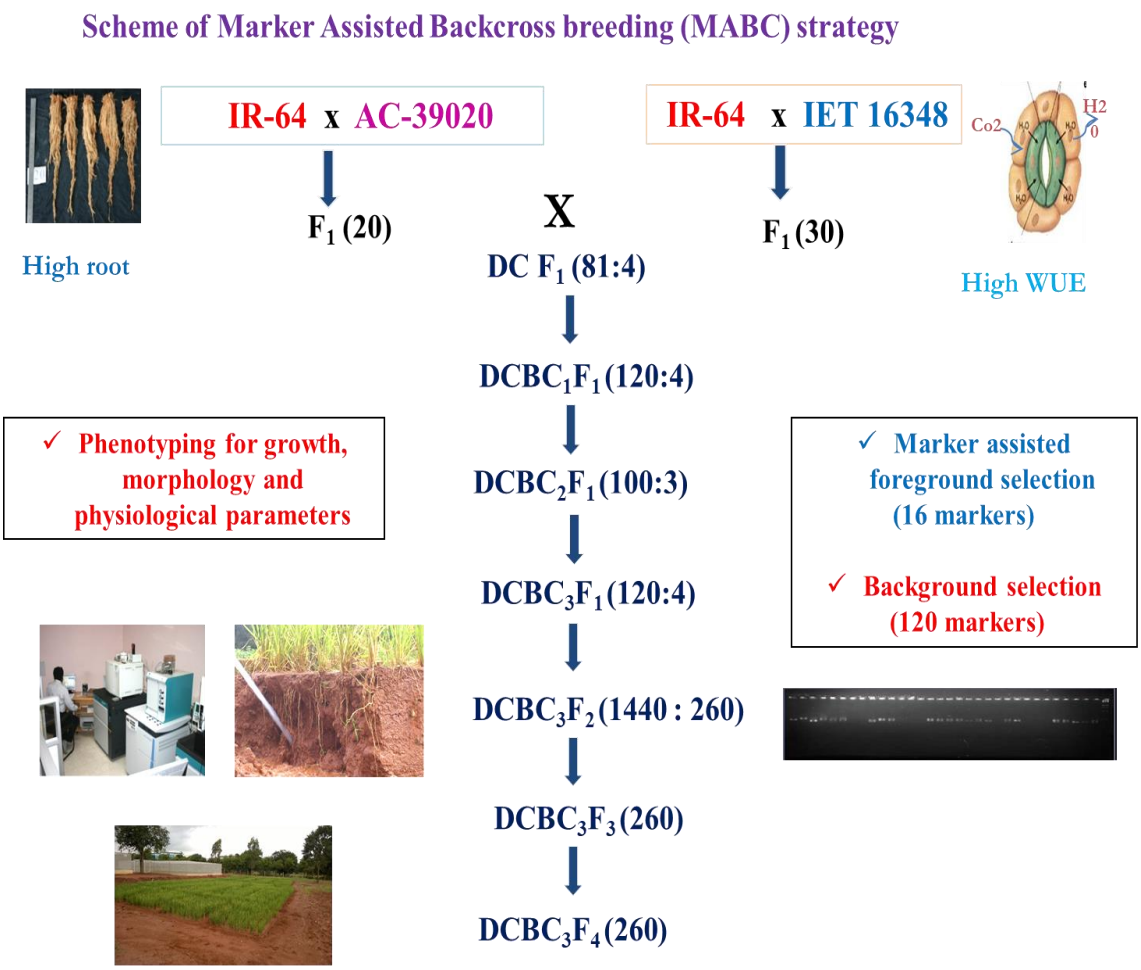

Figure 3.1: Developmental scheme of marker assisted backcross breeding strategy (Prathibha, 2016)

3.1.2 Experimental details

Phenotyping for drought adaptive traits was conducted in the experiment (Plate 3.1). The morpho-physiological traits recorded during experiments are listed in the Table 3.2 with abbreviations and units.

3.1.3 Crop husbandry

The trait introgressed lines along with its parents were grown in the field. The spacing of 20 cm was maintained in between plants and rows. Plants were grownup in augmented design using parents as checks. Plant was provided irrigation alternatively through surface level irrigation. The method of aerobic cultivation was according to Girish *et. al.,* (2006). Cultural operations such as protection measurements, accurate nutrient supply through fertilizer application were followed as per recommendation. All essential prophylactic measures were opted to ensure the development of healthy plants in the experiment.

METHODS USED FOR PHENOTYPING IN FIELD CONDITION

Carbon isotope discrimination (CID):

Carbon isotope discrimination, an important surrogate to measure WUE was measured using an Isotopic Ratio Mass Spectrometer (Thermo Fischer scientific, Germany) interfaced with an elemental analyzer (NA112, Italy) by a continuous flow device (Conflo-III, Thermo Fischer scientific) is installed at Dept of Crop Physiology, UAS, GKVK, Bengaluru. Dried leaf samples were homogenized to a fine powder using ball mill. CID ($\Delta^{13}C$) which is expressed in per mil (‰), was calculated as per by Fraquhar, *et, al.,* 1989.

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_p) / (1+\delta^{13}C_p/1000)$$

Where; $\delta^{13}C_a$ and $\delta^{13}C_p$ are the carbon isotope composition of atmospheric air and plant, respectively. Further, the $\delta^{13}C_a$ was measured as -8 parts per mill for the computation.

Table 3.2: Morpho-physiological traits recorded in field under aerobic condition

Sl. No.	Parameters	Method / formula	Abbreviation with Unit
1	Plant height	Height from soil surface/ground to leaf tip of main tiller	PH, cm
2	Stem weight	Dry weight of stem after oven drying	SW, g
3	Total Leaf weight	Dry weight of leaf after oven drying	TLW, g
4	Total Leaf Area	Product of specific leaf area (SLA) and total leaf dry weight	TLA, cm^2
5	SPAD chlorophyll meter reading	Transmittance of light from the leaf blade	SCMR (unit-less)
6	Specific Leaf Area	Ratio of area of leaf to leaf dry mass	SLA, cm^2/g
7	Total Dry Matter	Summation of above ground biomass and below ground biomass	TDM, g pl^{-1}
8	Carbon isotope discrimination	Measure of carbon isotopic composition in plant	$\Delta^{13}C$, per mill
9	Tiller number	Number of productive tillers per plant	NT, per hill
10	Days to fifty percent flowering	Number of days between sowing and 50% flowering	DFF, days
11	Leaf temperature	Temperature of the leaf	LT, degree Celsius
12	Grain yield	Weight of grains per plant	YPP, g
13	Spikelet fertility	The ratio of number of filled spikelets to total number of spikelets	SF, percentage (%)
14	Harvest index	Ratio of economical biomass to the biological biomass	HI, unitless
15	Panicle number	Number of panicles present in a plant	NPP, per hill
16	Number of filled grains	Number of filled seeds per plant	NFG, numbers
17	Number of chaffy grains	Number of unfilled seeds per plant	NCG, numbers
18	Weight of filled grains	The total weight of all the filled grains per plant	WFG, numbers
19	Weight of chaffy grains	The total weight of all the chaffy grains per plant	WCG, numbers

Plate 3.1: Field view of 260 DCBC$_3$F$_4$ plants grown under semi-irrigated aerobic condition. DCBC$_3$F$_4$ plants along with the parents were grown under semi-irrigated aerobic condition.

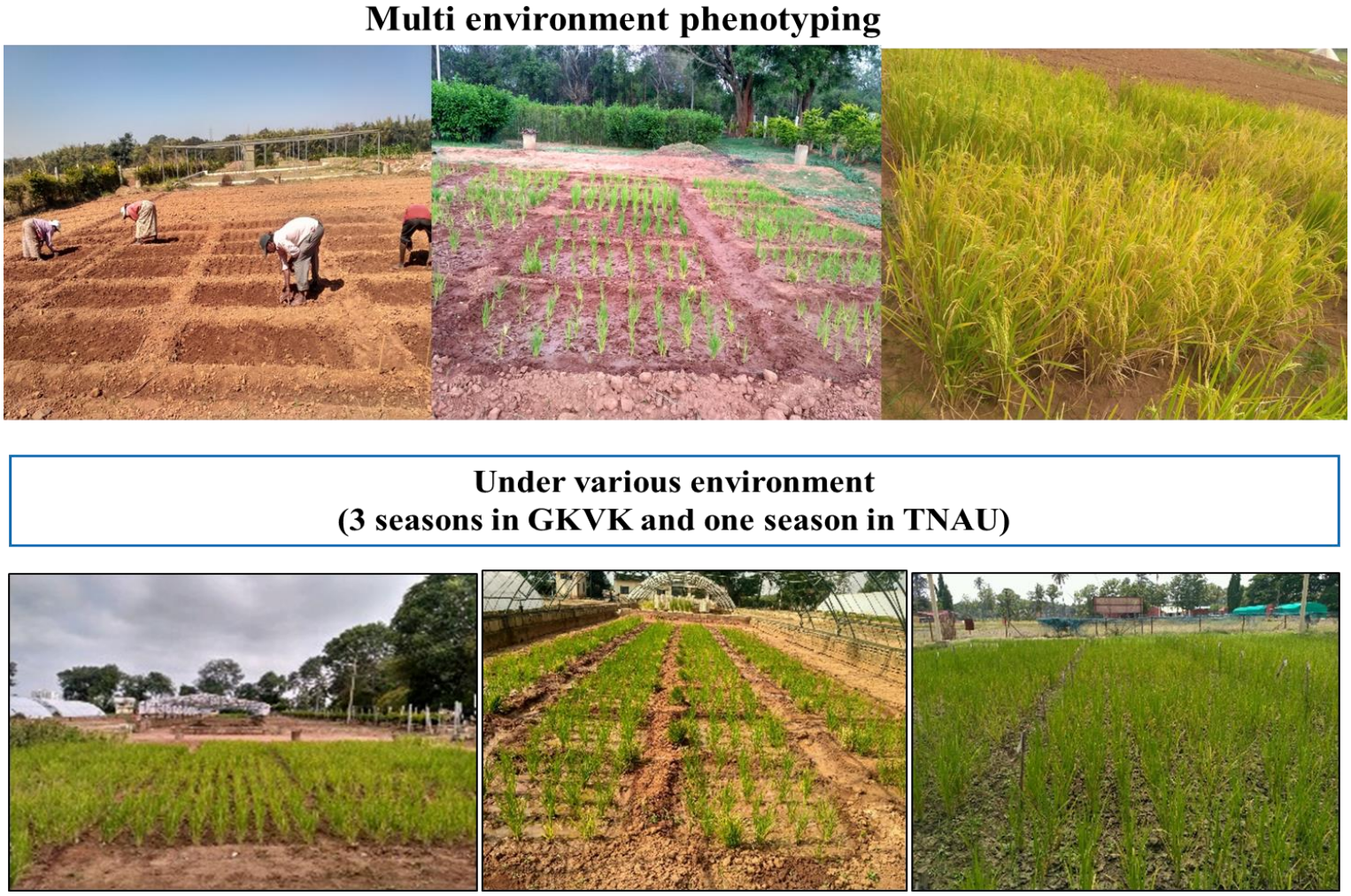

Plate 3.2: Field view of 25 TILs grown under semi-irrigated aerobic condition in various seasons. TILs along with the parents were grown under semi-irrigated aerobic condition in 1*1 m^2 plot size.

Leaf temperature:

Temperature of leaf was measured with the help of infra-red gun during the field experiment. The observation was recorded twice a day during the crop cycle. Leaf temperature (LT) was measured before irrigation. Gun was held focusing towards the leaf surface or canopy and the measurements were done at the midday when there was complete bright sunlight.

Soil Plant Analysis Device (SPAD) chlorophyll meter reading (SCMR) and Specific Leaf Area (SLA):

The leaf nitrogen content reveals us about chlorophyll content present in the leaf. An instrument developed to measure leaf nitrogen content using Minolta Corp., Ramsey, N.J., measures light attenuation at a wavelength of 430 nm (the peak wavelength for the absorption by chlorophyll a and b) and at 750 nm (near-infrared) with zero transmittance. The unit less measurement taken by chlorophyll meter (SPAD-502) is known as SCMR (SPAD Chlorophyll Meter Reading) and it is a good estimation of chlorophyll content and N content. The SPAD is a user-friendly hand-held device that is portable and works with DC energy (Volts).

Three observations were noted on each leaf (9 observations/3 leaves) using SCMR (Minolta SPAD-502 meter, Japan). Necessary precaution was taken to reassure and to avoid the interference with the midrib. The same leaves, i.e. 3 representative leaves from individual plant were collected by cutting at the joint of leaf petiole and further the leaf area was determined by multiplying maximum leaf length (LL, cm) and maximum leaf width (LW, cm) of each sample to record SLA (cm^2 g^{-1}). The same samples were gathered in distinct paper bags for drying and oven dried at 70 °C until sample reaches steady dry weight. SLA was calculated using the area of three leaves (cm^2) and the dry weight of the same three leaves (g).

OBSERVATIONS RECORDED IN FIELD EXPERIMENTS

Plant height:

Plant height was measured as height difference from base to the tip of longest leaf at the time of tillering stage and expressed in cm.

Specific leaf area (SLA):

SLA is the ratio of leaf area to leaf biomass which is measured at 50 per cent flowering. Leaves representing the entire canopy were picked and the area was recorded by l*b method. The same leaves were oven dried at 70 °C for 3 days and the constant dry weight was documented. The ratio of leaf area to leaf biomass had given SLA.

Carbon isotope discrimination:

The leaf samples collected for SLA was finely powdered and powder is used to estimate Carbon isotope discrimination at the National facility for stable isotopes at Department of Crop Physiology, UAS, GKVK, Bengaluru.

Shoot weight:

Shoots collected separately and oven dried at the temperature of 80^{o}C for 48 hrs to measure the dry weight in gram.

Total leaf area:

All the leaves collected separately and further oven dried at 80^{o}C for 48 hrs to determine the leaf dry weight. The total leaf area was computed by multiplying leaf dry weight with SLA.

Total dry matter accumulation (TDM):

The biomass accumulated over the experimental time was computed by summing up leaf, shoot dry weights and yield.

Per cent spikelet fertility:

Spikelet fertility can be calculated by means of both number of filled and chaffy spikelets and also the weight of filled and chaffy spikelets (Raju *et al.,* 2012).

$$\text{Spikelet Fertility (\%)} = \frac{\text{Number of filled seeds}}{\text{Number of chaffy seeds + Number of filled seeds}} * 100$$

Yield:

Grain yield per plant is measured at physiological maturity through measuring weigh of grains collected from each plant.

3.2 Plant Material

A set of 25 TILs/NILs along with the three parents was the plant material used in the study.

3.2.1 Phenotyping for drought adaptive traits

Selected 25 TILs with the parents were evaluated for various drought adaptive traits in various seasons and locations. Climatic conditions prevailed during the cropping seasons are given in Table 3.3.

Table 3.3: Details of the experimental locations for 25 TILs

Experiment	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Place	Bengaluru	Coimbatore	Bengaluru	Bengaluru
Year and season	2016 (Kharif)	2017 (Summer)	2017 (Summer)	2018 (Summer)
Latitude and longitude	130 05’N;770 34’E	11’ N ;77’ E	130 05’N;770 34’E	130 05’N;770 34’E
Altitude (mean sea level)	930	426.7	930	930
Site/conditions	Aerobic condition	Aerobic condition	Aerobic condition	Aerobic condition
Soil type	Red loamy	Clay loamy	Red loamy	Red loamy

3.2.2 Experimental details

Phenotyping for drought adaptive traits was carried out in the field experiment (Plate 3.2). All the morpho-physiological traits recorded during experiments are listed in the Table 3.2 with abbreviations and units.

3.2.3 Crop husbandry

The trait introgressed lines along with its parents were directly sown in the field. The spacing of 20 cm was maintained between plants and rows. In all four seasons TILs and parents were raised in blocks of 1m*1m size with three replications of blocks in Randomized Completely Block Design (RCBD). Plot was irrigated alternatively by surface irrigation. Aerobic cultivation was as per the method followed by Girish *et. al.*, (2006). Cultural operations such as protection measurements, accurate nutrient supply through fertilizer application were followed as per the recommendation. Necessary prophylactic measures were carried out to ensure the development of healthy plants in the experiment.

Details of morpho-physiological traits recorded in field under aerobic condition is listed in Table 3.2.

Statistical analysis for field experiments

The data obtained from above experiments were analyzed using statistical software packages like XLSTAT, SPAD and MS EXCEL.

Analysis of variance (ANOVA)

Analysis of variance for measured traits for parental lines and progenies was done using windostat. Variance components due to the influence of genotype (d^2g) and genotype x environment (d^2g_e) were predicted using the corresponding average of the variance coefficients.

Comparison of means and variances

For separate experiment, mean, range and variances were calculated for the whole set including (parental lines and progenies). For means comparisons throughout the study were based on Newman-Keuls test (Newman, 1939; Keuls, 1952) and the similarity of variances was tested using SAS / SAST2 ® 9.3 by the Levene (1960) test.

Correlation analysis

Phenotypic correlations for all the traits were calculated among them for entire population across each experiment using XLSTAT.

3.3 Marker class analysis

Based on previous genotypic data with 16 foreground markers (Table 3.4) and current phenotypic data, marker class analysis was carried out with 260 $DCBC_3F_4$ lines. Genotypic data coding was done with "Y" and "N" letters based on the alleles present from donor and other parents. Donor alleles (allele of AC-39020 for root traits and IET-16348 for WUE) was denoted as "Y" and other than donor alleles with "N". Coded data is given in plate 3.3.

3.4 Physiological characterization of TILs to evaluate the drought stress response

A mini-lysimeter based phenomics platform was conceptualized at our center (Department of Crop Physiology, UAS, GKVK, Bangalore) for the precise maintenance of soil and plant moisture status across large number of accessions. The approach involves the real time measurement of transpiration and soil moisture content in container grown plants exposed to natural field environment conditions. The evapotranspiration (ET) interfaced replenishment of water provides a additional to maintain the desired soil moisture level across the genotypes irrespective of variation in transpiration. It also consists of an indigenously built, remotely operated mobile rain out shelter.

The system continuously captures the weight loss in mini-lysimeters due to evapotranspiration, in real-time and brings it back to the original weight by automatically irrigating with equivalent water (w/w). Hence, the facility has a completely automated

ET- interfaced irrigation system. It has custom built 'drought simulator software' to monitor the field capacity of each MLM individually. The facility can execute a desired dry down rate in the MLMs to achieve gradual induction of stress, as it occurs in the natural field scenario. In the similar way the facility also has the ability to rehydrate the MLMs gradually at desired rate.

Table 3.4: List of markers used for marker class analysis

	Marker	**Chr. no**	**Position**	**Traits**	**R^2**
Root	RM80	8	103.7	RLD	19
	RM2584	8	45.8	RWT	14.5
	RM1388	4	77.9	RLD	17.2
	RM262	2	81.1	RLD	14.5
	RM239	10	25.2	R/S	13.8
	RM3825	1	143.7	RV	13
	RM16	3	131.5	RV	10.8
	RM3276	4	102.4	RL	13.3
	RM247	12	32.3	RV	12.1
	RM167	4	37.5	RLD	16.7
	RM4455	10	21.8	RV	20.4
	RM71	2	49.8	R/S	10.1
WUE	RM493	1	79.9	$\Delta^{13}C$	14.2
	RM586	6	7.4	$\Delta^{13}C$	17.4
	RM149	8	122.1	$\Delta^{13}C$	15.4
	RM131	4	148.8	$\Delta^{13}C$	18.3

	RLD	RWT	R/D	RLD	R/S	RV	RV	RL	RV	RLD	RV	R/S	D13C	D13C	D13C	D13C			
TIL#	RM80	RM2584	RM1388	RM262	RM239	RM3825	RM16	RM3276	RM247	RM167	RM4455	RM71	RM493	RM586	RM149	RM131	Root markers	D13C marker	Total
1	Y	N	N	Y	N	Y	Y	N	N	N	N	N	Y	N	N	N	3	1	4
2	Y	N	N	N	N	Y	N	N	N	N	N	N	Y	N	N	Y	2	2	4
3	N	N	N	N	N	N	N	N	N	Y	N	N	Y	Y	N	Y	1	3	4
4	Y	Y	N	N	N	Y	N	N	N	N	N	Y	Y	Y	N	Y	4	3	7
5	Y	Y	N	N	Y	Y	N	N	N	N	N	N	Y	N	Y	Y	4	3	7
6	N	Y	N	N	N	N	Y	Y	N	N	Y	N	Y	Y	N	Y	4	3	7
7	N	Y	N	N	N	Y	N	N	N	N	N	N	Y	Y	Y	Y	2	4	6
8	N	Y	N	N	N	N	Y	N	N	N	N	N	Y	Y	Y	Y	2	4	6
9	N	Y	N	N	N	Y	Y	N	N	N	Y	N	Y	Y	Y	Y	4	4	8
10	N	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	Y	N	Y	4	3	7
11	N	Y	Y	N	N	N	Y	N	N	N	N	N	Y	N	Y	Y	3	3	6
12	N	Y	Y	Y	N	Y	N	N	Y	N	N	N	Y	N	Y	Y	5	3	8
13	N	Y	Y	N	N	N	Y	N	N	N	N	N	Y	N	Y	Y	3	3	6
14	N	Y	Y	N	N	N	Y	N	N	Y	N	N	Y	N	N	Y	4	2	6
15	N	Y	Y	N	Y	N	N	N	N	Y	N	N	Y	N	Y	Y	4	3	7
16	N	N	N	N	N	N	N	N	N	Y	N	N	Y	N	N	Y	1	2	3
17	N	Y	N	N	N	N	N	N	N	Y	N	N	Y	N	N	Y	2	2	4
18	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	Y	N	N	Y	5	2	7
19	Y	N	Y	N	N	N	Y	N	Y	Y	N	N	Y	N	Y	Y	5	3	8
20	N	Y	Y	N	N	N	Y	N	Y	Y	N	N	Y	N	N	Y	5	2	7
21	Y	N	Y	Y	N	N	Y	N	N	N	N	N	Y	N	N	Y	4	2	6
22	Y	N	Y	N	N	N	N	N	N	N	N	N	Y	N	Y	Y	2	3	5
23	N	N	Y	N	N	N	Y	N	N	Y	N	N	Y	N	N	Y	3	2	5
24	Y	N	Y	N	N	N	N	N	Y	Y	N	N	Y	N	Y	Y	4	3	7
25	N	N	Y	Y	N	N	Y	N	Y	N	N	N	Y	N	N	N	4	1	5
26	N	Y	N	N	N	N	N	N	N	N	N	N	Y	Y	N	Y	1	3	4
27	N	Y	Y	N	N	N	Y	N	Y	Y	N	N	Y	N	Y	Y	5	3	8
28	N	Y	N	N	N	N	N	N	Y	Y	N	N	Y	N	N	Y	3	2	5
29	Y	Y	N	N	N	N	N	N	N	N	N	N	Y	N	N	Y	2	2	4
30	Y	N	N	Y	N	N	Y	N	N	N	N	N	Y	N	Y	Y	3	3	6
243	N	N	Y	N	N	N	N	Y	N	Y	N	N	N	Y	N	Y	3	2	5
244	N	Y	N	Y	N	N	N	N	N	Y	N	N	N	N	Y	N	3	1	4
245	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	0	2	2
246	N	N	N	N	N	N	N	N	Y	Y	Y	N	N	Y	N	Y	3	2	5
247	N	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N	N	N	N	8	0	8
248	N	N	N	N	N	N	N	N	N	Y	Y	N	N	Y	N	Y	2	2	4
249	Y	Y	N	N	N	N	N	Y	N	N	N	N	N	Y	Y	Y	3	3	6
250	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	3	0	3
251	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	0	2	2

Plate 3.3: Marker class analysis. Donor alleles (allele of AC-39020 for root traits and IET 16348 for WUE) was denoted as "Y" and other than donor alleles with "N"

Plate 3.4: Phenomics facility to screen TILs for drought stress. Selected TILs and the recurrent parent were grown in phenomics. Several traits were measured and analyzed under WW and WL condition.

3.4.1 Plant materials

From the 25 TILs studied under various seasons, 6 lines were selected based on the marker/QTL combination. These six TILs (TIL # 18, 54, 55, 84, 228, 248) along with the recurrent parent IR-64 were grown under various moisture regimes in phenomics experiment.

Apart from the six TILs, few more TILs has been selected from 260 $DCBC_3F_4$ lines (Table 4.15) in phenomics experiment 3. These TILs were having the same marker combinations with difference in alleles.

3.4.2 Phenotyping for drought adaptive traits

Selected TILs along with the recurrent parent were evaluated for various drought adaptive traits in various water regimes. Climatic conditions prevailed during the cropping seasons are given in Table 3.5.

3.4.3 Experimental details

Phenotyping for drought adaptive traits was carried out in the experiment (Plate 3.4) at various water regimes. All the morpho-physiological traits recorded during experiments are listed in the Table 3.6 with abbreviations and units.

Table 3.5: Details of the experimental location for phenomics experiment

Experiment	Details
Place	Bengaluru
Year and season	2017 (Summer) , 2017 (Kharif), 2018 (Kharif)
Latitude and longitude	130 05’N;770 34’E
Altitude (mean sea level)	930
Site/conditions	Aerobic condition
Soil type	Red loamy

3.4.4 Crop husbandry

Empty pot weights were measured before pot filling and dry soil was filled to the pots and weights were recorded. Seeds of the selected TILs and parents were soaked overnight in water and treated with bavistin for 15min before sowing. 10 seeds per pot were sown early morning. 100:50:50 kg/hectare N: P: K was applied as per the recommendation. The quantity of fertilizers was planned based on soil volume in the pots. The plant population was thinned down to two plants per pot. In order to circumvent iron deficiency, an equimolar concentration of Fe-EDTA solution was prepared at the concentration of 2 g/L and was added at an interval of every 7 days. All prophylactic plant protection measures were taken periodically as per the recommendation to get healthy plants.

OBSERVATIONS RECORDED IN PHENOMICS EXPERIMENTS

Shoot length:

The shoots were detached from individual plants and the shoot length was documented at tillering stage using a graduated scale in cm.

Root length:

The root was separated from each plant in the internodal region at maturity. The root length was measured with a graduated scale in cm.

Root volume:

Root volume was measured by water displacement method after harvesting at maturity stage. Known volume of water was taken in a graduated beaker and separated root was immersed into the beaker and then the increase in the volume of water was noted as a measure of root volume in ml.

Leaf area:

Leaf area was measured by (length × width × correction factor) method.

Table 3.6: Traits recorded in phenomics under aerobic condition in stress and well watered condition

Sl. No.	Parameters recorded	Method of measurement / formula	Abbreviation & Unit
1	Root Length	Maximum length of root in a plant using the graduated scale	RL, cm
2	Root weight	Root dry weight per plant after oven drying	RW, g
3	Root volume	Root volume measured by water displacement method	RV, cc
4	Root to Shoot ratio	Ratio of root dry weight over shoot dry weight	R/S, ratio
5	Shoot length	Plant height from soil surface to leaf tip of the main tiller	SL, cm
6	Stem weight	Stem dry weight per plant after oven drying	SW, g
7	Total Leaf weight	Leaf dry weight per plant after oven drying	TLW, g
8	Total Leaf Area	Product of specific leaf area and leaf dry weight	TLA, cm^2
9	SPAD chlorophyll meter reading	Transmittance of light from leaf blade	SCMR (unit less)
10	Specific Leaf Area	Leaf area to leaf dry mass	SLA, cm^2/g
11	Total Dry Matter	Sum of above and below ground biomasses	TDM, g pl^{-1}
12	Carbon isotope discrimination	Measure of carbon isotopic composition in plant material relative to the value of the same ratio in the air where the plant is grown (13C/12C)	$\Delta^{13}C$, per mill (‰)
13	Tiller number	Number of productive tillers per plant	NT, per hill
14	Days to fifty percent flowering	Number of days between sowing and 50% flowering	DFF, days
15	Relative water content	Leaf relative water content is an important indicator of plant water status	RWC, percentage (%)
16	Leaf temperature	Temperature of the leaf	LT, degree Celsius
17	Grain yield	Weight of grains per plant	YPP, g
18	Spikelet fertility	Ratio of number of filled spikelets per panicle over total number of spikelets per panicle	SF, percentage (%)
19	Harvest index	Ratio of grain yield to total biomass	HI, Unitless
20	Test weight	Weight of 1000 grains determined using electronic digital balance	TW, 1000 grains
21	Panicle number	Number of panicles present in a plant	NPP, per hill

Specific leaf area (SLA):

SLA is the ratio of leaf area to leaf biomass which is determined at 50 per cent flowering. Leaves representing the whole canopy were taken and the area was recorded by l*b*correction factor method. The same leaves were oven dried at 70 °C for 3 days and the constant dry weight was recorded. The ratio of leaf area to leaf biomass had given SLA.

Carbon isotope discrimination:

The leaf samples collected for SLA was finely powdered and used to estimate Carbon isotope ratios at the National facility for stable isotopes at Department of Crop Physiology, UAS, GKVK, Bengaluru.

Total leaf area:

The leaves were collected separately and oven dried at 80^{o}C for at least 48 hrs to determine the leaf dry weight. The leaves were weighed and the total leaf area was computed by multiplying leaf dry weight with SLA.

Total leaf area (cm^2 p^{-1}) = Total leaf dry weight (g p^{-1}) x SLA (cm^2g^{-1})

Shoot weight:

Shoots were collected separately and oven dried at 80^{o}C for two days to determine the dry weight.

Root weight:

Roots were washed thoroughly, then oven-dried at 80^{o}C for 48 hrs and dry weights were recorded.

Total dry matter accumulation (TDM):

The biomass accumulated during the experimental period was computed by summing up leaf, shoot dry weights, root dry weight and yield.

Per cent spikelet fertility:

Spikelet fertility was calculated by using both numbers of filled and chaffy spikelets as well as weight of filled spikelet and chaffy spikelets.

$$\text{Spikelet Fertility (\%)} = \frac{\text{Number of filled seeds}}{\text{Number of chaffy seeds + Number of filled seeds}} * 100$$

Yield:

Grain yield per plant was measured at physiological maturity by taking weigh of grains collected from each plant.

Leaf temperature:

Leaf temperature of plants was measured using thermal imaging (Fluke) across the phenomics experiments. The observation recorded twice during the crop cycle (mean values of the different timings observation were used for analysis). Leaf temperature (LT) was measured just before irrigation.

Relative Water Content (RWC):

Leaf relative water content is an important indicator of plant water status. The leaf discs from the plants were collected separately and the fresh weight was recorded. The leaf discs were floated on distilled water for 5 hours to attain full turgidity. The turgid weight was taken at the end of 5 hours when the leaf discs were fully turgid. Later, the samples were dried in hot air oven at 70 °C to attain a constant weight and then dry weight was recorded. Based on the observations, RWC was calculated (Barrs *et al.*, 1962).

$$\text{RWC (\%)} = \{(\text{FW-DW})/ (\text{TW-DW})\} \text{ X100}$$

where, FW- Fresh weight, DW- Dry weight, TW- Turgid weight

Based on these primary observations the following parameters were computed:

- Cumulative Water Transpired (CWT)
- Water Use Efficiency (WUE)
- Mean Transpiration Rate (MTR)
- Net Assimilation Rate (NAR)
- Leaf Area Duration (LAD)

Cumulative Water Transpired (CWT)

The amount of water added automatically daily to individual pot after weighing by weighing device at the platform, to bring back the soil to 100% FC and other water regimes was summated individually for each pot during the experimental period and was expressed as cumulative water added (CWA). The daily soil evaporation loss was determined by recording the weights of the empty pots. The soil evaporation was deducted from the CWA to arrive at the cumulative water transpired (CWT).

$$\mathbf{CWT = CWA - CWA^*}$$

Where,

CWA: cumulative water added to each pot

CWA*: cumulative water added to empty pot

Water Use Efficiency (WUE)

Measurement of Water use efficiency by phenomics experiment involves the measurement of total dry matter (TDM) accumulated over a specific period of time and the total water transpired by the plant throughout the same period. The ratio of total dry matter during the experimental period to the total water transpired was calculated to arrive at the whole plant WUE and expressed as g/L of water transpired.

$$\text{WUE} = \frac{\text{Total Dry Matter}}{\text{Cumulative water transpired}}$$

Mean transpiration rate (MTR)

Mean transpiration rate is the measure of rate of transpiration over the complete experimental period. It is expressed as gram or ml of water per $dm^{-2}LA.day^{-1}$. It is considered as the time combined measure of Transpiration.

$$\mathbf{MTR = CWT/LAD}$$

Where,

MTR: Mean Transpiration Rate

CWT: Cumulative Water Transpired

LAD: leaf area functions during the experimental period

Net Assimilation Rate (NAR)

NAR is a good indicator of assimilation capacity per unit leaf area. It is calculated using the dry matter accumulated throughout the experimental period and functional leaf area of the same period. It is expressed as mg. dm^{-2} day^{-1}.

$$NAR = \frac{\text{Total Dry Matter}}{\text{Leaf area functions during the experimental period (LAD)}}$$

Leaf Area Duration (LAD)

Leaf area duration (LAD) is a reflection of assimilation during the experimental period. LAD was calculated as following:

$$LAD = \text{Total Leaf Area} \times \text{Number of days}$$

Photosynthesis related traits

Gas exchange

Gas exchange traits such as net CO_2 assimilation rate (A) was used measured using portable photosynthesis system LI- 6400 (LICOR 6400, Lincoln, Nebraska, USA).

Principle of IRGA

Infrared Gas Analyzer (IRGA) is used for the measurement of hetero-atomic gas molecules including CO_2, H_2O, SO_2, N_2O. Hetero-atomic molecules have characteristic absorption spectra in infrared region. Therefore, absorption of radiation by a specific hetero-atomic molecule is directly proportional to its concentration in the given sample.

LICOR- 6400

The equipment, Li 6400 (LiCOR-Inc. Lincoln, Nebraska, USA), functions in the open mode, which facilitates the maintenance of constant CO_2 and water vapor concentration in the leaf chamber during measurements. The change in the CO_2 concentration and water vapor is determined by separating infrared gas analyzers, which are located in the leaf chamber. This ensures real time measurements of gas exchange parameters.

The system consists of the two parts, main console and a leaf chamber. The main console consists of a peristaltic pump and necessary software for the computation of gas exchange parameters. Software permits precise control of the flow rate, CO_2 and water vapor concentration in the leaf chamber. The leaf chamber which also houses the IRGA has an exterior as well as an internal quantum sensor to control photon flux density (PPFD) in the Photosynthetic Active Region (PAR) range. It has facility to expose a leaf area of 6 cm^2 at the sensor head. A thermocouple is placed in such a way that it would touch the leaf surface to determine leaf temperature. The speed variable mixing fan guarantees proper mixing of air in the leaf chamber. The leaf chamber is fitted with blue and red LEDs (Light Emitting Diode), which can give a PPFD (Photosynthetic Photon Flux Density) up to 2000 moles m^{-2} s^{-1}. The leaf chamber is also equipped with a Peltier cooling system that can preserve the chamber temperature. The operational option provided with the system also maintains a constant chamber RH around that of the ambient air.

Recording gas exchange parameters

The gas exchange parameters were documented for the second fully expanded leaf from the apex of the plant. The target leaf was clamped to the leaf chamber and the observations were recorded when A, gs, T and Ci touched a steady value. Gas exchange parameters were logged between 9 am and 12 pm on sunny days.

3.5 Mining of QTLs to identify genes

Three *in-silico* validated QTLs from the marker class analysis for root characteristics and WUE have been used for candidate gene mining. Identification and regulatory segmentation as well as candidate genes in such QTL regions appear to be significant indicators for the detection of superior QTL allelic variations. The IRGSP's high-quality rice genome sequence accelerated cloning, quantitative characteristic loci (QTLs) for both biotic and abiotic stress. Numerous public resources have been created to uncover important genes of rice.

3.5.1 Mining genes underlying QTL in a genome

Several public online tools for the rice genome for mining genes that underlie genome areas of concern or quantitative trait loci (QTL) are easily assessable. GRAMENE and RiceGeneThresher databases integrate a variety of information sources and offers strong web-based application as well as versatile instruments to deliver tailored rice biological data. While GRAMENE is a great resource for important plant genomes including rice, it alone does not deliver an easy way for QTL-based biological mining information. Selection of candidate genes depend on a wealth of information added through traditional genetics and molecular methods and this biological information on rice are stored on the public domain databases (Table 2.6). These databases were used to mine the genes within the decided flanking region. The genes within each region is automatically listed by GRAMENE with their annotated functions.

3.5.2 Identification of molecular network responsible

The genomic, coding sequence and protein sequence of these genes were collected from Gramene database. The annotated functions of the genes were checked for

relatedness to root and water use efficiency trait by checking the involvedness of the similar in various metabolic pathways by Ricefrnd database. Sequences were blasted using Gramene database against Arabidopsis sequence, the model plant. mi- RNAs present in the region were checked by using the Ricefrnd database.

Based on the position of markers, co-localization study was conducted using meta-analysis of QTLs by online tool phenogram.

IV RESULTS AND DISCUSSION

Rice (*Oryza sativa* L.) is one of the major staple foods for majority of the population in Asia. Its increased production is in urgent need to feed the exponentially growing population. Environmental stresses constrain rice production, it affects about 30% of the 700 million poor in Asia alone who live in rain-fed rice-growing areas (IRRI, 2016). Rice is a water intensive crop, which consumes about 80% of the total irrigation fresh water resources (Yang *et al.*, 2019). However, in the most parts of the world, it is being cultivated in diverse ecosystems with extreme variation in water availability. Drought stress is considered the most important limitation to production in rainfed lowlands. The crop fails during drought, and water scarcity affects 19- 23 million hectares of rainfed rice production areas only in South and Southeast Asia (Haefele *et al*., 2009). Water shortage is a challenge for rice production leading to yield losses globally.

To improve the productivity and the production of drought-prone systems, a combination of improved varieties and crop management is necessary. Sustaining productivity through development of improved cultivars more resilient to water limited conditions is the need of the hour (Reynolds *et al.,* 2011; Chapman *et al*., 2012).

Although selection of cultivar for absolute yield under water limited conditions was a successful approach, the narrow variability in yield *per se* among already improved cultivars and a high G X E interaction, may not provide the necessary impetus for improving productivity under water limited conditions. Various research clearly emphasizes the adoption of trait-based breeding over the empirical breeding by selection for yield *per se*. From the agronomic point of view, drought tolerance is associated with sustained productivity under water limited conditions.

Ludlow (1989) defined three principal mechanisms of genotypic adaptations contributing to increased yields in water limited environments: (a) drought escape, where the plant completes its life in the time where no water limitation occurs, (b) drought avoidance, where the plant maximizes its water uptake and minimizes its water loss, and (c) drought tolerance, where the plant continues to grow and function at reduced water

contents. Drought avoidance and drought tolerance are drought resistance mechanisms. Drought avoidance is the major primary factor in drought-resistant performance in plants and drought tolerance acts as the second line of defense subsequent to drought avoidance (Blum, 2005).

Plants have evolved various morpho-physiological mechanisms/traits that facilitate its growth and survival under drought stress. The morpho-physiological traits that supports plant under drought can be categorized as constitutive traits (also expressed under well-watered conditions) and induced traits or acquired traits (expressed under pronounced water shortage condition). Constitutive traits display a higher level of stress adaptation and functional conservation across environments (Sheshshayee *et al.,* 2018).

Among the large number of traits enumerated, water mining along with water conservation traits have great significance in maintaining water relations and cell metabolism under water limited conditions (Lynch, 2007). Water mining through well root system and efficient use of water by improved water use efficiency have been shown to be prominent traits for trait based breeding or physiological breeding (Sheshshayee *et al.,* 2012, Raju *et al.,* 2014).

Root facilitates numerous functions in plants including water and nutrient uptake. Because of its underground behavior, it is very difficult to overlook its importance to plant productivity. Root traits like root depth, length, thickness and root system architecture had been positively correlated with drought tolerance by enhanced water harvesting through deep and fast-growing root system (Tradieu, 2011). Although it is cumbersome to phenotype the root traits, with the advancement in technologies, many tools have been developed for root phenotyping. Leaf temperature (LT) has been considered a good method for field phenotyping of crops under drought and is used to calculate root traits in relation to drought tolerance. It is observed that transpiration through stomata results in cooler leaves (Wasaya *et al.,* 2018). Therefore, Cooler LT is linked with deeper roots.

Water use efficiency (WUE) is an important trait. But the relevance has been debated. While Passioura (1986) suggested the importance of WUE, Blum (2009) had other opinions. Research that attempts to comprehend the physiological factors which contribute to the observed differences in WUE, the concepts of photosynthetic capacity and stomatal conductance determining WUE emerged. Stomatal conductance plays a critical role in regulating water balance of the plant and determining $\Delta^{13}C$ and WUE (Sinclair *et al.,* 2010). It is clear that WUE determined by photosynthetic capacity has great relevance in sustaining productivity under water limited conditions. But in conditions where water availability is lower than required such as in semi-irrigated aerobic conditions, for a comprehensive improvement in growth and productivity introgressing root and WUE become most important. Sheshshayee *et al*., 2012 evaluated the WUE sub-components and debated how WUE could be regarded as a prospective characteristic for crop improvement. Scientists showed that growing WUE has tremendous importance after optimizing the use of water and/or light interception characters via canopy cover.

A successful effort was made to introgress root and WUE traits using the trait donor parents and the markers (discovered at UAS, GKVK, Bangalore by Raju *et al.*, 2016) by conventional breeding that has led to the development of a superior trait introgressed line which is now released for farmer's cultivation in Karnataka. To accelerate the success of trait introgression, another focused molecular breeding strategy was adapted to introgress root and WUE traits onto IR-64 background. IR-64 is one of the mega varieties which is cultivated extensively in India under puddled conditions with an average yield of >8 tons/ha. However, this cultivar is one of the most drought sensitive cultivars. In water limited conditions it leads to significant yield loss (Uga *et al.*, 2013). Therefore, maintaining the grain yield while improving drought resilience is important. Towards this an attempt was made to introgress complex physiological traits by Marker Assisted Selection (MAS). AC-39020 was selected as root donor parent and IET-16348 was selected as WUE donor parent in focused multi-parent marker assisted backcross breeding program to introgress root and WUE traits into IR-64 background. Based on polymorphism, 16 markers (for root traits and $\Delta^{13}C$) were used in foreground selection

and 120 markers (10 markers/chromosome) for background selection. 260 $DCBC_3F_3$ trait introgressed lines were developed by various molecular and physiological selection. Several transgressive trait introgressed lines were identified and evaluated for yield and drought adaptive traits. The selected five transgressive segregants out-performed the recurrent parent IR-64 (Dharmappa *et al.*, 2019). Foreground markers used in the study was trait associated markers from previous association mapping study (Raju *et al.*, 2014). Traditionally, QTLs are identified by extensive genotypic and trait characterization of a defined panel of germplasm by association mapping or using a trait specific bi-parental mapping population. Hence, these are QTLs for the specific trait, the program is QTL pyramiding. The effect of identified genetic loci in a pyramiding program is not always as expected because of the complexity of gene networks among genetic regions (Kumar *et al*., 2018). Apart from this, Quantitative traits are strongly modulated by the prevailing environmental conditions. Hence, discovery and validation of QTLs should be done across multiple environments and populations. This approach though would be useful, would only increase the cost and time of QTL discovery. Therefore, accessing the performance of TILs across various environments would be a strategic approach to circumvent the doubts arising from the environmental modulation of trait variability. Further understanding the genomic network among the TILs or QTL-NILs would be essential in clearly deciphering specific marker combinations that enhances the success of trait introgression through molecular breeding.

Towards this an attempt was made to analyze the interaction among traits and to assess the molecular network in marker assisted back cross progenies of rice. The results obtained in various experiments at multilocation are described and discussed in this chapter.

4.1 Phenotypic evaluation of $DCBC_3F_4$ lines (TILs) for growth, yield, WUE and other drought adaptive traits under aerobic condition (AC)

The main goal of the study was to phenotype 260 $DCBC_3F_4$ lines for drought adaptive traits along with their parents under semi-irrigated aerobic condition. Phenotypic data on various drought adaptive traits like $\Delta^{13}C$ (a surrogate for water use efficiency),

leaf temperature (LT), yield attributes, spikelet fertility, total leaf area, SCMR value were recorded for each line along with the parents under semi-irrigated aerobic condition.

4.1.1 Water Use Efficiency (WUE)

Water use efficiency that acts through avoidance mechanism is an important trait for drought tolerance in crop plants (Blum *et al.,* 2009). In regulating water balance of the plant, stomatal conductance plays a critical role, hence, in determining $\Delta^{13}C$ and water use efficiency of plants (Sinclair *et al.,* 2010). The plasticity of phenological development rather than the mean phenological stage distinctly influences plant adaptation to moisture stress. Sheshshayee *et al.,* (2012) suggested that high WUE when no substantial reduction in total water use can be a potential trait for sustaining productivity under semi-irrigated aerobic condition. Discrimination against carbon isotopes (CID) is one of the well-accepted surrogates to estimate water use efficiency on a time averaged scale. The theory explaining the relationship amongst $\Delta^{13}C$ and WUE has been well developed (Farquaqr, 1989) and widely adopted in various crops (Martin *et al.,* 1989, Mian *et al.,* 1996, Handley *et al.,* 1994, Impa *et al.,* 2005).

$\Delta^{13}C$ value of IR-64 (recurrent parent), AC-39020 (root trait donor) and IET-16348 (WUE donor) was 20.05, 19.56, and 18.93 parts per mill, respectively indicating that WUE donor IET-16348 was the highest WUE type and IR-64 was the lowest (Table 4.1). The range of $\Delta^{13}C$ value for 260 TILs was 16.31 to 21.56 per mill. The mean of $\Delta^{13}C$ value for 260 TILs was 19.58, which was significantly lesser than the recurrent parent IR-64 (Fig. 4.4). $\Delta^{13}C$ distribution was negatively skewed (Fig. 4.2). It suggests that the TILs had higher WUE than the recurrent parent.

WUE is an important element of Passioura's yield model. This suggests that, increase in WUE would result in increase in biomass. However, when selected for high WUE, a mixed response was noticed (Richards *et al.,* 1986, 2002). Analysis of literature describing the relationship between WUE and total dry matter (TDM) revealed highly inconsistent trends (Sheshshayee *et al.,* 2003). A positive or negative or neutral relationship among the parameters completely discouraged the breeders from using WUE as drought adaptive trait. This anomaly was analyzed by Sheshshayee *et al.,* 2012, who

comes out to a concept that WUE is still a useful parameter if variability in root and leaf area is optimized (Priyanka, 2015). It can be altered by changes in either carbon assimilation or by decreasing transpiration through stomatal behavior. Due to a strong link between transpiration and total biomass, any increase in WUE through reduced transpiration invariably leads to reduction in crop growth rates. Although increased WUE through reduced transpiration is relevant for survival under water scarce conditions, this strategy seems to have a detrimental effect on growth rates. Therefore, it becomes apparent that increased WUE is achieved without a significant reduction in transpiration. Such genotypes will have a superior growth rates and higher capacity for carbon assimilation.

Analysis by Sheshshayee *et al.,* (2012) also revealed that low root genotypes had relatively higher WUE. As per the earlier discussions, the low root genotypes would be associated with low transpiration and hence, low crop growth rates. Therefore, in the present study, an attempt was made to choose the high WUE and low WUE line in combination with low root type and high root types for further experiments.

4.1.2 Root traits

Root systems are essential for adaptation against various types of biotic and abiotic stresses. Root trait plasticity is considered as a drought avoidance strategy for plants as it enables absorption of water from deeper strata of soil (Price and Courtois, 1999). Well-endowed root system is the vital mechanism of crop plants to grow under water limited conditions (Serraj, *et al.,* 2004; Li, *et al.,* 2005; Lynch, 2007; Araus, *et al.,* 2008; Songsri, *et al.,* 2008). Leaf temperature (LT) acts as a surrogate for root traits. It is observed that transpiration through stomata results in cooler leaves if water is available for transpiration (Wasaya *et al.,* 2018). Therefore, Cooler LT is likely to be linked with deeper roots.

The leaf temperature (LT) value of IR-64, AC-39020 and IET-16348 was 34.8, 24.97 and 27.10 degree celsius. In this investigation, we measured leaf temperature as an alternative approach to assess the differences in root character. AC-39020 maintained the lowest leaf temperature among with the parents, which shows it is having better root

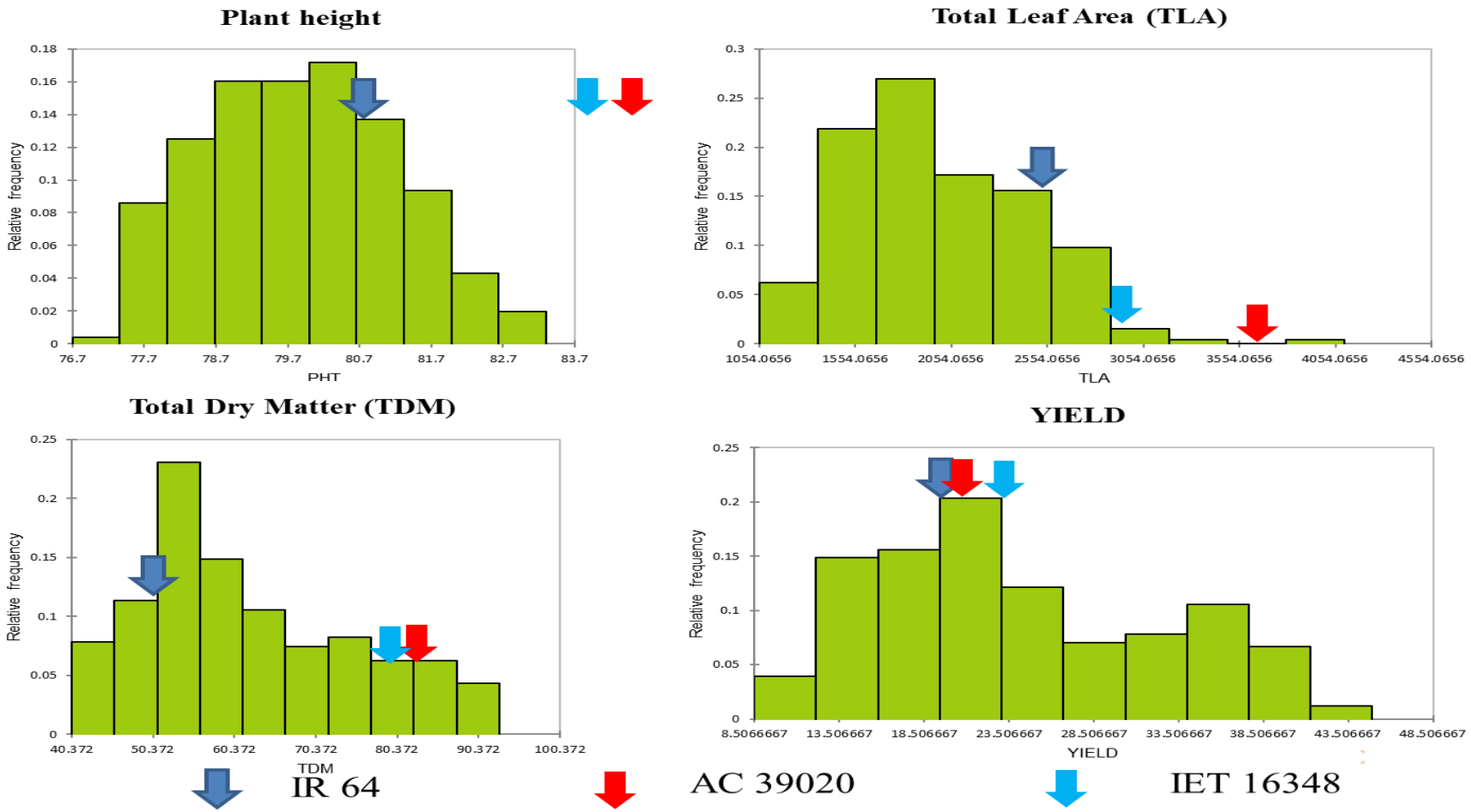

Figure 4.1: Frequency distribution of various traits such as Plant height, Total leaf area, TDM and Yield.

Plant height (cm); Total Leaf Area (cm^2); Total dry matter (g); Yield (g)

Note-Dark blue color indicates IR-64, light blue indicates IET-16348 and Red indicates AC-39020.

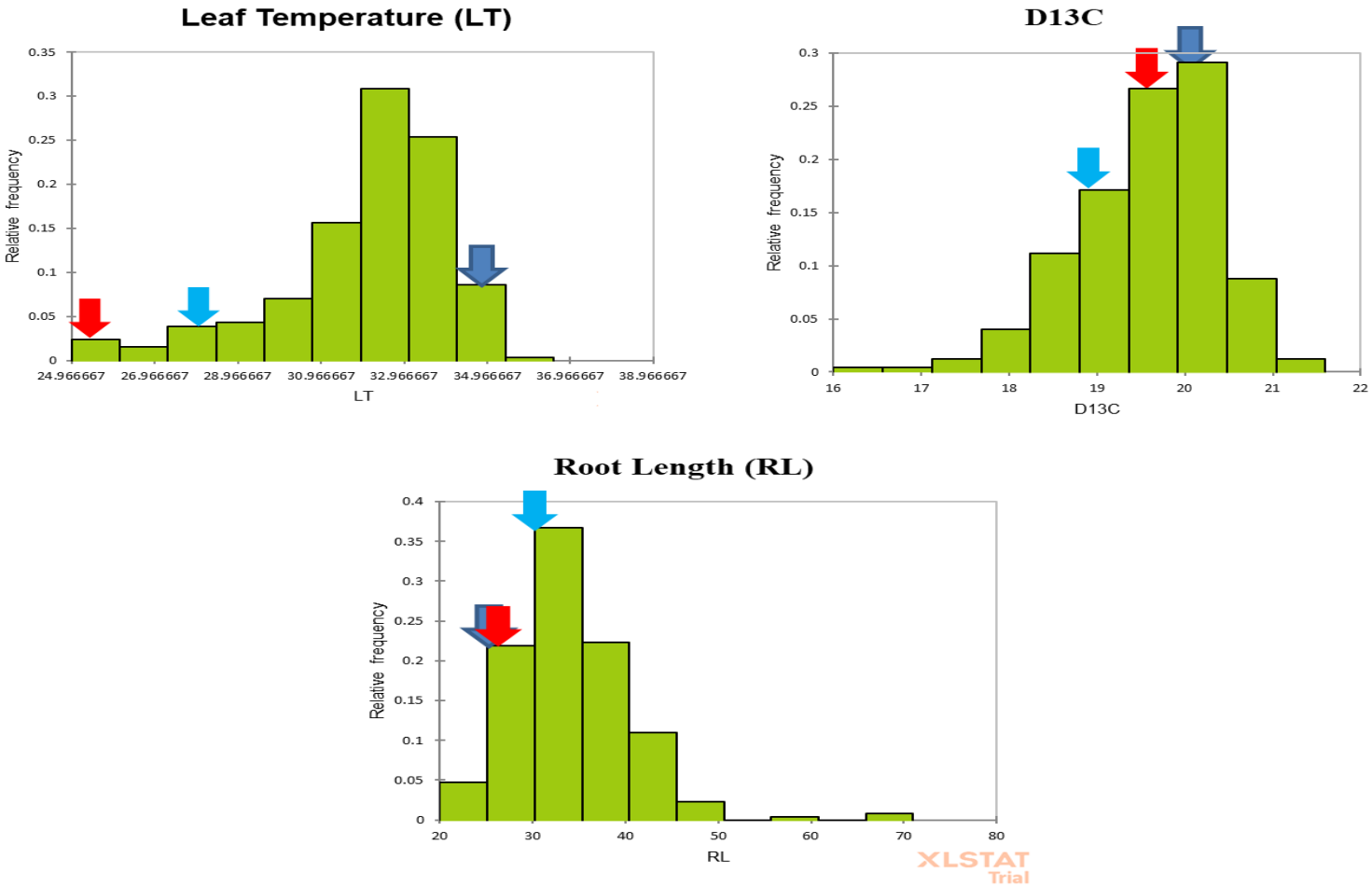

Figure 4.2: Frequency distribution of various traits such as Leaf temperature, $\Delta^{13}C$ and Root length.

LT (°C); D13C (per mill); Root length (cm)

Note-Dark blue color indicates IR-64, light blue indicates IET-16348 and Red indicates AC-39020.

system. The distribution of leaf temperature was negatively skewed (Fig. 4.2) which suggests that still genetic variability exists between $DCBC_3F_4$ lines. The range of leaf temperature for TILs was from 24.97 to 35.57 degree celsius (Table 4.1). Mean value for LT was 28.49, suggesting most of the TILs maintains its LT to the lower level. It indicates good root system among the TILs. The mean value of leaf temperature was 18.13 per cent lower than IR-64 (Fig. 4.3).

4.1.3 Yield and its attributes

In a plant, drought is a complex process involving various steps beginning with moisture and nutrient uptake from roots to panicle and grain formation. Screening of physiological traits is more precise than screening complex quantitative characteristics or traits. Each physiological characteristic or trait meets one or two of the various sequential elements required to generate greater yields. The suitable or appropriate combinations of these elements are not well understood to obtain enhanced output under drought. Yield which is a complicated quantitative feature was regarded as an appropriate breeding selection criterion (Campos *et al.,* 2004). However, exploitation of genetic variation using direct selection for the yield attributes for grain yield under drought and combining high yield potential with yield attribute has now been suggested as an appropriate alternative (Venuprasad *et al.,* 2007). Reduction in yield under aerobic conditions is an investable consequence of water limitations. Dissecting the yield components into attributes has relevance in understanding the causes for yield reduction under semi-irrigated aerobic conditions. Hence, several parameters viz., number of filled or chaffy grains, number of panicles, grain weight and number and spikelet fertility were measured as yield attributes among the parents and TILs under AC.

The mean performance of the parents (IR-64, IET -16348 and AC-39020) and TILs for the measured traits under aerobic condition are shown in Table 4.1. The parental lines recorded non-significant variability for tiller number under aerobic condition. Among the TILs, the variation was significant. The yield attributes that determine the output of grain viz., spikelet fertility also differed considerably among the lines. Table 4.1 shows the skewness and kurtosis values for these traits.

Table 4.1: Performance of parents and TILs for morpho-physiological traits

Parameters	IR 64	AC 39020	IET 16348	F>Pr	Mean	Minimum	Maximum	σ2g	Skewness	Kurtosis
PHT	80.10	106.77	91.73	*	80.03	76.70	106.77	**	0.14	-0.63
DFF	85.00	102.00	91.00	*	82.09	79.00	102.00	**	0.04	-1.12
SPAD	38.27	37.47	38.07	ns	38.46	33.30	46.53	**	1.22	1.44
TLA	2668.77	3678.78	2899.59	*	1993.53	1054.07	4046.02	**	0.59	0.52
TLWT	7.81	18.93	17.67	*	10.37	6.79	18.93	**	0.25	-1.13
YIELD	20.58	20.94	22.00	ns	35.32	8.51	43.92	**	0.45	-0.90
SWT	24.45	41.35	38.69	*	27.82	18.95	41.35	**	0.36	-0.56
TDM	52.84	81.21	78.35	*	79.95	40.37	91.91	**	0.56	-0.71
SF	75.40	80.38	85.89	*	84.75	82.40	95.60	**	-0.01	-0.97
HI	0.39	0.26	0.34	*	0.45	0.25	0.66	**	-0.15	0.18
LT	34.80	24.97	27.10	*	28.49	24.97	35.57	**	-1.27	1.58
SLA	163.73	238.58	193.72	*	192.75	142.08	289.67	**	0.63	0.90
TN	19.33	20.67	19.33	ns	20.76	15.00	30.33	**	0.88	0.48
RL	25.50	27.00	30.00	*	34.13	20.00	70.00	**	1.26	4.99
RWT	2.30	9.47	2.53	*	4.20	1.54	12.20	**	1.18	3.64
$\Delta^{13}C$	20.05	19.56	18.93	*	19.58	16.31	21.56	**	-0.73	1.03

* *and* ** significant P <0.05, and p<0.01 respectively. ns: is non- significant

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TLWT-Total leaf weight, SWT-Shoot Weight, TDM-Total Dry Matter, SF- Spikelet fertility, LT-Leaf Temp, SLA-Specific Leaf Area, HI- Harvest Index, TN-Tiller Number, RL-Root length, RWT-Root weight

Both spikelet fertility and total number of panicles per plant showed important inter-line grain yield positive association. Grain yield of the lines were statistically significant among the lines with the mean value of 35.32 g plant^{-1} which was ranged from 8.51 to 43.92 g plant^{-1} showing the diversity among the lines. Total dry matter or biological yield of the parents were also showing significance among the lines. The kurtosis values for all 16 traits was less than three except for two traits (RWT and RL) suggesting that distribution curves for the majority of the measured traits seems to be platykurtic. Yield components showed significant variability under aerobic condition among TIL lines. TILs recorded 71.62 per cent higher yield than IR-64 (Fig. 4.3). The increment in TDM was found to be 51.3 per cent and in spikelet fertility it was 25.42 per cent more than IR-64.

Canopy light interception is a function of the rates of leaf production, expansion and abscission as well as stand density and arrangement. In plants, leaf area development (leaf production and expansion) is more sensitive to water stress than leaf abscission (Muchow, 1985). There was significant difference in leaf area recorded among the parents and the TILs. Most of the TILs recorded very similar leaf area to that of IR-64.

4.1.4 Associated morphological traits

Plant morphology is the study of the physical form and external structure such as roots, stems, leaves, flowers, seeds, fruits etc. Plant morphological parameters analyzed in this study were plant height (cm), leaf weight (g), shoot weight (g), days to 50% flowering (number of days), specific leaf area (cm^2/g).

Several plant morphological traits differed significantly among the TILs (Table 4.1). However, it was noticed that most of these parameters in TILs were comparable to IR-64. Plant height ranged between 76.7 to 106.7 cm among TILs with a mean of 80.0 cm. AC-39020 had significantly taller phenotype than the other parent and IR-64 (Table 4.1). Similarly, shoot weight, leaf weight and SLA also showed significant variability among TILs but the respective mean value were similar to that of IR-64. The range of days to fifty percent flowering was 79 to 102 days (Table 4.1) with the average value of 82 days which showed statistical significance among different TILs.

4.1.5 Correlation among traits

Phenotypic characters are interdependent with wide range of magnitude. To study collective contribution and mechanism of trait development, their relation with other traits should be clear. Study of correlation among component traits for drought is important for effective selection.

Selection of a specific trait leads to an effective selection of the other correlated trait. However, identification of causative correlation would enhance the efficiency of a relevant dependent trait that can lead to improving drought adaptation. Interestingly, yield and total biomass showed a strong negative relationship with leaf temperature (Table 4.2). Canopy can remain cooler only when plants transpire through open stomata. This would enhance CO_2 entry for photosynthesis thus explaining the inverse relationship between leaf temperature and total biomass accumulation.

4.2 Identification of superior recombinant inbred lines for semi irrigated aerobic condition

Based on the phenotypic data of the present as well as earlier studies, 260 $DCBC_3F_4$ progenies with similar leaf area were grouped into 4 categories *viz.* High root with high $\Delta^{13}C$; High root with low $\Delta^{13}C$; Low root with high $\Delta^{13}C$ and Low root with low $\Delta^{13}C$. TILs from each group were selected, which had higher yield per plant in the respective group for further studies (Table 4.4). Although selected TILs were having different phenology, but these groups were not selected based on phenology. Therefore, difference in yield of the selected TILs was not due to duration/ phenology. TILs with high root low $\Delta^{13}C$ recorded highest total biomass and spikelet fertility resulting in highest grain yield per plant (Table 4.3). It is well known that maintenance of cooler canopies is possible only when root characteristics are better and can mine water from deeper layer of soil effectively. It is therefore possible that the deep root types would maintain better tissue water relations. Accordingly, the TILs belonging to high root category showed higher biomass. Interestingly, high root types recorded significantly higher spikelet fertility (Table 4.3).

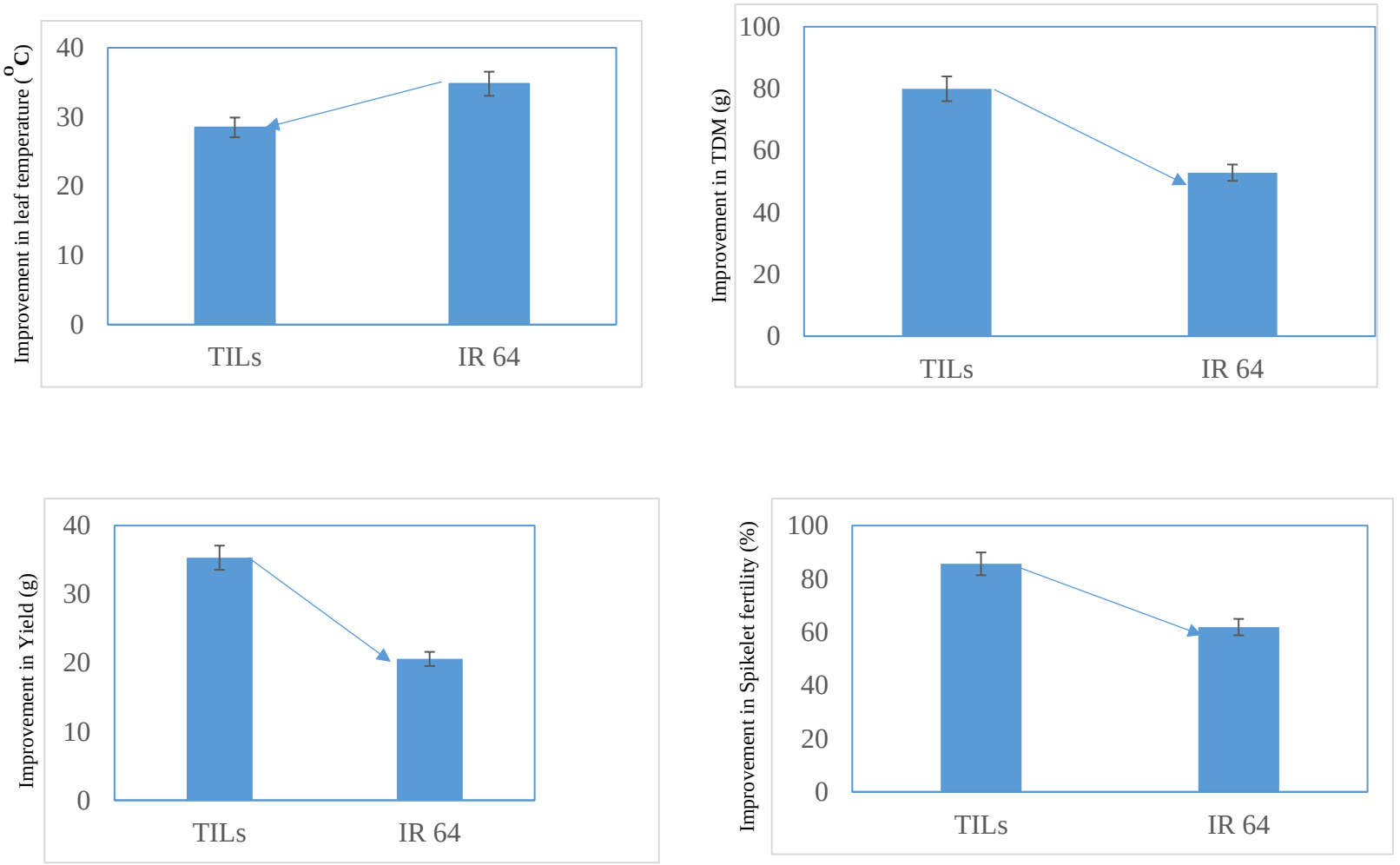

Figure 4.3: Improvement over IR-64 for Leaf temperature, Total dry matter, Yield and Spikelet fertility

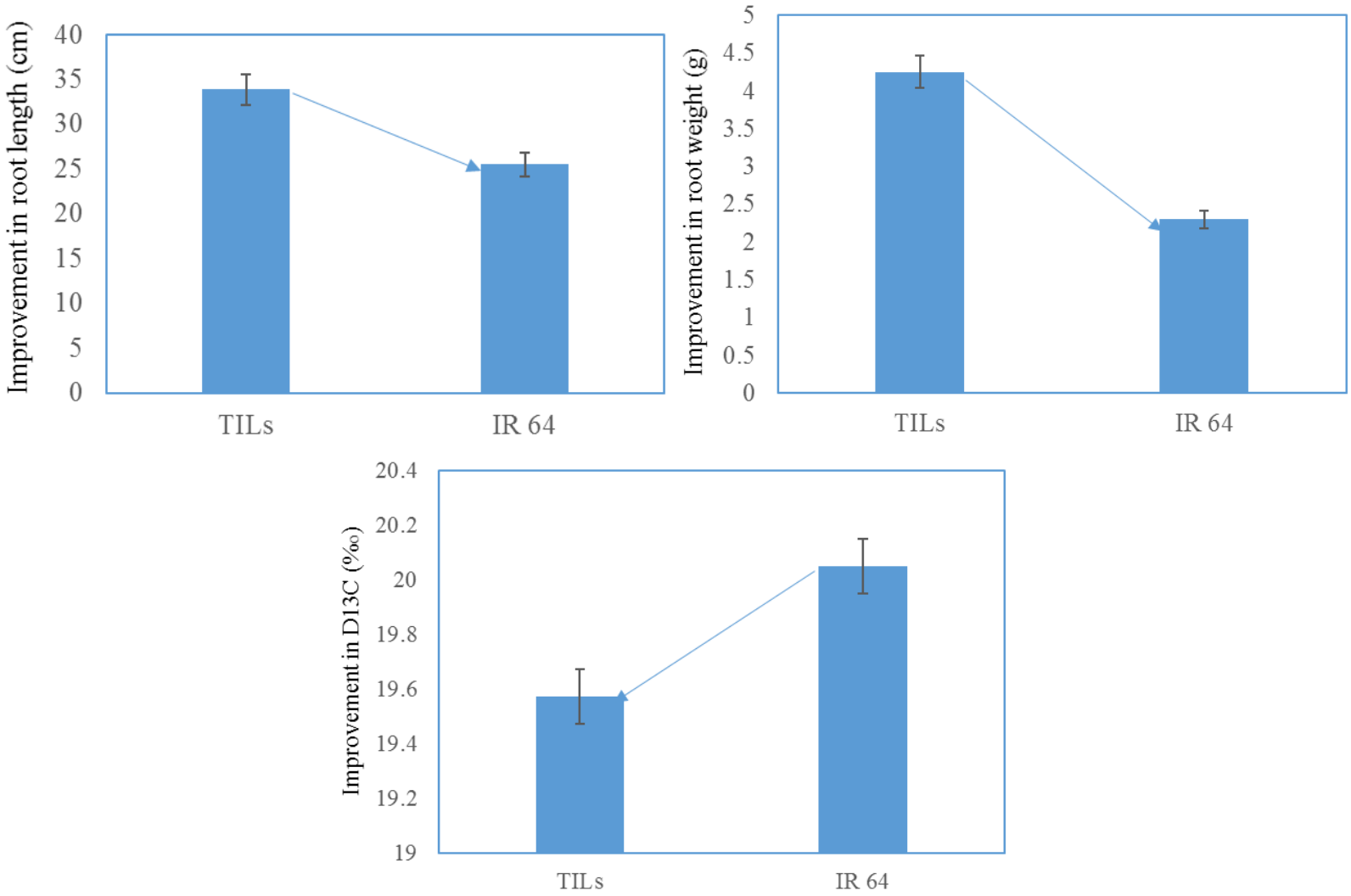

Figure 4.4: Improvement over IR-64 for root length, root weight and $\Delta^{13}C$

Table 4.2: Correlation matrix for various traits for 260 DCBC$_3$F$_4$ TILs

Variables	PHT	DFF	SCMR	TLA	TLWT	Yield	SWT	TDM	LT	SLA	TN	RL	RWT	$\Delta^{13}C$	HI
PHT	**1**	-0.044	0.083	**0.152**	**0.148**	0.076	0.108	0.109	-0.068	0.026	-0.115	-0.045	0.018	0.003	0.032
DFF	-0.044	**1**	**-0.186**	-0.052	-0.045	**-0.139**	-0.045	-0.112	**0.166**	-0.035	0.046	-0.028	-0.030	0.023	-0.114
SCMR	0.083	**-0.186**	**1**	**0.421**	**0.424**	**0.548**	**0.459**	**0.572**	**-0.687**	0.099	-0.062	0.069	**0.224**	-0.065	**0.410**
TLA	**0.152**	-0.052	**0.421**	**1**	**0.822**	**0.589**	**0.523**	**0.684**	**-0.356**	**0.489**	**-0.190**	0.026	0.061	0.024	**0.367**
TLWT	**0.148**	-0.045	**0.424**	**0.822**	**1**	**0.631**	**0.542**	**0.746**	**-0.378**	-0.054	**-0.157**	0.039	0.077	-0.013	**0.358**
Yield	0.076	**-0.139**	**0.548**	**0.589**	**0.631**	**1**	**0.622**	**0.951**	**-0.497**	0.074	**-0.159**	0.040	0.114	-0.084	**0.879**
SWT	0.108	-0.045	**0.459**	**0.523**	**0.542**	**0.622**	**1**	**0.815**	**-0.449**	0.082	0.032	-0.057	0.065	-0.039	**0.250**
TDM	0.109	-0.112	**0.572**	**0.684**	**0.746**	**0.951**	**0.815**	**1**	**-0.528**	0.066	-0.118	0.014	0.107	-0.069	**0.707**
LT	-0.068	**0.166**	**-0.687**	**-0.356**	**-0.378**	**-0.497**	**-0.449**	**-0.528**	**1**	-0.070	-0.001	-0.060	**-0.154**	0.062	**-0.353**
SLA	0.026	-0.035	0.099	**0.489**	-0.054	0.074	0.082	0.066	-0.070	**1**	-0.078	-0.020	-0.014	0.058	0.098
TN	-0.115	0.046	-0.062	**-0.190**	**-0.157**	**-0.159**	0.032	-0.118	-0.001	-0.078	**1**	-0.026	-0.087	0.073	**-0.162**
RL	-0.045	-0.028	0.069	0.026	0.039	0.040	-0.057	0.014	-0.060	-0.020	-0.026	**1**	**0.158**	-0.041	0.020
RWT	0.018	-0.030	**0.224**	0.061	0.077	0.114	0.065	0.107	**-0.154**	-0.014	-0.087	**0.158**	**1**	-0.020	0.070
$\Delta^{13}C$	0.003	0.023	-0.065	0.024	-0.013	-0.084	-0.039	-0.069	0.062	0.058	0.073	-0.041	-0.020	**1**	-0.096
HI	0.032	-0.114	**0.410**	**0.367**	**0.358**	**0.879**	**0.250**	**0.707**	**-0.353**	0.098	**-0.162**	0.020	0.070	-0.096	**1**

Values in bold are different from 0 with a significance level alpha=0.05

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TLWT-Total leaf weight, SWT-Shoot Weight, TDM-Total Dry Matter, LT-Leaf Temp, SLA-Specific Leaf Area, TN-Tiller Number, RL-Root length, RWT-Root weight, HI-Harvest Index

Table 4.3: Details of traits in selected groups of TILs (Trait Introgressed Lines)

Group	RL (cm)	RWT (g)	$\Delta^{13}C$ (per mil)	TLA (cm2)	Yield (g)	TDM (g)	SF (%)
HR+LD	39.67	6.42	18.58	2269.71	36.10	85.68	86.2
HR+HD	36.86	4.49	20.08	2277.19	35.16	80.84	84.1
LR+LD	30.50	2.93	19.26	2379.71	33.63	78.12	78.6
LR+HD	28.43	2.70	20.01	2300.24	34.40	71.04	75.3

HR-High Root, LR-Low Root, HD-High Delta 13C, LD-Low Delta

TLA-Total Leaf Area, TDM-Total Dry Matter, SF- Spikelet fertility, RL-Root length, RWT-Root weight

Table 4.4: List of selected trait introgressed lines from the four groups

Group	TIL #	Group	TIL #
HR+LD	55	**LR+LD**	21
	93		219
	227		253
	228		262
	237		
HR+HD	12	**LR+HD**	3
	54		18
	57		97
	84		233
	86		145
	87		
	139		
	142		
	6		
	221		
	248		

4.2.1 Characterization of selected TILs in various seasons and locations for various morpho-physiological traits

The selected TILs along with the parents were extensively studied in various seasons and locations. These were widely phenotyped in field conditions for multiple adaptive features of drought such as leaf temperature (surrogate for root traits), $\Delta^{13}C$ (surrogate for WUE), yield and other morpho-physiological traits. This section outlines the outcomes for the performance of the selected TILs.

4.2.1.1 Performance of parents and TILs for morpho-physiological traits in GKVK in Kharif 2016

Total dry matter of TILs ranged from 59.8 to 69.7 g per plant with a mean of 65.9 g per plant. Similarly, yield of these lines were also not significantly different, as all the selected TILs were higher yield types in all the four groups. The value of $\Delta^{13}C$ for IR-64, AC-39020 and IET-16348 was 22.67, 21.46 and 19.27 per mil, respectively. The mean of $\Delta^{13}C$ for TILs was 19.95 per mil which ranges from 18.15 to 21.83 per mil. Leaf temperature for IR-64, AC-39020 and IET-16348 was 33.6, 24.58 and 26.4 degree Celsius, respectively. The mean value for the selected 25 TILs was 28.12 degree Celsius and range was from 27.3 to 28.7 degree Celsius (Table 4.5). The improvement over IR-64 for yield was ~28 per cent, for $\Delta^{13}C$ was ~11 per cent and for LT the improvement was ~18 per cent (Fig. 4.5).

Correlation Matrix

There was strong positive correlation between total leaf area and yield, although the selected TILs were of similar leaf area types. It suggests that even marginal increase in leaf area contributes to yield in significant manner. $\Delta^{13}C$ and spikelet fertility showed strong positive correlation (Table 4.6).

4.2.1.2 Performance of parents and TILs for morpho-physiological traits in GKVK in Summer season 2017

IR-64, AC-39020 and IET-16348 flowered at 85, 106 and 92 days respectively. Average number of days to flowering for TILs was 82 days with a range of 78 to 87 days.

Table 4.5: Performance of parents and TILs for morpho-physiological traits in GKVK (Kharif, 2016)

Parameters	IR-64	AC-39020	IET-16348	F>Pr	Mean	Minimum	Maximum	σ2g
PHT	81.22	108.45	78.93	*	81.83	79.25	83.62	ns
DFF	88.00	105.00	93.00	*	82.48	77.00	88.00	*
SCMR	43.78	44.27	43.87	ns	43.13	42.05	44.77	ns
TLA	2793.91	3637.58	2929.81	*	2499.40	1883.04	3036.21	*
YIELD	21.70	20.95	21.89	ns	28.12	22.41	32.86	*
TDM	71.27	96.49	66.16	*	65.99	59.80	69.71	*
SF	74.47	76.26	76.64	ns	77.74	72.52	84.03	*
HI	0.30	0.22	0.33	ns	0.32	0.30	0.34	*
LT	33.60	24.58	26.40	*	28.12	27.30	28.70	*
SLA	180.86	243.78	198.56	*	184.45	165.47	194.48	*
TN	27.50	30.17	28.50	ns	27.40	24.83	31.00	*
$\Delta^{13}C$	22.67	21.46	19.27	*	19.95	18.15	21.83	*

* *and* ** significant P <0.05, and p<0.01 respectively. ns: is non- significant

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TDM-Total Dry Matter, SF-Spikelet fertility, LT-Leaf Temp, SLA-Specific Leaf Area, HI- Harvest Index, TN-Tiller Number

Table 4.6: Correlation matrix for various traits (GKVK, Kharif 2016)

Variables	Yield	TDM	LT	SCMR	TN	PHT	SLA	DFF	SF%	TLA	$\Delta^{13}C$
Yield	**1**	**0.728**	0.039	-0.033	0.038	-0.028	0.172	-0.145	0.053	**0.402**	-0.132
TDM	**0.728**	**1**	0.041	0.037	-0.101	0.074	0.204	0.004	0.016	**0.601**	0.171
LT	0.039	0.041	**1**	0.123	0.209	0.101	-0.016	-0.358	-0.081	-0.072	0.157
SCMR	-0.033	0.037	0.123	**1**	-0.061	0.263	0.259	0.015	-0.132	0.203	0.205
TN	0.038	-0.101	0.209	-0.061	**1**	0.237	0.025	-0.144	0.291	-0.192	0.247
PHT	-0.028	0.074	0.101	0.263	0.237	**1**	0.173	0.138	-0.039	0.144	0.229
SLA	0.172	0.204	-0.016	0.259	0.025	0.173	**1**	-0.059	-0.287	**0.766**	0.104
DFF	-0.145	0.004	-0.358	0.015	-0.144	0.138	-0.059	**1**	-0.096	0.038	-0.041
SF%	0.053	0.016	-0.081	-0.132	0.291	-0.039	-0.287	-0.096	**1**	-0.262	**0.378**
TLA	**0.402**	**0.601**	-0.072	0.203	-0.192	0.144	**0.766**	0.038	-0.262	**1**	0.112
$\Delta^{13}C$	-0.132	0.171	0.157	0.205	0.247	0.229	0.104	-0.041	**0.378**	0.112	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Note: Correlation coefficient for various traits like Yield, TDM-Total Dry Matter, LT-Leaf Temperature, SCMR- SPAD Chlorophyll Meter Reading, TN-Tiller Number, PHT-Plant height, SLA-Specific Leaf Area, DFF-Days to 50%flowering, SF- Spikelet fertility, TLA-Total Leaf Area and $\Delta^{13}C$ -Carbon Isotope Discrimination are given in the table.

The average number of days to flowering for selected lines was statistically non-significant. Total leaf area (TLA) for parents was significantly different. The value of TLA for IR-64, AC-39020 and IET-16348 was 2044.18, 3371.58 and 2536.52 cm^2 respectively. Selected TILs were of similar leaf area and there were no significant differences in leaf area of TILs. The value of $\Delta^{13}C$ for IR-64, AC-39020 and IET-16348 was 21.12, 20.21 and 19.74 per mil respectively. Mean $\Delta^{13}C$ for TILs was 20.47 per mil which ranges from 19.21 to 21.80 per mil. Leaf temperature for IR-64, AC-39020 and IET-16348 was 34.45, 30.02 and 31.5 degree Celsius, respectively. The mean value for 25 selected TILs was 28.38 degree Celsius and range was from 25.06 to 29.8 degree Celsius (Table 4.7). It suggests that the selected trait introgressed lines maintains cooler leaf temperature by using water effectively. Hence, there is no penalty of yield reduction. The improvement over IR-64 for yield was ~20 per cent, for $\Delta^{13}C$ was ~4 per cent and for LT was ~17 per cent (Fig. 4.5).

Correlation Matrix

Yield was positively correlated with spikelet fertility, tiller number, total dry matter, harvest index, total leaf area and negatively correlated with $\Delta^{13}C$ value. $\Delta^{13}C$ value was strongly positively correlated with leaf temperature. Leaf temperature was strongly negatively correlated with yield and yield attributes. Specific leaf area positively correlated with total leaf area (Table 4.8). Total leaf area showed significant association with yield per plant, total dry matter, tiller number and filled seeds per plant, of which all the traits had positive relationship.

4.2.1.3 Performance of parents and TILs for morpho-physiological traits in TNAU in Summer season 2017

There was no significant variation among TILs for plant height. It ranges from 75.4 to 83.8 cm with the mean of 78.96 cm. Total leaf area of the selected 25 TILs were similar, hence it was not significantly different. Significant variation was found in parents for TLA. Yield was found to be significantly different for the selected TILs. The value of $\Delta^{13}C$ for IR-64, AC-39020 and IET-16348 was 22.61, 21.88 and 19.60 per mil respectively.

Table 4.7: Performance of TILs and parents for morpho-physiological traits in GKVK (Summer, 2017)

Parameters	IR 64	AC 39020	IET 16348	F>Pr	Mean	Minimum	Maximum	σ2g
PHT	80.83	103.55	78.65	*	75.49	72.00	79.11	ns
DFF	85.00	106.00	92.00	*	82.68	78.00	87.00	*
SCMR	44.15	47.12	45.97	ns	44.90	42.80	47.13	ns
TLA	2044.18	3371.58	2536.52	**	2031.79	2128.61	2337.54	ns
Yield	25.18	23.65	21.84	ns	30.29	25.91	33.52	*
TDM	74.26	91.33	72.39	*	75.84	69.13	81.44	*
SF	78.67	74.86	73.41	ns	76.92	70.00	87.13	*
HI	0.34	0.26	0.30	ns	0.40	0.37	0.41	*
LT	34.45	30.02	31.50	*	28.38	25.06	29.80	*
SLA	178.09	285.53	197.18	*	180.54	172.47	196.21	*
TN	31.67	24.17	33.17	*	32.31	30.50	34.83	*
$\Delta^{13}C$	21.12	20.21	19.74	*	20.47	19.21	21.80	*

* *and* ** significant P <0.05, and p<0.01 respectively. ns: is non- significant

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TDM-Total Dry Matter, SF-Spikelet fertility, LT-Leaf Temp, SLA-Specific Leaf Area, HI- Harvest Index, TN-Tiller Number

Table 4.8: Correlation matrix for various traits (GKVK, Summer 2017)

Variables	Yield	TDM	LT	SCMR	TN	PHT	SLA	DFF	$\Delta^{13}C$	SF	HI	TLA
Yield	**1**	**0.666**	**-0.702**	0.138	**0.436**	-0.202	0.054	-0.095	**-0.405**	**0.443**	**0.893**	**0.473**
TDM	**0.666**	**1**	**-0.421**	-0.119	0.342	-0.070	-0.014	-0.152	-0.135	**0.470**	0.260	**0.443**
LT	**-0.702**	**-0.421**	**1**	-0.136	-0.175	0.370	-0.020	-0.059	**0.512**	**-0.638**	**-0.645**	**-0.439**
SCMR	0.138	-0.119	-0.136	**1**	0.038	0.245	-0.230	0.045	-0.107	0.018	0.260	-0.203
TN	**0.436**	0.342	-0.175	0.038	**1**	0.100	0.316	-0.159	0.008	0.041	0.350	0.328
PHT	-0.202	-0.070	0.370	0.245	0.100	**1**	0.152	-0.186	**0.484**	-0.333	-0.220	-0.140
SLA	0.054	-0.014	-0.020	-0.230	0.316	0.152	**1**	-0.372	0.123	-0.174	0.076	**0.552**
DFF	-0.095	-0.152	-0.059	0.045	-0.159	-0.186	-0.372	**1**	0.040	-0.090	-0.026	-0.231
$\Delta^{13}C$	**-0.405**	-0.135	**0.512**	-0.107	0.008	**0.484**	0.123	0.040	**1**	-0.378	**-0.434**	-0.117
SF	**0.443**	**0.470**	**-0.638**	0.018	0.041	-0.333	-0.174	-0.090	-0.378	**1**	0.278	0.266
HI	**0.893**	0.260	**-0.645**	0.260	0.350	-0.220	0.076	-0.026	**-0.434**	0.278	**1**	0.338
TLA	**0.473**	**0.443**	**-0.439**	-0.203	0.328	-0.140	**0.552**	-0.231	-0.117	0.266	0.338	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Note: Correlation coefficient for various traits like Yield, TDM-Total Dry Matter, LT-Leaf Temperature, SCMR- SPAD Chlorophyll Meter Reading, TN-Tiller Number, PHT-Plant height, SLA-Specific Leaf Area, DFF-Days to 50%flowering, $\Delta^{13}C$ -Carbon Isotope Discrimination, SF-Spikelet fertility, HI- Harvest Index and TLA-Total Leaf Area are given in the table.

$\Delta^{13}C$ mean for TILs was 21.50 per mil which ranges from 18.93 to 22.24 per mil. Leaf temperature for IR-64, AC-39020 and IET-16348 was 39.52, 35.93 and 37.87 degree Celsius respectively. Specific leaf area (SLA) ranged from 150.02 to 177.54 cm^2/g with mean of 160.39 cm^2/g (Table 4.9). Per cent improvement over IR-64 was much more for yield (~50 per cent). Reduction in leaf temperature among TILs was observed. These all suggests the improvement of TILs over IR-64 (Fig. 4.5).

Correlation Matrix

Negative correlation was found between spikelet fertility and $\Delta^{13}C$. Leaf temperature showed negative correlation with yield attributes. $\Delta^{13}C$ was highly negatively correlated with total dry matter. Yield attributes such as tiller number, panicle number, spikelet fertility, harvest index was strongly positively correlated with yield (Table 4.10).

4.2.1.4 Performance of parents and TILs for morpho-physiological traits in GKVK in Summer season 2018

Mean performance of the parents (IR-64, AC-39020 and IET-16348) and TILs for the measured traits under semi-irrigated aerobic condition is shown in Table 4.11. SCMR of the selected plants statistically did not vary compared to IR-64 whereas leaf temperature of the selected plants was statistically significant compared to IR-64. Days to fifty per cent flowering of TILs was similar to the IR-64. TILs had mean grain yield of 32.79 g per plant which ranged 20.04 to 35.34 g per plant. IR-64, AC-39020 and IET-16348 had grain yield of 26.91, 19.09 and 27.16 g per plant, respectively. The value of $\Delta^{13}C$ for IR-64, AC-39020 and IET-16348 was 22.45, 20.56 and 20.67 per mil, respectively. $\Delta^{13}C$ for TILs ranges from 18.19 to 22.75 per mil. Leaf temperature for IR-64, AC-39020 and IET-16348 was 34.5, 30.40 and 31.2 degree Celsius, respectively (Table 4.11). The per cent improvement over IR-64 for yield ~22, LT ~6 and $\Delta^{13}C$ was ~9 (Fig. 4.5).

Table 4.9: Performance of TILs along with parents for morpho-physiological traits in TNAU (Summer, 2017)

Parameters	IR-64	AC-39020	IET-16348	F>Pr	Mean	Minimum	Maximum	σ2g
PHT	80.85	106.30	80.18	*	78.96	75.40	83.80	ns
DFF	78.00	95.00	77.00	*	78.76	72.00	85.00	*
SCMR	41.53	41.82	39.87	ns	41.45	39.43	43.32	ns
TLA	1915.78	3257.01	2217.25	**	1914.93	1883.61	1937.54	ns
Yield	13.56	11.49	13.40	ns	20.89	18.91	22.29	*
TDM	46.64	53.85	49.39	*	53.50	50.02	58.39	*
SF	70.31	68.86	69.50	ns	67.76	63.18	71.43	*
HI	0.29	0.24	0.27	ns	0.39	0.37	0.41	*
LT	39.52	35.93	37.87	*	35.61	34.60	36.43	*
SLA	158.83	192.59	180.34	*	160.39	150.02	177.54	*
TN	15.50	12.50	17.17	*	18.01	16.00	19.83	*
$\Delta^{13}C$	22.61	21.88	19.60	*	21.50	18.93	22.24	*

* *and* ** significant P <0.05, and p<0.01 respectively. ns: is non- significant

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TDM-Total Dry Matter, SF-Spikelet fertility, LT-Leaf Temp, SLA-Specific Leaf Area, HI- Harvest Index, TN-Tiller Number

Figure 4.5: Per cent improvement over IR-64 for Yield, $\Delta^{13}C$, LT under different seasons

Table 4.10: Correlation matrix for various traits (TNAU, Summer 2017)

Variables	Yield	TDM	LT	SCMR	TN	PN	PHT	SLA	DFF	$\Delta^{13}C$	SF	TLA	HI
Yield	**1**	**0.917**	**-0.611**	0.323	**0.449**	0.236	**-0.417**	-0.172	-0.084	-0.333	**0.444**	0.099	**0.698**
TDM	**0.917**	**1**	**-0.485**	0.372	0.239	0.328	-0.172	0.015	0.031	**-0.376**	**0.557**	**0.376**	**0.386**
LT	**-0.611**	**-0.485**	**1**	-0.085	**-0.548**	0.130	0.004	0.103	0.171	0.287	-0.268	-0.192	**-0.494**
SCMR	0.323	0.372	-0.085	**1**	0.046	0.092	0.115	0.249	**0.460**	-0.046	**0.399**	0.344	0.122
TN	**0.449**	0.239	**-0.548**	0.046	**1**	-0.187	**-0.455**	-0.164	-0.299	-0.284	0.324	-0.177	**0.534**
PN	0.236	0.328	0.130	0.092	-0.187	**1**	-0.157	0.056	-0.149	-0.092	-0.057	0.077	-0.102
PHT	**-0.417**	-0.172	0.004	0.115	**-0.455**	-0.157	**1**	**0.547**	0.279	-0.071	0.119	**0.658**	**-0.693**
SLA	-0.172	0.015	0.103	0.249	-0.164	0.056	**0.547**	**1**	0.210	-0.230	0.186	**0.767**	**-0.520**
DFF	-0.084	0.031	0.171	**0.460**	-0.299	-0.149	0.279	0.210	**1**	-0.074	0.127	0.254	-0.135
$\Delta^{13}C$	-0.333	**-0.376**	0.287	-0.046	-0.284	-0.092	-0.071	-0.230	-0.074	**1**	**-0.437**	-0.348	-0.031
SF	**0.444**	**0.557**	-0.268	**0.399**	0.324	-0.057	0.119	0.186	0.127	**-0.437**	**1**	**0.478**	-0.010
TLA	0.099	**0.376**	-0.192	0.344	-0.177	0.077	**0.658**	**0.767**	0.254	-0.348	**0.478**	**1**	**-0.481**
HI	**0.698**	**0.386**	**-0.494**	0.122	**0.534**	-0.102	**-0.693**	**-0.520**	-0.135	-0.031	-0.010	**-0.481**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Note: Correlation coefficient for various traits like Yield, TDM-Total Dry Matter, LT-Leaf Temperature, SCMR- SPAD Chlorophyll Meter Reading, TN-Tiller Number, PN-Panicle Number, PHT-Plant height, SLA-Specific Leaf Area, DFF-Days to 50%flowering, D13C-Carbon Isotope Discrimination, SF- Spikelet fertility, HI- Harvest Index and TLA-Total Leaf Area are given in the table.

Table 4.11: Performance of TILs and parents for morpho-physiological traits in GKVK (Summer, 2018)

Parameters	IR 64	AC 39020	IET 16348	F>Pr	Mean	Minimum	Maximum	σ2g
PHT	79.20	113.80	82.40	*	84.63	81.80	88.40	ns
DFF	82.00	95.00	81.00	*	81.36	78.00	84.00	*
SCMR	47.32	47.52	43.93	ns	44.06	41.66	47.56	*
TLA	2209.00	3115.00	2258.30	**	2248.64	1910.54	2256.74	ns
Yield	26.91	19.09	27.16	*	32.79	20.04	35.34	*
TDM	68.99	87.60	76.41	*	75.31	68.99	89.90	*
SF	79.50	84.60	84.10	ns	82.37	78.90	86.10	*
HI	0.39	0.22	0.36	*	0.38	0.30	0.43	*
LT	34.50	30.40	31.20	*	32.66	30.30	35.40	*
SLA	185.10	218.20	172.60	*	194.90	180.02	202.71	*
TN	25.72	19.73	20.15	*	30.21	24.87	37.34	*
$\Delta^{13}C$	22.45	20.56	20.67	*	20.64	18.19	22.75	*

* *and* ** significant P <0.05, and p<0.01 respectively. ns: is non- significant

PHT-Plant height, DFF-Days to 50%flowering, SCMR-SPAD Chlorophyll Meter Reading, TLA-Total Leaf Area, TDM-Total Dry Matter, SF-Spikelet fertility, LT-Leaf Temp, SLA-Specific Leaf Area, HI- Harvest Index, TN-Tiller Number

Correlation Matrix

Yield was positively correlated with yield attributes. Total leaf area was negatively correlated with leaf temperature. Leaf temperature showed negative correlation with yield attributes (Table 4.12).

4.2.2 Consistency of traits across seasons

Phenotype of a plant is a dynamic interaction between its genotype and the prevailing environmental conditions. Quantitative traits are controlled by many genes exhibiting a string of modulation by the environment. Although an inconsistent phenotypic expression would not be useful in crop improvement due to lack of stability. Hence, it becomes essential that environmental and genetic control of any given trait is assessed before it can be exploited. The mean values of a few important traits are compared to assess the effects of the environment. Stability of the phenotypic expression across environment or location or season is a good indicator of strong genetic control of the traits. Heritability of such traits will be generally high. Majority of the traits exhibited continuous variation indicating a Quantitative inheritance pattern. Regressions analysis was carried out to assess the consistency of a few traits across seasons and environment. The results of the analysis are described under this section.

To examine the consistency of WUE, regression analysis was done between $\Delta^{13}C$ values among the selected 25 TILs measured in field experiment across various seasons and locations. Data indicated that there was a consistency between the experiments for CID values (Fig. 4.6, 4.7, 4.8, 4.9) indicating that $\Delta^{13}C$ is a consistent parameter in terms of its relative ranking. Similarly, consistency analysis was done for grain yield and leaf temperature to verify whether there is consistency among the seasons or not. The results implicate that these traits are stable across the season or location in these lines (Fig. 4.6, 4.7, 4.8, 4.9). It further suggests that the QTLs effects are stable across the season and location.

Table 4.12: Correlation matrix for various traits (GKVK, Summer 2018)

Variables	Yield	TDM	LT	SCMR	TN	PHT	SLA	DFF	$\Delta^{13}C$	SF	HI	TLA
Yield	**1**	**0.548**	**-0.492**	-0.114	**0.603**	-0.030	0.173	-0.020	-0.299	0.195	**0.712**	0.333
TDM	**0.548**	**1**	-0.314	**0.429**	**0.436**	0.123	0.372	-0.059	-0.283	0.102	-0.194	**0.394**
LT	**-0.492**	-0.314	**1**	0.149	**-0.496**	0.069	-0.247	-0.008	-0.056	-0.205	-0.320	**-0.512**
SCMR	-0.114	**0.429**	0.149	**1**	-0.131	0.211	0.042	-0.174	-0.043	-0.050	**-0.494**	0.161
TN	**0.603**	**0.436**	**-0.496**	-0.131	**1**	0.107	0.307	-0.138	-0.128	0.310	0.344	0.079
PHT	-0.030	0.123	0.069	0.211	0.107	**1**	-0.022	-0.105	0.002	0.032	-0.158	-0.149
SLA	0.173	0.372	-0.247	0.042	0.307	-0.022	**1**	0.016	**-0.454**	**0.405**	-0.121	0.135
DFF	-0.020	-0.059	-0.008	-0.174	-0.138	-0.105	0.016	**1**	-0.012	0.047	0.034	0.138
$\Delta^{13}C$	-0.299	-0.283	-0.056	-0.043	-0.128	0.002	**-0.454**	-0.012	**1**	0.006	-0.081	0.052
SF	0.195	0.102	-0.205	-0.050	0.310	0.032	**0.405**	0.047	0.006	**1**	0.145	0.157
HI	**0.712**	-0.194	-0.320	**-0.494**	0.344	-0.158	-0.121	0.034	-0.081	0.145	**1**	0.074
TLA	0.333	**0.394**	**-0.512**	0.161	0.079	-0.149	0.135	0.138	0.052	0.157	0.074	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Note: Correlation coefficient for various traits like Yield, TDM-Total Dry Matter, LT-Leaf Temperature, SCMR- SPAD Chlorophyll Meter Reading, TN-Tiller Number, PHT-Plant height, SLA-Specific Leaf Area, DFF-Days to 50%flowering, $\Delta^{13}C$ -Carbon Isotope Discrimination, SF-Spikelet fertility, HI- Harvest Index and TLA-Total Leaf Area are given in the table.

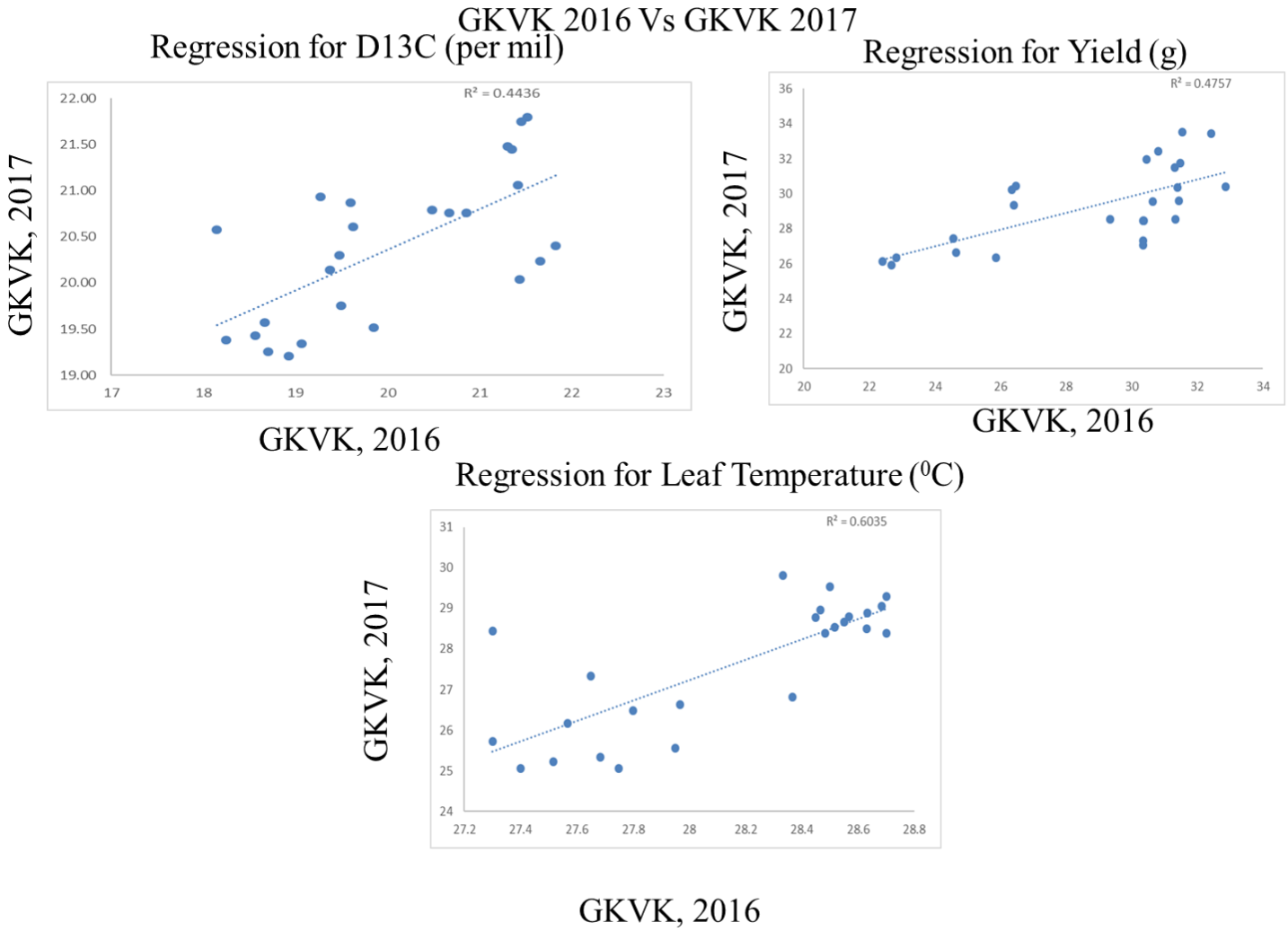

Figure 4.6: Consistency of traits such as $\Delta^{13}C$, Yield and Leaf temperature under GKVK 2016 Vs GKVK 2017

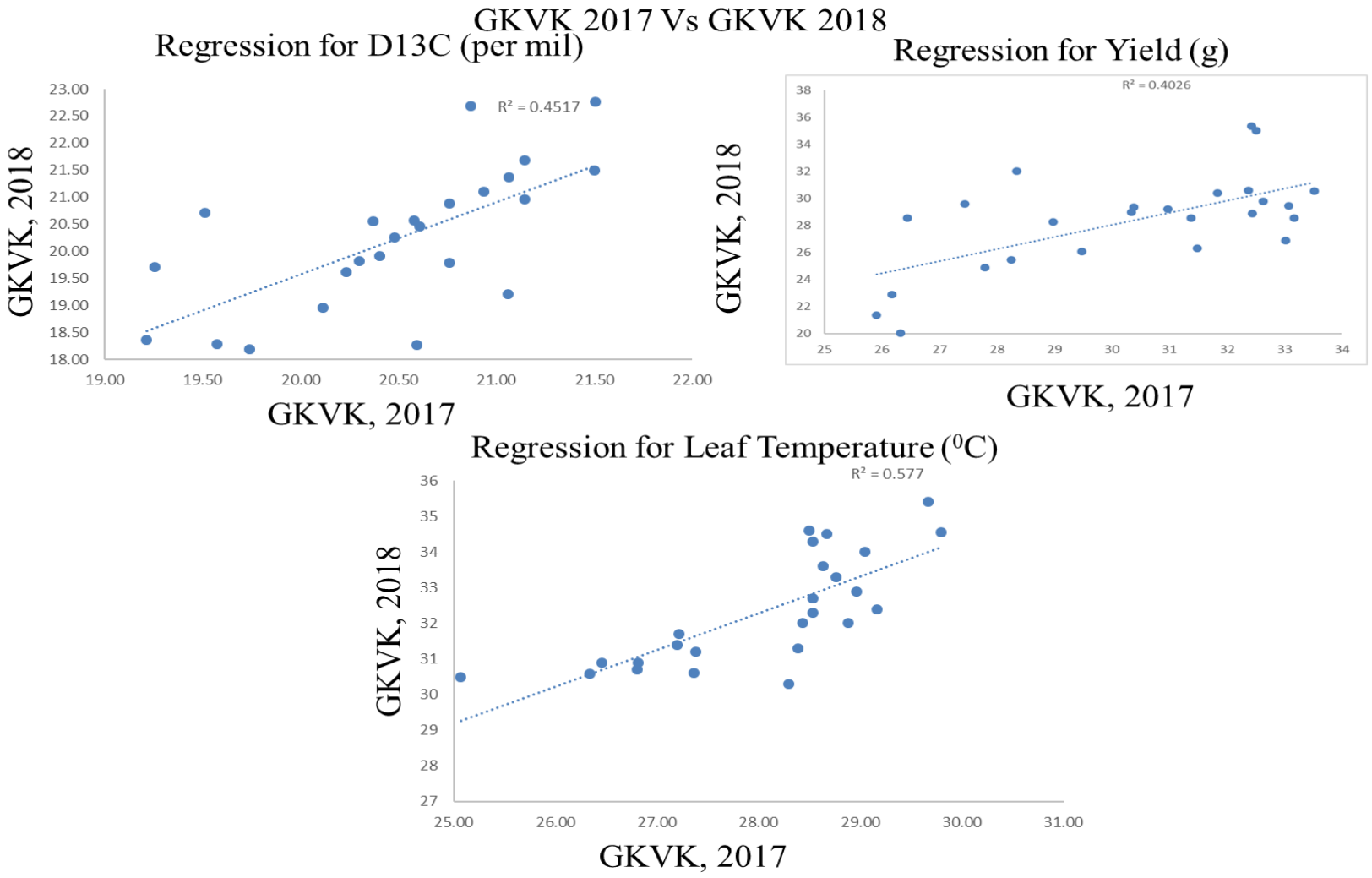

Figure 4.7: Consistency of traits such as $\Delta^{13}C$, Yield and Leaf temperature under GKVK 2017 Vs GKVK 2018

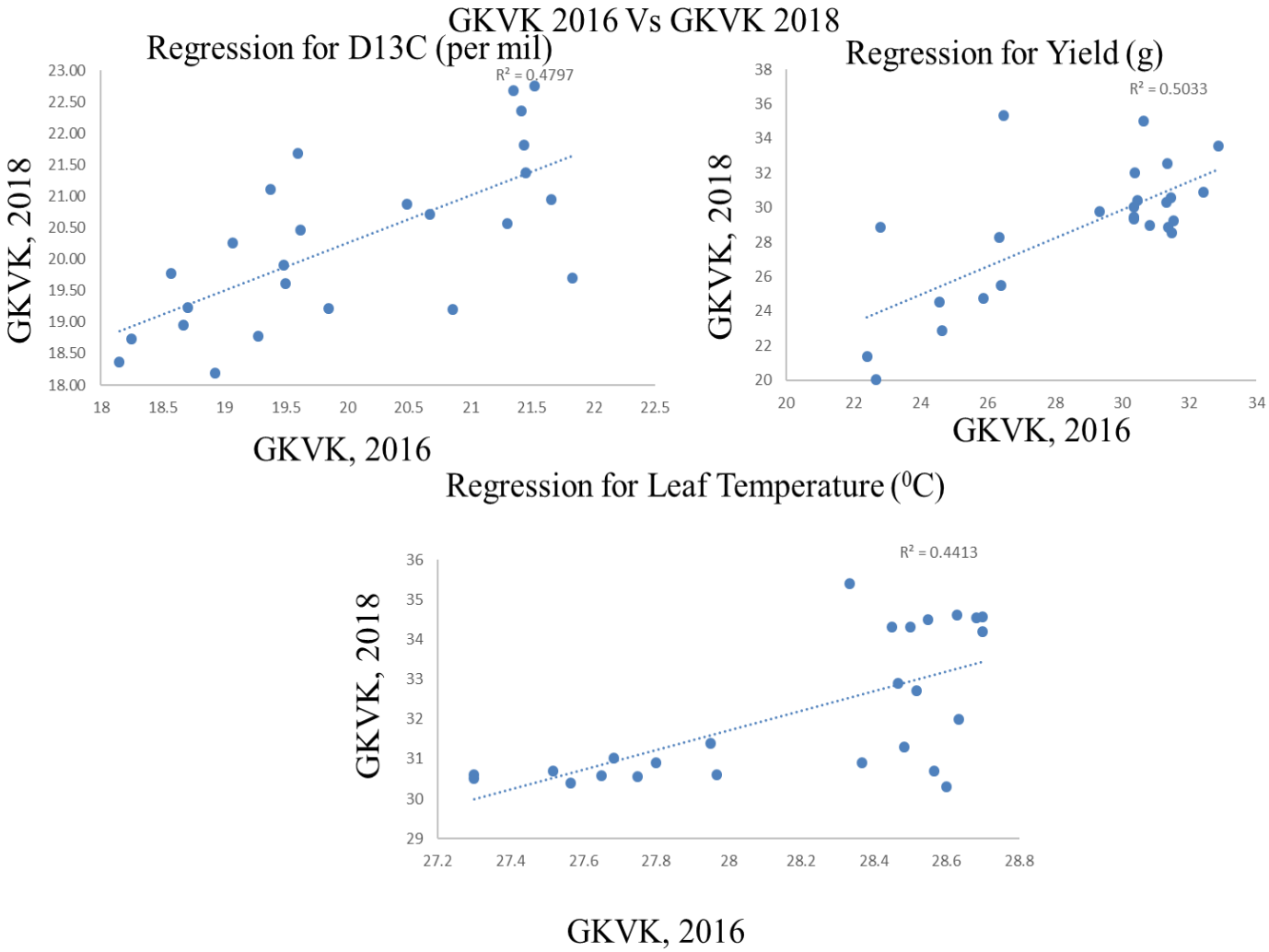

Figure 4.8: Consistency of traits such as $\Delta^{13}C$, Yield and Leaf temperature under GKVK 2016 Vs GKVK 2018

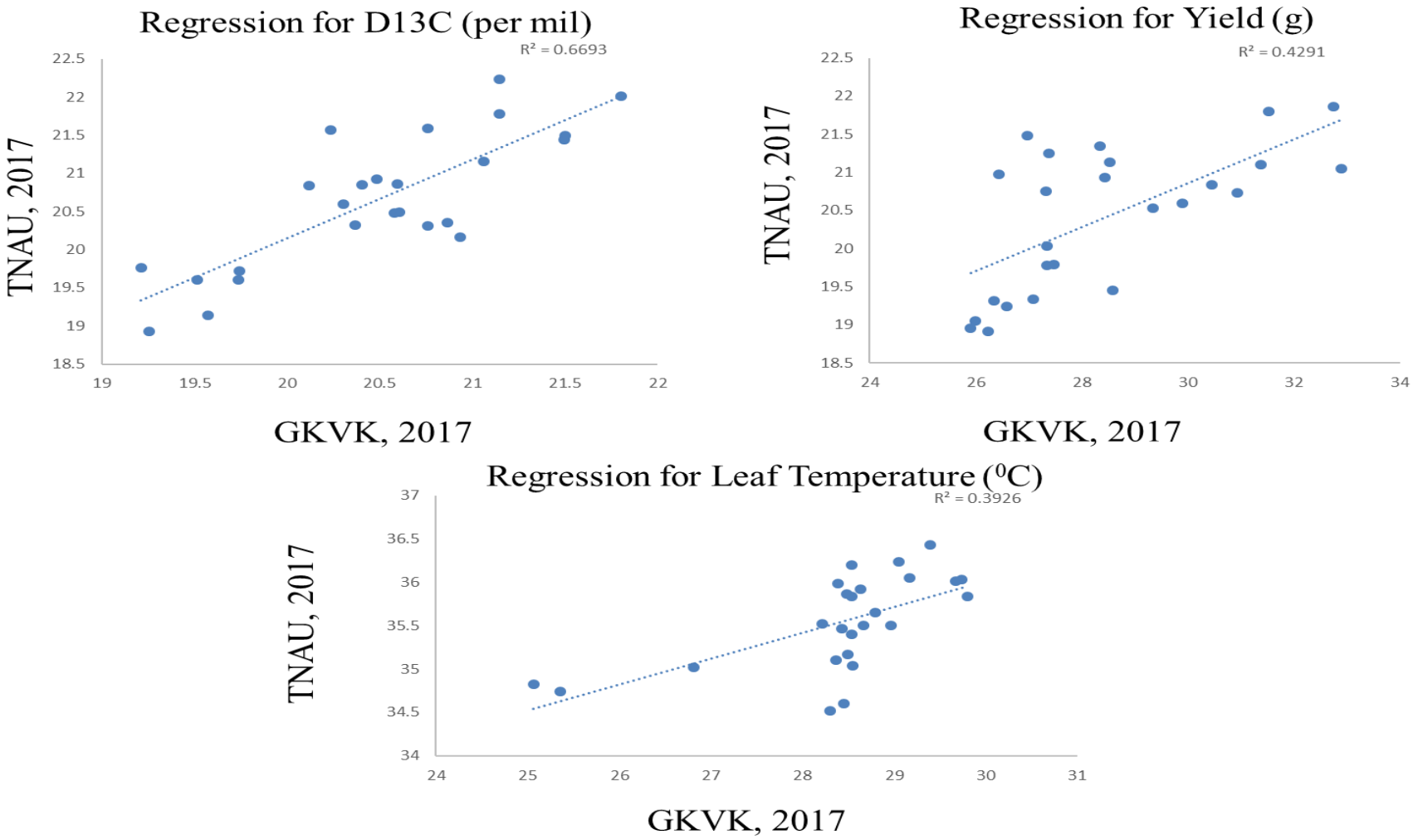

Figure 4.9: Consistency of traits such as $\Delta^{13}C$, Yield and Leaf temperature under GKVK 2017 Vs TNAU 2017

4.3 Marker class analysis

Based on previous genotypic data with 16 foreground markers (Prathibha, 2016) (Table 3.4) and current phenotypic data, marker class analysis was carried out with 260 $DCBC_3F_4$ lines. Coding of genotypic data was done with "Y" and "N" letters based on the alleles present from donor and other parents. Donor alleles (allele of AC-39020 for root traits and IET-16348 for $\Delta^{13}C$ was denoted as "Y" and other than donor alleles with "N". Coded data is given in plate 3.3. The phenotypic performance of the TILs was analysed with the presence of marker loci from trait donor genotypes.

The lines were grouped into low root and high root type with low $\Delta^{13}C$ value and higher yield. Low root lines with low $\Delta^{13}C$ had a particular combination of markers/QTLs and high root lines with low $\Delta^{13}C$ had different combination pattern. This assessment of marker combination is all that more important as root and WUE traits have an intrinsic trade-off (Table 4.13, Table 4.14). This suggests that a specific combination of marker loci would be important to achieve greater success in introgressing relevant traits. Low root types were having the alleles of RM 2584, RM 1388 and RM 16 from AC-39020. However, high root types had RM 2584 and RM 1388 alleles from AC-39020 and RM 16 alleles from non-donor parent.

4.4 Validation of the combination by physiological characterization

4.4.1 Selection of best trait introgressed lines based on marker combination

From the 25 TILs studied under various seasons, six lines were selected based on the marker/QTL combination given in Table 4.15. These six TILs (TIL # 18, 54, 55, 84, 228, 248) along with the recurrent parent IR-64 were grown under various moisture regimes in phenomics experiment.

Developing aerobic worthy rice varieties with yield potential will help in maximizing rice production in the challenging environment of water scarcity. Mean yield and relative yield performance under well-watered and water limited conditions are the most widely used criteria for selecting genotypes for water limited environments.

Table 4.13: Number of markers and traits variability of TILs

TIL#	Root markers	D13C marker	Total	D13C	RL	RWT
1	3	1	4	19.80	33.33	2.80
2	2	2	4	19.93	37.00	2.23
3	1	3	4	20.08	20.00	1.55
4	4	3	7	19.20	32.50	3.55
5	4	3	7	18.94	39.50	2.67
6	4	3	7	19.94	41.50	4.60
7	2	4	6	18.86	32.33	3.40
8	2	4	6	19.41	32.00	6.30
9	4	4	8	20.23	31.33	4.05
10	4	3	7	18.43	36.00	3.88
11	3	3	6	19.86	42.00	5.20
12	5	3	8	19.73	43.67	4.25
24	4	3	7	20.32	28.00	4.50
25	4	1	5	20.19	28.33	3.95
26	1	3	4	19.12	34.50	4.60
27	5	3	8	19.38	40.00	3.00
28	3	2	5	20.40	31.50	3.17
29	2	2	4	19.58	30.00	2.40
30	3	3	6	19.68	26.50	4.50
243	3	2	5	20.17	33.00	2.53
244	3	1	4	18.75	31.00	6.85
245	0	2	2	19.71	33.50	4.65
246	3	2	5	20.26	30.00	7.13
247	8	0	8	16.31	32.00	2.73
248	2	2	4	20.44	22.50	4.07
249	3	3	6	20.15	29.00	5.05
250	3	0	3	18.50	32.67	4.15
251	0	2	2	20.36	32.33	6.83
140	6	3	9	19.75	34.67	3.10

RL- Root Length; RWT-Root Weight

Table 4.14: Marker combination and the trait value of the TILs

Low root, Low yield

TIL	RL	RWT	RM80	RM2584	RM1388	RM262	RM239	RM3825	RM16	RM3276	RM247	RM167	RM4455	RM71	# of root markers
122	25.33	1.65	N	Y	Y	N	Y	N	Y	N	N	Y	N	N	5
56	33.67	2.15	Y	Y	Y	Y	N	N	Y	N	N	Y	N	N	6
156	43.00	2.37	N	Y	Y	N	N	Y	Y	N	N	N	N	N	4
20	22.50	2.78	N	Y	Y	N	N	N	Y	N	Y	Y	N	N	5
38	32.67	2.90	Y	Y	Y	N	N	N	Y	N	N	Y	N	N	5
27	40.00	3.00	N	Y	Y	N	N	N	Y	N	Y	Y	N	N	5

High root, High yield

TIL	RL	RWT	RM80	RM2584	RM1388	RM262	RM239	RM3825	RM16	RM3276	RM247	RM167	RM4455	RM71	# of root markers
15	33.00	3.70	N	Y	Y	N	Y	N	N	N	N	Y	N	N	4
219	33.00	3.75	N	Y	Y	N	N	Y	N	N	N	N	N	N	3
10	36.00	3.88	N	Y	Y	Y	N	N	N	N	N	Y	N	N	4
108	32.33	3.90	N	Y	Y	N	Y	N	N	N	N	N	N	N	3
232	35.67	4.05	N	Y	Y	N	N	N	N	N	N	N	N	N	2
215	24.50	4.13	N	Y	Y	N	N	N	N	N	N	N	Y	N	3
250	32.67	4.15	N	Y	Y	Y	N	N	N	N	N	N	N	N	3
12	43.67	4.25	N	Y	Y	Y	N	Y	N	N	Y	N	N	N	5
55	40.00	4.25	Y	Y	Y	N	N	N	N	N	N	N	N	N	3
134	35.00	4.25	N	Y	Y	N	Y	N	N	N	N	Y	N	N	4
78	44.67	4.65	Y	Y	Y	N	N	N	N	Y	N	N	N	N	4
35	26.00	4.90	N	Y	Y	N	N	N	N	N	N	Y	N	N	3
213	30.50	4.93	N	Y	Y	N	N	Y	N	N	N	N	Y	N	4
199	44.00	7.95	N	Y	Y	N	Y	N	N	N	N	N	N	N	3
77	41.00	7.50	Y	Y	Y	N	N	N	N	N	N	Y	N	N	4
161	37.00	6.85	N	Y	Y	Y	Y	Y	N	N	N	Y	Y	N	7
124	33.50	6.27	N	Y	Y	Y	Y	N	N	N	N	Y	Y	N	6
43	24.33	6.10	N	Y	Y	Y	N	N	N	N	N	N	N	N	3
50	36.00	5.78	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	5
210	35.00	5.43	N	Y	Y	Y	N	Y	N	Y	N	Y	Y	N	7
167	40.00	5.33	N	Y	Y	Y	Y	N	N	N	N	N	N	N	4
159	41.33	4.97	N	Y	Y	N	N	Y	N	N	N	Y	Y	N	5

RL-Root Length, RWT-Root Weight

Table 4.15: List of selected TILs for phenomics experiment 1

# TIL	Indicated for phenomics experiment
18	1
248	2
228	3
84	4
54	5
55	6

The ability of crop cultivars to perform reasonably well in water limited condition is paramount for stability of production. The combination of high yield stability and high relative yield under water limited condition has been proposed as useful selection criteria for characterizing genotypic performance under varying degrees of water stress (Pinter *et al.*, 1990).

A Mini-Lysimeter based phenomics platform conceptualized at our center was used for the precise maintenance of soil and plant moisture status. The approach involves the real time measurement of transpiration and soil moisture status in container grown plants exposed to natural field environment conditions. The evapotranspiration (ET) interfaced renewal of water provides an option to maintain the desired soil moisture status across the genotypes irrespective of variation in transpiration. Non-invasive phenotyping tools like Fieldspec (Spectral reflectance characteristics), Planteye (imaging growth), IR Camara (thermal imaging) made the platform highly accurate in terms of real time and high through put phenotyping.

Phenomics experiment 1:

In this experiment, plants were phenotyped under three moisture regimes (100%FC, 65% FC and 45% FC during kharif 2017. Six TILs were renamed as follows (Table 4.15).

The morpho-physiological parameters like total dry matter, total leaf area, yield, spikelet fertility, root traits, $\Delta^{13}C$, relative water content (RWC) and gas exchange parameters were recorded for the six TILs along with IR-64.

Relative water content (RWC) is the suitable measure of plant water status in terms of the physiological consequence of cellular water deficit. Relative water content of the tissue is primary factor affected under moisture stress. Relative water content of IR-64 and TILs did not differ significantly under 100% FC (well-watered) condition (Fig 4.10). But relative water content of the TILs under 65% FC and 45% FC (water limited) condition were higher compared to IR-64. RWC of IR-64 under water limited condition

was 68.2 per cent (65%FC) & 64.75 per cent (45% FC) and for TILs it was in the range of 80-85per cent under the two regimes of water limitation (Fig 4.10).

Drought stress suppresses leaf expansion, tillering and midday photosynthesis (Kramer and Boyer, 1995) and reduces photosynthetic rates and leaf area due to early senescence (Nooden, 1988). All of these factors are responsible for a reduction in dry matter accumulation and grain yield under drought. The instantaneous photosynthetic rate

was low for IR-64 compared to TILs even under well-watered condition (Fig 4.10). Although, the reduction in leaf area was not significantly for TILs as well as IR-64, the yield and its attributes reduced in case of IR-64 compared to the TILs under water limited conditions (Fig 4.11). The $\Delta^{13}C$ value was not significantly different for TILs and IR-64 under all water regimes (Fig 4.11).

IR-64 had lower root biomass compared with TILs at all three water regimes. It shows that all the trait introgressed lines have better root growth than IR-64 at all water regimes (Fig 4.11).

Per cent improvement over IR-64 for the traits like root weight, yield, leaf temperature and $\Delta^{13}C$ signifies the importance of trait based breeding (Fig 4.12).

Phenomics experiment 2:

The same TILs along with IR-64 were grown in two water regimes (100% FC and 65% FC during summer season of 2017). The morpho-physiological parameters total dry matter, total leaf area, spikelet fertility, root traits, $\Delta^{13}C$, relative water content (RWC) and gas exchange parameters and also yield were recorded. Relative water content at water limited condition (65% FC) decreased for all the TILs as well as for IR-64. But the reduction was more for IR-64 (reduced from 72.8 to 43.7 %) (Fig. 4.13). It suggests that at same water regimes, IR-64 experienced greater tissue dehydration than TILs.

Water deficit reduces cell expansion and hence photosynthesis. The photosynthetic rate of IR-64 was lower than TILs even under well-watered condition (Fig. 4.13). As IR-64 is an irrigated rice variety, at 100% FC also it experiences stress.

Therefore, the total leaf area under well-watered treatment was less, compared to TILs. There was no significant difference in $\Delta^{13}C$ value of IR-64 and TILs under both water regimes (Fig. 4.14).

Per cent improvement of yield over IR-64 was 22.3% and 38.6% at 100% and 65% FC respectively. Per cent improvement over IR-64 for $\Delta^{13}C$ was -9.7 and -17.3% at 100% and 65% FC respectively. Leaf temperature improvement over IR-64 suggests root traits has been improved for TILs (Fig. 4.14).

Phenomics experiment 3:

Apart from the six TILs, few more TILs were selected from the set of 260 $DCBC_3F_4$ lines (Table 4.16) to reconfirm the interaction effect of makers/QTLs. These TILs had the same marker combinations. The total lines that were phenotyped in this experiment was 14 (13 TILs and IR-64). These lines were phenotyped for various drought adaptive traits such as root weight, $\Delta^{13}C$, yield and its attributes, mean transpiration rate (MTR), relative water content (RWC), Net Assimilation Rate (NAR), Water use efficiency on whole plant basis, Leaf temperature etc.

Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure (Taiz and Zeiger, 2006). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Impaired mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under drought (Nonami, 1998; Kaya *et al*., 2006; Hussain *et al.*, 2008).

The yield of IR-64 in control/well-watered condition was 68.4 g per plant and in stressed condition 63.3 g per plant. Mean yield of TILs was 80.22 and 74.62 g under control (well-watered) and stressed (water limited) conditions, respectively (Table 4.17).

The stable isotope ratio of carbon was determined using isotope ratio mass spectrometer with samples collected under both the water regimes. Significant genotypic variation in $\Delta^{13}C$ was noticed among the TILs.

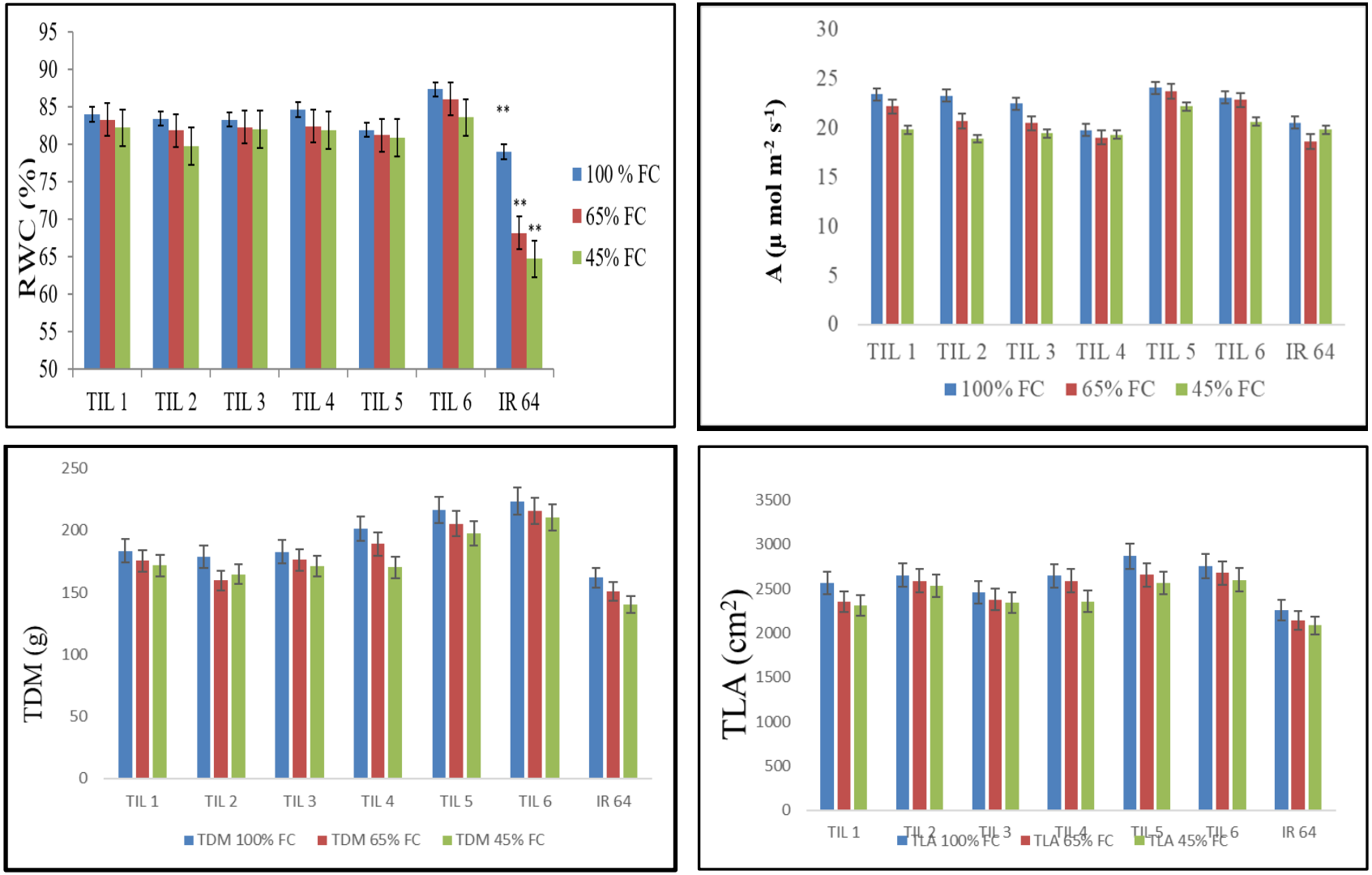

Figure 4.10: Various parameters under different water regimes for TILs along with recurrent parent

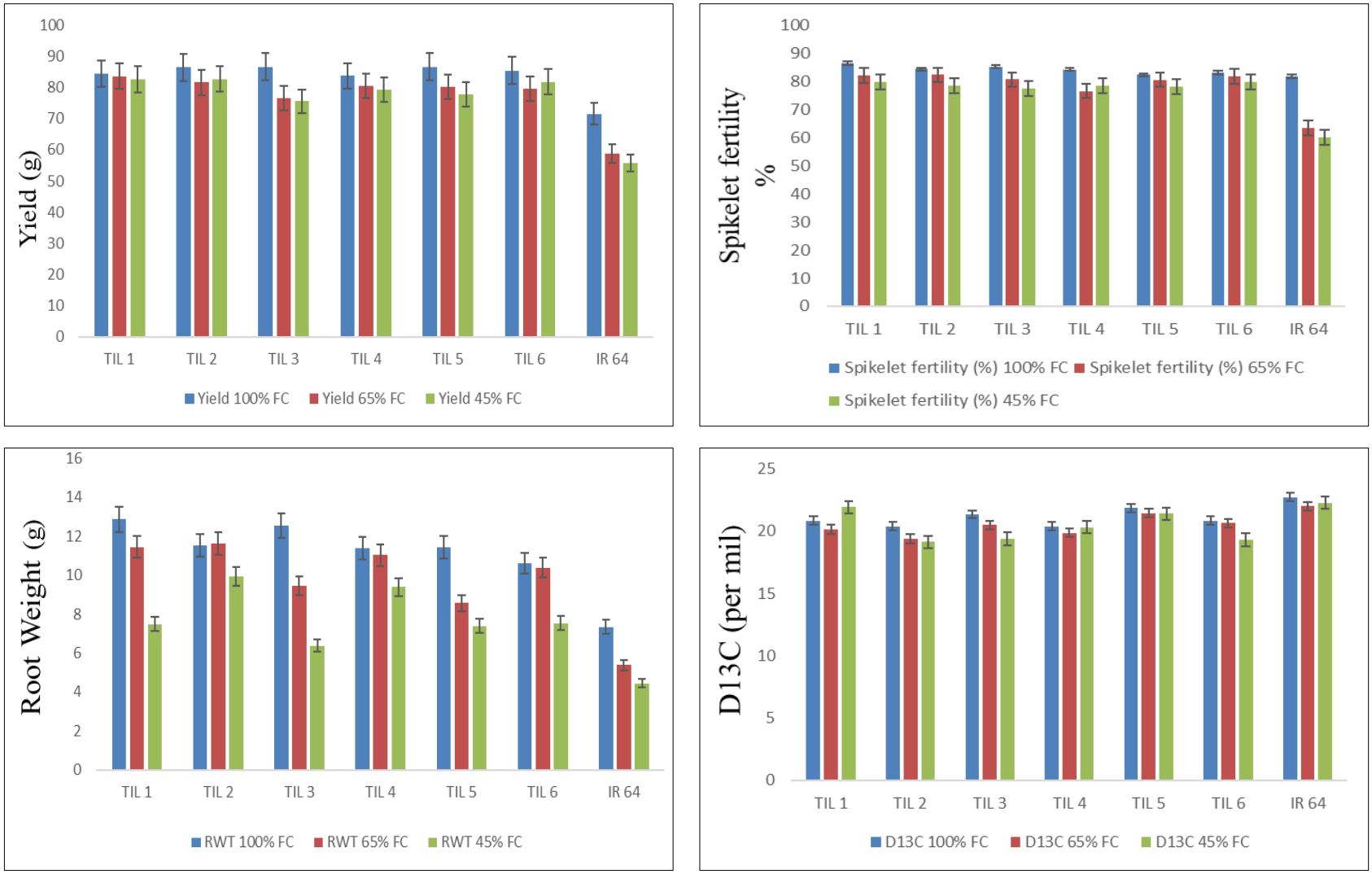

Figure 4.11: Various parameters under different water regimes for TILs along with recurrent parent

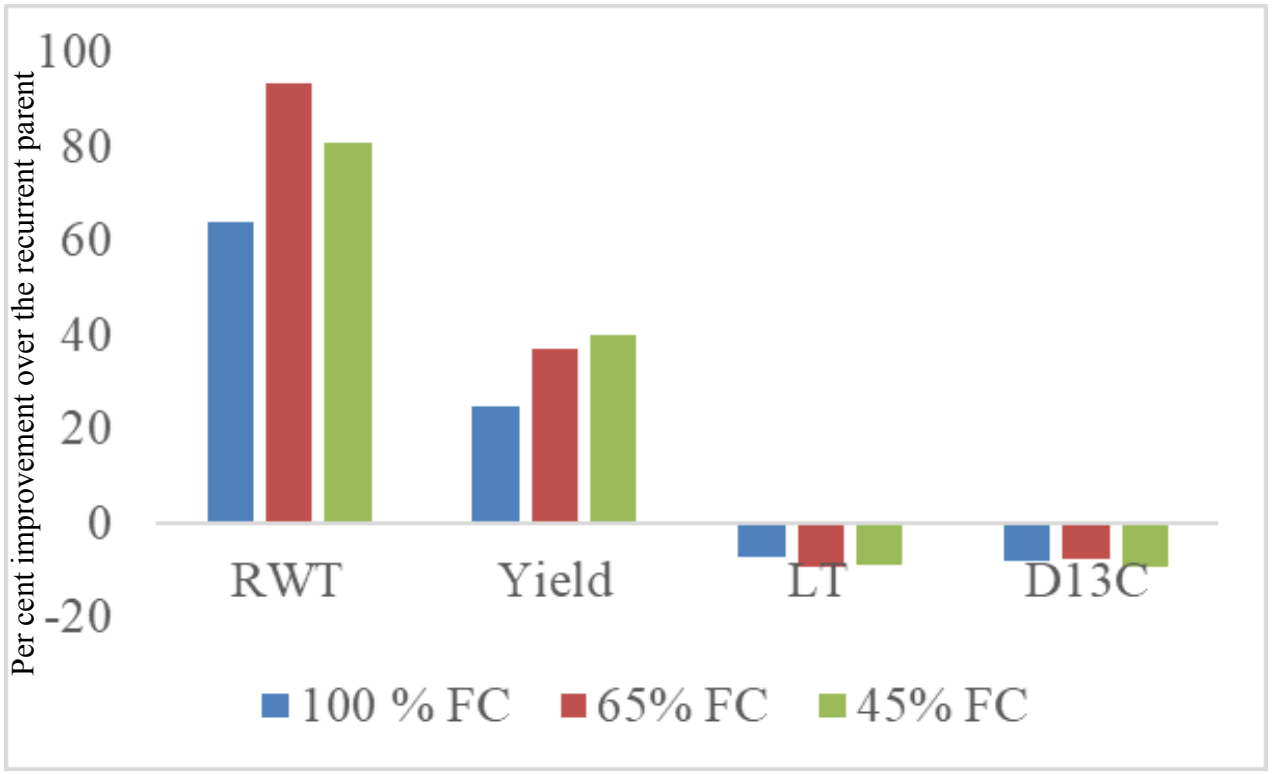

Figure 4.12: Per cent improvement over the recurrent parent

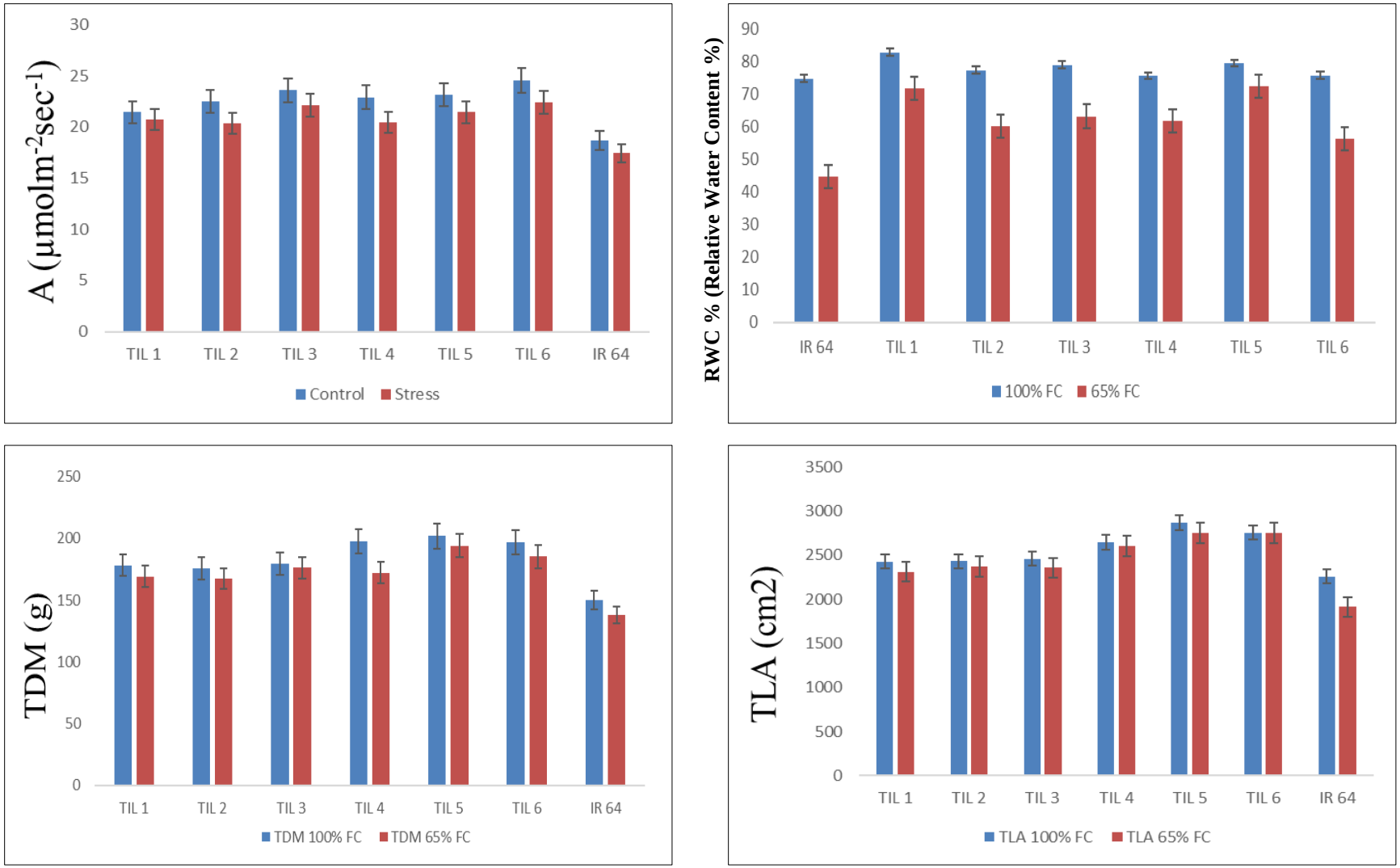

Figure 4.13: Various parameters under different water regimes for TILs along with recurrent parent

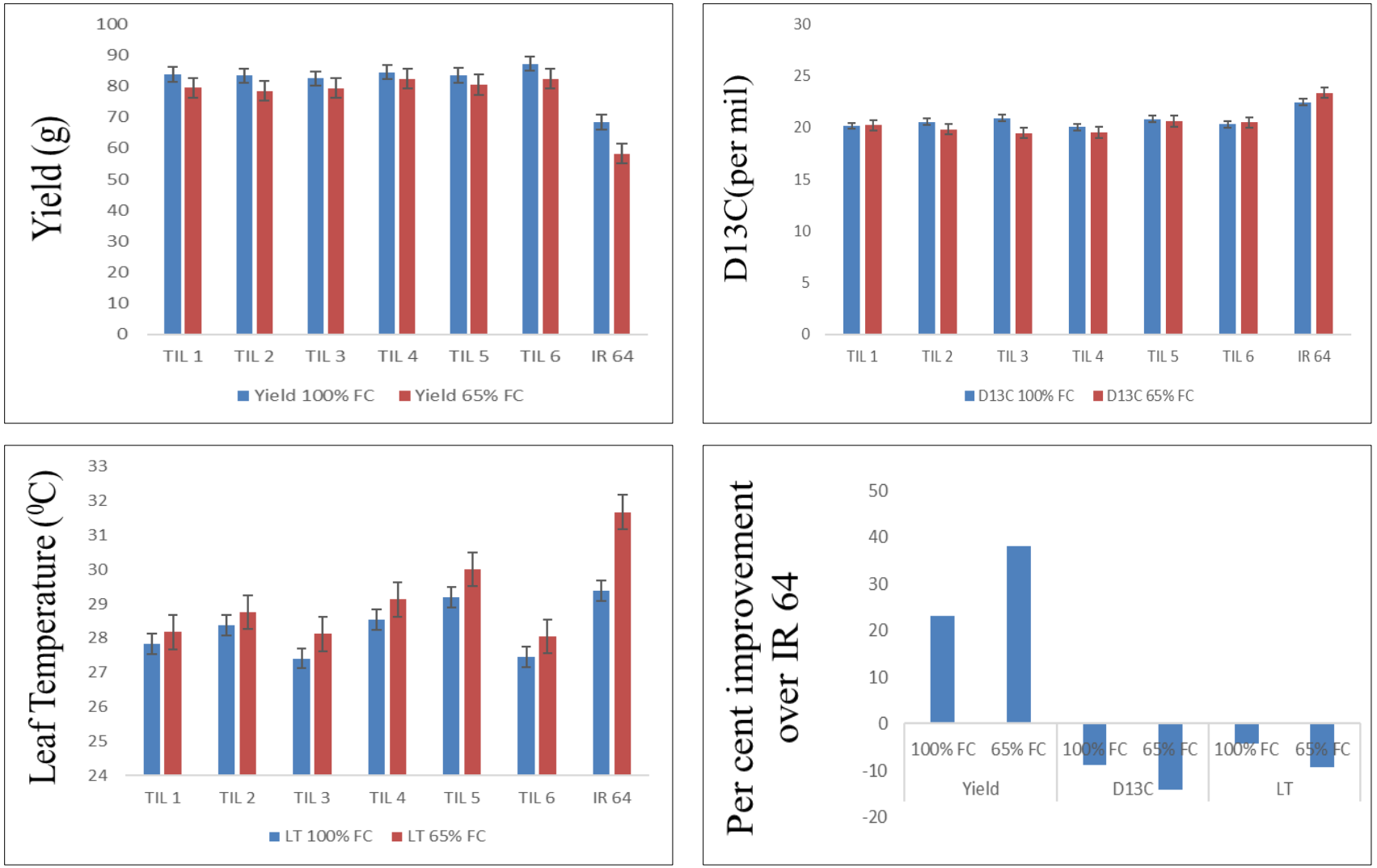

Figure 4.14: Various parameters under different water regimes for TILs along with recurrent parent

Table 4.16: Selection of trait introgressed lines based on marker combination

Marker profile	Phenomics	RM80	RM2584	RM1388	RM262	RM239	RM3825	RM16	RM3276	RM247	RM167	RM4455	RM71
18	1	Y	Y	Y	Y	N	N	N	N	Y	N	N	N
54	5	N	Y	Y	N	N	N	Y	N	N	N	N	N
55	6	Y	Y	Y	N	N	N	N	N	N	N	N	N
84	4	Y	N	Y	N	Y	N	N	N	N	Y	N	N
228	3	N	N	N	N	N	N	Y	N	Y	N	N	N
248	2	N	N	N	N	N	N	N	N	N	Y	Y	N
122	7	N	Y	Y	N	Y	N	Y	N	N	Y	N	N
56	9	Y	Y	Y	Y	N	N	Y	N	N	Y	N	N
20	8	N	Y	Y	N	N	N	Y	N	Y	Y	N	N
199	11	N	Y	Y	N	Y	N	N	N	N	N	N	N
77	10	Y	Y	Y	N	N	N	N	N	N	Y	N	N
215	12	N	Y	Y	N	N	N	N	N	N	N	Y	N

Note-Marker profile column contains the generic number of the TILs. Phenomics column contains the number provided to the TILs in phenomics.

Table 4.17: Performance in terms of root weight of TILs and IR-64 under stress and control conditions and its improvement

Genotype	Root weight (g)		% increase over control	% increase over IR 64
	Control	Stress		
TIL 1	11.57	15.10	23.43	54.85
TIL 2	14.44	17.12	15.63	60.16
TIL 3	22.20	16.34	-35.87	58.25
TIL 4	11.56	16.25	28.82	58.02
TIL 5	14.21	13.21	-7.60	48.37
TIL 6	13.24	17.20	22.99	60.34
TIL 7	9.29	15.64	40.56	56.38
TIL 8	11.61	22.56	48.52	69.77
TIL 9	12.32	13.23	6.90	48.45
TIL 10	13.17	13.32	1.16	48.80
TIL 11	11.88	13.21	10.08	48.37
TIL 12	12.99	18.24	28.81	62.61
TIL 13	10.98	14.68	25.22	53.55
IR 64	9.04	6.82	-32.42	

An inverse relationship between WUE and $\Delta^{13}C$ (Fig. 4.15) are in accordance with expected trends, confirming the relevance of stable isotope ratios as a time averaged surrogate for WUE and transpiration rate.

Total water transpired (CWT) during the entire period of crop growth was monitored on a real time basis at the MLM platform. CWT varied significantly among the TILs. The CWT of IR-64 was 44.9 L which was higher than TILs. The ratio of total biomass and CWT over the entire period of crop growth was computed to arrive at the time averaged whole plant water use efficiency. This parameter ranged between 5.3 and 6.9 g per litre among TILs and it was 4.8 g per litre in IR-64. WUE was increased under water limited conditions among the TILs. Root weight of IR-64 in control condition was 9.03 g and in stressed condition was 6.82g. The mean of root weight of TILs was 13.04 g and 15.85g under controlled and stresses conditions respectively (Table 4.17). The root weight of the line with RM 2584 and RM 1388 donor allele & RM 16 with non-donor allele was having higher root weight compared to other TILs (Fig. 4.17). RM 16 seems to act like negative regulator of root growth. Literature also suggests RM 16 as negative regulator (Table 4.18).

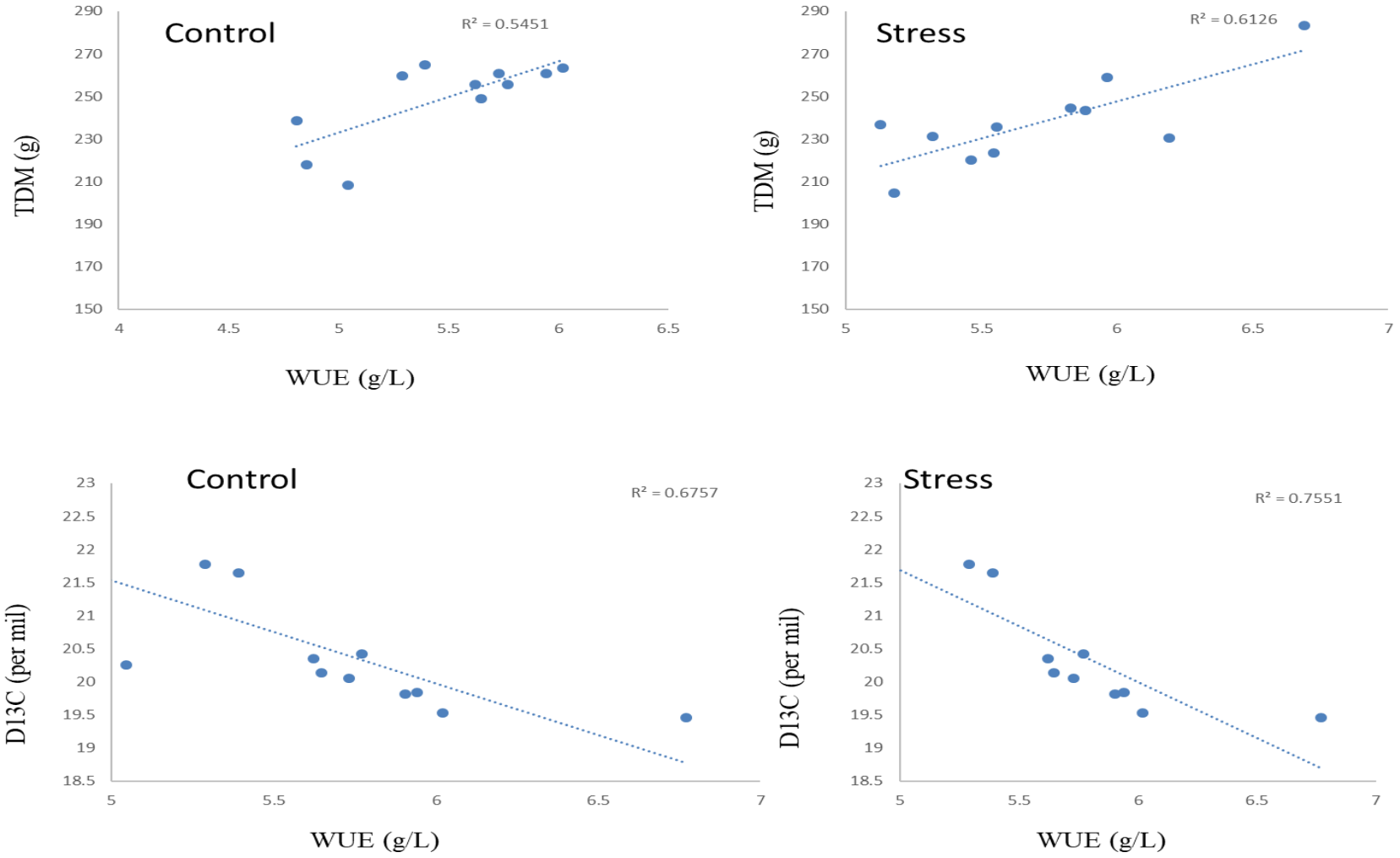

Figure 4.15: Relationship between WUE and TDM & WUE and $\Delta^{13}C$ under control and stress conditions

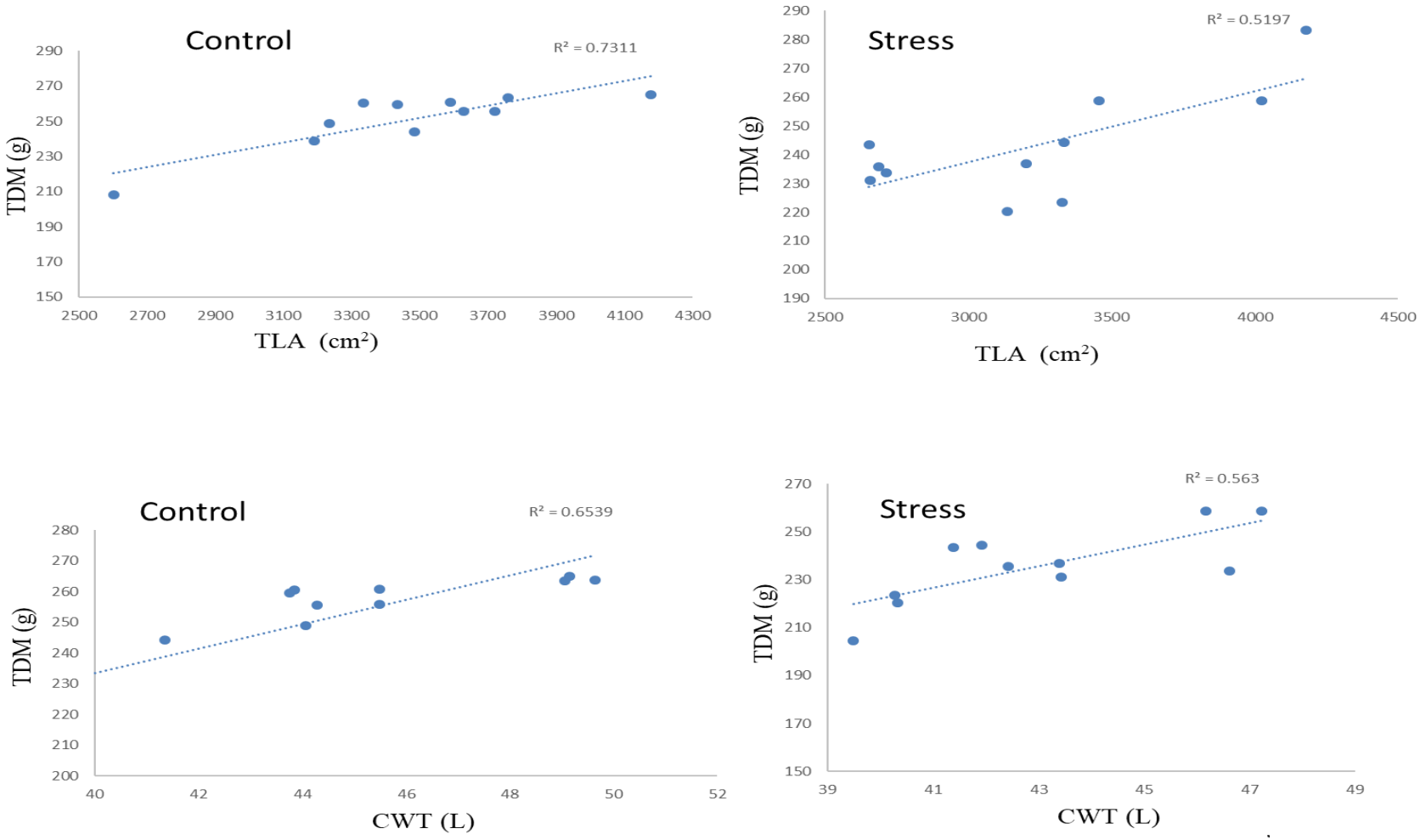

Figure 4.16: Relationship between TLA and TDM & CWT and TDM under control and stress conditions

TLA-Total Leaf Area; TDM-Total Dry Matter; CWT-Cumulative Water Transpired

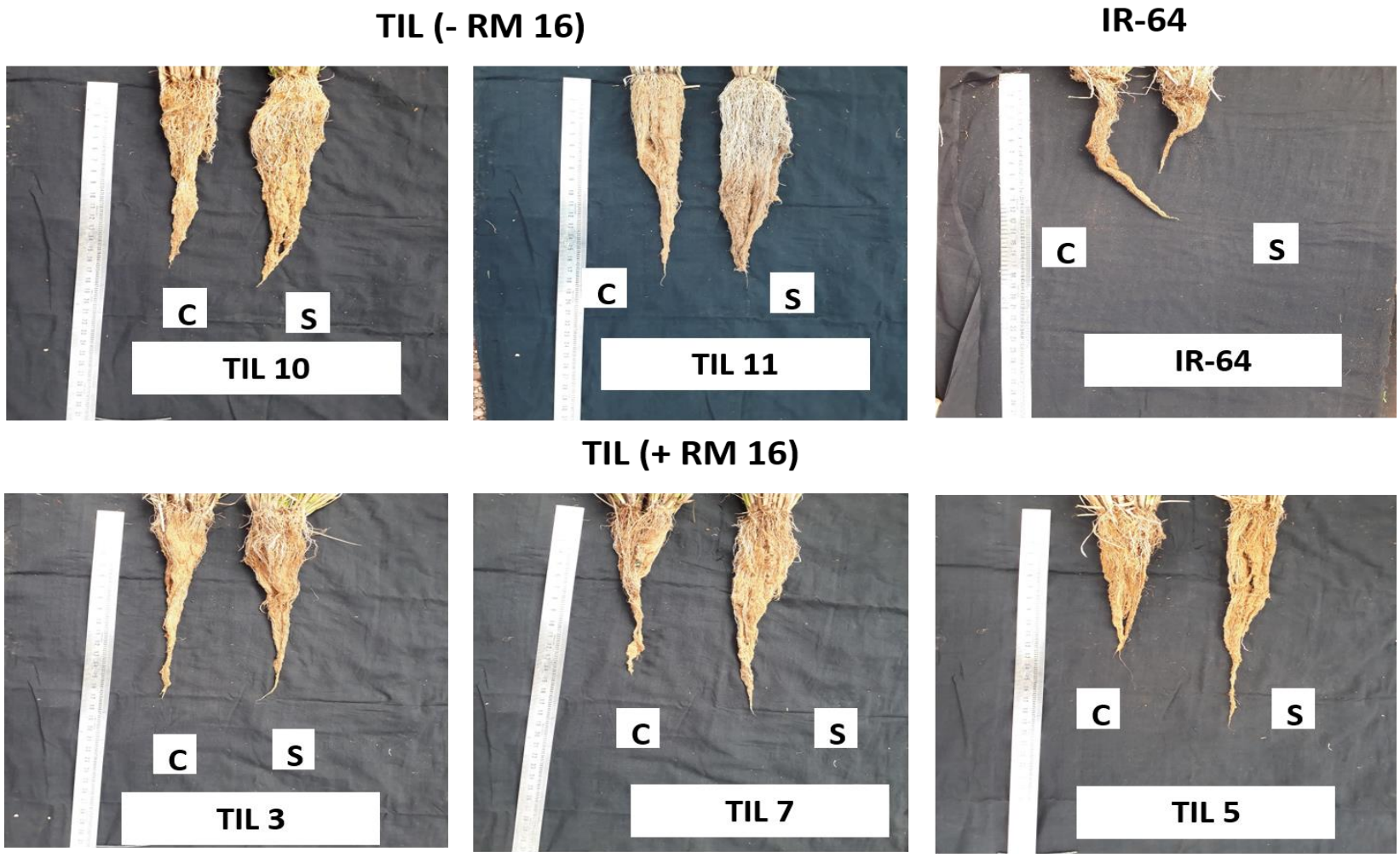

Figure 4.17: Representative picture depicting the presence and absence of RM 16 alleles and its effect

C- Control; S- Stress

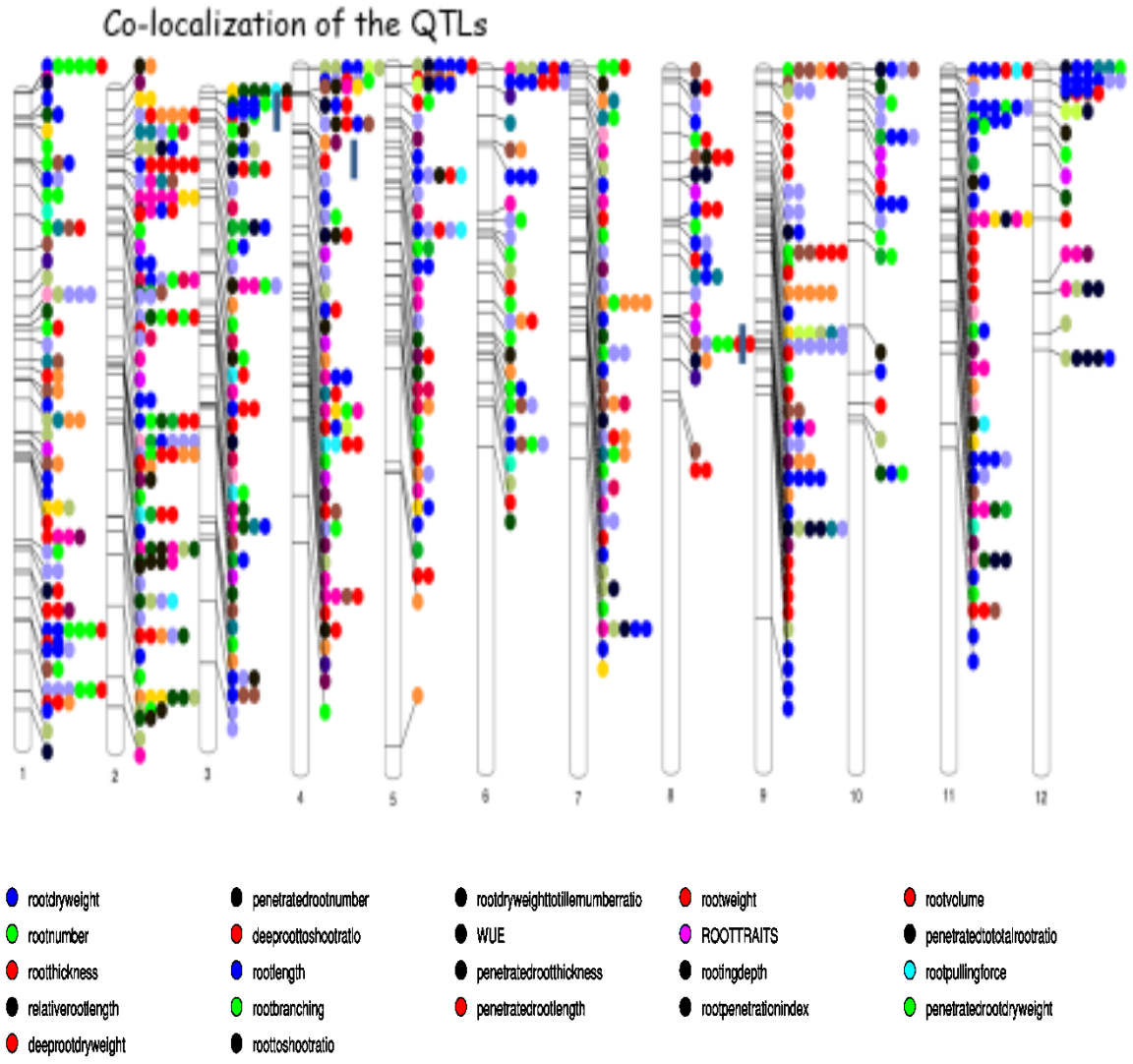

Figure 4.18: Co-localization study of root traits associated QTLs

Table 4.18: RM 16 acts as negative regulator in previous studies

Trait	QTL	Interval	LOD	Additive effect	Phenotypic variance	Reference
Grain length	qGL-3	RM 6283-RM 16	90.13	-0.99	48.45	Lou *et al.*, 2009
Length width ratio	qLWR-3	RM 6283-RM 16	43.13	-0.32	36.09	Lou *et al*., 2009
Spikelet fertility	qSF3	RM 15303-RM 16		-1.28	3.84	Zhu *et al.*, 2016
R/S ratio	qRS-3-1	RM16-RM237	3.23	-0.06	50.18	Smitharani, 2019
Leaf length	qLL-3-1	RM16-RM237	4.39	-4.19	54.08	Smitharani, 2019
Total leaf area	qTLA-3-1	RM16-RM237	4.18	-809.83	47.94	Smitharani, 2019
Grain weight per plant	qGW-3-1	RM 563-RM16	3.09	-3.81	38.8	Sangodele *et al*., 2014
Grain weight per plant	qGW-3-2	RM 16-RM 130	3.11	-3.62	43.87	Sangodele *et al*., 2014
Number of grains per plant	qNGP-3	RM 15303-RM 16		-6.68	3.42	Zhu *et al.*, 2016
Leaf blast disease	qLB3	RM 563-RM 16	4.05	-0.8	41.67	Sangodele *et al*., 2014

TDM is correlated with TLA in control as well as in stressed conditions. TDM is also correlated with CWT (Fig 4.16).

Outcome

All the TILs identified as superior transgressive seggregants, performed better than the recurrent parent. RM 16 seems to act like negative regulator of root growth.

4.5 Molecular network responsible for variation in traits

The panel of germplasm used previously for association mapping revealed that LD decay was 5cM (Raju *et al.,* 2016). Considering this limit PCR primers were designed to amplify genomic DNA in the range of 1.2755 Mb. This stretch of genomic DNA was computed as per the equation cM/3.92. For designing primers for the associated SSR marker loci, the physical location of the SSR markers was ascertained by comparing with the rice physical map at Gramene database (Table 4.19). The selected marker/QTL combination (RM 2584, RM 1388 and RM 16) with in the flanking region was searched for the respective physical positions in the genome (Table 4.20).

Table 4.19: Physical positions of markers on chromosome

Marker name	Chromosome number	Start position	End position
RM16	3	231,26,064 bp	231,26,256 bp
RM2584	8	75,64,586 bp	75,64,979 bp
RM1388	4	250,35,173 bp	250,35,422 bp

Table 4.20: Flanking regions converted to base pair

New positions		
Flanking region	5 cM down	5 cM up
Marker name		
RM 16	218,50,554 bp	244,01,766 bp
RM 2584	62,89,076 bp	88,40,489 bp
RM 1388	237,59,663 bp	263,10,932 bp

Co-localization study

QTLs reported in rice for drought adaptive traits till date were collected from the available literature and available databases. A meta-analysis was performed for 8000 QTLs collected and analysed for its function. Total number of root QTLs were 810 out of 8000 reported QTLs. The phenogram was developed with the help of these QTLs along with the QTLs being studied in this investigation (Fig. 4.18). The phenogram developed, a digital linkage map, revealed several QTL hotspots. The three marker loci, viz., RM16, RM2584 and RM1388 were also found to co-localise with important root QTL hotsopts (Fig. 4.18).

Gene mining

The foremost goal of mapping QTL is to identify genes underlying polygenic traits and gain a better understanding of their physiology and biochemistry (Korstanje *et al.,* 2002). The process of selecting candidate gene/s relie/s on wealth of information gained through traditional genetics and molecular approaches (Borevitz *et al.,* 2004). Many publicly useful biological data, especially, the high-throughput technologies are significantly increasing the volume of biological information to assist gene function identification. Most of these biological data are published on the public domain databases. Scientists target these databases to apply bioinformatics approaches and data integration systems to find the most promising candidate genes and to elucidate functions of rice genes.

Although a large amount of data available in public database have been precious in molecular biology and functional genomics in particular (Kurata and Yamazaki 2006, Liang *et al.,* 2008), only a few databases have been developed so far to facilitate more efficient utilization of genomics data particularly in applied aspects of genomics. Most of these databases such as the RiceGeneThresher (Thongjuea *et al.,* 2009), Gramene QTL Database (Ni *et al.,* 2009) and Q-TARO Database (Yonemaru *et al.,* 2010) focused on quantitative trait locus (QTL) information available for rice.

In the present study, Gramene QTL Database was used to identify the genes underlying QTLs. Now it has been designed and developed to integrate catalogs from public domain databases on rice that involve genetic information, genome annotation, expressed sequence tags (ESTs), protein information such as protein domains, gene ontology (GO), metabolic pathway information, prediction of protein–protein interaction and stress-responsive genes. In our findings from QTL combination, three QTL regions around RM16, RM 2584 and RM 1388 were considered to detect candidate genes. Candidate genes have been successfully used in genetic and association mapping, molecular marker-assisted selection and development of transgenic plants for various traits in crop plants. Generally, candidate genes are known with its biological function regulating the investigated traits, which are involved directly or indirectly in the development stages of the trait and are confirmed by evaluating the effects.

For instance, a gene or transcription factors like bZIP, Myb, AP2 domain, DREB factor, and WRKY showed the relevance in mitigating stress effects by altering root growth parameters and their function analysis has been confirmed by transgenic approach (*www.plantstress.com*). Though, fine mapping of specific QTL regions is a widely adopted approach for "gene mining", since the whole genome sequence of rice is available along with an accurate physical map, locating the actual gene position is now highly feasible (Thongjuea *et al*., 2009). When validated, such genes could help in fine mapping the QTL regions besides being useful in improving a trait through genetic transformation or molecular breeding.

A total of 368 genes found associated with RM 16, 393 genes were found associated with RM 2584 and 440 genes found associated with RM 1388.

Attempts to map metabolic networks using known functions of identified genes present in the selected marker loci or miRNA interactions did not reveal any novel discovery (Table 4.21). However, the marker locus around RM 16 that always showed a negative association with root traits led to the identification of a gene.

Table 4.21: mi RNA in the region and their targets

RM2584 miRNA on chromosome 8 and their target

Model	**Start**	**Stop**	**Chr8_RM2584**	**locus**	miRNA_Acc.	
LOC_Os03g39830.1	22150323	22151612	Chr3	LOC_Os03g39830	osa-miR1854-5p	Network forming
LOC_Os03g41780.1	23201176	23207379	Chr3	LOC_Os03g41780	osa-miR1862b	
LOC_Os04g41250.1	24471709	24479505	Chr4	LOC_Os04g41250	osa-miR1862b	Network forming
LOC_Os04g41560.4	24647901	24649396	Chr4	LOC_Os04g41560	osa-miR1874-3p	Network forming
LOC_Os04g42860.1	25368373	25372115	Chr4	LOC_Os04g42860	osa-miR1874-5p	Network forming
LOC_Os08g11040.1	6496646	6500101	Chr8	LOC_Os08g11040	osa-miR1874-5p	
LOC_Os08g11190.1	6573875	6576641	Chr8	LOC_Os08g11190	osa-miR1874-5p	Network forming
LOC_Os08g12020.1	7054851	7072109	Chr8	LOC_Os08g12020	osa-miR1854-5p	
LOC_Os08g12210.1	7190774	7195324	Chr8	LOC_Os08g12210	osa-miR1854-5p	
LOC_Os08g13320.1	7911799	7916504	Chr8	LOC_Os08g13320	osa-miR1854-5p	Network forming
LOC_Os08g14290.1	8560660	8564463	Chr8	LOC_Os08g14290	osa-miR1854-5p	

Table 4.21 Contd….

RM1388 miRNA on chromosome 4 and their target

Model	Start	Stop	Chr4_RM1388	locus	miRNA_Acc.	
LOC_Os03g42320.1	23545317	23547668	Chr3	LOC_Os03g42320	osa-miR156e	Network forming
LOC_Os04g42760.1	25307143	25308653	Chr4	LOC_Os04g42760	osa-miR156e	Network forming
LOC_Os04g43916.1	26023936	26034503	Chr4	LOC_Os04g43916	osa-miR5532	Network forming
LOC_Os08g10800.1	6358775	6359320	Chr8	LOC_Os08g10800	osa-miR162b	
LOC_Os08g12430.1	7338826	7348077	Chr8	LOC_Os08g12430	osa-miR156e	Network forming
LOC_Os08g12880.1	7643663	7646302	Chr8	LOC_Os08g12880	osa-miR162b	
LOC_Os08g14050.1	8415405	8418565	Chr8	LOC_Os08g14050	osa-miR156e	Network forming

RM16 miRNA on chromosome 3 and their target

Model	Start	Stop	Chr3_RM16	locus	miRNA_Acc.	
LOC_Os03g40690.1	22624156	22628484	Chr3	LOC_Os03g40690	osa-miR1319b	
LOC_Os03g40920.1	22739267	22743374	Chr3	LOC_Os03g40920	osa-miR2880	Network forming
LOC_Os04g40230.1	23932699	23933966	Chr4	LOC_Os04g40230	osa-miR2880	
LOC_Os04g40860.1	24243897	24247750	Chr4	LOC_Os04g40860	osa-miR821a	Network forming
LOC_Os04g42670.1	25244008	25246628	Chr4	LOC_Os04g42670	osa-miR821a	Network forming
LOC_Os04g43600.1	25799856	25802057	Chr4	LOC_Os04g43600	osa-miR5485	
LOC_Os08g11430.1	6700177	6704358	Chr8	LOC_Os08g11430	osa-miR1428e-3p	
LOC_Os08g11480.1	6734408	6739686	Chr8	LOC_Os08g11480	osa-miR821a	Network forming
LOC_Os08g11830.1	6934977	6937612	Chr8	LOC_Os08g11830	osa-miR1428e-3p	
LOC_Os08g12430.1	7338826	7348077	Chr8	LOC_Os08g12430	osa-miR1428d	Network forming
LOC_Os08g12680.1	7488653	7510517	Chr8	LOC_Os08g12680	osa-miR821a	Network forming

The functional characterization of this gene has been reported elsewhere and is shown to be a negative regulator of root growth. This gene is found to be auxin responsive that belonged to the AUX/IAA gene families.

Bioinformatics analysis suggested that OsIAA 11 is the molecular candidate which is responsible for root growth regulation in QTL-NILs/TILs. *In-silico* expression of the gene indicates that it is expressed in root during vegetative stage in day as well as night time (Plate 4.1, 4.2). INDELS present in the gene may be responsible for variation in phenotype for different alleles (Table 4.22). The well explained model of IAA11 in inhibition of lateral root growth is given in the plate 4.3. It suggests that RM 16 acts via auxin mediated mechanism to control the root growth in rice.

Table 4.22: INDELS present in the gene *Os03g43400*

INDEL ID	Start Position	End Position	ALT	Effect
vf0324197128	24197128	24197130	GT	LOC_Os03g43400.1(-T) INTRON
vf0324197130	24197130	24197134	TGAGAGA	LOC_Os03g43400.1(+GA) INTRON
vf0324197348	24197348	24197356	TAAAAAAA TAAAAAAAAA	LOC_Os03g43400.1(+A) INTRON LOC_Os03g43400.1(-A) INTRON
vf0324197355	24197355	24197355	AT	LOC_Os03g43400.1(+T) INTRON
vf0324197356	24197356	24197357	ATT ATTT	LOC_Os03g43400.1(+T) INTRON LOC_Os03g43400.1(+TT) INTRON
vf0324197821	24197821	24197823	C CATAG	LOC_Os03g43400.1(+TA) INTRON LOC_Os03g43400.1(-AG) INTRON
vf0324197825	24197825	24197836	CTATATATAT CTATATATATATAT	LOC_Os03g43400.1(+AT) INTRON LOC_Os03g43400.1(-AT) INTRON
vf0324198086	24198086	24198099	AATATATATATA	LOC_Os03g43400.1(-TA) INTRON
vf0324198448	24198448	24198457	TTTTT	LOC_Os03g43400.1(-ATTTT) INTRON
vf0324198449	24198449	24198456	TTTT	LOC_Os03g43400.1(-ATTT) INTRON
vf0324198450	24198450	24198455	TTT	LOC_Os03g43400.1(-ATT) INTRON
vf0324198451	24198451	24198454	TT	LOC_Os03g43400.1(-AT) INTRON
vf0324198452	24198452	24198453	T	LOC_Os03g43400.1(-A) INTRON
vf0324198526	24198526	24198534	GAAAAAAAAA GAAAAAAAAAA	LOC_Os03g43400.1(+A) INTRON LOC_Os03g43400.1(+AA) INTRON
vf0324198754	24198754	24198762	TAAAAAAA TAAAAAAAAA	LOC_Os03g43400.1(+A) INTRON LOC_Os03g43400.1(-A) INTRON
vf0324199156	24199156	24199162	CATAT	LOC_Os03g43400.1(-AT) INTRON
vf0324199692	24199692	24199693	CTT	LOC_Os03g43400.1(+T) INTRON
vf0324199884	24199884	24199912	TCGTCGGC	LOC_Os03g43400.1(-GGCCGCCGCCGCCGCGTCGGC) CODON_DELETION

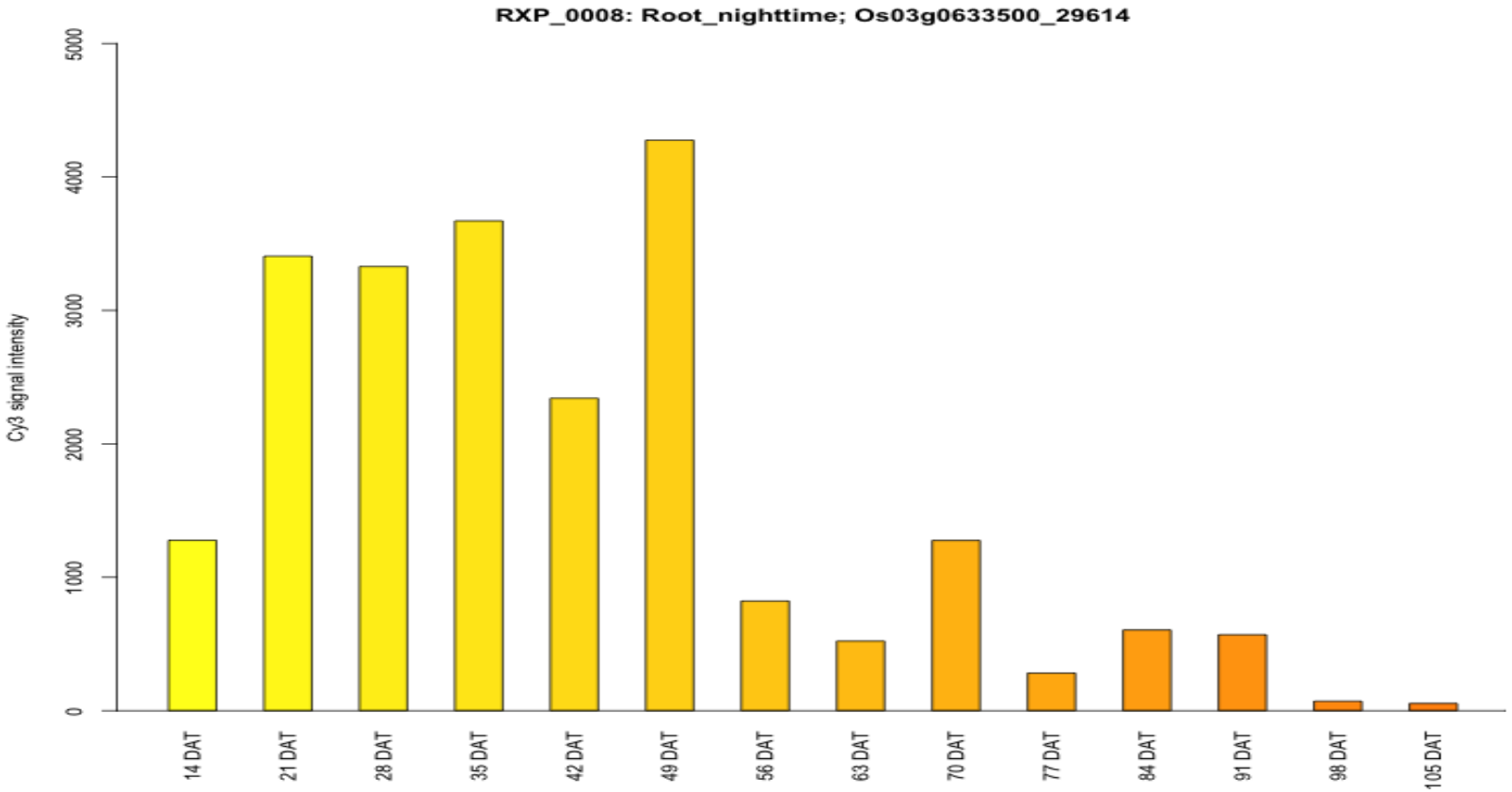

Plate 4.1: *Insilico* expression of gene *Os03g43400* in root during night time

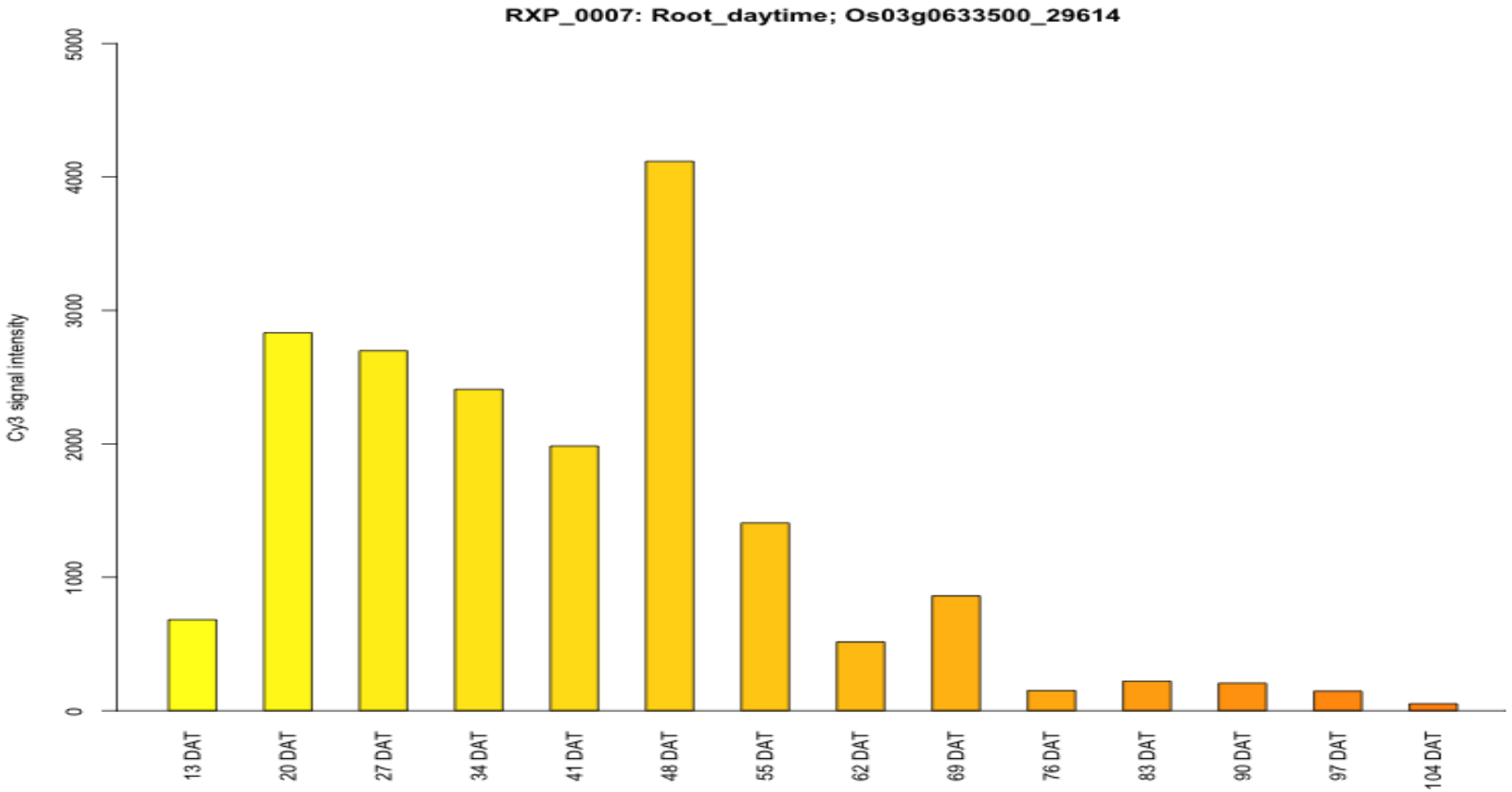

Plate 4.2: *Insilico* expression of gene *Os03g43400* in root during day time

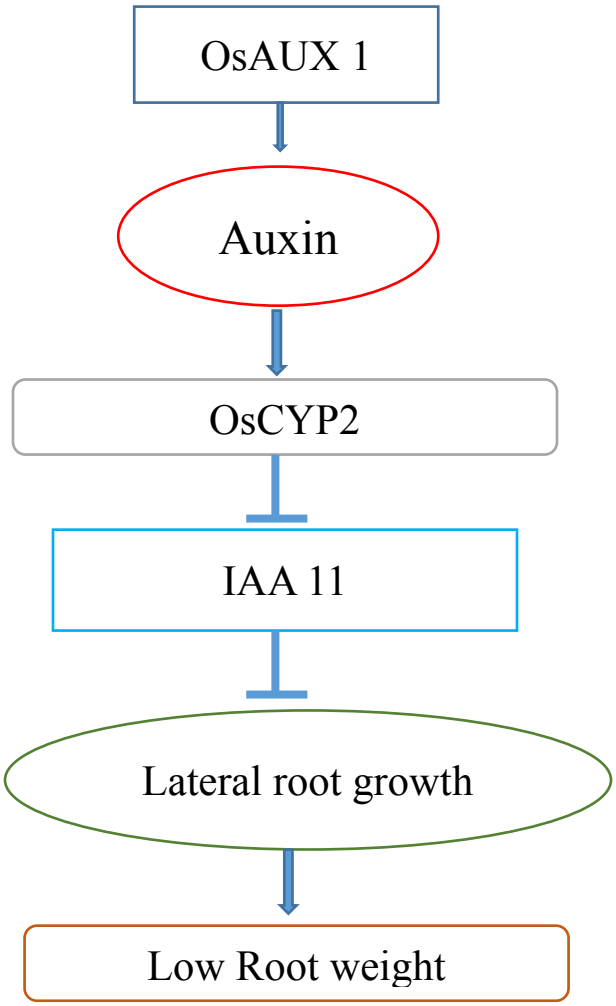

Plate 4.3: Model representing involvement of IAA 11 gene in controlling root weight (Reference: Meng et al., 2019)

V SUMMARY

Rice (*Oryza sativa* L.), the staple crop that requires enormous quantity of water during its growth period. It accounts for more than 50 per cent of fresh water diverted for agriculture. Water, which is a non-renewable is becoming a major constraint. Therefore, the effort is to produce more grain per drop of water used. Several water saving management practices (namely alternate wetting and drying, SRI method etc.) are being practiced. Aerobic rice has been credited as one of the water saving method. Although it saves 60 per cent of water required, significant reduction in yield has been also noticed. Therefore, sustaining productivity under aerobic condition is more important. Rice cultivar with increased tolerance for water limited conditions is need of the hour. The cultivars that can mine water from various soil strata and use it more efficiently for growth and development are extremely vital to sustain productivity under water scarce conditions. Efforts are needed to bring together distinct traits on an elite background. Several relevant traits must be pyramided to develop drought tolerant crop varieties through **"trait-based"** breeding approach. Traits involved in maintaining leaf water relations and metabolism have greatest pertinence. Improvement of plant traits linked with water relations and water conservation besides cellular level tolerance is needed. There are evidences signifying introgression of drought adaptive traits such as root trait and WUE trait has improved the yield level of rice varieties under semi-irrigated aerobic conditions.

Roots and WUE traits are complex morpho-physiological traits that are controlled by multiple genes. It rendered conventional breeding for trait introgression almost difficult. Considering the importance of trait pyramiding, highly focused molecular breeding is the most appropriate approach. In this direction, numerous QTLs were identified by both LD (Linkage Disequilibrium) based association mapping and bi-parental mapping methods. Though there are several published data available online on QTLs and trait specific candidate genes related to drought related traits, validation of linked markers (QTLs) and genes are still in the preliminary stage. In spite of high information on QTLs linked to different drought adaptive traits they are not frequently used in MAS. This is because of their inconsistency due to large G×G and G×E

interaction. Hence, the major focus of the present study was to analyze the interaction of traits among QTL-NILs/ TILs/ CSSLs, to identify the best trait introgressed line/s consistent across environments and to find out the molecular candidate responsible for the variation.

A set of 260 $DCBC_3F_4$ lines developed by introgressing root traits and WUE via marker assisted back cross were phenotyped for different drought adaptive traits like leaf temperature (surrogate for root traits), $\Delta^{13}C$ (surrogate for WUE), yield, spikelet fertility and other morpho-physiological traits along with the parents under semi-irrigated aerobic conditions. The phenotypic data indicated that variability for the traits still exists in $DCBC_3F_4$ TILs for most of the traits. Transgressive segregants were having improved traits indicating the introgression of genomic region by donor parents. It indicated improvement over recurrent parent for drought adaptive traits. Based on traits of interest and yield data, 25 TILs were shortlisted for further recharacterization which came under four groups (HD+HR, HD+LR, LD+HR and LD+LR). These 25 lines were of similar leaf area and high yield types with difference in root and $\Delta^{13}C$ value.

The performance of these selected TILs/NILs were evaluated in various seasons and locations. These TILs and the parents grown in GKVK (three seasons) and TNAU (one season) in $1*1m^2$ plots. The TILs performed much superior than the recipient parent IR-64. Multi season experiments were conducted to check the stability of the lines for traits and also yield. The result indicated that TILs/NILs performed consistently better than IR-64. The consistency of the trait across seasons demonstrated the stability of the QTLs along with the stability of TILs.

To analyze the interaction among traits in QTL-NILs/TILs marker class analysis was performed by using previous genotypic data (Prathibha, 2016) and current phenotypic data in 260 lines and selected 25 lines. The analysis highlighted that the number of QTLs present did not tally with the trait value, suggesting the interaction among loci. The lines were divided into low root type and high root type with low $\Delta^{13}C$ value and higher yield. Low root types were having the alleles of RM 2584, RM 1388

and RM 16 from AC-39020. However, high root types had RM 2584 and RM 1388 alleles from AC-39020 and RM 16 alleles from non-donor parent.

From the 25 TILs studied under various seasons, six lines selected based on the above marker/QTL combination and additional seven lines were selected out of 260 $DCBC_3F_4$ with the marker combination. These lines were physiologically characterized under phenomics platform. The result specified that root weight of the line with RM 2584 and RM 1388 donor allele & RM 16 with non-donor allele was having higher root weight compared to other TILs. RM 16 seems to act like negative regulator of root growth. Various literature also suggests RM 16 as negative regulator. All the 13 TILs identified as superior transgressive seggregants, performed better than the recurrent parent.

To know the genic network responsible for variation in traits, the selected marker/QTL combination was digged to determine the genes within the flanking region. The flanking region was selected based on the LD decay value. Co-localization study suggested that those regions which had been taken to find the candidate gene was correct.

The genes inside the regions were searched for their role in controlling of the trait or related by using bioinformatics online tools. Several public databases were used to find the candidate genes that controlled the traits. RiceFREND database was used to get the network for the gene forms with any metabolic pathway. The gene *Os03g43400* present within RM 16 region was selected as the candidate gene as it has a clear role in inhibiting lateral root growth. *In-silico* expression of the gene highlighted that it is expressed in root throughout the vegetative stage.

The salient features of work are summarized as follows

- 260 $DCBC_3F_4$ lines were phenotyped for drought adaptive and growth parameters
- These 260 $DCBC_3F_4$lines were classified into four sub classes (HR+LD, HR+HD, LR+LD, LR+HD)
- 25 TILs corresponding to all groups together selected for further study

- 25 TILs were grown in 4 different seasons (three in GKVK and one in TNAU) and various morpho-physiological and growth parameters were examined
- Marker class analysis for 260 $DCBC_3F_4$ suggests that there is interaction among traits in the TILs
- 13 trait introgressed lines identified as superior transgressive seggregants. All performed superior over the recurrent parent IR-64.
- TILs with RM 16 allele from AC-39020 showed lower root biomass compared with TILs which contain RM 16 allele from either IR 64 or IET 16348
- RM 16 seems to act like negative regulator of root growth

Future line of work

- Validation of the gene for its function by wet lab experiments
- Identification and mining of superior alleles for trait specific candidate genes is crucial in developing focused translational research activities
- Reconfirmation of the marker/QTL
- Extensive field trial of the selected promising lines as a step towards releasing as a variety
- TILs having consistent and superior performance in diverse environment can be used as improved IR-64 variety

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APPENDIX-I: Morpho-physiological parameters recorded among trait introgressed lines in semi-irrigated aerobic condition

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
1	78.83	81.00	34.83	1532.07	8.66	23.53	32.14	64.34	30.9
2	79.67	83.00	37.57	1908.90	12.36	17.93	29.47	59.77	33.0
3	82.67	82.00	44.57	2720.85	10.01	33.32	30.50	73.83	27.7
4	82.07	85.00	39.13	2827.20	14.00	28.65	32.61	75.26	34.1
5	81.40	81.00	36.83	2068.94	11.26	26.61	31.77	69.65	32.7
6	80.17	85.00	44.30	1952.07	9.82	38.17	32.79	80.78	28.9
7	80.03	85.00	38.33	2478.71	12.42	14.41	32.62	59.45	33.6
8	79.50	82.00	40.13	1651.60	9.21	18.81	24.95	52.97	34.8
9	79.60	83.00	36.70	1447.62	7.87	16.90	27.22	51.99	31.0
10	81.30	81.00	37.63	2069.94	11.42	22.65	31.79	65.86	30.6
11	79.97	85.00	36.87	2050.64	10.85	22.54	29.44	62.83	33.9
12	77.87	79.00	44.50	2542.44	12.68	34.73	33.54	80.95	29.8
13	80.23	82.00	38.03	1779.72	7.10	14.46	25.69	47.25	34.0
14	80.97	82.00	39.90	1783.14	10.28	20.38	24.48	55.14	33.0
15	80.77	83.00	43.70	2167.31	11.58	36.82	25.56	73.96	29.4
16	79.27	80.00	38.63	1398.57	8.75	22.42	23.55	54.72	34.2
17	82.03	83.00	36.60	1790.77	7.77	17.55	29.19	54.51	34.1
18	82.50	79.00	45.83	2745.66	14.44	30.21	30.66	75.31	27.7
19	78.40	82.00	36.30	1595.34	8.94	24.67	31.35	64.95	32.6
20	80.80	80.00	34.23	1389.64	7.34	20.00	26.60	53.94	31.2
21	80.37	79.00	42.30	2593.28	12.87	30.09	28.28	71.24	28.7
22	80.20	80.00	39.90	1506.40	7.74	25.92	31.33	64.99	34.4
23	80.03	79.00	38.07	1843.35	10.58	20.91	27.21	58.70	33.5
24	82.73	85.00	36.57	1442.11	7.69	17.01	32.94	57.64	33.7
25	79.10	82.00	36.60	2561.94	11.77	21.37	28.16	61.30	32.7
26	82.97	85.00	33.30	1054.07	7.47	21.12	26.05	54.64	33.4
27	77.57	82.00	35.27	1527.45	6.99	21.67	23.56	52.22	33.4
28	79.97	80.00	35.33	1699.36	7.64	11.91	26.13	45.68	34.2
29	78.53	84.00	36.53	1619.39	7.70	14.15	26.77	48.62	33.7
30	77.47	85.00	35.70	1763.04	9.42	21.74	22.78	53.94	32.0
31	80.77	81.00	39.40	2201.31	9.88	21.27	32.88	64.02	31.5
32	77.63	83.00	36.23	1835.19	8.83	18.47	26.50	53.80	34.4
33	80.57	82.00	38.87	1673.03	9.87	23.47	30.44	63.78	32.8
34	78.60	85.00	35.33	1649.33	9.08	19.13	29.60	57.82	34.3
35	79.33	83.00	44.97	2404.22	13.15	34.30	35.05	82.50	25.0
36	79.30	81.00	37.40	1719.43	10.21	37.29	33.68	81.17	33.7

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
37	79.47	81.00	37.53	1476.27	8.22	34.10	31.60	73.92	33.3
38	82.60	79.00	35.50	1573.61	8.72	18.13	26.92	53.77	34.3
39	80.07	84.00	35.53	1578.98	9.79	13.03	33.30	56.13	31.6
40	79.07	84.00	36.07	1282.63	8.47	22.54	28.04	59.05	33.5
41	78.00	84.00	37.77	1569.22	7.77	20.79	28.50	57.06	30.7
42	78.80	82.00	36.90	1691.03	7.54	14.10	30.22	51.87	33.9
43	78.67	85.00	44.33	2965.06	13.21	32.22	33.66	79.09	28.8
44	80.43	81.00	36.40	1492.01	7.20	20.79	26.19	54.19	32.9
45	78.90	85.00	38.43	1399.52	8.35	21.15	26.71	56.21	33.1
46	78.73	83.00	36.20	1646.62	8.17	13.57	26.87	48.60	32.9
47	77.77	83.00	37.97	2675.34	13.84	17.96	26.28	58.08	30.9
48	80.90	83.00	35.57	1698.00	9.06	27.29	30.41	66.77	31.3
49	79.80	84.00	37.03	1512.44	10.13	21.52	30.84	62.50	32.4
50	78.33	79.00	46.53	2381.47	14.06	38.95	34.90	87.90	26.1
51	80.60	83.00	35.67	1909.87	8.18	16.98	27.88	53.04	32.0
52	80.07	81.00	37.93	1762.02	11.84	24.16	29.23	65.23	32.6
53	79.70	80.00	39.00	2263.94	10.23	23.58	23.88	57.69	32.9
54	80.47	80.00	45.87	2378.40	12.79	35.46	32.38	80.64	28.5
55	80.27	80.00	44.73	1733.82	10.67	34.87	32.57	78.11	29.5
56	79.73	83.00	35.30	2030.80	8.43	15.85	25.11	49.39	33.3
57	77.90	81.00	41.43	2161.39	11.24	31.67	32.59	75.49	28.4
58	80.40	85.00	34.97	2152.61	11.64	24.58	30.02	66.24	32.0
59	81.33	82.00	36.13	2419.58	13.78	32.87	23.95	70.60	34.0
60	81.17	79.00	38.23	2435.78	7.72	23.99	24.16	55.87	33.1
61	80.47	83.00	35.40	1450.49	7.27	20.37	23.52	51.16	31.7
62	79.70	85.00	38.73	1493.61	7.27	23.83	27.43	58.53	33.0
63	80.10	83.00	37.40	1734.83	9.18	22.19	24.95	56.32	32.7
64	81.63	85.00	36.40	1885.87	10.44	19.11	24.59	54.14	32.7
65	78.23	84.00	36.73	1722.06	8.56	20.42	24.26	53.24	32.2
66	77.67	82.00	38.57	1821.84	9.49	18.43	26.16	54.09	34.4
67	81.77	80.00	37.63	1418.79	7.92	23.27	28.02	59.22	33.0
68	80.80	83.00	36.53	1295.86	8.90	14.43	27.72	51.04	33.3
69	79.07	83.00	37.83	1975.15	12.31	28.92	27.37	68.60	34.3
70	78.10	81.00	36.80	1532.81	7.05	16.92	22.27	46.24	33.2
71	79.20	83.00	35.00	1771.91	9.34	24.01	24.05	57.40	33.6
72	78.63	85.00	37.80	1741.31	8.51	19.72	24.48	52.70	32.0
73	78.90	81.00	35.03	2126.90	12.00	30.47	26.65	69.12	33.0
74	80.93	79.00	37.93	1843.23	12.65	35.94	30.73	79.32	31.2

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
75	79.37	79.00	36.20	1807.92	7.58	33.83	27.80	69.21	32.5
76	77.70	81.00	37.97	1819.41	8.73	25.39	27.71	61.83	32.2
77	81.30	79.00	44.57	2497.40	12.82	40.46	36.67	89.95	26.0
78	78.33	82.00	42.10	2353.08	14.47	34.32	37.40	86.19	25.5
79	80.07	83.00	35.80	1787.14	9.22	12.08	25.35	46.65	33.0
80	78.03	84.00	39.87	1899.32	9.28	19.09	28.04	56.41	31.5
81	79.23	80.00	37.73	1602.43	9.15	27.73	24.48	61.36	31.2
82	80.20	81.00	38.53	1698.75	8.62	20.61	29.63	58.86	32.9
83	80.13	82.00	36.57	1854.95	10.00	34.03	22.65	66.68	32.2
84	80.37	83.00	44.90	2363.06	12.03	39.27	36.69	87.98	29.4
85	79.87	80.00	35.00	1716.36	9.67	24.29	30.30	64.26	31.3
86	81.63	84.00	45.83	2428.75	13.76	37.74	29.01	80.51	28.6
87	80.77	80.00	43.77	2007.36	12.83	34.02	30.01	76.86	27.2
88	81.63	84.00	37.03	2357.66	12.94	15.49	23.83	52.26	31.9
89	79.53	83.00	36.07	2329.79	12.97	15.61	28.17	56.75	33.9
90	79.27	79.00	37.03	1831.57	9.65	16.83	26.47	52.95	34.3
91	78.47	81.00	34.70	1218.88	7.60	20.80	30.04	58.44	35.4
92	79.40	81.00	37.50	1729.15	7.64	19.92	23.36	50.92	32.5
93	77.93	79.00	43.57	1963.40	10.74	31.86	30.36	72.95	28.3
94	81.97	83.00	37.50	1738.04	9.64	20.88	22.92	53.44	35.6
95	81.97	84.00	37.07	1948.19	9.91	19.82	27.78	57.51	33.9
96	80.70	81.00	38.07	1150.51	7.06	25.64	30.78	63.48	34.2
97	80.83	83.00	42.33	1812.92	9.41	32.89	30.84	73.14	27.5
98	82.10	81.00	37.93	2668.55	14.05	13.87	31.64	59.56	31.1
99	81.20	83.00	36.73	1901.36	8.34	15.50	25.41	49.25	32.9
100	78.60	79.00	38.57	1441.04	7.29	11.77	23.22	42.28	32.4
101	83.27	83.00	38.13	2285.67	12.77	26.63	27.36	66.77	32.3
102	79.67	82.00	36.17	1160.01	6.79	8.51	28.57	43.87	33.1
103	78.23	85.00	36.63	1720.33	8.38	21.72	30.45	60.55	32.9
104	81.17	81.00	39.57	2252.39	13.83	18.13	23.37	55.33	33.4
105	80.47	79.00	34.73	1912.24	9.04	20.88	21.62	51.54	30.7
106	79.03	80.00	38.23	1939.78	9.86	17.77	25.68	53.31	32.0
107	76.70	81.00	36.67	1857.94	9.64	18.99	23.39	52.03	32.7
108	77.53	82.00	37.00	2137.05	11.37	15.39	27.73	54.50	32.1
109	81.40	79.00	38.30	1625.00	9.34	11.96	23.07	44.37	34.2
110	77.83	84.00	36.00	2226.89	9.08	16.39	23.52	48.99	31.9
111	79.37	80.00	34.57	1365.85	7.86	11.52	25.80	45.18	33.0
TIL	**PH**	**DFF**	**SPAD**	**TLA**	**TLWT**	**YIELD**	**SWT**	**TDM**	**LT**

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
112	79.10	83.00	37.77	1595.20	8.52	16.51	27.89	52.92	32.0
113	78.40	84.00	34.63	1640.99	9.08	19.65	22.70	51.44	32.5
114	81.10	82.00	36.57	2072.54	12.11	28.34	23.60	64.06	33.5
115	80.50	83.00	38.70	2040.90	9.60	24.96	25.55	60.12	31.3
116	79.20	84.00	37.57	2108.29	12.89	15.56	24.30	52.75	35.2
117	79.13	79.00	38.83	1856.99	7.98	14.32	29.81	52.12	31.0
118	82.07	82.00	38.57	1558.32	9.17	12.28	32.05	53.50	31.8
119	80.90	83.00	36.33	1653.47	8.17	12.90	24.70	45.77	32.5
120	80.57	83.00	38.77	2457.31	10.21	12.12	30.35	52.68	34.3
121	80.10	79.00	37.83	2100.69	9.81	17.53	33.47	60.80	31.8
122	80.37	81.00	36.90	1449.20	7.87	13.20	25.54	46.61	33.7
123	80.77	85.00	37.70	2798.88	12.13	20.00	25.30	57.43	32.5
124	81.33	79.00	42.10	2574.29	13.78	36.68	33.12	83.58	27.5
125	79.83	82.00	36.60	2629.51	13.23	13.67	26.73	53.63	33.1
126	78.83	80.00	36.57	2155.72	12.05	23.02	26.32	61.40	33.1
127	79.53	84.00	38.67	1776.36	12.10	23.12	27.98	63.20	32.0
128	81.20	82.00	37.97	1483.22	8.18	14.81	24.29	47.28	33.0
129	79.47	82.00	38.20	1311.08	7.63	12.30	25.20	45.13	31.3
130	78.90	85.00	38.17	1431.99	8.59	21.67	24.35	54.62	33.2
131	77.67	81.00	39.40	2602.43	11.14	26.81	22.96	60.90	34.8
132	79.03	80.00	39.27	1924.32	11.01	25.79	26.07	62.87	31.9
133	78.37	81.00	39.07	1717.99	7.65	11.80	25.64	45.09	32.2
134	78.33	79.00	37.10	2058.44	12.92	10.88	25.93	49.73	31.4
135	78.83	80.00	38.17	2461.81	11.73	14.54	24.29	50.56	33.3
136	80.20	85.00	39.80	1468.86	7.60	17.18	23.94	48.72	33.2
137	79.30	80.00	39.47	1516.52	7.41	17.02	27.08	51.51	32.9
138	79.67	82.00	37.07	1901.25	10.13	20.65	25.07	55.86	33.6
139	79.77	83.00	43.73	2546.16	12.62	32.70	32.78	78.09	29.2
140	80.27	82.00	36.70	1912.36	11.91	24.88	26.73	63.53	30.2
141	78.90	79.00	38.37	1097.50	7.61	15.39	21.31	44.31	34.6
142	78.67	81.00	41.47	1981.22	12.33	30.50	30.16	72.99	27.7
143	78.67	80.00	36.67	1434.08	7.20	16.18	23.62	47.00	32.8
144	77.47	84.00	38.03	1222.07	7.58	20.64	22.29	50.50	31.2
145	80.77	80.00	44.93	2964.57	12.01	36.17	28.48	76.66	27.8
146	77.97	83.00	37.87	1641.00	9.30	15.22	22.59	47.10	34.7
147	79.23	83.00	36.53	1408.07	8.27	19.91	23.24	51.42	32.3
148	80.07	80.00	39.33	1584.43	9.10	19.69	24.13	52.92	31.9
149	81.23	83.00	36.80	1992.37	10.96	20.31	22.82	54.09	31.7

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
150	80.17	81.00	39.63	1881.87	9.60	13.59	21.73	44.93	30.8
151	78.43	80.00	37.90	3054.73	13.40	29.35	30.49	73.24	33.0
152	78.83	83.00	39.03	2520.63	12.60	25.29	26.90	64.79	33.5
153	81.77	83.00	33.53	1338.16	8.76	12.66	18.95	40.37	34.3
154	78.53	83.00	37.77	1776.56	8.91	13.61	18.97	41.49	30.9
155	79.40	82.00	37.43	1591.95	10.51	14.09	22.40	46.99	34.3
156	80.17	83.00	34.73	1403.92	7.82	14.61	19.78	42.20	34.1
157	77.60	79.00	38.73	1920.64	11.57	29.78	25.17	66.52	32.7
158	78.17	85.00	38.03	2113.70	10.28	17.38	31.16	58.82	34.2
159	80.27	82.00	45.63	2585.70	13.02	43.01	32.52	88.54	26.9
160	81.27	85.00	37.23	2646.63	12.59	37.07	29.48	79.14	32.6
161	79.83	83.00	40.27	1452.36	8.82	20.17	26.65	55.64	33.3
162	77.60	82.00	37.77	1482.60	7.71	13.13	22.97	43.81	32.5
163	81.07	80.00	39.67	2003.28	9.72	18.86	27.04	55.62	33.6
164	79.37	81.00	39.03	1317.89	8.30	20.65	26.78	55.74	34.0
165	77.73	85.00	37.17	1778.77	10.15	23.85	24.10	58.10	34.0
166	78.80	84.00	38.20	1745.37	10.13	18.84	27.02	56.00	33.0
167	79.77	80.00	40.93	2605.13	12.27	41.69	33.53	87.50	27.3
168	81.63	85.00	37.40	1928.75	10.09	13.39	29.88	53.35	30.6
169	81.37	80.00	37.47	1970.15	8.39	14.15	20.84	43.38	33.9
170	80.63	79.00	36.07	1314.40	8.52	14.75	21.12	44.40	30.6
171	81.83	79.00	39.20	2809.05	14.63	33.69	25.70	74.02	31.7
172	81.63	84.00	39.27	2169.65	9.55	14.32	20.83	44.70	31.7
173	80.27	83.00	38.80	1544.84	8.60	15.90	21.42	45.92	31.4
174	81.73	80.00	37.63	1863.46	9.40	18.51	21.39	49.30	30.5
175	78.17	82.00	38.03	1990.13	9.48	21.97	22.46	53.90	31.2
176	79.83	84.00	38.20	2248.91	10.35	25.73	28.42	64.51	32.3
177	80.23	85.00	38.23	2066.26	10.70	21.53	28.02	60.25	32.3
178	81.03	79.00	39.60	2442.17	12.35	33.60	29.53	75.48	33.8
179	81.03	83.00	36.10	2152.03	12.80	38.60	28.50	79.90	32.4
180	81.40	81.00	37.10	1629.84	9.60	22.79	23.00	55.39	33.9
181	79.00	85.00	38.77	2446.43	12.43	34.07	31.81	78.32	31.6
182	80.90	83.00	38.57	2234.91	12.81	26.34	28.57	67.73	33.9
183	80.20	85.00	40.37	1595.39	9.74	13.22	26.21	49.18	32.0
184	81.40	84.00	39.07	1861.55	10.34	11.86	27.85	50.05	32.6
185	80.43	81.00	37.17	2496.63	11.85	31.46	28.32	71.64	31.9
186	78.87	80.00	38.60	1920.89	10.34	26.84	27.64	64.82	33.5
187	81.97	80.00	36.43	1811.87	9.34	19.97	22.94	52.24	34.3

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
188	78.20	85.00	37.73	1545.43	9.86	21.40	28.76	60.02	31.1
189	78.40	84.00	39.23	1740.00	8.52	20.30	22.33	51.16	31.1
190	81.10	82.00	36.90	1953.00	9.93	23.30	23.85	57.08	33.8
191	78.90	81.00	38.03	2216.92	11.90	25.97	33.75	71.62	34.7
192	79.50	84.00	39.23	2672.47	12.94	30.65	31.48	75.07	33.1
193	79.30	83.00	37.73	2100.93	7.85	22.60	22.28	52.73	33.5
194	82.07	79.00	37.87	1926.53	9.38	13.00	22.32	44.70	32.8
195	80.67	85.00	35.93	1574.35	9.30	16.65	23.14	49.09	30.0
196	79.90	80.00	37.93	1770.05	7.15	17.16	21.18	45.50	30.6
197	79.87	83.00	36.83	1924.59	9.11	16.78	22.23	48.12	33.3
198	81.10	80.00	37.73	2672.77	14.26	35.62	33.13	83.00	30.4
199	82.73	80.00	46.33	2562.27	13.75	39.68	36.64	90.07	25.0
200	79.10	84.00	37.73	1101.52	7.44	21.32	24.08	52.84	32.7
201	81.10	83.00	39.17	1487.42	7.59	20.34	25.12	53.05	33.2
202	79.10	80.00	37.43	2338.35	11.75	36.25	32.11	80.11	33.9
203	78.00	80.00	38.63	1311.81	7.47	13.98	20.30	41.75	33.6
204	79.67	81.00	37.30	1509.45	9.48	16.35	23.37	49.19	32.6
205	79.80	82.00	38.10	1759.89	9.14	26.49	26.50	62.14	33.1
206	82.33	84.00	39.20	2477.57	12.96	35.34	33.10	81.40	31.5
207	79.40	84.00	39.77	1603.95	7.30	17.88	23.17	48.35	32.1
208	81.87	83.00	37.27	4046.02	14.06	34.54	36.98	85.59	30.9
209	78.40	82.00	37.70	1740.13	10.09	29.18	22.37	61.64	32.6
210	80.30	82.00	37.37	1457.09	8.44	13.51	23.25	45.20	34.7
211	81.70	79.00	39.10	1614.85	8.66	21.53	27.51	57.70	33.1
212	79.30	83.00	37.80	1154.44	7.23	19.34	21.10	47.67	31.5
213	78.60	81.00	37.07	2648.33	13.10	40.75	36.87	90.72	33.1
214	78.17	83.00	39.23	2181.53	10.94	22.75	34.90	68.60	32.5
215	78.73	79.00	44.63	2512.58	12.28	39.78	37.61	89.67	25.6
216	79.90	80.00	36.87	2601.28	10.98	32.53	27.80	71.31	32.2
217	77.80	81.00	36.87	2428.94	12.65	36.23	34.40	83.28	32.7
218	79.33	85.00	38.90	1809.89	9.91	21.44	27.55	58.90	35.2
219	79.77	82.00	41.57	2166.14	12.15	37.16	35.68	85.00	30.7
220	80.37	81.00	37.93	2268.12	13.84	28.37	29.30	71.51	31.4
221	80.87	79.00	42.97	2034.12	12.53	33.25	33.05	78.83	29.8
222	79.90	79.00	40.10	1709.87	8.44	13.54	22.58	44.55	33.8
223	78.97	82.00	37.10	2392.39	12.30	38.50	26.01	76.81	33.4
224	79.17	85.00	36.37	2407.18	12.33	22.87	29.80	65.00	31.9
225	79.40	80.00	38.70	2071.71	13.67	39.37	29.58	82.62	32.3

TIL	PH	DFF	SPAD	TLA	TLWT	YIELD	SWT	TDM	LT
226	78.43	82.00	38.53	1714.08	8.66	26.26	23.74	58.67	34.8
227	81.27	82.00	44.17	2505.78	11.63	38.68	33.19	83.50	30.0
228	82.07	82.00	42.20	2384.32	11.67	37.46	33.49	82.61	28.9
229	77.47	85.00	39.17	2147.08	12.14	32.36	26.28	70.78	31.2
230	79.03	81.00	38.73	2482.39	10.73	29.32	29.75	69.80	32.1
231	79.17	85.00	39.33	2230.86	12.22	27.15	28.65	68.02	34.4
232	80.10	85.00	38.33	2482.30	12.77	33.55	27.68	74.00	33.4
233	78.63	80.00	42.93	2757.22	13.82	39.40	38.05	91.27	27.2
234	80.47	81.00	36.80	2606.28	14.28	38.44	34.29	87.01	33.6
235	78.27	79.00	40.03	1891.64	9.46	29.70	24.34	63.50	32.5
236	79.73	83.00	39.23	2533.46	13.28	36.28	26.29	75.85	32.0
237	78.43	83.00	45.37	2761.25	11.71	37.63	35.52	84.87	27.5
238	80.57	85.00	39.10	2654.10	13.38	34.79	33.06	81.24	33.7
239	80.80	85.00	37.43	2316.04	12.34	30.20	31.57	74.11	32.6
240	81.13	79.00	40.23	2718.01	11.38	32.26	32.02	75.66	33.0
241	81.90	82.00	39.87	2814.96	14.00	38.50	38.85	91.35	32.1
242	79.57	83.00	39.37	1999.81	9.60	26.28	24.95	60.84	32.6
243	79.43	83.00	36.80	1310.78	8.83	17.29	24.72	50.85	33.1
244	79.43	79.00	39.63	2426.50	12.25	37.60	33.12	82.96	32.8
245	79.93	84.00	38.60	3325.19	12.78	36.45	34.27	83.50	32.4
246	81.47	81.00	38.27	1499.73	7.72	18.62	24.67	51.01	32.3
247	78.67	80.00	37.93	1701.79	9.00	25.33	26.65	60.98	31.3
248	82.60	81.00	42.07	2654.06	12.06	39.23	33.90	85.19	28.6
249	79.33	80.00	39.23	1979.62	9.96	16.31	26.44	52.70	31.0
250	80.53	80.00	37.90	2539.02	11.35	22.32	29.22	62.88	33.7
251	80.17	82.00	38.07	2068.75	9.52	18.52	24.11	52.15	33.0
252	77.73	81.00	37.70	2371.89	11.64	27.85	31.44	70.93	30.0
253	82.03	81.00	42.00	2994.58	14.57	41.19	36.15	91.91	26.4
254	81.10	85.00	36.67	2276.60	12.48	32.24	29.69	74.40	30.7
255	80.30	81.00	39.33	1406.49	8.99	24.20	26.20	59.39	30.4
256	81.50	81.00	40.07	2772.48	13.26	43.92	33.94	91.12	30.4

APPENDIX-II: List of known function genes found in the genome region of RM 16

Sl. No.	Start	Stop	locus	Annotation
1	21849343	21853782	LOC_Os03g39330	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
2	21866682	21874515	LOC_Os03g39350	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
3	21874849	21877521	LOC_Os03g39356	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
4	21878910	21879224	LOC_Os03g39362	retrotransposon protein, putative, unclassified
5	21880770	21881621	LOC_Os03g39370	expressed protein
6	21881948	21885333	LOC_Os03g39380	expressed protein
7	21885995	21886435	LOC_Os03g39390	expressed protein
8	21893388	21900472	LOC_Os03g39400	retrotransposon protein, putative, Ty1-copia subclass, expressed
9	21900414	21904231	LOC_Os03g39407	expressed protein
10	21910225	21913786	LOC_Os03g39432	CPuORF31 - conserved peptide uORF-containing transcript, expressed
11	21912140	21913786	LOC_Os03g39432	CPuORF31 - conserved peptide uORF-containing transcript, expressed
12	21921579	21926957	LOC_Os03g39450	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
13	21927810	21928475	LOC_Os03g39460	retrotransposon, putative, centromere-specific
14	21933715	21934736	LOC_Os03g39490	retrotransposon protein, putative, Ty3-gypsy subclass
15	21937196	21941799	LOC_Os03g39500	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
16	21944298	21944624	LOC_Os03g39510	expressed protein
17	21950474	21953062	LOC_Os03g39520	plant protein of unknown function domain containing protein, expressed
18	21955115	21956457	LOC_Os03g39530	expressed protein
19	21959933	21960490	LOC_Os03g39540	cytochrome P450, putative, expressed
20	21963215	21963968	LOC_Os03g39550	expressed protein
21	21966674	21967615	LOC_Os03g39560	retrotransposon protein, putative, unclassified, expressed
22	21969710	21973153	LOC_Os03g39570	transposon protein, putative, CACTA, En/Spm sub-class, expressed
23	21975258	21978550	LOC_Os03g39580	retrotransposon protein, putative, unclassified, expressed
24	21979178	21983610	LOC_Os03g39590	retrotransposon protein, putative, unclassified, expressed

Sl. No.	Start	Stop	locus	Annotation
25	21988968	21990035	LOC_Os03g39594	transposon protein, putative, CACTA, En/Spm sub-class, expressed
26	21990590	21993965	LOC_Os03g39598	transposon protein, putative, CACTA, En/Spm sub-class, expressed
27	21995993	21998021	LOC_Os03g39602	expressed protein
28	21999942	22001714	LOC_Os03g39610	chlorophyll A-B binding protein, putative, expressed
29	22008351	22009220	LOC_Os03g39620	expressed protein
30	22011080	22011546	LOC_Os03g39629	expressed protein
31	22016151	22029740	LOC_Os03g39640	CBS domain containing protein, expressed
32	22048470	22052542	LOC_Os03g39650	cytochrome P450, putative, expressed
33	22053827	22056431	LOC_Os03g39655	expressed protein
34	22056764	22058465	LOC_Os03g39660	expressed protein
35	22069041	22070159	LOC_Os03g39670	retrotransposon protein, putative, unclassified
36	22075719	22076960	LOC_Os03g39680	retrotransposon protein, putative, LINE subclass
37	22083215	22086166	LOC_Os03g39690	cytochrome P450, putative, expressed
38	22089623	22090102	LOC_Os03g39700	expressed protein
39	22091999	22095372	LOC_Os03g39710	transporter family protein, putative, expressed
40	22099661	22100952	LOC_Os03g39720	expressed protein
41	22103750	22105110	LOC_Os03g39730	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
42	22110421	22116473	LOC_Os03g39740	expressed protein
43	22123330	22125643	LOC_Os03g39760	cytochrome P450, putative, expressed
44	22132011	22135108	LOC_Os03g39780	retrotransposon protein, putative, unclassified, expressed
45	22135801	22138216	LOC_Os03g39790	retrotransposon protein, putative, unclassified, expressed
46	22147967	22149973	LOC_Os03g39820	expressed protein
47	22150323	22151612	LOC_Os03g39830	expressed protein
48	22160246	22161494	LOC_Os03g39850	glutathione S-transferase, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
49	22164612	22169919	LOC_Os03g39860	acyl carrier protein, putative, expressed
50	22171726	22174047	LOC_Os03g39870	expressed protein
51	22173113	22174949	LOC_Os03g39880	expressed protein
52	22175331	22177113	LOC_Os03g39890	retrotransposon, putative, centromere-specific
53	22179255	22184032	LOC_Os03g39900	retrotransposon protein, putative, unclassified, expressed
54	22184199	22187943	LOC_Os03g39910	XH domain containing protein, expressed
55	22187208	22193170	LOC_Os03g39920	retrotransposon protein, putative, unclassified, expressed
56	22193676	22195637	LOC_Os03g39930	retrotransposon protein, putative, unclassified, expressed
57	22197895	22200121	LOC_Os03g39940	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
58	22203679	22207213	LOC_Os03g39960	expressed protein
59	22210884	22212880	LOC_Os03g39970	conserved hypothetical protein
60	22213575	22214408	LOC_Os03g39980	expressed protein
61	22217150	22218152	LOC_Os03g40000	expressed protein
62	22219243	22227221	LOC_Os03g40010	DEK C terminal domain containing protein, expressed
63	22233268	22238894	LOC_Os03g40020	PPR repeat containing protein, expressed
64	22239456	22239986	LOC_Os03g40030	expressed protein
65	22243187	22244649	LOC_Os03g40040	expressed protein
66	22253571	22254887	LOC_Os03g40060	expressed protein
67	22255208	22259154	LOC_Os03g40070	transposon protein, putative, unclassified, expressed
68	22262511	22267163	LOC_Os03g40080	GRAS family transcription factor containing protein, expressed
69	22268809	22270021	LOC_Os03g40084	expressed protein
70	22271274	22272394	LOC_Os03g40090	expressed protein
71	22281686	22284642	LOC_Os03g40100	ACT domain containing protein, expressed
72	22285683	22292562	LOC_Os03g40110	nop14-like family protein, expressed
73	22295371	22295889	LOC_Os03g40120	hypothetical protein

Sl. No.	Start	Stop	locus	Annotation
74	22298590	22300150	LOC_Os03g40130	universal stress protein domain containing protein, putative, expressed
75	22314403	22321662	LOC_Os03g40160	transposon protein, putative, unclassified, expressed
76	22332262	22335094	LOC_Os03g40170	zinc finger, C3HC4 type domain containing protein, expressed
77	22336536	22338324	LOC_Os03g40180	60S ribosomal protein L15, putative, expressed
78	22347660	22351524	LOC_Os03g40194	disease resistance RPP13-like protein 1, putative, expressed
79	22353967	22354876	LOC_Os03g40210	expressed protein
80	22357722	22359717	LOC_Os03g40220	retrotransposon protein, putative, unclassified
81	22361179	22361714	LOC_Os03g40230	expressed protein
82	22365732	22366824	LOC_Os03g40240	hypothetical protein
83	22369319	22371345	LOC_Os03g40250	Leucine Rich Repeat family protein, expressed
84	22372924	22380193	LOC_Os03g40260	Regulator of chromosome condensation domain containing protein, expressed
85	22381320	22383933	LOC_Os03g40270	alpha-1,4-glucan-protein synthase, putative, expressed
86	22399872	22400594	LOC_Os03g40290	expressed protein
87	22402435	22403164	LOC_Os03g40300	expressed protein
88	22413557	22418672	LOC_Os03g40310	RNA recognition motif containing protein, putative, expressed
89	22423119	22423738	LOC_Os03g40320	expressed protein
90	22424025	22424841	LOC_Os03g40330	glycosyl hydrolases family 17, putative, expressed
91	22430238	22431881	LOC_Os03g40360	retrotransposon protein, putative, unclassified, expressed
92	22434875	22436110	LOC_Os03g40370	OsFBDUF15 - F-box and DUF domain containing protein, expressed
93	22441160	22441552	LOC_Os03g40380	expressed protein
94	22443273	22445121	LOC_Os03g40390	expressed protein
95	22445964	22447733	LOC_Os03g40400	interferon-induced, double-stranded RNA-activated protein kinase, putative, expressed
96	22451553	22453961	LOC_Os03g40410	expressed protein

Sl. No.	Start	Stop	locus	Annotation
97	22462307	22462933	LOC_Os03g40430	expressed protein
98	22464518	22470146	LOC_Os03g40440	B12D protein, putative, expressed
99	22474543	22478130	LOC_Os03g40460	expressed protein
100	22494223	22494507	LOC_Os03g40470	expressed protein
101	22497208	22504375	LOC_Os03g40480	transposon protein, putative, CACTA, En/Spm sub-class, expressed
102	22512261	22512545	LOC_Os03g40490	expressed protein
103	22513652	22515991	LOC_Os03g40500	transposon protein, putative, CACTA, En/Spm sub-class, expressed
104	22519106	22522201	LOC_Os03g40510	transposon protein, putative, CACTA, En/Spm sub-class, expressed
105	22532290	22532535	LOC_Os03g40520	hypothetical protein
106	22538341	22542520	LOC_Os03g40540	cytochrome P450, putative, expressed
107	22547513	22552046	LOC_Os03g40550	kinase, pfkB family, putative, expressed
108	22554950	22559774	LOC_Os03g40560	retrotransposon protein, putative, unclassified, expressed
109	22559946	22560944	LOC_Os03g40570	retrotransposon protein, putative, unclassified, expressed
110	22562357	22564715	LOC_Os03g40580	retrotransposon protein, putative, unclassified, expressed
111	22567670	22568685	LOC_Os03g40600	cytochrome P450 78A11, putative, expressed
112	22572706	22574008	LOC_Os03g40610	cytochrome P450, putative, expressed
113	22593142	22594058	LOC_Os03g40630	transposon protein, putative, unclassified
114	22595701	22604033	LOC_Os03g40650	bromodomain associated family protein, expressed
115	22615886	22619553	LOC_Os03g40670	glycerophosphoryl diester phosphodiesterase family protein, putative, expressed
116	22624156	22628484	LOC_Os03g40690	PPR repeat containing protein, expressed
117	22628794	22630343	LOC_Os03g40700	presenilin, putative, expressed
118	22635142	22640881	LOC_Os03g40710	ZOS3-13 - C2H2 zinc finger protein, expressed
119	22641452	22657252	LOC_Os03g40720	UDP-glucose 6-dehydrogenase, putative, expressed
120	22659157	22664290	LOC_Os03g40725	expressed protein

Sl. No.	Start	Stop	locus	Annotation
121	22666722	22669037	LOC_Os03g40770	expressed protein
122	22670460	22675791	LOC_Os03g40780	transport protein-related, putative, expressed
123	22683996	22684274	LOC_Os03g40790	hypothetical protein
124	22689139	22690325	LOC_Os03g40800	expressed protein
125	22694126	22696715	LOC_Os03g40820	retrotransposon protein, putative, unclassified, expressed
126	22702919	22706164	LOC_Os03g40830	OsSub30 - Putative Subtilisin homologue, expressed
127	22710115	22713187	LOC_Os03g40840	expressed protein
128	22721921	22722178	LOC_Os03g40860	expressed protein
129	22723013	22729444	LOC_Os03g40870	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
130	22730603	22732967	LOC_Os03g40880	retrotransposon protein, putative, unclassified, expressed
131	22736696	22737199	LOC_Os03g40900	expressed protein
132	22737749	22738570	LOC_Os03g40910	retrotransposon protein, putative, unclassified, expressed
133	22739267	22743374	LOC_Os03g40920	expressed protein
134	22744328	22746916	LOC_Os03g40930	expressed protein
135	22758451	22759095	LOC_Os03g40950	expressed protein
136	22766735	22767868	LOC_Os03g40960	retrotransposon protein, putative, unclassified
137	22768487	22774452	LOC_Os03g40970	retrotransposon protein, putative, unclassified, expressed
138	22775104	22775430	LOC_Os03g40980	retrotransposon protein, putative, Ty3-gypsy subclass
139	22786883	22793943	LOC_Os03g41000	retrotransposon protein, putative, Ty1-copia subclass, expressed
140	22797204	22797865	LOC_Os03g41010	hypothetical protein
141	22803576	22803995	LOC_Os03g41030	expressed protein
142	22815635	22816468	LOC_Os03g41050	expressed protein
143	22816933	22817775	LOC_Os03g41060	GASR2 - Gibberellin-regulated GASA/GAST/Snakin family protein precursor, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
144	22823772	22824543	LOC_Os03g41064	natural resistance-associated macrophage protein, putative, expressed
145	22825897	22826541	LOC_Os03g41070	metal transporter Nramp2, putative, expressed
146	22827426	22833017	LOC_Os03g41080	seed maturation protein PM23, putative, expressed
147	22845483	22847345	LOC_Os03g41100	cyclin, putative, expressed
148	22850839	22852132	LOC_Os03g41110	ZOS3-14 - C2H2 zinc finger protein, expressed
149	22856683	22861392	LOC_Os03g41120	expressed protein
150	22863494	22864072	LOC_Os03g41130	expressed protein
151	22870904	22871934	LOC_Os03g41140	transposable element protein, putative, containing Pfam profile: PF04195, Transposase_28, expressed
152	22880011	22880454	LOC_Os03g41150	expressed protein
153	22882427	22882627	LOC_Os03g41154	hypothetical protein
154	22884800	22885632	LOC_Os03g41160	RNA-directed DNA polymerase, putative
155	22892396	22895558	LOC_Os03g41170	expressed protein
156	22900882	22903977	LOC_Os03g41180	transposon protein, putative, CACTA, En/Spm sub-class
157	22911337	22918539	LOC_Os03g41200	retrotransposon protein, putative, unclassified, expressed
158	22912599	22918539	LOC_Os03g41200	retrotransposon protein, putative, unclassified, expressed
159	22930270	22937157	LOC_Os03g41229	expressed protein
160	22944456	22944740	LOC_Os03g41260	hypothetical protein
161	22947541	22950581	LOC_Os03g41280	expressed protein
162	22959065	22959955	LOC_Os03g41290	expressed protein
163	22960920	22967160	LOC_Os03g41300	expressed protein
164	22969255	22969500	LOC_Os03g41310	expressed protein
165	22971491	22971736	LOC_Os03g41320	expressed protein
166	22976955	22979936	LOC_Os03g41330	DUF260 domain containing protein, putative, expressed
167	22984760	22985227	LOC_Os03g41339	expressed protein

Sl. No.	Start	Stop	locus	Annotation
168	22990165	22996334	LOC_Os03g41350	BTBN7 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain, expressed
169	23021091	23022920	LOC_Os03g41380	expressed protein
170	23036228	23037437	LOC_Os03g41390	ZOS3-15 - C2H2 zinc finger protein, expressed
171	23045392	23046942	LOC_Os03g41400	EBNA-1, putative, expressed
172	23048222	23053499	LOC_Os03g41419	serpin domain containing protein, putative, expressed
173	23054583	23068315	LOC_Os03g41438	serpin domain containing protein, putative, expressed
174	23068745	23071303	LOC_Os03g41460	CAMK_CAMK_like.20 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
175	23072297	23074935	LOC_Os03g41470	expressed protein
176	23077832	23080251	LOC_Os03g41480	expressed protein
177	23082698	23082964	LOC_Os03g41500	expressed protein
178	23086275	23090291	LOC_Os03g41510	oxidoreductase, aldo/keto reductase family protein, putative, expressed
179	23093139	23096386	LOC_Os03g41530	single-stranded DNA-binding protein, putative, expressed
180	23100472	23101690	LOC_Os03g41540	hypothetical protein
181	23119044	23122456	LOC_Os03g41570	transposon protein, putative, unclassified, expressed
182	23123059	23123593	LOC_Os03g41580	transposon protein, putative, CACTA, En/Spm sub-class
183	23127983	23128906	LOC_Os03g41600	DUF260 domain containing protein, putative, expressed
184	23135473	23140230	LOC_Os03g41612	ribosomal protein L25, putative, expressed
185	23142781	23147227	LOC_Os03g41624	retrotransposon, putative, centromere-specific, expressed
186	23149433	23150566	LOC_Os03g41640	GRF zinc finger family protein, expressed
187	23151315	23153673	LOC_Os03g41650	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
188	23155593	23156099	LOC_Os03g41662	expressed protein
189	23157870	23159127	LOC_Os03g41675	expressed protein
190	23165458	23165841	LOC_Os03g41690	expressed protein

Sl. No.	Start	Stop	locus	Annotation
191	23167124	23170084	LOC_Os03g41700	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
192	23173062	23176315	LOC_Os03g41710	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
193	23176661	23177506	LOC_Os03g41720	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
194	23183608	23187458	LOC_Os03g41740	retrotransposon protein, putative, unclassified, expressed
195	23189236	23190465	LOC_Os03g41750	retrotransposon protein, putative, unclassified
196	23192525	23198427	LOC_Os03g41770	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
197	23201176	23207379	LOC_Os03g41780	retrotransposon, putative, centromere-specific, expressed
198	23208034	23210281	LOC_Os03g41790	retrotransposon, putative, centromere-specific, expressed
199	23213785	23217778	LOC_Os03g41800	transposon protein, putative, unclassified, expressed
200	23218781	23219212	LOC_Os03g41810	hypothetical protein
201	23221526	23221927	LOC_Os03g41820	expressed protein
202	23227125	23232645	LOC_Os03g41830	retrotransposon protein, putative, unclassified, expressed
203	23234414	23235754	LOC_Os03g41840	retrotransposon protein, putative, unclassified, expressed
204	23239272	23240408	LOC_Os03g41850	transposon protein, putative, unclassified
205	23244333	23247136	LOC_Os03g41860	retrotransposon, putative, centromere-specific, expressed
206	23248780	23249588	LOC_Os03g41870	nucleic acid binding protein, putative
207	23262074	23262832	LOC_Os03g41890	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
208	23264430	23264876	LOC_Os03g41879	retrotransposon, putative, centromere-specific
209	23278859	23279095	LOC_Os03g41910	hypothetical protein
210	23280702	23285567	LOC_Os03g41920	expressed protein
211	23290579	23291191	LOC_Os03g41932	expressed protein
212	23294200	23294912	LOC_Os03g41940	expressed protein
213	23303545	23318841	LOC_Os03g41960	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
214	23320512	23321492	LOC_Os03g41970	retrotransposon protein, putative, unclassified
215	23322635	23328795	LOC_Os03g41980	retrotransposon protein, putative, unclassified, expressed

Sl. No.	Start	Stop	locus	Annotation
216	23329181	23332413	LOC_Os03g41990	retrotransposon protein, putative, unclassified, expressed
217	23333353	23333676	LOC_Os03g42000	retrotransposon protein, putative, Ty3-gypsy subclass
218	23334978	23340914	LOC_Os03g42010	DNA-directed polymerase, putative, expressed
219	23341805	23347331	LOC_Os03g42020	calcium-transporting ATPase, plasma membrane-type, putative, expressed
220	23351131	23351813	LOC_Os03g42030	expressed protein
221	23353232	23360680	LOC_Os03g42040	HEAT repeat family protein, putative, expressed
222	23368991	23382556	LOC_Os03g42050	exportin-7-A, putative, expressed
223	23387051	23387693	LOC_Os03g42060	expressed protein
224	23410207	23412753	LOC_Os03g42070	cyclin, putative, expressed
225	23417451	23417831	LOC_Os03g42080	expressed protein
226	23424832	23425245	LOC_Os03g42090	expressed protein
227	23433316	23434712	LOC_Os03g42100	helix-loop-helix DNA-binding domain containing protein, expressed
228	23438157	23442337	LOC_Os03g42110	semialdehyde dehydrogenase, NAD binding domain containing protein, putative, expressed
229	23444572	23446715	LOC_Os03g42120	expressed protein
230	23450164	23452171	LOC_Os03g42130	gibberellin 20 oxidase 2, putative, expressed
231	23453396	23453889	LOC_Os03g42140	hypothetical protein
232	23455130	23457864	LOC_Os03g42150	retrotransposon protein, putative, Ty1-copia subclass, expressed
233	23460943	23461605	LOC_Os03g42170	retrotransposon protein, putative, Ty1-copia subclass, expressed
234	23462933	23463301	LOC_Os03g42180	hypothetical protein
235	23464716	23466453	LOC_Os03g42190	expressed protein
236	23472278	23475827	LOC_Os03g42200	dof zinc finger domain containing protein, putative, expressed
237	23484619	23484996	LOC_Os03g42210	expressed protein
238	23485491	23490741	LOC_Os03g42220	T-complex protein, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
239	23487817	23490741	LOC_Os03g42220	T-complex protein, putative, expressed
240	23491995	23496324	LOC_Os03g42230	B3 DNA binding domain containing protein, expressed
241	23496334	23497401	LOC_Os03g42235	expressed protein
242	23499435	23503720	LOC_Os03g42240	B3 DNA binding domain containing protein, expressed
243	23512612	23513579	LOC_Os03g42259	hypervariable Bacillus group-specific protein, putative, expressed
244	23516352	23521799	LOC_Os03g42270	retrotransposon protein, putative, unclassified, expressed
245	23523494	23528315	LOC_Os03g42280	B3 DNA binding domain containing protein, expressed
246	23523560	23528315	LOC_Os03g42280	B3 DNA binding domain containing protein, expressed
247	23530061	23530602	LOC_Os03g42284	hypothetical protein
248	23533463	23538865	LOC_Os03g42290	B3 DNA binding domain containing protein, expressed
249	23541015	23542945	LOC_Os03g42310	expressed protein
250	23545317	23547668	LOC_Os03g42320	Sec1 family transport protein, putative, expressed
251	23555365	23555901	LOC_Os03g42334	expressed protein
252	23556180	23562967	LOC_Os03g42350	ankyrin, putative, expressed
253	23565984	23566454	LOC_Os03g42360	expressed protein
254	23567544	23577244	LOC_Os03g42370	B3 DNA binding domain containing protein, expressed
255	23577861	23588045	LOC_Os03g42380	expressed protein
256	23592134	23592585	LOC_Os03g42400	expressed protein
257	23595409	23597429	LOC_Os03g42410	B3 DNA binding domain containing protein
258	23609771	23611885	LOC_Os03g42420	B3 DNA binding domain containing protein, expressed
259	23613955	23616375	LOC_Os03g42430	B3 DNA binding domain containing protein, expressed
260	23616738	23620260	LOC_Os03g42440	expressed protein
261	23620827	23621303	LOC_Os03g42450	expressed protein
262	23623464	23630649	LOC_Os03g42464	expressed protein
263	23633268	23640082	LOC_Os03g42480	expressed protein

Sl. No.	Start	Stop	locus	Annotation
264	23641888	23642211	LOC_Os03g42490	expressed protein
265	23644298	23645268	LOC_Os03g42500	expressed protein
266	23647892	23651394	LOC_Os03g42510	transposon protein, putative, CACTA, En/Spm sub-class, expressed
267	23655935	23658301	LOC_Os03g42520	expressed protein
268	23658542	23667612	LOC_Os03g42530	retrotransposon, putative, centromere-specific, expressed
269	23671609	23674781	LOC_Os03g42540	transposon protein, putative, CACTA, En/Spm sub-class
270	23689494	23691731	LOC_Os03g42550	expressed protein
271	23694358	23704806	LOC_Os03g42569	expressed protein
272	23705188	23705874	LOC_Os03g42590	expressed protein
273	23715172	23715759	LOC_Os03g42600	expressed protein
274	23718070	23723733	LOC_Os03g42610	retrotransposon protein, putative, unclassified, expressed
275	23734580	23736564	LOC_Os03g42630	No apical meristem protein, putative, expressed
276	23738885	23746139	LOC_Os03g42650	vegetative storage protein, putative, expressed
277	23754704	23755198	LOC_Os03g42654	expressed protein
278	23765062	23768125	LOC_Os03g42660	retrotransposon protein, putative, unclassified
279	23768899	23771242	LOC_Os03g42670	transposon protein, putative, unclassified, expressed
280	23774090	23778184	LOC_Os03g42690	transposon protein, putative, CACTA, En/Spm sub-class, expressed
281	23780175	23780484	LOC_Os03g42700	hypothetical protein
282	23781111	23782726	LOC_Os03g42710	WD-40 repeat family protein, putative, expressed
283	23785316	23787756	LOC_Os03g42720	expressed protein
284	23789242	23791395	LOC_Os03g42730	expressed protein
285	23794410	23795359	LOC_Os03g42740	F-box/LRR-repeat protein 16, putative, expressed
286	23797161	23808349	LOC_Os03g42750	roothairless 1, putative, expressed
287	23812565	23816582	LOC_Os03g42760	zinc finger protein-related, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
288	23817641	23824229	LOC_Os03g42770	expressed protein
289	23827633	23832767	LOC_Os03g42780	zinc finger protein-related, putative, expressed
290	23836063	23839588	LOC_Os03g42790	zinc finger protein-related, putative, expressed
291	23841709	23844608	LOC_Os03g42799	expressed protein
292	23849970	23852188	LOC_Os03g42810	endonuclease/exonuclease/phosphatase family domain containing protein, expressed
293	23855187	23861941	LOC_Os03g42820	LIM domain-containing protein, putative, expressed
294	23870183	23876307	LOC_Os03g42830	MATE efflux family protein, putative, expressed
295	23888729	23895481	LOC_Os03g42840	calcineurin B, putative, expressed
296	23898856	23899879	LOC_Os03g42850	expressed protein
297	23900148	23900540	LOC_Os03g42860	pentatricopeptide, putative, expressed
298	23901880	23902191	LOC_Os03g42870	hypothetical protein
299	23907648	23913211	LOC_Os03g42880	nucleotide-binding protein-like, putative, expressed
300	23915676	23917302	LOC_Os03g42890	retrotransposon protein, putative, unclassified, expressed
301	23925619	23934205	LOC_Os03g42900	KH domain containing protein, putative, expressed
302	23937445	23937999	LOC_Os03g42910	hypothetical protein
303	23938325	23938825	LOC_Os03g42920	expressed protein
304	23940036	23943060	LOC_Os03g42930	expressed protein
305	23947041	23954477	LOC_Os03g42950	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
306	23954085	23954870	LOC_Os03g42954	conserved hypothetical protein
307	23957825	23961086	LOC_Os03g42960	expressed protein
308	23962964	23965814	LOC_Os03g42970	transposon protein, putative, unclassified, expressed
309	23977568	23978287	LOC_Os03g42990	hypothetical protein
310	23981542	23981781	LOC_Os03g43000	expressed protein
311	23989140	23997536	LOC_Os03g43010	expressed protein

Sl. No.	Start	Stop	locus	Annotation
312	23999212	24003075	LOC_Os03g43020	prefoldin subunit, putative, expressed
313	24009227	24010121	LOC_Os03g43030	expressed protein
314	24010375	24013822	LOC_Os03g43040	retrotransposon protein, putative, unclassified, expressed
315	24014790	24015912	LOC_Os03g43050	LTPL90 - Protease inhibitor/seed storage/LTP family protein precursor, putative, expressed
316	24018612	24023409	LOC_Os03g43060	OsFBX96 - F-box domain containing protein, expressed
317	24028263	24031101	LOC_Os03g43070	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
318	24035639	24036309	LOC_Os03g43080	expressed protein
319	24036836	24044082	LOC_Os03g43090	transposon protein, putative, CACTA, En/Spm sub-class, expressed
320	24046089	24047243	LOC_Os03g43100	expressed protein
321	24049172	24053785	LOC_Os03g43114	expressed protein
322	24055470	24058548	LOC_Os03g43130	transposon protein, putative, unclassified
323	24060188	24061232	LOC_Os03g43140	expressed protein
324	24076287	24076643	LOC_Os03g43150	transposon protein, putative, unclassified, expressed
325	24079290	24082825	LOC_Os03g43160	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
326	24084048	24084550	LOC_Os03g43169	retrotransposon protein, putative, Ty3-gypsy subclass
327	24085073	24085396	LOC_Os03g43180	transposon protein, putative, unclassified, expressed
328	24087079	24087718	LOC_Os03g43190	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
329	24092341	24093516	LOC_Os03g43200	retrotransposon protein, putative, unclassified, expressed
330	24095243	24098440	LOC_Os03g43210	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
331	24098772	24105769	LOC_Os03g43220	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
332	24106025	24110042	LOC_Os03g43230	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
333	24110456	24111678	LOC_Os03g43235	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
334	24111938	24113250	LOC_Os03g43240	retrotransposon protein, putative, Ty3-gypsy subclass

Sl. No.	Start	Stop	locus	Annotation
335	24114766	24119958	LOC_Os03g43250	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
336	24121819	24126219	LOC_Os03g43270	retrotransposon protein, putative, unclassified, expressed
337	24129970	24133662	LOC_Os03g43280	transposon protein, putative, CACTA, En/Spm sub-class, expressed
338	24136043	24140389	LOC_Os03g43290	transposon protein, putative, CACTA, En/Spm sub-class, expressed
339	24143221	24145696	LOC_Os03g43309	retrotransposon protein, putative, unclassified, expressed
340	24154395	24156863	LOC_Os03g43330	keratin, type I cytoskeletal 9, putative, expressed
341	24160652	24162189	LOC_Os03g43340	expressed protein
342	24168644	24171622	LOC_Os03g43350	expressed protein
343	24174317	24180460	LOC_Os03g43360	zinc finger, C3HC4 type domain containing protein, expressed
344	24184421	24185066	LOC_Os03g43374	retrotransposon protein, putative, unclassified
345	24189817	24197398	LOC_Os03g43390	F-box/LRR domain containing protein, putative, expressed
346	24198649	24202021	LOC_Os03g43400	OsIAA11 - Auxin-responsive Aux/IAA gene family member, expressed
347	24212554	24213860	LOC_Os03g43410	OsIAA12 - Auxin-responsive Aux/IAA gene family member, expressed
348	24215221	24219448	LOC_Os03g43420	single-stranded DNA-binding protein, putative, expressed
349	24220021	24222271	LOC_Os03g43430	mttA/Hcf106 family protein, putative, expressed
350	24226224	24227930	LOC_Os03g43440	CAMK_KIN1/SNF1/Nim1_like.17 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
351	24240070	24240501	LOC_Os03g43460	hypothetical protein
352	24246840	24248814	LOC_Os03g43464	hypothetical protein
353	24250816	24252399	LOC_Os03g43470	pentatricopeptide, putative, expressed
354	24255997	24256851	LOC_Os03g43480	AGG2, putative, expressed
355	24261349	24262664	LOC_Os03g43490	retrotransposon protein, putative, unclassified, expressed
356	24264677	24266783	LOC_Os03g43500	expressed protein
357	24271404	24277905	LOC_Os03g43510	expressed protein
358	24283867	24284737	LOC_Os03g43524	expressed protein

Sl. No.	Start	Stop	locus	Annotation
359	24308939	24313541	LOC_Os03g43540	expressed protein
360	24315573	24320015	LOC_Os03g43550	retrotransposon protein, putative, unclassified
361	24326109	24328372	LOC_Os03g43560	hypothetical protein
362	24335387	24337564	LOC_Os03g43564	hypothetical protein
363	24341243	24344785	LOC_Os03g43570	UBX domain-containing protein 1, putative, expressed
364	24347498	24349006	LOC_Os03g43580	IQ calmodulin-binding motif family protein, putative, expressed
365	24351611	24357284	LOC_Os03g43590	LSTK-1-like kinase, putative, expressed
366	24371063	24376085	LOC_Os03g43610	retrotransposon protein, putative, Ty1-copia subclass, expressed
367	24389529	24390916	LOC_Os03g43620	hypothetical protein
368	24400926	24402895	LOC_Os03g43650	uncharacterized protein At4g06744 precursor, putative, expressed

APPENDIX-III: List of known function genes found in the genome region of RM 1388

Sl. No.	Start	Stop	locus	Annotation
1	23762322	23768245	LOC_Os04g39900	Os4bglu13 - beta-glucosidase homologue, similar to Os4Bglu12 exoglucanase/b-glucosidase, expressed
2	23770072	23772456	LOC_Os04g39910	receptor-like protein kinase, putative, expressed
3	23779181	23781586	LOC_Os04g39930	receptor-like protein kinase, putative, expressed
4	23782369	23784821	LOC_Os04g39940	brevis radix, putative, expressed
5	23790789	23792871	LOC_Os04g39970	vacuolar sorting protein 9 domain-containing protein, putative, expressed
6	23795409	23797531	LOC_Os04g39980	gibberellin 20 oxidase 2, expressed
7	23800784	23806938	LOC_Os04g39990	retrotransposon protein, putative, unclassified, expressed
8	23807559	23810542	LOC_Os04g40000	retrotransposon protein, putative, unclassified, expressed
9	23815413	23817050	LOC_Os04g40010	PPR repeat domain containing protein, putative, expressed
10	23826525	23829383	LOC_Os04g40030	OsFBO17 - F-box and other domain containing protein, expressed
11	23829705	23836276	LOC_Os04g40040	copper methylamine oxidase precursor, putative, expressed
12	23843544	23847934	LOC_Os04g40050	ribonuclease H2 subunit B, putative, expressed
13	23849329	23853944	LOC_Os04g40060	transposon protein, putative, unclassified, expressed
14	23859220	23863737	LOC_Os04g40070	GRAM and C2 domains containing protein, putative, expressed
15	23864150	23867322	LOC_Os04g40080	leucine rich repeat containing protein, expressed
16	23867457	23872309	LOC_Os04g40090	zinc finger, ZZ type family protein, expressed
17	23876327	23878958	LOC_Os04g40100	BTBN11 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain, expressed
18	23882661	23885227	LOC_Os04g40130	Rf1, mitochondrial precursor, putative, expressed
19	23892683	23896476	LOC_Os04g40150	glycosyl transferase family 17 protein, putative, expressed
20	23915728	23917010	LOC_Os04g40190	circumsporozoite protein, putative, expressed
21	23919033	23920878	LOC_Os04g40200	HNH endonuclease family protein, putative, expressed
22	23923507	23924362	LOC_Os04g40210	transposon protein, putative, CACTA, En/Spm sub-class, expressed

Sl. No.	Start	Stop	locus	Annotation
23	23927513	23930310	LOC_Os04g40220	transposon protein, putative, CACTA, En/Spm sub-class, expressed
24	23932699	23933966	LOC_Os04g40230	transposon protein, putative, CACTA, En/Spm sub-class, expressed
25	23934260	23936479	LOC_Os04g40240	transposon protein, putative, CACTA, En/Spm sub-class
26	23944139	23947759	LOC_Os04g40260	retrotransposon protein, putative, unclassified, expressed
27	23955470	23959975	LOC_Os04g40290	AAA-type ATPase family protein, putative, expressed
28	23960070	23963329	LOC_Os04g40300	transglutaminase, putative, expressed
29	23963996	23967809	LOC_Os04g40310	dehydrogenase, putative, expressed
30	23976077	23979533	LOC_Os04g40330	OsFBX141 - F-box domain containing protein, expressed
31	23990892	23991709	LOC_Os04g40350	GRF zinc finger family protein
32	23992130	23998033	LOC_Os04g40360	transposon protein, putative, unclassified, expressed
33	23998827	24002250	LOC_Os04g40370	OsFBX142 - F-box domain containing protein, expressed
34	23999562	24002250	LOC_Os04g40370	OsFBX142 - F-box domain containing protein, expressed
35	24013077	24016880	LOC_Os04g40400	eukaryotic translation initiation factor 4B, putative, expressed
36	24018289	24019456	LOC_Os04g40410	high affinity nitrate transporter, putative, expressed
37	24021401	24026205	LOC_Os04g40420	SWIRM domain containing protein, expressed
38	24026632	24029053	LOC_Os04g40430	surfeit locus protein, putative, expressed
39	24036670	24041085	LOC_Os04g40450	retrotransposon protein, putative, unclassified, expressed
40	24043880	24047054	LOC_Os04g40460	cytochrome P450, putative, expressed
41	24049070	24051142	LOC_Os04g40470	cytochrome P450, putative, expressed
42	24054885	24058958	LOC_Os04g40490	glycosyl hydrolase family 5 protein, putative, expressed
43	24060045	24063819	LOC_Os04g40500	glycosyl hydrolase family 5 protein, putative, expressed
44	24064718	24068505	LOC_Os04g40510	glycosyl hydrolase family 5 protein, putative, expressed
45	24068037	24072124	LOC_Os04g40520	uncharacterized glycosyltransferase, putative, expressed
46	24073335	24074729	LOC_Os04g40530	methyltransferase domain containing protein, expressed

Sl. No.	Start	Stop	locus	Annotation
47	24075211	24077600	LOC_Os04g40540	protein-L-isoaspartate O-methyltransferase, putative, expressed
48	24086334	24088075	LOC_Os04g40560	WD domain, G-beta repeat domain containing protein, expressed
49	24093981	24098250	LOC_Os04g40570	ABC transporter, ATP-binding protein, putative, expressed
50	24098305	24099665	LOC_Os04g40580	O-acyltransferase, putative, expressed
51	24101138	24102493	LOC_Os04g40590	wax synthase isoform 1, putative, expressed
52	24102870	24104925	LOC_Os04g40600	peptide methionine sulfoxide reductase, putative, expressed
53	24108926	24109758	LOC_Os04g40620	peptide methionine sulfoxide reductase, putative, expressed
54	24115971	24118216	LOC_Os04g40630	BTBZ4 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-binding domains, expressed
55	24129561	24133242	LOC_Os04g40650	metalloprotease ATP23, putative, expressed
56	24133854	24138557	LOC_Os04g40660	MA3 domain containing protein, expressed
57	24187919	24188910	LOC_Os04g40750	expressed protein
58	24189434	24191259	LOC_Os04g40760	OsFBX143 - F-box domain containing protein, expressed
59	24192009	24195350	LOC_Os04g40770	OsFBX144 - F-box domain containing protein, expressed
60	24199124	24200930	LOC_Os04g40780	OsFBX145 - F-box domain containing protein, expressed
61	24202313	24204684	LOC_Os04g40790	conserved hypothetical protein
62	24206249	24210114	LOC_Os04g40800	OsFBL18 - F-box domain and LRR containing protein, expressed
63	24229225	24236905	LOC_Os04g40830	cullin family domain containing protein, putative, expressed
64	24237082	24240761	LOC_Os04g40840	elongator complex protein 3, putative, expressed
65	24240921	24243770	LOC_Os04g40850	26S proteasome non-ATPase regulatory subunit 6, putative, expressed
66	24241295	24243770	LOC_Os04g40850	26S proteasome non-ATPase regulatory subunit 6, putative, expressed
67	24243897	24247750	LOC_Os04g40860	transposon protein, putative, unclassified, expressed
68	24253308	24257461	LOC_Os04g40870	alveolar soft part sarcoma chromosome region, candidate 1, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
69	24261369	24267801	LOC_Os04g40874	glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform, putative, expressed
70	24267885	24269926	LOC_Os04g40880	nucleotide-binding protein 1, putative, expressed
71	24276590	24278318	LOC_Os04g40900	exonuclease, putative, expressed
72	24279585	24283277	LOC_Os04g40910	OsFBX146 - F-box domain containing protein, expressed
73	24296450	24301211	LOC_Os04g40930	MYB family transcription factor, putative, expressed
74	24301441	24303182	LOC_Os04g40940	mitotic spindle checkpoint protein MAD2, putative, expressed
75	24305451	24309169	LOC_Os04g40950	glyceraldehyde-3-phosphate dehydrogenase, putative, expressed
76	24309580	24310149	LOC_Os04g40960	OsFBX147 - F-box domain containing protein, expressed
77	24312618	24319913	LOC_Os04g40970	DEAD-box ATP-dependent RNA helicase, putative, expressed
78	24321375	24325746	LOC_Os04g40980	Divergent PAP2 family domain containing protein, expressed
79	24325764	24328254	LOC_Os04g40990	malate synthase, glyoxysomal, putative, expressed
80	24339609	24343784	LOC_Os04g41020	kelch repeat protein, putative, expressed
81	24348022	24350872	LOC_Os04g41030	inactive receptor kinase At1g27190 precursor, putative, expressed
82	24352391	24355421	LOC_Os04g41040	DNA-directed RNA polymerases I, II, and III subunit RPABC1, putative, expressed
83	24356759	24357616	LOC_Os04g41050	zinc finger, C3HC4 type domain containing protein, expressed
84	24359394	24360538	LOC_Os04g41060	zinc finger C-x8-C-x5-C-x3-H type family protein, expressed
85	24363537	24364130	LOC_Os04g41070	zinc finger, C3HC4 type domain containing protein, expressed
86	24366152	24366736	LOC_Os04g41080	zinc finger, C3HC4 type domain containing protein, expressed
87	24368547	24369678	LOC_Os04g41090	retrotransposon protein, putative, unclassified, expressed
88	24371795	24378071	LOC_Os04g41100	cyclin-dependent kinase G-2, putative, expressed
89	24379793	24386408	LOC_Os04g41110	Rad21 / Rec8 like protein, putative, expressed
90	24389132	24390928	LOC_Os04g41120	pentatricopeptide, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
91	24391002	24395755	LOC_Os04g41130	osFTL6 FT-Like6 homologous to Flowering Locus T gene; contains Pfam profile PF01161: Phosphatidylethanolamine-binding protein, expressed
92	24399162	24402461	LOC_Os04g41140	PPR repeat containing protein, expressed
93	24402658	24405107	LOC_Os04g41150	DUF565 domain containing protein, putative, expressed
94	24416321	24418474	LOC_Os04g41160	protein kinase, putative, expressed
95	24420285	24421910	LOC_Os04g41170	retrotransposon protein, putative, unclassified, expressed
96	24431480	24440347	LOC_Os04g41200	lipase, putative, expressed
97	24455226	24459050	LOC_Os04g41220	transposon protein, putative, unclassified, expressed
98	24461146	24468949	LOC_Os04g41229	helix-loop-helix DNA-binding domain containing protein, expressed
99	24471709	24479505	LOC_Os04g41250	armadillo/beta-catenin repeat family protein, putative, expressed
100	24480757	24488863	LOC_Os04g41260	amine oxidase, flavin-containing, domain containing protein, expressed
101	24497616	24505401	LOC_Os04g41280	ankyrin repeat domain containing protein, expressed
102	24505544	24508402	LOC_Os04g41300	ribosome recycling factor, putative, expressed
103	24512414	24515768	LOC_Os04g41310	STRUBBELIG-RECEPTOR FAMILY 8 precursor, putative, expressed
104	24514932	24521631	LOC_Os04g41320	nucleotide-sugar transporter family protein, putative, expressed
105	24527897	24530505	LOC_Os04g41340	4-nitrophenylphosphatase, putative, expressed
106	24534461	24536254	LOC_Os04g41350	amino acid transporter, putative, expressed
107	24538462	24541590	LOC_Os04g41370	Leucine Rich Repeat family protein, expressed
108	24541955	24545637	LOC_Os04g41380	Leucine Rich Repeat family protein, expressed
109	24546341	24549172	LOC_Os04g41400	peptide transporter PTR2, putative, expressed
110	24557253	24558614	LOC_Os04g41430	retrotransposon protein, putative, unclassified
111	24560986	24566456	LOC_Os04g41450	TGF-beta receptor, type I/II extracellular region, putative, expressed
112	24567103	24571704	LOC_Os04g41460	transporter family protein, putative, expressed
113	24589351	24590058	LOC_Os04g41480	skin secretory protein xP2 precursor, putative, expressed
114	24593234	24604876	LOC_Os04g41490	DNA-directed RNA polymerase III subunit RPC1, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
115	24615554	24624651	LOC_Os04g41510	serine/threonine-protein kinase GCN2, putative, expressed
116	24625390	24630182	LOC_Os04g41520	retrotransposon protein, putative, unclassified, expressed
117	24631230	24635840	LOC_Os04g41530	serine/threonine-protein kinase GCN2, putative, expressed
118	24640382	24641575	LOC_Os04g41540	OsCML22 - Calmodulin-related calcium sensor protein, expressed
119	24644170	24647863	LOC_Os04g41550	CRS1/YhbY domain containing protein, putative, expressed
120	24647901	24649396	LOC_Os04g41560	B-box zinc finger family protein, putative, expressed
121	24655105	24660068	LOC_Os04g41570	ethylene-responsive protein related, putative, expressed
122	24667261	24670983	LOC_Os04g41580	glycine-rich protein, putative, expressed
123	24675324	24685375	LOC_Os04g41599	transposon protein, putative, CACTA, En/Spm sub-class, expressed
124	24687707	24689307	LOC_Os04g41620	CHIT2 - Chitinase family protein precursor, expressed
125	24692677	24693326	LOC_Os04g41640	HEV2 - Hevein family protein precursor, expressed
126	24696653	24697516	LOC_Os04g41650	transposon protein, putative, CACTA, En/Spm sub-class
127	24700214	24702288	LOC_Os04g41660	transposon protein, putative, CACTA, En/Spm sub-class, expressed
128	24704669	24708297	LOC_Os04g41670	transposon protein, putative, CACTA, En/Spm sub-class, expressed
129	24708716	24709910	LOC_Os04g41680	CHIT3 - Chitinase family protein precursor, expressed
130	24712476	24716983	LOC_Os04g41690	transposon protein, putative, unclassified, expressed
131	24731431	24732927	LOC_Os04g41720	retrotransposon, putative, centromere-specific, expressed
132	24734341	24737996	LOC_Os04g41730	retrotransposon protein, putative, Ty1-copia subclass, expressed
133	24769903	24773347	LOC_Os04g41820	transcription factor RF2a, putative, expressed
134	24775664	24779580	LOC_Os04g41830	myb-like DNA-binding domain containing protein, expressed
135	24787424	24794732	LOC_Os04g41840	transposon protein, putative, unclassified, expressed
136	24795660	24802127	LOC_Os04g41850	NIN, putative, expressed
137	24815364	24816067	LOC_Os04g41875	oxidoreductase, short chain dehydrogenase/reductase domain containing protein, expressed

Sl. No.	Start	Stop	locus	Annotation
138	24822028	24823293	LOC_Os04g41880	transposon protein, putative, unclassified, expressed
139	24834401	24839784	LOC_Os04g41910	RNA recognition motif containing protein, putative, expressed
140	24841303	24843711	LOC_Os04g41920	protein FAM133, putative, expressed
141	24846903	24853939	LOC_Os04g41950	calcium-binding mitochondrial protein anon-60Da, putative, expressed
142	24858040	24859661	LOC_Os04g41960	NADP-dependent oxidoreductase, putative, expressed
143	24863964	24867824	LOC_Os04g41970	endoglucanase, putative, expressed
144	24874021	24875977	LOC_Os04g41980	ATOZI1, putative, expressed
145	24875259	24875977	LOC_Os04g41980	ATOZI1, putative, expressed
146	24876009	24877897	LOC_Os04g41990	autophagy-related protein 10, putative, expressed
147	24878141	24881728	LOC_Os04g42000	6,7-dimethyl-8-ribityllumazine synthase, chloroplast precursor, putative, expressed
148	24882776	24886711	LOC_Os04g42010	RNA binding protein, putative, expressed
149	24889983	24891487	LOC_Os04g42020	CCT/B-box zinc finger protein, putative, expressed
150	24915054	24918786	LOC_Os04g42090	CPuORF7 - conserved peptide uORF-containing transcript, expressed
151	24915889	24918786	LOC_Os04g42090	CPuORF7 - conserved peptide uORF-containing transcript, expressed
152	24920323	24924654	LOC_Os04g42100	retrotransposon protein, putative, unclassified, expressed
153	24932085	24936078	LOC_Os04g42110	cell division control protein 48 homolog B, putative, expressed
154	24935457	24938782	LOC_Os04g42120	N-acetyltransferase ESCO2, putative, expressed
155	24940461	24943098	LOC_Os04g42130	integral membrane transporter family protein, putative, expressed
156	24943121	24945222	LOC_Os04g42134	enhancer of rudimentary protein, putative, expressed
157	24946091	24952282	LOC_Os04g42140	eukaryotic initiation factor iso-4F subunit p82-34, putative, expressed
158	24993910	24999600	LOC_Os04g42250	transferase family protein, putative, expressed
159	25001492	25006621	LOC_Os04g42260	protein phosphatase 2C, putative, expressed
160	25007808	25009995	LOC_Os04g42270	60S ribosomal protein L23A, putative, expressed
161	25011981	25015314	LOC_Os04g42280	CorA-like magnesium transporter protein, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
162	25017214	25020556	LOC_Os04g42290	methyladenine glycosylase, putative, expressed
163	25034970	25036582	LOC_Os04g42310	B4-BTB3 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with B4 subfamily conserved sequence, expressed
164	25038921	25045802	LOC_Os04g42320	AT hook motif family protein, expressed
165	25048428	25054483	LOC_Os04g42330	Spc97 / Spc98 family protein, putative, expressed
166	25059789	25061979	LOC_Os04g42350	heavy metal-associated domain containing protein, expressed
167	25076764	25079041	LOC_Os04g42380	ribosomal protein S17, putative, expressed
168	25082797	25089333	LOC_Os04g42399	retrotransposon protein, putative, Ty1-copia subclass, expressed
169	25102101	25107672	LOC_Os04g42420	nodulin, putative, expressed
170	25104771	25107672	LOC_Os04g42420	nodulin, putative, expressed
171	25111035	25115188	LOC_Os04g42430	EF hand family protein, putative, expressed
172	25123382	25125264	LOC_Os04g42460	G-patch domain containing protein, expressed
173	25126715	25134362	LOC_Os04g42470	regulatory subunit, putative, expressed
174	25128146	25134362	LOC_Os04g42470	regulatory subunit, putative, expressed
175	25140210	25145158	LOC_Os04g42480	receptor-like protein kinase At3g46290 precursor, putative, expressed
176	25145877	25149607	LOC_Os04g42490	DIE2/ALG10 family, putative, expressed
177	25150360	25152873	LOC_Os04g42500	trafficking protein particle complex subunit, putative, expressed
178	25165690	25170764	LOC_Os04g42520	phosphoribosyl transferase, putative, expressed
179	25182201	25186675	LOC_Os04g42570	AP2/EREBP transcription factor BABY BOOM, putative, expressed
180	25187851	25192745	LOC_Os04g42580	carboxyvinyl-carboxyphosphonate phosphorylmutase, putative, expressed
181	25191054	25192947	LOC_Os04g42590	expressed protein
182	25194025	25201272	LOC_Os04g42600	polyadenylate-binding protein, putative, expressed
183	25194790	25201272	LOC_Os04g42600	polyadenylate-binding protein, putative, expressed
184	25207809	25213918	LOC_Os04g42610	plant protein of unknown function DUF869 domain containing protein, expressed

Sl. No.	Start	Stop	locus	Annotation
185	25217850	25219732	LOC_Os04g42620	uncharacterized protein At4g06744 precursor, putative, expressed
186	25232433	25234689	LOC_Os04g42650	plant protein of unknown function domain containing protein, expressed
187	25244008	25246628	LOC_Os04g42670	OsFBL19 - F-box domain and LRR containing protein, expressed
188	25251409	25253149	LOC_Os04g42690	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed
189	25253589	25258424	LOC_Os04g42700	receptor-like protein kinase precursor, putative, expressed
190	25263750	25265626	LOC_Os04g42710	retrotransposon protein, putative, unclassified, expressed
191	25280907	25285098	LOC_Os04g42720	TMS membrane protein/tumour differentially expressed protein, putative, expressed
192	25287663	25293334	LOC_Os04g42730	retrotransposon protein, putative, unclassified
193	25297188	25299569	LOC_Os04g42740	serine/threonine-protein kinase receptor precursor, putative, expressed
194	25302993	25304831	LOC_Os04g42750	retrotransposon protein, putative, unclassified, expressed
195	25307143	25308653	LOC_Os04g42760	sialyltransferase family domain containing protein, expressed
196	25323642	25340031	LOC_Os04g42784	DNA mismatch repair protein, putative, expressed
197	25340375	25341989	LOC_Os04g42800	photosystem-II repair protein, putative, expressed
198	25342960	25344751	LOC_Os04g42810	transposon protein, putative, unclassified
199	25346878	25348319	LOC_Os04g42830	transferase family protein, putative, expressed
200	25349743	25361092	LOC_Os04g42840	HEAT repeat family protein, putative, expressed
201	25368373	25372115	LOC_Os04g42860	GDSL-like lipase/acylhydrolase, putative, expressed
202	25372496	25374580	LOC_Os04g42870	methyltransferase domain containing protein, putative, expressed
203	25374638	25376829	LOC_Os04g42880	WD domain, G-beta repeat domain containing protein, expressed
204	25396939	25399827	LOC_Os04g42930	OsGrx_C2.2 - glutaredoxin subgroup I, expressed
205	25404933	25407689	LOC_Os04g42950	MYB family transcription factor, putative, expressed
206	25417063	25418733	LOC_Os04g42960	Lung seven transmembrane receptor domain containing protein, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
207	25423949	25429277	LOC_Os04g42980	zinc finger family protein, putative, expressed
208	25435287	25438940	LOC_Os04g42990	suppressor of stem-loop protein 1, putative, expressed
209	25451638	25451961	LOC_Os04g43010	retrotransposon protein, putative, unclassified
210	25453449	25459806	LOC_Os04g43020	protein kinase, putative, expressed
211	25460247	25468576	LOC_Os04g43030	lipase class 3 family protein, putative, expressed
212	25492137	25493273	LOC_Os04g43060	enzyme of the cupin superfamily protein, putative, expressed
213	25500158	25502633	LOC_Os04g43070	ammonium transporter protein, putative, expressed
214	25535300	25541426	LOC_Os04g43140	DEAD-box ATP-dependent RNA helicase, putative, expressed
215	25543324	25548914	LOC_Os04g43150	nuclear transport factor 2, putative, expressed
216	25554735	25556460	LOC_Os04g43170	caleosin related protein, putative, expressed
217	25559011	25559448	LOC_Os04g43180	transposon protein, putative, unclassified, expressed
218	25563393	25565204	LOC_Os04g43200	caleosin related protein, putative, expressed
219	25567693	25571247	LOC_Os04g43210	transporter family protein, putative, expressed
220	25573548	25579413	LOC_Os04g43220	zinc finger, C3HC4 type domain containing protein, expressed
221	25584582	25585379	LOC_Os04g43240	retrotransposon protein, putative, unclassified, expressed
222	25586050	25590654	LOC_Os04g43250	retrotransposon protein, putative, unclassified, expressed
223	25591244	25594230	LOC_Os04g43260	retrotransposon protein, putative, unclassified, expressed
224	25598959	25606215	LOC_Os04g43270	WAX2, putative, expressed
225	25608130	25611089	LOC_Os04g43290	ARPC2B, putative, expressed
226	25611673	25615824	LOC_Os04g43300	BRCA1 C Terminus domain containing protein, expressed
227	25627113	25631440	LOC_Os04g43324	G-patch domain containing protein, putative, expressed
228	25631835	25636842	LOC_Os04g43340	disease resistance RPP13-like protein 1, putative, expressed
229	25640270	25644607	LOC_Os04g43360	Os4bglu14 - monolignol beta-glucoside homologue without catalytic acid/base, expressed

Sl. No.	Start	Stop	locus	Annotation
230	25646120	25646676	LOC_Os04g43370	pectinesterase, putative, expressed
231	25650132	25652646	LOC_Os04g43380	Os4bglu15 - GH1 gene fragment, expressed
232	25658402	25666730	LOC_Os04g43390	Os4bglu16 - monolignol beta-glucoside homologue, expressed
233	25672571	25674982	LOC_Os04g43400	Os4bglu17 - GH1 gene fragment, expressed
234	25681557	25693918	LOC_Os04g43410	Os4bglu18 - monolignol beta-glucoside homologue, expressed
235	25700861	25703587	LOC_Os04g43420	PTAC5, putative, expressed
236	25702074	25707426	LOC_Os04g43430	pentatricopeptide, putative, expressed
237	25703701	25707426	LOC_Os04g43430	pentatricopeptide, putative, expressed
238	25708299	25712533	LOC_Os04g43440	NB-ARC/LRR disease resistance protein, putative, expressed
239	25727283	25731803	LOC_Os04g43490	CK1_CaseinKinase_1.7 - CK1 includes the casein kinase 1 kinases, expressed
240	25737467	25743088	LOC_Os04g43500	transposon protein, putative, unclassified
241	25753700	25756577	LOC_Os04g43550	MazG nucleotide pyrophosphohydrolase domain containing protein, expressed
242	25773341	25776243	LOC_Os04g43560	no apical meristem protein, putative, expressed
243	25790014	25791567	LOC_Os04g43580	DUF640 domain containing protein, putative, expressed
244	25793153	25798426	LOC_Os04g43590	transposon protein, putative, CACTA, En/Spm sub-class
245	25799856	25802057	LOC_Os04g43600	transposon protein, putative, CACTA, En/Spm sub-class, expressed
246	25803356	25806887	LOC_Os04g43610	transposon protein, putative, CACTA, En/Spm sub-class, expressed
247	25816497	25816985	LOC_Os04g43630	retrotransposon protein, putative, unclassified
248	25822676	25825480	LOC_Os04g43640	hypothetical protein
249	25826665	25830703	LOC_Os04g43650	L-allo-threonine aldolase, putative, expressed
250	25843038	25844912	LOC_Os04g43680	MYB family transcription factor, putative, expressed
251	25855652	25860031	LOC_Os04g43690	DUF647 domain containing protein, putative, expressed
252	25860364	25865216	LOC_Os04g43700	glycosyl transferase 8 domain containing protein, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
253	25867807	25869189	LOC_Os04g43710	CAMK_CAMK_like.3 - CAMK includes calcium/calmodulin depedent protein kinases, expressed
254	25873036	25878414	LOC_Os04g43720	retrotransposon protein, putative, unclassified, expressed
255	25884745	25888285	LOC_Os04g43730	OsWAK51 - OsWAK receptor-like protein kinase, expressed
256	25896733	25897489	LOC_Os04g43740	OsSAUR18 - Auxin-responsive SAUR gene family member, expressed
257	25898097	25902359	LOC_Os04g43750	kinase, pfkB family, putative, expressed
258	25906849	25910074	LOC_Os04g43760	phenylalanine ammonia-lyase, putative, expressed
259	25920463	25921383	LOC_Os04g43780	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
260	25923951	25925448	LOC_Os04g43790	expressed protein
261	25926988	25929732	LOC_Os04g43800	phenylalanine ammonia-lyase, putative, expressed
262	26013418	26016187	LOC_Os04g43910	auxin response factor, putative, expressed
263	26036773	26041443	LOC_Os04g43922	exosome complex exonuclease rrp4, putative, expressed
264	26074682	26075869	LOC_Os04g43990	DUF584 domain containing protein, putative, expressed
265	26079566	26084308	LOC_Os04g44000	transposon protein, putative, CACTA, En/Spm sub-class, expressed
266	26084656	26086810	LOC_Os04g44010	transposon protein, putative, CACTA, En/Spm sub-class, expressed
267	26090648	26093773	LOC_Os04g44030	PPR repeat domain containing protein, putative, expressed
268	26099273	26101782	LOC_Os04g44060	aquaporin protein, putative, expressed
269	26114801	26118126	LOC_Os04g44110	hydrolase, putative, expressed
270	26118498	26118866	LOC_Os04g44120	collagen triple helix repeat, putative, expressed
271	26120333	26120839	LOC_Os04g44130	DEF12 - Defensin and Defensin-like DEFL family, expressed
272	26130707	26134356	LOC_Os04g44150	gibberellin 2-beta-dioxygenase 7, putative, expressed
273	26167892	26168538	LOC_Os04g44200	oxygen-evolving enhancer protein 3, chloroplast precursor, putative, expressed
274	26179449	26184228	LOC_Os04g44220	MIF4G domain containing protein, putative, expressed
275	26184978	26186908	LOC_Os04g44224	brain acid soluble protein 1, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
276	26199152	26203656	LOC_Os04g44230	cytokinin dehydrogenase precursor, putative, expressed
277	26206039	26207903	LOC_Os04g44240	cytokinin-O-glucosyltransferase 3, putative, expressed
278	26213702	26215406	LOC_Os04g44250	cytokinin-O-glucosyltransferase 3, putative, expressed
279	26218187	26218732	LOC_Os04g44260	thioredoxin reductase 2, putative, expressed
280	26227217	26228435	LOC_Os04g44280	OsRR5 type-A response regulator, expressed
281	26260450	26262268	LOC_Os04g44354	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed
282	26260664	26267392	LOC_Os04g44354	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed
283	26263781	26267430	LOC_Os04g44354	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed
284	26284657	26285880	LOC_Os04g44400	OsClp7 - Putative Clp protease homologue, expressed
285	26287757	26292362	LOC_Os04g44410	OsSCP25 - Putative Serine Carboxypeptidase homologue, expressed
286	26296166	26296639	LOC_Os04g44420	isochorismatase family protein, putative, expressed
287	26299432	26307749	LOC_Os04g44430	transporter, major facilitator family, putative, expressed
288	26310603	26311673	LOC_Os04g44440	TCP family transcription factor, putative, expressed

APPENDIX-IV: List of known function genes found in the genome region of RM 2584

Sl. No.	Start	Stop	locus	Annotation
1	7338826	7348077	LOC_Os08g12430	VERNALIZATION-INSENSITIVE, putative, expressed
2	7488653	7510517	LOC_Os08g12680	zinc finger domain, LSD1 subclass family protein, expressed
3	7539941	7551975	LOC_Os08g12740	disease resistance protein RGA3, putative, expressed
4	7545583	7551989	LOC_Os08g12740	disease resistance protein RGA3, putative, expressed
5	7553769	7558834	LOC_Os08g12750	serine/threonine-protein kinase HT1, putative, expressed
6	7559098	7563188	LOC_Os08g12760	YT521-B, putative, expressed
7	7568534	7574295	LOC_Os08g12780	chloroplast envelope membrane protein, putative, expressed
8	7571731	7574295	LOC_Os08g12780	chloroplast envelope membrane protein, putative, expressed
9	7576906	7577904	LOC_Os08g12790	cytokinin inducible protein, putative, expressed
10	7583446	7588419	LOC_Os08g12800	glucan endo-1,3-beta-glucosidase precursor, putative, expressed
11	7591562	7597012	LOC_Os08g12820	proteasome/cyclosome repeat containing protein, expressed
12	7605599	7610227	LOC_Os08g12830	cytidylyltransferase domain containing protein, expressed
13	7610695	7614807	LOC_Os08g12840	FabA-like domain containing protein, expressed
14	7616771	7620292	LOC_Os08g12850	pentatricopeptide, putative, expressed
15	7693822	7694865	LOC_Os08g12960	speckle-type POZ protein-like, putative, expressed
16	7735492	7737096	LOC_Os08g13020	BTB and MATH domain containing protein, putative, expressed
17	7982559	7985538	LOC_Os08g13420	S-domain receptor-like protein kinase, putative, expressed
18	8061312	8063491	LOC_Os08g13570	exo70 exocyst complex subunit family protein, putative, expressed
19	8153369	8155981	LOC_Os08g13690	60S ribosomal protein L7, putative, expressed
20	8216427	8219047	LOC_Os08g13780	U-box domain-containing protein, putative, expressed
21	8230111	8236739	LOC_Os08g13800	NB-ARC domain containing protein, expressed
22	8258575	8259595	LOC_Os08g13840	WRKY25, expressed
23	8282962	8285334	LOC_Os08g13870	S-locus lectin protein kinase family protein, putative, expressed
24	8327866	8329399	LOC_Os08g13920	glycosyl hydrolases family 16, putative, expressed

Sl. No.	Start	Stop	locus	Annotation
25	8342181	8344712	LOC_Os08g13930	mannose-1-phosphate guanyltransferase, putative, expressed
26	8355403	8360496	LOC_Os08g13950	transposon protein, putative, CACTA, En/Spm sub-class
27	8367120	8372818	LOC_Os08g13960	retrotransposon protein, putative, Ty1-copia subclass, expressed
28	8375647	8383493	LOC_Os08g13970	transposon protein, putative, unclassified, expressed
29	8384570	8385799	LOC_Os08g13980	glycosyl hydrolases family 16, putative, expressed
30	8389386	8393585	LOC_Os08g13990	pentatricopeptide, putative, expressed
31	8424284	8425291	LOC_Os08g14070	LYR motif containing protein, putative, expressed
32	8464913	8469510	LOC_Os08g14180	flavonol sulfotransferase, putative, expressed
33	8472521	8475589	LOC_Os08g14190	flavonol sulfotransferase, putative, expressed
34	8488370	8489922	LOC_Os08g14200	glycosyl hydrolases family 16, putative, expressed
35	8493861	8495316	LOC_Os08g14210	glycosyl hydrolases family 16, putative, expressed
36	8508449	8516435	LOC_Os08g14230	villin, putative, expressed
37	8582061	8584105	LOC_Os08g14320	zinc finger, C3HC4 type domain containing protein, expressed
38	8589037	8594478	LOC_Os08g14330	aldose 1-epimerase, putative, expressed
39	8590592	8594478	LOC_Os08g14330	aldose 1-epimerase, putative, expressed
40	8668896	8672887	LOC_Os08g14440	uridylyltransferase-related, putative, expressed
41	8677955	8681990	LOC_Os08g14450	RNA polymerase sigma factor, putative, expressed
42	8703799	8708988	LOC_Os08g14490	dehydrogenase-phosphopantetheinyltransferase, putative, expressed
43	8760073	8765208	LOC_Os08g14570	NADPH reductase, putative, expressed
44	8767726	8771583	LOC_Os08g14580	haloacid dehalogenase-like hydrolase domain-containing protein 1A, putative, expressed
45	8784440	8793976	LOC_Os08g14610	DRD1, putative, expressed
46	8802818	8806882	LOC_Os08g14640	syntaxin 6, N-terminal domain containing protein, expressed
47	8804473	8806882	LOC_Os08g14640	syntaxin 6, N-terminal domain containing protein, expressed
48	8811774	8816699	LOC_Os08g14660	SET domain containing protein, expressed

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Analysis of Trait Introgressed Back Cross Progenies to Identify Superior Lines for Aerobic Cultivation in Rice (*Oryza sativa* L.)

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Abstract

Markers assisted back cross ($DCBC_3F_2$, Double Cross Back Cross) progenies introgressing root traits and WUE,were screened for yield performance under semi-irrigated aerobic cultivation.Based on the initial vigour 100 plants were selected for phenotyping for yield related traits. Effective surrogates such as leaf temperature for root traits, SCMR for WUEwere used for the identification of promising trait introgressed lines. The trait introgressed plants showed better yield than the recurrent parent, IR-64. Based on the variability in trait introgression, a few highly promising lines were selected using 16 traitsassociated polymorphic SSR markers. The molecular analysis suggests that the selected transgressiveseggregantswere having allelesintrogressed from the donor parents of the respective traits.

Keywords : Rice, introgression, SSR marker, superior, root, WUE.

Rice (*Oryza sativa* L.) is the major staple food for more than half of the world population. The consumption is projected to increase to 490 million tons in 2020 and to about 650 million tons by 2050 globally (Annon., 2016). These environmental crises have been the factors that decrease rice production around the world (IPCC, 2013). Drought is one of the major factors that target the rice yield (Fischer *et al*., 2012). Therefore, the challenge before the plant scientists is to increase the yield by minimizing the impacts of climate change through the adoption of modern molecular breeding approaches.

Plants have adopted several drought tolerance mechanisms to overcome drought effects ranging from cellular level to whole plant level. It maintains growth by maintaining tissue water relations and positive carbon gain by mining and providing water from deeper soil profiles (Farooq *et al.,* 2009). Water content of the leaf (turgor) and carbon gain under water limited conditions strongly dictates crop productivity. Turgidity of the leaf is strongly associated with water mining properties (Deep root, cooler canopy) and water use efficiency of the plant. It has been proven that, if these traits are pyramided together, the crop productivity increases (Raju *et al.,* 2014). This can be achieved by bringing together various drought adaptive traits with reasonably high acquired tolerance traits also referred to as cellular level tolerance (CLT) on to an elite genetic background.Although a conventional breeding approach to combine drought adaptive traits such as root and WUE led to the development of a significantly higher yielding cultivar namely KMP-175 (Sheshshayee *et al.,* 2011), introgressing such physiological traits are extremely difficult through the conventional breeding programmes. Therefore, a more focused marker assisted breeding strategy needs to be adopted.

Material and Methods

The major goal of the present study was to analyse backcross progenies and identify the superior lines with improved WUE and root traits for aerobic cultivation. Plant vigour is one of the main characters which are selected under semi-irrigated condition. Based on the initial vigour, 100 $DCBC_3F_2$ plants (the scheme for the development of $DCBC_3F_2$ is given in Fig. 1.) were selected for phenotyping. The parameters measured were chlorophyll content (SCMR value), leaf temperature (surrogate for root traits), tiller number, yield per plant (YPP) and total dry matter (TDM).

Chlorophyll content was measured using the SPAD meter (Soil Plant Analysis Development). The instrument measures the light attenuation at 430 nm (the peak wavelength for chlorophyll a and b absorption) and that at 750 nm (near infrared) with no

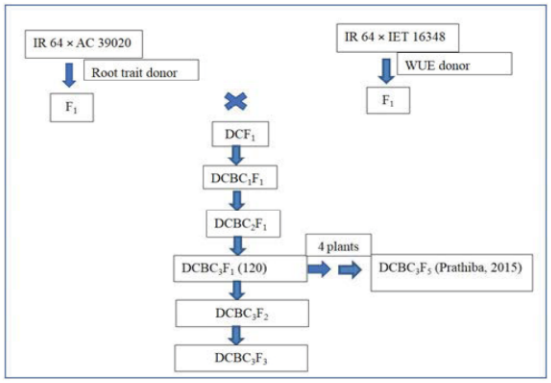

Fig. 1: The scheme for development of Marker Assisted Back Cross progenies

transmittance. The unit less value measured by the chlorophyll meter is termed as SCMR (SPAD Chlorophyll Meter Reading) which is a good estimate of chlorophyll content and therefore N content. Three readings were recorded on each leaves using SPAD chlorophyll meter. Necessary care was taken to avoid the interference of the midrib and sensor fully cover the leaf surface. Leaf temperature of the plants was measured using infra-red gun and expressed in degree celsius. Observations recorded just before irrigation twice during the crop cycle (mean values ofobservations were used for analysis). Infra-red gun was held pointing towards the leaf surface and the measurements were made at the midday when there was bright sunlight. Tiller number was computed by counting the number of productive tillers per plant. Grain yield per plant (YPP) was measured at physiological maturity by taking weight of all the grains from the plant. Total dry matter (TDM) the biomass accumulated during the experimental period was computed by summing up leaf dry weight, stem dry weights and yield.

10 $DCBC_3F_2$ plants were selected based on yield for molecular analysis. Genomic DNA was extracted from the leaves of 4 weeks old plants of the donor (AC-39020 for root and IET-16348 for WUE) and recipient parents (IR-64) and the selected progenies by using the CTAB (CetylTrimethyl Ammonium Bromide) method. Using Biospec-nano (Spectrophotometer for life science), advanced automated DNA quantifier, the ratios between 260 nm and 280 nm was estimated and used to estimate the DNA purity. A ratio of 1.8 to 2.0 for pure DNA samples is standard. PCR was performed in 15µl reactions containing 25ng of DNA template, 1.5µl Taq buffer (1X), 1.5µl of dNTPs (3.0mM), 0.3µl of $MgCl_2$ (2mM), 1.5 µL (5 pmole. μL^{-1}) each forward primer and reverse SSR primers, 1U of Taq polymerase and 7.40 µL of sterile water. Based on polymorphism among the three parents, 16 associated SSR markers were selected from previous study (Prathiba, 2015) as foreground markers for trait introgression. The 16 SSR markers (12 for roots and 4 for $\Delta^{13}C$) were used to screen the selected progenies along with the parents in the present study (Table I).

TABLE I
List of markers used for foreground selection

Trait	Trait component	Marker	Chr. No.	Position on chromosome (cM)
Root	RLD	RM80	8	103.7
	RWT	RM2584	8	45.8
	RLD	RM1388	4	77.9
	RLD	RM262	2	81.1
	R/S	RM239	10	25.2
	RV	RM3825	1	143.7
	RV	RM16	3	131.5
	RL	RM3276	4	102.4
	RV	RM247	12	32.3
	RLD	RM167	4	37.5
	RV	RM4455	10	21.8
	/S	RM71	2	49.8
Ä13C	Ä13C	RM493	1	79.9
	Ä13C	RM586	6	7.4
	Ä13C	RM149	8	122.1
	Ä13C	RM131	4	148.8

A total of 800 $DCBC_3F_2$ plants were screened under semi irrigated aerobic conditions. Based on the early seedling vigour 100 $DCBC_3F_2$plants were selected for phenotyping.The parameters considered for phenotyping were SCMR value (chlorophyll content), canopy temperature (surrogate parameter for root traits), number of tillers, yield per plant and

total dry matter. Further, advancing and characterisation of TILs depends on the identification of appropriate promising lines. Therefore, trait introgressed lines were selected based on marker data (foreground selection) and phenotyping data (grain yield and total dry matter).

Results and Discussion

The SCMR value of 100 plants varied from 31.7 to 49.5 with a mean of 40.96. Tiller number of IR-64, AC-39020 and IET-16348 was 20, 11 and 25 per plant, respectively. The tiller number of 100 plants ranged from 9 to 37 per plant with a mean of 23 per plant. Yield per plant of IR-64, AC-39020 and IET-16348 was 20.45, 17.25 and 19.15 g pl^{-1}, respectively. Yield of 100 plants varied from 10 to 23 g pl^{-1} with a mean of 18.92. Total dry matter of IR-64, AC-39020 and IET-16348 was 51.05, 83.45 and 58.65 g pl^{-1}, respectively. The total dry matter of 100 plants ranged from 15 to 79 g pl^{-1} with a mean of 55.84 g pl^{-1}. Leaf temperature of IR-64, AC-39020 and IET-16348 was 35.7, 31.1 and 33.3 ^{0}C, respectively. Leaf temperature of 100 plants ranged from 30.90 to 35.90 ^{0}C with a mean of 33.91^{0}C.

Based on the yield per plant 10 lines were selected which were having lower leaf temperature, as leaf temperature is surrogate for root traits, and higher total dry matter. The selected lines were having better drought adaptive traits as these lines are having more SCMR value, cooler leaf and also enhanced yield associated parameters than the recurrent parent IR-64 and other lines of the same population (Fig. 2).

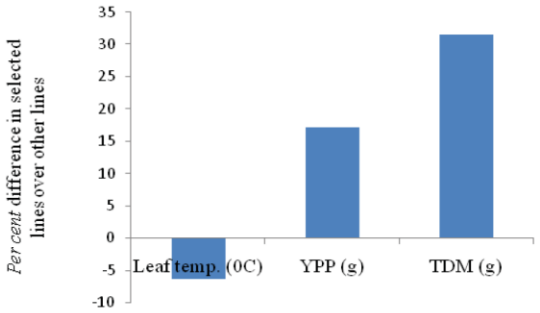

Fig. 2: Per cent difference in traits of the selected lines in comparison with non-selected lines

SCMR values varied from 34.7 to 46.7, leaf temperature 30.9 to 32.9^{0}C, TDM 64.90 to 78.65 g and YPP 20.95 to 23.05 g (Table II). It implies that the selected introgressed lines have better root system and cellular level tolerance mechanisms due to which these lines were performed better under semi-irrigated aerobic cultivation and shows the improved traits values. The

Table II

Comparison of selected trait introgressed lines with the parents for biomass and yield related parameters in DCBC$_3$F$_2$ generation

Plant No.	SCMR	Leaf temp. (°C)	Panicle per plant (#)	TDM (g)	YPP (g)
IR-64	39.9	35.7	20	51.05	20.45
AC-39020	37.6	31.1	11	83.45	17.25
IET-16348	38.5	33.3	25	58.65	19.15
37	46.7	31.6	26	64.90	21.05
38	43.9	31.8	24	75.35	20.95
40	41.9	30.9	37	69.05	22.30
45	41.7	32.2	33	72.40	22.05
49	43.7	31.3	32	68.85	21.70
61	42.5	32.7	32	74.65	20.95
65	44.9	31.8	29	73.55	23.05
69	34.7	31.8	28	69.55	22.55
72	41.5	32.5	25	78.65	21.60
75	34.9	32.9	21	65.35	21.75

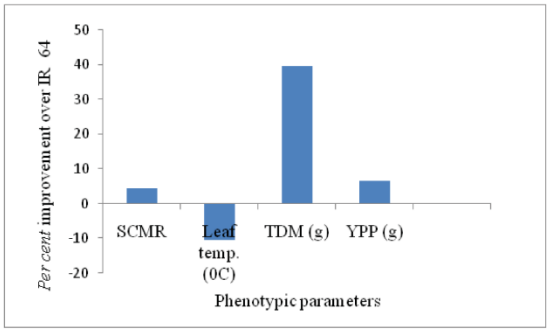

Fig. 3: Per cent improvement of traits for selected lines over the recurrent parent

parental lines differed significantly for all the traits specially leaf temperature and total dry matter.

The selected lines (mean of the 10 lines) show 10 per cent lower leaf temperature than the recurrent parent IR-64 (Fig. 3). This signifies that the selected lines were having improved root traits, due to which these lines were able to explore more water from the deeper layer of soil and hence maintain lower leaf temperatures by transpiring more. Ability to extract water from deeper soil profiles is an extremely important determinant of crop growth under water limited conditions. Identification of TILs with good root system under semi-irrigated aerobic condition holds the key for success in crop improvement programmes.

Further, these lines show 40 per cent improvement over recurrent parent for total dry matter. It implies that these lines were able to gain more carbon than IR-64 either by means of more water use efficiency or by keeping stomata opens for longer time or combination of both. There was significant improvement for SCMR value of the selected TILs over recurrent parent. This suggests that the TILs were able to maintain better chlorophyll content under aerobic conditions, which is also needed for more vigorous growth. Hence, the phenotypic characterization proved that the selected lines were having drought adaptive traits.

TABLE III

Foreground marker distribution of the selected $DCBC_3F_2$ lines

Trait	Marker	45	61	49	37	40	65	75	69	38	72
Root	RM3825	-	✓	✓	-	-	-	-	✓	-	-
Root	RM262	✓	-	-	-	✓	-	-	✓	✓	✓
Root	RM71	✓	✓	-	✓	✓	✓	-	-	-	-
Root	RM16	-	-	✓	✓	-	✓	✓	-	-	-
Root	RM1388	-	✓	-	✓	✓	-	-	-	-	-
Root	RM3276	✓	✓	-	-	✓	✓	✓	-	-	-
Root	RM167	✓	-	-	✓	✓	-	-	✓	✓	-
Root	RM80	✓	-	✓	-	-	✓	✓	-	-	✓
Root	RM2584	-	-	✓	✓	✓	✓	✓	-	-	-
Root	RM4455	-	✓	-	-	-	-	-	-	✓	-
Root	RM239	✓	-	-	✓	-	✓	✓	✓	-	-
Root	RM247	✓	✓	✓	-	✓	-	-	-	-	-
$\Delta^{13}C$	RM493	✓	-	✓	✓	-	-	-	-	✓	-
$\Delta^{13}C$	RM131	-	✓	✓	-	-	-	-	-	-	✓
$\Delta^{13}C$	RM586	✓	✓	-	-	-	✓	✓	✓	-	-
$\Delta^{13}C$	RM149	-	✓	✓	-	-	-	✓	-	-	✓
	Total (#)	**9**	**9**	**8**	**7**	**7**	**7**	**7**	**5**	**4**	**4**

The marker distribution indicated that the number of foreground markers varied from four alleles to nine alleles per plant. The foreground marker distribution of the selected 10 lines is given in Table III. The plants with higher foreground as well as higher yield and cooler leaf temperature were selected. It suggests that the lines with more proportion of introgressed traits / markers performed better in terms of yield performance under semi-irrigated aerobic condition.

The marker distribution indicated that the number of foreground markers varied from four alleles to nine alleles per plant. The foreground marker distribution of the 10 lines is given in Table III. The plants with higher foreground as well as higher yield were selected.

Molecular characterization leads to the identification of plants with higher foreground markers. Physiological parameters support the results of molecular characterization. Based on molecular and phenotyping data, 10 TILs with improved drought tolerance were identified.

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Research Article

Physiological Characterization of Trait Introgressed Lines of Rice (*Oryza sativa* L.) for Drought Stress Tolerance

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ABSTRACT

Twenty-five lines of rice which were introgressed for root traits and water use efficiency (WUE) were physiologically characterized for drought adaptive traits in the present study. These lines along with the parents were screened for yield and other trait performance under semi-irrigated aerobic cultivation. Leaf temperature was selected as surrogate for root traits and SCMR for WUE. Apart from these traits, yield and yield associated traits, relative water content (RWC), membrane stability index and chlorophyll content were measured. The lower leaf temperature of trait introgressed lines than IR-64 suggests lines have better root biomass which enabled them to maintain its temperature. The yield performance of trait introgressed lines were better than recurrent parent, IR-64 under semi-irrigated aerobic cultivation. It indicates that the introgression of the respective traits onto an elite background, IR-64 improved the efficacy of the introgressed lines under water limited condition. Comparative analysis of introgressed lines for drought adaptive traits suggested G 25, G 4, G 12 and G 18 performed better than other lines. Therefore, these lines introgreseed for drought tolerance traits could be utilized for other breeding programs for further improvement in drought tolerance in rice for the development of elite genotypes.

Keywords: *Rice, Drought, Root, WUE, RWC.*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food crops of Asia. It belongs to Poaceae family with chromosome number 2n=24. Out of the 25 species of genus *Oryza* known so far, only 2 species are being cultivated *i.e., Oryza sativa* and *Oryza glaberrima.* On view of its importance as one of the staple foods, its enhanced production is needed to feed the world's growing population. Rice is water intensive crop (Bouman, 2007). Due to shortage of fresh water, various technologies have been developed to grow rice in considerable less amount of water. Among the various technologies semi-irrigated aerobic approach had shown great potential to save water with a significant compromise in yield (Bouman et al., 2000).

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Therefore, to avoid such compromise, one needs to develop or breed for varieties which grow well under aerobic condition without penalty in yield.

Several attempts have been made so far, but plant breeders selected yield as a trait under water limited conditions. Due to high G X E interaction and narrow variability for yield among the improved lines, further selection for yield was not effective (Araus et al., 2008, & Reynolds & Tuberosa, 2008). Targeting its component traits led to a great deal of success (Araus et al., 2008). Genetic enhancement of rice by component trait based breeding is one of the most appropriate strategies to tackle drought. Among the various component traits, water acquisition by roots, WUE, water conservation through epicuticle wax load and cellular level tolerance (CLT) proved to have great impact during water limited conditions. Therefore, introgression of root and WUE traits onto an elite background appears to improve the yield under semi irrigated aerobic conditions.

Developing aerobic worthy rice varieties with yield potential will help in maximizing rice production in the challenging environment of water scarcity. Mean yield and relative yield performance under well-watered and water limited conditions are the most widely used criteria for selecting genotypes for water limited environments. The ability of crop cultivars to perform reasonably well in water limited condition is paramount for stability of production. The combination of high yield stability and high relative yield under water limited condition has been proposed as useful selection criteria for characterizing genotypic performance under varying degrees of water stress.

MATERIALS AND METHODS

The experimental material compromised of 25 trait introgressed lines (the scheme for the development of trait introgressed lines is given in Fig. A) and the parental lines (AC-39020 (Root donor), IET-16348 (WUE donor) and IR-64 (recurrent parent). These materials were raised in blocks of 1m*1m size with three replications of blocks in Randomized Completely Block Design (RCBD) at Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore during kharif 2017. Irrigation was provided alternatively by means of surface irrigation. Cultural operations such as protection measurements, accurate nutrient supply by fertilizer application were followed as per the recommendation. All necessary measures were taken to ensure the development of healthy plants.

The major goal of the present study was to physiologically characterize these lines and identify the superior lines with improved WUE and root traits. The parameters selected to meet the objective were chlorophyll content (SCMR value) (Surrogate for WUE), Relative water content (RWC), cell membrane stability (CMS), leaf temperature (surrogate for root traits) (LT), spikelet fertility (SF), yield per plant (YPP) and total dry matter (TDM). Nitrogen/Chlorophyll content was measured using the SPAD (Soil Plant Analysis Development) meter. This instrument measures the light attenuation at 430 nm (peak wavelength for chlorophyll a and b absorption) and at 750 nm (near infrared) with no transmittance. The value measured by chlorophyll meter is termed as SCMR (SPAD Chlorophyll Meter Reading) which is a unit less parameter. Three readings were recorded on each leaves using SPAD meter. Care was taken to avoid the interference of midrib and sensor should fully cover the leaf surface. Relative water content (RWC) is an important indicator of leaf water status. The leaf discs from the lines were collected separately and the fresh weight was measured and recorded. Leaf discs were floated on distilled water for 5 hours to attain full turgidity. Turgid weight was taken when the leaf discs were fully turgid. Later, leaf discs were dried in hot air oven at 70 °C to attain a constant weight and dry weight was recorded. RWC was calculated by equation as given by Barrs et al. 1962.

RWC (%) = {(FW-DW)/ (TW-DW)} X100

where, FW- Fresh weight, DW- Dry weight, TW- Turgid weight

Cell membrane stability index/ membrane stability index (MSI) was calculated as protocol given by Sairam et al. (2002) as follows:

$$MSI= [1- (EC1 / EC2)] \times 100$$

Leaf temperature (surrogate for root traits) was measured using infra-red gun and expressed in degree celsius. Spikelet fertility was calculated by considering ratio of number of filled spikelets per panicle over total number of spikelets per panicle and expressed in percentage. Grain yield per plant (YPP) was measured at physiological maturity stage by taking weight of all the grains from one plant. Total dry matter (TDM) the biomass accumulated during the experimental period was computed by summing up leaf dry weight, stem dry weights and yield. The data were statistically analyzed by XLSTAT software. The means of trait introgressed lines were compared by Least significance difference (LSD) at 5 % level of significance.

RESULTS AND DISCUSSION

The main goal of the study was to phenotype 25 $DCBC_3F_6$ lines for drought adaptive traits along with the parents under semi-irrigated aerobic condition. Phenotypic data on various drought adaptive traits like SCMR (a surrogate for water use efficiency and chlorophyll content), leaf temperature (LT), yield attributes, spikelet fertility. relative water content and membrane stability index were recorded for each line along with the parents under semi-irrigated aerobic condition. The SCMR value of lines varied from 41.40 to 48.5 with a mean of 44.64. Yield per plant of IR-64, AC-39020 and IET-16348 was 15.43, 12.54 and 13.28 g pl-1, respectively. Yield of introgressed lines varied from 18.26 to 24.66 g pl-1. Total dry matter of IR-64, AC-39020 and IET-16348 was 65.10, 81.73 and 59.62 g pl-1, respectively. However, total dry matter of introgressed lines ranged from 56.72 to 72.6 g pl-1with a mean of 65.10 g pl-1. The yield attributes which determine the output of grain viz., spikelet fertility also differed considerably among the lines and parents. The spikelet fertility percentage of lines varied from 80.71 to 94.54 *per cent* with a mean of 86.37 *per cent*. However, IR-64 had 75.65 % spikelet fertility. Relative water content (RWC) is the suitable measure of plant water status in terms of the physiological consequence of cellular water deficit. Relative water content of the tissue is primary factor affected under moisture stress. Relative water content of IR-64 and TILs differ significantly. RWC of lines varied from 85.24 to 89.92 *per cent* with a mean of 88.18 *per cent*. RWC value for IR-64 was 81.04 *per cent*. Leaf temperature (LT) of lines varied from 28.5 to 29.5 degree celsius with a mean of 28.98 degree celsius. LT value for IR-64 was 31.2 degree celsius. MSI also differed significantly between lines and IR-64.

Fig. B shows percent improvement of traits of trait introgressed lines over IR-64. IR-64 is recurrent parent, therefore, the traits has been compared with IR-64. Yield per plant for trait introgressed lines was 35.82, TDM (43.33), SF (14.17), RWC (8.81), SCMR (9.04), MSI (10.35) *per cent* improved than IR-64. Leaf temperature for introgressed lines was reduced to 7.10 *per cent* than IR-64. *Per cent* improvement over IR-64 for the traits like SCMR value, yield, leaf temperature and cell membrane stability signifies the importance of trait based breeding. Leaf temperature improvement over IR-64 suggests root traits has been improved for introgressed lines.

Drought stress/ Water limited condition suppresses leaf expansion and photosynthesis (Kramer & Boyer, 1995) and reduces photosynthetic rates and leaf area due to early senescence (Nooden, 1988). Chlorophyll pigment dictates photosynthetic capability of genotypes and generally used to quantify leaf senescence. The reduction in chlorophyll content reduces the carbon fixation which ultimately affects the

photosynthesis of genotype. Plasma membranes are one of the primary sites of water stress and MSI measure the electrolyte leakage due to cell membrane injury. Electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of drought tolerant plants. All of these factors are responsible for a reduction in dry matter accumulation and grain yield under drought. The drought tolerant variety expected to perform better under water limited condition by developing these traits. Higher RWC and a lower electrolyte leakage can be used as markers of membrane integrity under drought stress condition.

Comparative study of trait introgressed lines for physiological traits under aerobic condition had identified superior lines based on ranking of physiological traits. The trait introgressed lines G 25, G 4, G 12 and G 18 scored higher ranks followed by G 3, G6, G 13, G 114, G 15, G 21 and G 22 based on performance ranks (Table 1). Therefore, trait introgressed lines G 25, G 4, G 12 and G 18 identified for drought tolerance in the present study based on comparative study which could be used for further improvement in rice for drought tolerance in future breeding programme.

FIGURES

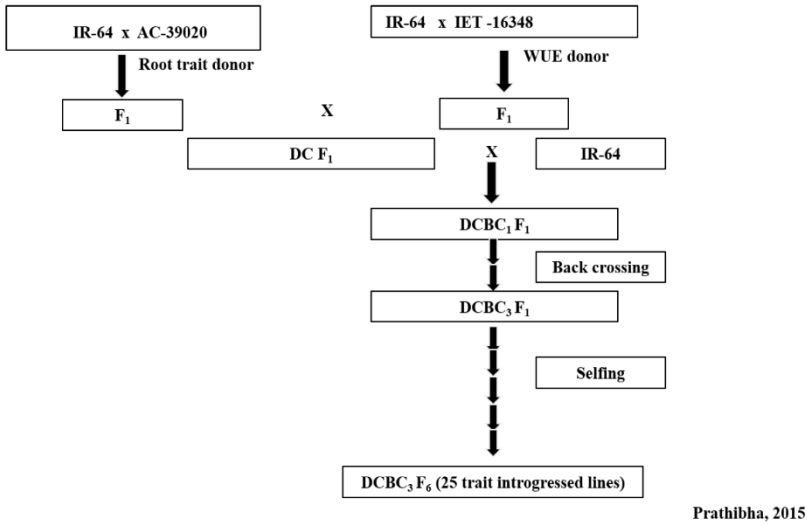
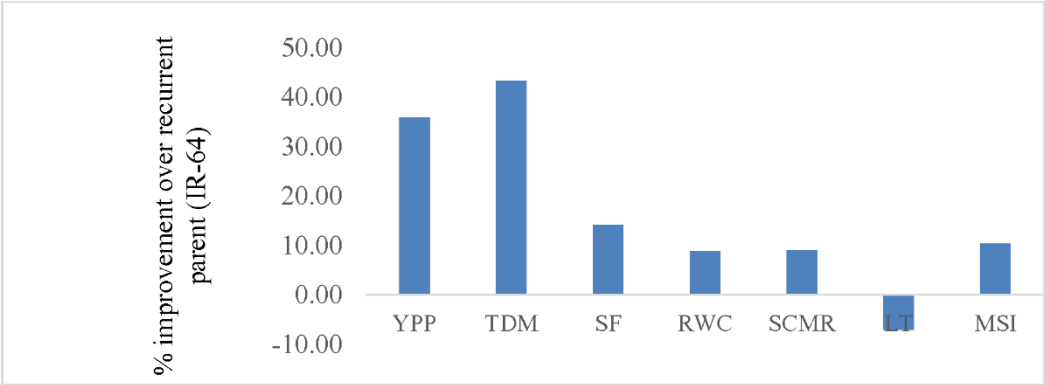


Fig. (A) The scheme for development of 25 Trait Introgressed Line



YPP-Yield per plant; TDM-Total Dry Matter; SF-Spikelet fertility; RWC-Relative Water Content; SCMR-SPAD value; LT-Leaf Temperature; MSI-Membrane Stability Index

Fig. (B) *Per cent* improvement of traits in trait introgressed lines over recurrent parent (IR-64)

Table 1: Comparative study of ranks of trait introgressed lines of rice based on physiological performance under aerobic conditions as compare to IR-64

% I- Per cent Improvement

Lines	RWC		SCMR		CMS		LT		SF		YPP		TDM		Repeated ranks					Total
	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	1	2	3	4	5	
G 1	10.51	3	12.81	8	8.61	20	-6.41	17	89.67	6	42.06	7	25.74	23			1			1
G 2	9.48	11	7.64	17	10.6	6	-7.69	10	91.57	4	40.44	10	33.47	21				1		1
G 3	10.25	5	5.67	20	11.3	1	-6.73	16	88.62	8	20.29	24	25.69	24	1				1	2
G 4	8.00	18	19.46	1	10.3	7	-5.77	21	92.89	3	38.43	12	54.45	5	1		1		1	3
G 5	9.07	14	2.22	24	10.7	3	-6.41	18	83.56	19	26.31	21	40.31	16			1			1
G 6	9.87	8	11.08	10	9.46	13	-8.65	2	85.22	13	59.82	1	44.76	11	1	1				2
G 7	7.90	19	6.65	19	8.37	21	-8.01	7	83.97	18	45.69	4	41.30	15				1		1
G 8	7.60	20	8.87	15	8.23	24	-7.69	9	85.12	14	44.98	6	24.88	25						0
G 9	10.25	4	13.55	7	9.24	15	-8.01	8	84.76	15	35.71	13	36.06	20				1		1
G 10	9.37	12	4.68	23	8.31	22	-8.33	6	81.50	22	34.48	14	39.08	18						0
G 11	9.83	10	10.10	14	9.15	18	-7.37	11	84.23	17	23.14	22	31.66	22						0
G 12	9.16	13	5.42	22	10.6	5	-6.73	15	90.64	5	27.74	18	59.11	2		1			2	3
G 13	10.70	2	7.64	16	9.21	16	-7.37	12	88.17	9	51.98	2	53.35	6		2				2
G 14	9.86	9	5.67	21	7.17	25	-8.33	4	93.21	2	21.84	23	53.04	7		1		1		2
G 15	9.01	16	1.97	25	9.05	19	-8.65	1	94.52	1	18.34	25	42.51	13	2					2
G 16	5.18	25	15.02	4	9.39	14	-5.77	20	89.35	7	40.51	9	39.63	17				1		1
G 17	6.19	23	11.08	11	10	9	-5.45	25	86.34	11	29.94	17	42.12	14						0
G 18	10.96	1	10.34	12	8.28	23	-8.33	5	85.59	12	50.36	3	46.48	9	1		1		1	3
G 19	10.02	7	13.79	5	9.72	11	-5.45	24	84.33	16	26.51	20	43.57	12					1	1
G 20	8.64	17	7.14	18	9.2	17	-5.77	19	82.22	21	38.95	11	51.70	8						0
G 21	5.45	24	13.79	6	10.6	4	-7.37	14	87.45	10	41.35	8	59.84	1	1			1		2
G 22	7.10	21	11.82	9	10.9	2	-5.77	22	80.71	25	30.59	16	57.75	3		1	1			2
G 23	9.06	15	16.26	2	9.62	12	-5.45	23	83.41	20	27.41	19	44.85	10		1				1
G 24	10.13	6	10.34	13	9.94	10	-7.37	13	80.77	24	33.12	15	37.14	19						0
G 25	6.69	22	15.52	3	10.1	8	-8.65	3	81.34	23	45.50	5	54.82	4			2	1	1	4

CONCLUSION

In the present study G25, G 4, G 12 and G18 performed better than the other introgressed lines and IR-64 under aerobic condition. These lines could be used for further breeding programmes or released as variety after field trials in drought prone areas.

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