Identification of Genotypic Similarities Having QTLS for Drought Tolerance by Using SSR Markers

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ABSTRACT

Rice is one of the most important crops, providing staple food for about half population of the world. Rice production must be increased 60% so as to meet for the contention by the year 2025.Rice belongs to the ***genus Oryza*** *and has* ***two cultivated*** *and* ***22 wild species.*** *The cultivated species are Oryza sativa and Oryza glaberrima. Oryza sativa is grown all over the world while Oryza glaberrima has been cultivated in west Africa for the last ~3500 years. Rice is the principal food for over half of the production of the world. Drought is the most important abiotic stress limiting rice yields in almost all major ecosystems where it is presently grown. Drought is a critical abiotic stress that severely restrict crop production, under drought conditions plant accumulate reactive oxygen species.**Abiotic stress** is defined as the negative impact of non-living factors on the living organisms in a specific environment. The region within genome that contains gene associated with a particular trait are known as quantitative trait loci (QTLs).Different SSR markers are associated with different QTLs for drought tolerant.RM 3825 is associated with QTL $_{DTY\ 1.1}$,RM 279 is associated with QTL $_{DTY\ 2.2}$. Microsatellites are simple repeated motifs consisting of 1 to 6 base pairs, and they can be found in both coding and non-coding regions. The mutation rate of this type of genetic marker has been estimated to be between 10^{-2} and 10^{-4} per generation. The primary advantage of microsatellites as genetic markers is that they are inherited in a Mendelian fashion as co dominant markers. High polymorphism rates, high abundance and a broad distribution throughout the genome have made microsatellites one of the most popular genetic markers for use in plant breeding programs. Microsatellites are also known as simple sequence repeats (SSR), and they are typically composed of 1–6 nucleotide repeats[16].Recent advances in rice genomics have now made it possible to identify and map a number of genes through linkage to existing DNA markers. The ideal type of genetic marker should be highly polymorphic, show*
Co-dominant inheritance and be evenly distributed throughout the genome. A number of factors should be considered when choosing between the various molecular markers[16]:
(a) Marker system availability.
(b) Complexity of the technique and time investment.
(c) Estimated polymorphism levels within the study population.
(d) Quantity and quality of available DNA available.
(e) Transferability between laboratories, populations, pedigrees and species.
(f) The size and structure of the population to be studied.
(g) Availability of skilled workers and equipment
(h) Cost per data-point and funding availability.
During this experiment we got some of rice varieties from CRURRS-CRRI, Hazaribagh, Jharkhand. In which few of the samples having known QTL while the few of the samples which has unknown QTL. These all samples are known by this experiment.

I INTRODUCTION

Rice is one of the most important crops, providing staple food for about half population of the world. Rice production must be increased 60% so as to meet for the contention by the year 2025. Rice belongs to the **genus Oryza** and has **two cultivated** and **22 wild species.** The cultivated species are Oryza sativa and Oryza glaberrima. Oryza sativa is grown all over the world while Oryza glaberrima has been cultivated in west Africa for the last ~3500 years. Rice is the principal food for over half of the production of the world. With a compact genome spanning approximately 430 MB pairs, an extensive genetic map (Harushima et. Al. 1998) and established synteny with other cereal crops (Gale & Devos 1998), the cultivated rice species Oryza sativa represents a model for cereal as well as other monocot plants. Drought is one of the most important constraints in crop resulting in large yield losses and limiting the average yield increase. Rice is central to the lives of billions of people around the world. Rice provides 21% of global human per capita energy and 15% of per capital protein. Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake. Asia accounts for over 90% of the world's production of rice, with China, India and Indonesia producing the most.

Soil moisture deficit that leads to reduction in growth, development and yield of crops.

Drought tolerance mechanism is genetically controlled and genes or QTL responsible for drought tolerance have been discovered in several crops which opens avenue for molecular breeding for drought tolerance.

The region within genome that contains gene associated with a particular trait are known as quantitative trait loci (QTLs).Several QTLs were identified at IRRI and breeding programmes at Hazaribagh focused on the transfer of these QTLs to productive background, in collaboration with IRRI. Molecular tools facilitate the identification and genomic locations of genes controlling traits related to drought tolerance using quantitative trait loci (QTL) analysis.

Agriculture is the backbone of Indian economy and rice crops undoubtedly occupies a pivotal position in Indian agriculture.

Rice is the important crop of India occupying 23.3% of gross cropped area of the country and contribution 43% of the total food grain production. Rice is cultivated under diverse agro climate conditions, as its cultivation extends from 8-35°C latitude and 0 to 3000 m altitude. The productivity as well as pests and disease in different ecosystems are likely to be influenced by climate variability and climate change. it is necessary to understand the basics of the climate change and its projections .The effects of climate change on productivity and pests/disease of crops in general and rice in particular.

India being mainly an agricultural country the economy and further its growth purely depends on the vagaries of the weather and in particular the extreme weather events. In this paper present a factual and a brief review of the extreme weather events that occurred in India during the last 100 years (1991-2004).**Drought also occurs in this year 2016**, almost 70% of Indian states. The table show below is the table of temperature. The socio-economic impacts of the extreme weather events such as floods, droughts, cyclones, hail storm, thunderstorm, heat and cold waves have been increasing due to large growth of population and its migration towards urban areas which has led to greater vulnerability. In recent years as per WMO review global losses from such extreme weather events is about US $ 50-100 billion annually with loss of life of about 2,50,000. Thus, greater efforts are needed to improve the forecast skill and use these better forecasts in disaster management.

Table:-1
(in 2016),Different temperatures that cause drought in India, Red shows high temperatures.

Lucknow	47°C
Delhi	47°C
Agra	45°C
Nagpur	49°C
Kota	48°C
Hyderabad	45°C
Pune	42°C
Ahemdabad	46°c
Mumbai	42°c
Gwalior	40°c
Khandwa	42°c
Nashik	40°c
Bangalore	40°c
Chennai	45°c

The drought, in particular, an unusually dry, is matter of concern for scientists and planners.

Of all the major natural disasters, droughts account for nearly 22% of significant damages though the number of deaths is only 3% worldwide (De & Joshi 1998).

Droughts have a wide range of effects on the masses in a developing country like India. The impact of droughts is specifically conspicuous in view of the tropical monsoon character of the country. Rainfall by the south-west monsoon is notorious for its vagaries.

II IMPACT ON AGRICULTURE

Indian agriculture still largely depends upon monsoon rainfall where about two-thirds of the arable land lack irrigation facilities and is termed as rained. The effect is manifested in the shortfalls of agricultural production in drought years. History is replete with examples of serious shortfall in cultivated areas and drop in agricultural productivity.

Severe shortage of food-grains had been felt and the country had to resort to import of food-grains to save the poor people from hunger and starvation. However, India has been able to build a buffer stock of food–grains and threat from droughts is not as serious as it used to be before the Green Revolution. It is worth mentioning here that the shortfall in agricultural production may be the direct impact of meteorological droughts but the succeeding hydrological and agricultural droughts have a long range and far reaching impact on agriculture. This impact may be in the form of changes in the cropping patterns and impoverishment in cattle.

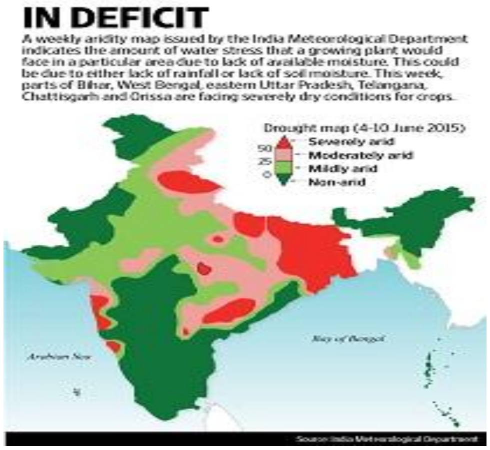

Fig. 1:- Effect of drought in year 2015.

Fig. 2:- Effected land by drought.

III EFFECT OF DROUGHT ON RICE ORYZA SATIVA (RICE)

Rice (Oryza sativa L.), the major staple food crop of the world, faces a severe threat from widespread drought. Drought is the most important limiting factor for crop production in many regions of the world. It is often unpredictable and does not occur in all years in a target environment. Rice (Oryza sativa L.) is a major staple crop on which about half of the world's population depends. Drought stress during the vegetative growth, flowering, and terminal stages of rice cultivation can cause spikelet sterility and unfilled grains. Usually, drought during the grain-filling process induces early senescence and shortens the grain-filling period, but increases remobilization of assimilates from the straw to the grains. Thus, the manner in which drought influences grain yield is not straightforward and it is necessary to understand the mechanism of plant responses to drought conditions, with the ultimate goal of improving crop performance for the vast rice cultivation areas of the world where rainfall is unreliable.

IV QTLS FOR DROUGHT IN RICE

Drought is a key factor affecting food security worldwide; its effect 70% in crop`s yield generally. Conventional plant breeding approaches for yield improvement under drought condition is time consuming and laborious, because carefully managed field conditions are required. Selection for a well-developed root system with long thick roots should improve the drought tolerance in upland rice because the plant would avoid water stress by absorbing stored water in the deep soil layer. Phenotypic selection for root morphological trait in conventional breeding programs is unfeasible. The use of molecular marker could provide a useful tool to support phenotypic selection. So several mapped population were developed to detect QTLs influencing root morphology and other drought related traits that could be used in marker assisted selection to improve upland rice varieties.

V QTL`s FOR ROOT CHARACTERS

Root is a vital organ for absorbing soil moisture and nutrients and influence drought resistance. The root provides, among others, anchoring and water/ nutrients absorption to the plant. Breeding for strong root systems is an important strategy for improving drought avoidance in rice. A quantitative trait locus (QTL), controlling root thickness and root length. Atchley and Zhu (1997) demonstrate that the genetic mechanism of controlling complex quantitative trait changed distinctly. Conditional QTL mapping may be a valid way to reveal dynamic gene expression for the development of quantitative traits, especially the epistatic effect. Mapping QTL with genetic main effects and QTLs X environmental interaction effects could help understand the nature of the quantitative trait.

The methods of soil chambers and hydroponics under greenhouse conditions played an important role for morphological characterization for root traits, but these methods are were insufficient to estimate environment effects on root traits.

VI PHYSIOLOGICAL PARAMETERS ASSOCIATED WITH DROUGHT IN RICE

Drought is one of the major limitations to plant productivity worldwide. Identifying suitable screening tools and quantifiable traits would facilitate the crop improvement process for drought tolerance. The physiological and biochemical experiments are in good agreement with the classification into drought-tolerant and drought-sensitive genotypes based on field experiments under natural drought stress. The results, especially the measurements of the osmotic potential and chlorophyll fluorescence, were close to and in some cases even better than those of the tolerant cultivars. The lack of agreement with the field trials in this case could be due to the effect of stress conditions other than drought working simultaneously on the plant. In field conditions, it is impossible to eliminate the effects of other negative environmental factors and the indicator of tolerance examined for example the crop yield, is a resultant indicator of the influence of many stress factors during the growth and development of plants.

(a) Materials and Methods

(i) **Methods**

- DNA Extraction.
- DNA Purification.
- NANO Drop.
- PCR.
- 2-D Gel Electrophoresis.

(ii) **Materials**

- Rice Leaf Samples.
- Motar & Pistal.
- Liquid Nitrogen.
- CTAB Extraction Buffer and other chemicals

Table 2:-
List of samples provided by CRURRS, Hazaribag, Jharkhand.

S.NO.	SAMPLE`S
1	CRR-693-28-B-1-B-B
2	CRR-696-42-B-1-B-B
3	CRR-616-1005-B-1-B-B
4	CRR-616-1022-B-1-B-B
5	CRR-697-76-B-1-B-B
6	ABHISHEK
7	CRR-708-1-B-2-B-B
8	CRR-708-7-B-1-B-B
9	VANDANA
10	VANDANA NIL
11	ANJALI
12	ANJALI NIL
13	SAHBHAGI DHAN
14	CR DHAN 103
15	IR 64 NIL
16	CRR680
17	Virendra
18	Sneha
19	MTU 1010
20	KALINGA 3
21	CR DHAN 40

VII PCR CONDITION

Initial denaturation 94^{o}C for 2 minutes

- 35 cycles of
- Denaturation 94^{o}C for 30 seconds
- Anneling** for 45 seconds
- Elongation 72^{o}C for 45 seconds
- Final elongation 72^{o}C for 10 minutes
- **Storage** 4^{o}C for initiate

**Annealing temperature depends on primer used. (Due to GC content of provided primer).

2-D Gel Electrophoresis

Milli Q = 130ml
Agarose (HI- PURE) = 2.5 %
Mix them and add 1.7 µl of Ethidium Bromide.
Mixed agarose transferred to casting gel plate.

Results

Table 3:-
Positions of rice samples with respect to electrophoresis well.

First two electrophoresis result	
DNA Ladder	L
Sahbhagi dhan	1
3.CR Dhan 40	2
Abhishek	3
Virender	4
Sneha	5
Kalinga 3	6
IR64 NIL	7
MTU 1010	8
Vandana	9
Vandana NIL	10
Anjali	11
Anjali NIL	12

List of primers have used to detect QTLs are as follows:-

1. RM 3825

2. RM 279.

RM 279 associated with $QTL_{DTY\ 2.2}$ and RM 3825 associated with $QTL_{DTY\ 1.1.}$

RM 431 Annealing temperature is **51.8** for forward and **51.8** for reverse.

REVERSE SEQUENCE: - 5` **AGAGCAAAACCCTGGTTCAC**

FORWARD SEQENCE: - 5` **TCCTGCGAACTGAAGAGTTG**

VIII RESULTS

RM 3825 which having $QTL_{DTY1.1.}$ Out of 12 samples only four samples contain $QTL_{DTY1.1,}$ CR Dhan 40 [2], Vandana NIL[10], Anjali[11]and Anjali NIL[12] and DNA ladder used for this electrophoresis is of 100kb.

RM 3825 electrophoresis result	
DNA Ladder	L
Sahbhagi dhan	1
CR Dhan 40	2
Abhishek	3
Virender	4
Sneha	5
Kalinga 3	6
IR64 NIL	7
MTU 1010	8
Vandana	9
Vandana NIL	10
Anjali	11
Anjali NIL	12

RM 279 associated with $QTL_{DTY\ 2.2}$ and only one sample IR 64 NIL contain $QTL_{DTY\ 2.2.}$

RM 279 electrophoresis result	
DNA Ladder	L
Sahbhagi dhan	1
CR Dhan 40	2
Abhishek	3
Virender	4
Sneha	5
Kalinga 3	6
IR64 NIL	7
MTU 1010	8
Vandana	9
Vandana NIL	10
Anjali	11
Anjali NIL	12

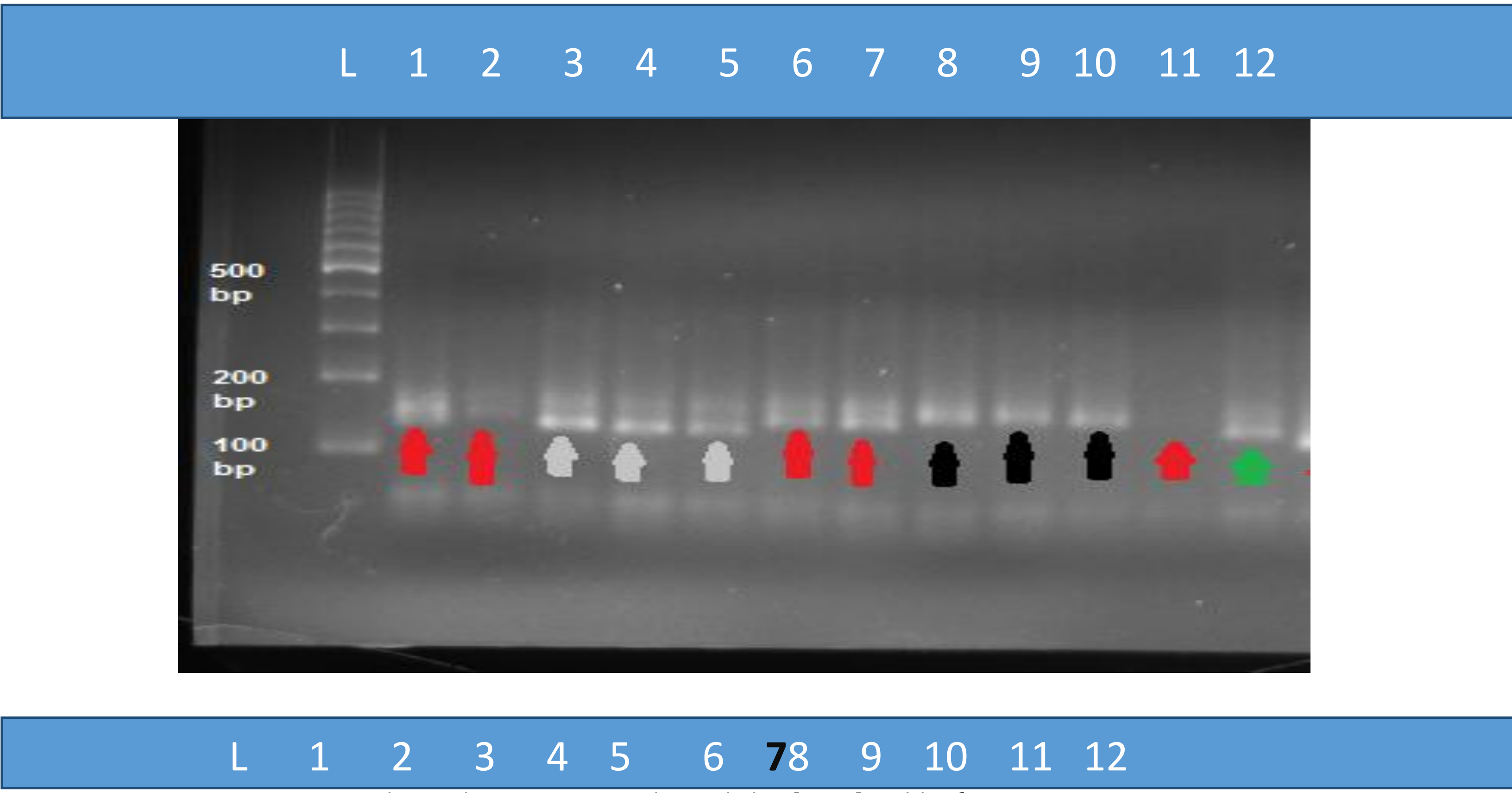

Fig 3. Primer RM 3825 :-Five varieties showed positive for QTL DTY 1.1

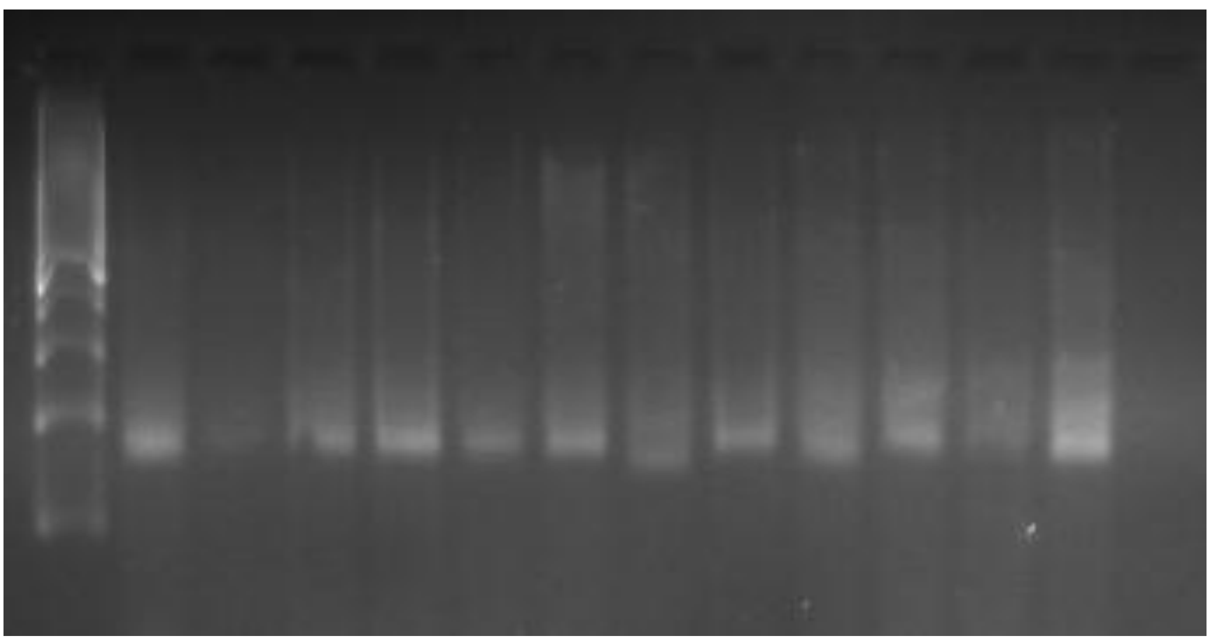

Fig. 4 RM 279 :-Out of 12 varieties tested, only IR64 NIL(No.7) possesses QTL DTY 2.2

IX DISCUSSION AND CONCLUSION

All markers are associated with its specific QTL, in this experiment we are using two markers RM 3825 which is associated with $QTL_{DTY\ 1.1}$ and another is marker RM 279 which associates with $QTL_{DTY\ 2.2}$, here we identified only four popular rice samples having $QYL_{DTY\ 1.1}$ and these are CR Dhan 40, Vandana NIL, Anjali and Anjali NIL. RM 279 associated with $QTL_{DTY\ 2.2}$ and only one sample has $QTL_{DTY\ 2.2}$ which is IR 64 NIL.

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