International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 11, Issue 5, 2019, pp.-597-600.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

IN-VITRO SELECTION OF TRADITIONAL LANDRACES FOR THE DEVELOPMENT OF CLIMATE RESILIENT RICE GENOTYPES

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Received: May 02, 2019; Revised: May 25, 2019; Accepted: May 26, 2019; Published: May 30, 2019

Abstract: Rice production in India depends on low land ecosystem and Rice varieties that can grow and produce well on upland are indispensable. Increasing the rice productivity remains a major concern due to change in climate variables and related factors. The use of *in- vitro* selection method for crop breeding has been widely used to improve the tolerant properties against biotic and abiotic factors. Germination of seeds and establishment of seedlings assume great significance for effective screening of genotypes for biotic and abiotic stresses. With this background, this study was aimed to screen traditional rice landraces for drought tolerance under *in-vitro* condition. Evaluation for drought tolerance during germination was accomplished by placing 100 seeds in germination paper with three replications in completely randomized design (CRD) using PEG 6000, at three levels of osmotic potential *viz*., (-) 10 bars, (-) 12.5 bars, and (-) 15 bars against a control. Significant mean sum of squares obtained for all the traits studied indicated the existence of variation among the genotypes. Under *in-vitro* condition, 15% of genotypes germinated at maximum osmotic potential (-) 15 bars, of which only six genotypes possessed > 40% germination efficiency. Among the six number of genotypes tested, Kuliyadichan recorded the highest root-shoot ratio (R/S) and signifies better source-sink relationship followed by Chandaikar, Mallikar, Mattaikar, Rajalakshmi and Sivappumalli. Hence it was concluded that these landraces, with highest drought tolerant potential, may be utilized in breeding programme to evolve drought tolerant varieties or hybrids.

Keywords: *Rice, Drought, Osmotic Stress, Landraces, PEG6000, R/S Ratio*

Citation: Pravin Kumar K., *et al*., (2019) *In-Vitro* Selection of Traditional Landraces for the Development of Climate Resilient Rice Genotypes. International Journal of Genetics, ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 11, Issue 5, pp.- 597-600.

Introduction

Rice production heavily depends on low land ecosystem and in such system, increasing the rice productivity remains a major concern due to change in climate variables and related factors. Drought is one of the most important constraints adversely affecting the rice productivity in Asia [9]. According to a survey, it has been estimated that by 2025, 15 million hectares of irrigated land and 22 million hectares in Asia will be under physical and economic water scarcity respectively [12].Drought adaptation mechanisms have been shown to be a complex phenomenon, as governed by different physio - biochemical processes at cell and organism level [17]. Early researchers proposed indirect selection based on secondary traits related to tissue water content and water potential for improved yield [4, 10, 13]. This approach was however unsuccessful for improving grain yield under drought whereas, current research programmes highlight the advantage of selecting high yielding cultivars [3], 10]. In order to achieve high yielding cultivars, grain yield has to be adopted as a predominant selection criterion, and evaluating the selected lines under both stress and irrigation [10]. However, the root system plays a pivotal role in plant development. A root system that is strong, healthy and has an adequate architecture for the environment in which it develops, provide numerous benefits to the plant. They provide access to below ground water and nutrients, facilitate anchorage of plants in soils [14-15] mediate chemical defense belowground [1, 23], serve as sites for rhizopheric microbiome and resilience to both biotic and abiotic soil. Hitherto, breeding of rice for more efficient root systems has traditionally been neglected due to the difficulties in phenotyping and of linking root form and function to crop productivity.

In order to breed for efficient root systems, it is necessary to have a genetic pool for the favorable traits.

The quantitative nature of root traits, their complex genetic control, and the strong environmental effects on those traits are some of the bottlenecks for classical genetic studies [8]. Many reports are available on the quantitative trait loci (QTLs) for the existing natural variations that control different aspects of root system architecture (RSA) and development. However, the studies on the architecture of plant root systems and their relationship to overall plant functions are relatively scarce. Unfortunately, RSA traits aimed to select for new climate resilient new cultivars, are in general performed under optimal nutrient conditions, which has led to mis-selection of smaller and plastic roots [26]. Hence the genetic improvement of root system has been slowed by the complex nature of drought and its strong interaction with the environment. Since drought is predicted to increase in near future, concerted efforts have been made to develop drought adapted rice varieties/hybrids. In the perspective of contributing to a better understanding on responses of to drought, we studied the physiology of root system in response to drought intensities in rice traditional landraces for better livelihood of farming communities.

Materials and Methods

The experiment was conducted at Biotechnology Lab, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam during 2018.

Genetic materials

Experimental material comprised of 50 traditional landraces with wide genetic base.

A detailed survey was conducted in the drought prone regions of southern parts of Tamil Nadu and different landraces were collected from farmers' fields. The passport data of 50 genotypes were prepared and catalogued (data not shown).

In-vitro screening for drought tolerance (DT)

The use of *in vitro* selection method for crop breeding has been widely used to improve the tolerant properties against biotic and abiotic factors. The *in vitro* screening for drought tolerance (DT) was carried out using Polyethylene Glycol (PEG 6000 MW) according to [2] Evaluation for drought tolerance during germination was accomplished by placing 100 seeds in germination paper with three replications in completely randomized design (CRD) four levels of osmotic potential *viz*., (-) 10 bars, (-) 12.5 bars, and (-) 15 bars with a control. were created using PEG 6000, A horizontal line was drawn at 3 cm from the top of germination paper and was marked with 25 points at 1 cm intervals. Hundred seeds of each genotype were placed on the marked points of the moistened paper towel, ensuring that the seeds do not touch each other and a moistened second paper towel was carefully placed over the seeds. The paper towels along with a polythene sheet below it was then rolled loosely to form a tube and held firmly. The germination paper rolls were placed in containers of different moisture stress levels *viz*., (-) 10 bars, (-) 12.5 bars, and (-) 15 bars the data were recorded at 14DAS on germination percentage, root length, shoot length, root / shoot (R/S) ratio.

Germination index

The germination index was calculated after final germination using the following equation:

$$Germination\ index\ = \frac{Germination\ percent\ in\ each\ treatment}{Germination\ percent\ in\ the\ control} \times 100$$

Germination rate

The germination rate was calculated after final germination using the following equation:

$$Germination\ rate\ = \frac{No\ of\ germinated\ seeds}{No\ of\ germination\ days}$$

Statistical analysis

The compiled data for all the parameters were subjected to analysis of variance (ANOVA) and the means were compared using CRD analysis to interpret the result.

Results and discussion

Effect of moisture stress on Germination Percentage

Under *in vitro* condition, 15% of the genotypes alone recorded germination of 40% at (-) 15 bars. (Fig.1) Other genotypes failed to record germination at this highest concentration and are considered sensitive to drought. At (-) 12.5 bars, 30% of genotypes recorded more than 60% germination. Only six genotypes showed more than 40% germination at (-) 15 bars. The Significant mean sum of squares obtained for all the traits under different osmotic potential levels, (control, (-) 10 bars, (-) 12.5 bars, and (-) 15 bars) studied indicated the existence of variation among the genotypes.

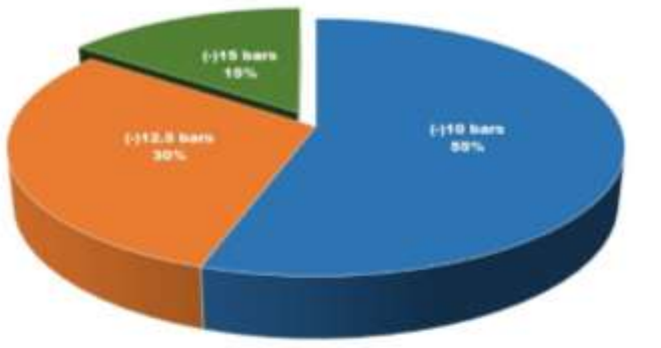

Fig-1 Germination of landraces under different levels of moisture stress

Different levels of water stress induced by PEG 6000 had significant effects on the seed germination. The germination capacity significantly varied (> 10% in -15.0 bars to 80.00% in control) between the treatments than the genotypic differences. Out of the 50 genotypes studied 23 recorded significant germination percent in control while 12 genotypes recorded significant germination at (-) 15.0 bars. Moreover, almost all the genotypes were affected by the osmotic water stress of (-)15.0 bars. Highest germination in most of the concentration was recorded by the genotype kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi, sivappumalli. The decline in germination percentage with decreasing water potential might be due to low hydraulic conductivity of the environment. Results of the current study were in agreement with [2,12,18,21]. PEG 6000 makes water unavailable to seeds, affecting the imbibition processes of the seed which is a fundamental phenomenon for germination [7]. The lowest germination percentage was observed at (-) 15.0 bars. This larger reduction in germination percent with PEG solution could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to control. At water potential (-) 15.0 bars, the rate of germination of the rice genotypes was below 50% which may be the consequence of hampered water and nutrient absorption due to decreasing water potential or greatly suppressed cell elongation as a result of the low turgor pressure which is similar to the results obtained by [2, 15] Among the 13 genotypes, six genotypes namely, kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi, sivappumalli, recorded significant germination percentage. Higher rate of germination even under water deficit conditions is a considered as a desirable feature by [7, 11-12]

Effect of moisture stress on Germination Rate (GR)

Germination rate is the average of number of seeds that germinate over the 14-day periods. Out of the 50 genotypes studied 23 recorded significant germination percent in control while 12 genotypes recorded significant germination at (-) 15.0 bars. Moreover, almost all the genotypes were affected by the osmotic water stress of (-)15.0 bars. The genotype Kuliyadichan, Chandaikar, Mallikar, Mattaikar, Rajalakshmi, Sivappumalli, recorded highest germination rate, the decline in germination percentage with decreasing water potential might be due to low hydraulic conductivity of the environment. Results of the current study were in agreement [2, 12,18]. In which PEG 6000 makes water unavailable to seeds, affecting the imbibition processes of the seed which is fundamental for germination [6-7]. In the present study lowest germination rate was observed at (-) 15.0 bars which might be attributed due to high viscosity of PEG concentration, where solubility and diffusion of oxygen were reduced compared to control. At water potential (-) 15.0 bars, the rate of germination of the rice genotypes was below 50% which may be the consequence of hampered water and nutrient absorption due to a decreasing water potential or greatly suppressed cell elongation as a result of the low turgor pressure which is similar to the results obtained [2, 15]. Among the 12 genotypes, six genotypes namely, Kuliyadichan, Chandaikar, Mallikar, Mattaikar, Rajalakshmi, Sivappumalli, recorded significant germination rate. Higher rate of germination even under water deficit conditions is a considered as a desirable attribute for rice breeding programme towards climate resilience [7, 12].

Effect of moisture stress on Germination Index (GI)

Germination is one of the most critical periods in the life cycle of plants. The effect of increasing drought intensity on seed germination was measured to determine the tolerance of rice genotypes to water deficit. Under moisture stress the highest GI was recorded with control and lowest at (-)15 bar. Out of the 50 genotypes studied, 17 recorded significant germination index (GI) in lower concentration (-) 10 bars while 12 genotypes recorded significant germination at higher concentration (-) 15.0 bars. Moreover, almost all the genotypes were affected by the osmotic water stress of (-) 15.0 bars. The six genotypes namely, Kuliyadichan, Chandaikar, Mallikar, Mattaikar, Rajalakshmi, Sivappumalli, recorded significant germination index, and followed the same trend as in GR, under water deficit condition. The results further indicated that these materials showed superiority over rest of the selected genotypes in relation to germination. In the present investigation drought stress greatly affects seed germination, but the response intensity and adverse effect of stress depend on the inherent potential of the genotypes.

It has been reported that drought stress adversely affects the seed germination, and seedling growth [23-24]. Under moisture stress, low water potential is a determining factor for inhibiting seed germination [20, 25]. It seems that lowering the osmotic potential with PEG6000 decrease water availability for seeds and then caused low germination. The physical process of water uptake leads to activation of metabolic process, as the dormancy of the seed is broken following hydration. Elevated drought stress slows down water uptake by seeds, thereby inhibiting their germination and root-shoot elongation.

Effect of moisture stress on root length

Early and rapid elongation of root is an important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions [5, 13]. In the present investigation, the root length also significantly declined with increased external water potential and consequently, all treatments caused a decrease in root elongation in all genotypes compared to their controls. Out of the 50 genotypes studied, 22 recorded significant root length in control while only 13 genotypes recorded significant root length at (-) 15.0 bars. The mean root length varied from 1.13 cm (15.0 bars) to 12.46 cm (Control). The genotype surakuruvai recorded highest root length in control alone while chandaikar had maximum root length of 12.43 cm at (-) 12.5.0 bars. In addition, thirteen genotypes recorded significant root length at maximum moisture stresses of (-) 15 bars. Long roots were reported as a component trait for drought tolerance by [2] [15]. as they play a direct role with high penetration ability and have large xylem vessel radii and lower axial resistance to water flux aiding in greater water acquition. Among the 13 genotypes, rajalakshmi recorded highest root length of 7.48 cm when compared to the control chandaikar (24.54 cm). on the contrary genotype milagi was distinguished from the other population by its marked reduction in root length (2.17 cm)., which suggests it inefficacy to tolerate high drought intensity.

Effect of moisture stress on above ground parameters

Though root length is more affected by drought than shoot length, the effect of drought is exhibited mostly on the above ground parameters (shoot as well as aerial parts of the plant), which will bear most economic parts of the crops. Hence, the shoot parameters will also help the breeder while selecting the superior genotypes against drought. In the present investigation, of the 50 genotypes studied only 20 recorded significant shoot length in control while only thirteen genotypes recorded significant values at (-) 15.0 bars and the shoot length was decreased with an increase in external water stress.However, this retardation in shoot length was found to be high in kuliyadichan (2.32 cm) at high drought intensity and low in kalinga-3 and rajalakshmi with the shoot length of 13.30 and 6.64 cm, respectively. These genotypes also showed highest individual mean shoot length of 12.78 and 14.29 cm in all the treatments conducted in the laboratory. These results corroborate with the findings [2] [7] [13] had also observed the retardation in growth of shoot and root length in response to increasing moisture stress under field as well as laboratory condition. In this study, the genotypes vanapraba and virendra recorded significant value in control alone, while 17 genotypes show mild tolerance (-.10.0 bars) and 15 genotypes exhibited moderate tolerance (-) 12.5 bars). Varietal difference for drought tolerance at seedling and vegetative state has also been reported by [10].

Effect of moisture stress on root/shoot ratio (R/S)

In addition to root and shoot lengths, root to shoot ratio also plays a major role in selecting drought tolerant lines. Genotypes with high root/shoot ratio under drought are much preferred. The present study revealed significant variations for the R/S ratio among the genotypes studied. The ratio ranged from 0.32 for maranellu to 2.18 for rajalakshmi in the control. Among the treatments, the highest ratio was observed in kichali samba (1.97) while the lowest ratio was recorded by the genotype jaya (0.24). The results are represented in [Table-1]. In control, high R/S ratio was exhibited by several genotypes which failed to record the same under drought stress. However, DT genotypes recorded significant root/shoot ratio under stressed condition. High R/S has been reported as a component trait for drought avoidance by [2] [18] [27] The results showed that the plants have a high capacity to vary the allocation of nutrients and the activities of different metabolic pathways for producing biomass in both shoots and roots.

Genotype	Germination percent	root length	shoot length	RS ratio
Chandaikar	80.85	6.97	4.14	1.75
Kuliyadichan	80.85	4.03	2.32	1.8
Mallikar	76.81	4.71	3.23	1.51
Mattaikar	65.69	6.01	4.87	1.28
Rajalakshmi	58.62	7.49	6.64	1.17
Sivappu Malli	40.43	3.24	5.68	0.98

The studies of [26, 19] revealed that under drought conditions the plasticity of the plants allow increased allocation of several primary metabolites to roots while decreasing allocation to shoots needs to be augmented by field evaluation methods to validate drought resistant genotypes identified by *in vitro* screening. In general, the findings of this study revealed that PEG-induced drought stress reduced germination percentage, shoot and root lengths while increased the R/S in DT genotypes when compared to susceptible ones. This corroborates with the findings of [2] [5] [18] [22]. Though many factors influence the drought tolerance of crops, these factors are not independent, but interact with one another. Hence, to study crop resistance to drought, attention should not only be focused in identifying typical characteristics, but also to their relationship and interaction of those characteristics.

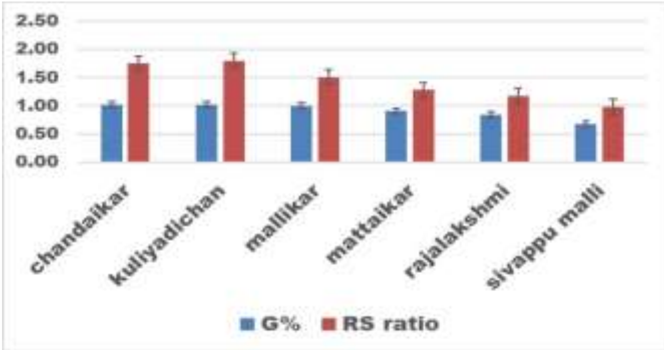

Fig-2 Graphical representation of Germination percent (arc sin transformation) and root/shoot ratio of rice land races under extreme moisture stress.

It has been reported that PEG-induced osmotic stress can cause hydrolysis of storage compounds that further lower the internal osmotic potentials of the seed. In this study, all the genotypes showed significant differences at various concentrations, but the genotypes namely Kuliyadichan, Chandaikar, Mallikar, Mattaikar, Rajalakshmi and Sivappumalli are identified as drought tolerant lines [Fig-2].

Effect of moisture stress on seed vigour (SV)

Seed vigour (SV) is sum total of those properties of seed which determine the level of activity performance of the seed during germination and seedling emergence. Seed vigor index declined with the increase in drought intensity. Out of the 50 genotypes studied 21 recorded significant germination per cent in control while 10 genotypes recorded significant germination at (-) 15.0 bars. In drought stress the highest SV was recorded at control and lowest at (-) 15 bars PEG concentration Among the significant genotypes surakuruvai has highest seed vigour (64.88) in control and rajalakshmi (8.36) in (-) 15 bars. Among the significant genotypes pokali (25.66) has lowest seed vigour in control and jaya (1.55) in (-) 15 bars. Moisture content of seedlings plays an important role in various physiological processes including growth. It has also been reported that drought suppresses the uptake of essential nutrients like P and K, which could adversely affect seedlings growth and vigor. This results in physiological and biochemical changes in both anabolic and catabolic organs of the seeds and seedlings[16] [9].

Conclusion

In the present study, the results proved the use of PEG 6000 for the experimental control of external osmotic potential, as an effective method for studying the effect of moisture stress on seed germination and seedling growth characters. It can also be concluded that with increasing levels of moisture stress, seed germination and early seedling growth were adversely affected in all rice landraces.

However, for the agronomic traits like germination percentage, root and shoot length, significant variations were observed, with maximum towards higher stress intensity. Furthermore, the landrace Kuliyadichan recorded the highest R/S ratio and germination percentage under severe moisture stress, followed by Chandaikar, Mallikar, Mattaikar, Rajalakshmi and Sivappumalli. The highest root-shoot ratio (R/S) signifies better source-sink relationship. Hence it was concluded that these landraces, with highest drought tolerant potential, may be utilized in breeding programme to evolve drought tolerant varieties or hybrids

Application of research: Screening landraces for drought tolerance enables the identification of potential donor parent for pyramiding in existing popular varieties. Understanding the root system architecture (RSA) is immensely important to study the root associated genes dictating drought tolerance in least exploited traditional landraces.

Research Category: Traditional landraces, Moisture stress tolerance

Abbreviations:
PEG: Poly Ethylene Glycol, DT: Drought tolerance, GI: Germination index,
GR: Germination rate, R/S: Root/shoot ratio, SV: Seed vigour

Acknowledgement / Funding: Authors are thankful to NADP-GOI phase IV by Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India for financial support

*** Research Guide or Chairperson of research: Dr Asish K Binodh**
University: Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu
Research project name or number: B27CP163 (Studies on landraces under high risk of climate variability for development of climate resilient varieties/hybrids)

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Biotechnology Lab, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam

Cultivar / Variety name: Rice- Kuliyadichan, Chandaikar, Mallikar, Mattaikar, Rajalakshmi, Sivappumalli

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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