Screening of genotypes for drought tolerance using PEG 6000 in different landraces of Rice (*Oryza sativa* L.)

Abstract

Drought stress restricts crop productivity in the face of climate change. The loss of water resources for food production is a global concern as the world's population continues to expand. Drought-tolerant cultivars must be developed to assure food and nutritional security. The drought response of rice landraces is poorly understood. Rice genotypes Sengalpattu sirumani, Mappillai sambha, N22, and IR64 were used to standardize drought stress using polyethylene glycol (PEG 6000). The PEG 6000 concentration which caused 50% mortality in the experiment was -0.8 MPa and this was used to impose drought stress to evaluate the total set of rice genotypes in the laboratory of the Department of Crop Physiology, Adhiparasakthi Agricultural College. The experiments were carried out by a factorial randomized complete block design in five replications. Germination percentage, shoot length, root length, vigor index, chlorophyll content, and proline content were used to screen and select the rice landraces. The rice landraces Sengalpattu sirumani, Poongar, Karuppukavuni, and Iluppaipoo sambha recorded higher germination percentages under drought stress conditions. It was observed from the physiological and biochemical parameters in the present study that the landraces Sengalpattu sirumani, Poongar, Karuppukavuni, and Iluppaipoo sambha withstood drought stress at the seedling stage. Furthermore, These four rice landraces could be used as donor parents in the breeding program to develop drought-tolerant rice varieties.

Key words: Drought Screening, PEG, Rice landraces

Introduction:

Rice (*Oryza sativa* L.) is the most significant food crop on the planet, particularly in Asia. Rice and wheat were grown in 124 and 126 countries, respectively, in 2013, with global production totaling 745 and 713 million tons [1]. Asia has 90 percent of the world's rice area and produces 92 percent of the world's rice. India is the most important rice-growing country in Asia, accounting for 23.3 percent of gross cultivated land and 43 percent of total food grain production, and 46 percent of overall cereal production [2]. After sugarcane and maize, rice is the third most widely produced agricultural commodity on the planet XX [1]. Rice is a good source of thiamine, riboflavin, niacin, and dietary fiber, and contributes 20% of the world's dietary energy and 13% of per capita protein [3]. Asia consumes 90% of the world's rice, and

the region's total rice demand continues to climb. Rice consumption per capita continues to rise outside of Asia, where it is not a staple diet. The ever-increasing human population, together with the loss of agricultural land owing to urbanization processes and reduced water availability due to climate change, pose major problems to global agriculture [4]. To meet the estimated population needs by 2050, large increases in grain yields of main crop species such as rice, wheat, and maize are necessary [5].

Rice production accounts for more than 40% of total food grain production in India. Rice output was predicted to be at 104.8 million tonnes in 2019-20, a decrease of 1.85 million tonnes from the previous year. New tactics must be devised in the framework of achieving food security, regardless of the harsh climate conditions predicted for the near future. Drought is one of the most obvious factors that limit rice output in South India's vulnerable areas. Existing current rice varieties do not fare well in drought-stricken situations. India is home to a diverse range of rice cultivars, landraces, and lesser-known types that have been cultivated by farmers and local entrepreneurs for centuries. However, the current climate change events are posing a significant barrier to increased rice productivity. Over the past three decades, the overall yield variability due to climate change was 53% of the total rice harvest. [6].
Drought or flood stress, nutrient deficiency, excess salt, osmotic stress, high or low-temperature stress, metal toxicity, and excessive light stress are some of the abiotic problems that plants confront. Drought stress is perhaps the most important of all the stresses to which plants are subjected for reduced yield, both in natural and agricultural settings [7]. Areas under drought have already expanded and this is expected to increase further with the advent of climate change.

Rice is grown in a variety of environments, including irrigated, rainfed upland, rainfed lowland, and deep water. Around 50% of rice-growing land is irrigated, with rainfed lowlands accounting for 34% of total rice planted area, rainfed uplands for 9%, and flooded systems accounting for 7%. Irrigated rice alone accounts for 75% of global rice output [8]. Rice is ranked top among the most irrigated crops in the world since it requires more water to grow [9]. Rice is suited to a wide range of settings, although its semi-aquatic nature makes paddy production more efficient at high soil moisture levels. In India, there are 22.0, 14.4, and 6.3 million ha of irrigated, rainfed lowland, and highland rice, respectively (Singh, 2009). About 6.3 million ha of the upland area and 7.3 million ha of lowland region are very drought-prone out of the total 20.7 million ha of rainfed rice area reported in India [10].

Droughts have apparent yield impacts, especially if they occur during critical times of the rice growth cycle when plant development is highly sensitive to water requirements. Similarly, drought scans limit the amount of land that can be cultivated, as in the case of delayed monsoon. There are various landraces accessible in Tamil Nadu, some of which are particularly resilient to environmental conditions such as drought and heat, and are traditionally used by the people in that area. Although the yielding capacity of traditional varieties is limited this is compensated by other appreciable characteristics such as high nutritional value, good cooking qualities including pleasurable aroma, and sufficient volume of a cooked meal with less quantity of raw rice. On-farm and in-market management responsiveness of landraces and high-yielding traditional varieties area about 30–35% more than modern varieties. The seed of traditional varieties costs 2.5 times lesser than that of modern varieties.

As a result, improving the history of traditional rice varieties and landraces could serve as a platform for future study, particularly in agricultural disciplines, resulting in validated outcomes for future food demands. These rice landraces need to be identified before they become extinct. Knowing about their existence and significance through ancient literature may pave the path for a successful collection and characterization of these traditional rice genotypes. There is a future need to expand the genetic base of the rice crop by introgressing genes from diverse sources. As a result, there is a pressing need to collect, use, and analyze untapped germplasm. Hence, we aim to select the drought tolerant plant by various factors viz., morphological, biochemical, and physiological.

Materials and methods

The present investigation was carried out during 2021-2022 under laboratory conditions. The experiment was conducted at the Department of Crop Physiology, Adhiparasakthi Agricultural College, Kalavai, Ranipet. A brief account of the materials used and methodologies followed in the experiment conducted to achieve the objectives of the study are given below.

Seed of rice varieties (Table 1) collected from Department of Rice, Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore-3 were used for this study.

Table 1. Detail of studied genotypes with their origin and special character

Sl. No.	Variety	Origin	Specific note

1	Athur kichilisambha	Athur, Tamil Nadu.	Low glycemic index, medicinal benefits, enhance muscles.
2	Illupai poo sambha	Tamilnadu	Resistant to water stress
3	Karuppukavani	Sivagangai, Tamil Nadu.	Rich in anti-oxidant, nonlodging.
4	Kitchidi sambha	Tamil Nadu	Suitable for dry sowing, pest, and disease-resistant.
5	kullakar	Tamil Nadu	Resistant to drought, pests, and disease, resistant to alkaline, saline soil, and water-logged areas.
6	Mappilai sambha	Thiruvanamalai, Tamil Nadu.	Medicinal benefits (use in mouth ulcers, improves digestion).
7	Poongar	Ranganadhapuram, Tamil Nadu.	Resistant to drought, tall variety.
8	Sengalpattu sirumani	Sengalpattu, TamilNadu	High yield, resistant to waterlogging, suitable for a south Indian meal.
9	Thanga sambha	Kanchipuram, TamilNadu	Very long matured grains look like gold, fine grains, and long earhead.
10	N 22	Eastern India	The short duration of maturity (80-95 days), deep-rooted, drought and heat tolerant as rice cultivar
11	IR 64	IRRI, Philippines	Maturity duration (115 days), hybrid variety with high yield, rainfed lowland areas, semi-dwarf, susceptible to abiotic stress.

Standardization of drought stress using Polyethylene glycol (PEG 6000)

Polyethylene glycol (PEG 6000) is an inert, water-binding polymer, which accurately mimics drought stress under dry soil conditions [11]. The prerequisite for screening the rice genotypes using PEG is the standardization of the optimum concentration of PEG. PEG is known to reduce the water potential and induce plant water deficits, causing physiological disorders and resulting in less water uptake and the loss of cell turgor.

Rice genotypes Sengalpattu sirumani, Iluppaipoo sambha, N22 and IR64 were used to standardize drought stress using polyethylene glycol (PEG 6000). Healthy seeds of uniform size were surface sterilized with 0.1% Mercuric chloride (HgCl2) for 2-3 min and then washed thoroughly with distilled water. Twenty-five sterilized seeds were sown in a rolled paper towel with various water potentials *viz*., 0.0 (control), -0.4, -0.5, -0.7, and -0.8 MPa of PEG 6000, and five replications were maintained for each treatment.

Number of seeds germinated was counted every alternate days from day-2 to day-15 after sowing to determine the germination percentage. Seedling growth parameters such as shoot length and root length were recorded on the 15^{th} day after sowing in randomly selected seedlings. Root length stress index (RLSI) and Seed vigor (SV) were measured using the following formula.

RLSI (%) = [Root length of stressed plant / Root length of control plants] x 100

SV (%) = Germination percentage × Seedling length.

Screening of rice genotypes for drought stress tolerance at seedling stage

The PEG 6000 concentration which caused 50% mortality in the previous experiment was -0.8 MPa and the same was used to impose the drought stress to evaluate the total set of rice genotypes. Uniform-sized seeds of each rice genotype were selected and disinfected with 0.1% mercuric chloride ($HgCl_2$) for 5 min and then thoroughly washed with distilled water three times. Fifteen seeds of each cultivar were placed in a rolled paper towel and transferred to an incubator with a photoperiod of 12 h light and 12 h dark with a daily maximum photosynthetic photon flux density (PPFD) of 380 ± 40 µmol/(m^2·s) at 25 ºC in the laboratory of Department of Crop Physiology, Adhiparasakthi Agricultural College, Kalavai, Ranipet (12.55° N, 79.24° E). The experiments were carried out by a factorial randomized complete block design in five replications.

Germination percentage

Twenty-five seeds of each rice cultivar were placed in a roll paper towel and the germinability was recorded on the fourteenth day after placing. The number of seeds germinated out of 25 was expressed as a percent.

Shoot length

The shoot length of the seedlings was measured from five randomly selected seedlings in each variety from each replication on the fourteenth day after sowing and the mean was calculated and expressed in cm.

Root length

The root length of the seedlings was measured from five randomly selected seedlings in each variety from each replication on the fourteenth day after sowing and the mean was calculated and expressed in cm.

Vigour index

Vigour index of the seedlings was worked out by using the following formula.

Vigour index = Germination percentage x (Root length (cm) + Shoot length (cm)).

Chlorophyll content

Chlorophyll content in leaves was estimated by using the method described by [12] and expressed in mg g^{-1} fresh weight.

Proline content

Proline accumulation in the leaf was estimated by the method of [13]. The proline content was expressed in mg g^{-1} fresh weight.

Statistical Analysis

The experimental design for the laboratory experiment was factorial with a completely randomized design (FCRD) replicated five times for each of the standardization and screening experiments. The data were statistically analyzed as suggested by Gomez and Gomez (1984). Wherever statistical significance was observed, the critical difference (CD) at 0.05 level of probability was used for mean comparison.

Results and Discussion

Drought stress at seedling stage in rice genotypes

Drought stress is a major stumbling block for crop development and growth. Drought stress has the principal consequence of reducing plant development [14]. PEG 6000 was used to test rice genotypes for drought stress. Seed germination is inhibited or seedling growth and development are suppressed when the water potential is low during germination [15]. Due to osmotic stress, the germination percentage decreases as the dose of water potential is raised. With an increasing dose of water potential, injured cellular organelles result in a lower germination percentage [16]. In rice treated with polyethylene glycol, [17] found that germination percentage reduced as the amount of water potential rose.

Shoot length normally decreased with an increased amount of water potential in all the genotypes, but some genotypes (Sengalpattu sirumani, Iluppaipoo sambha, Poongar, Karuppukavuni) exhibited a reduced reduction of shoot length at higher water potential and some genotypes reported a higher reduction of shoot length even at lower potential. Seedling shoot length increased and reduced with increased water potential, according to [17], and it mostly varied dependent on the genotype-specific effect. Shoot length is reduced due to stress

generated during water stress, while shoot length is increased due to growth stimulation during stress conditions [18].

Root length was found to decrease as water potential was increased; however, the reduction in root length varied among genotypes; some rice genotypes are highly sensitive and have a large reduction in root length at low water potential, while others tolerate it and have a small reduction in root length even at high water potential. Root length reduction was found to be positively related to water potential, according to [19]. With increased water potential, cell division and elongation are likely to be disrupted, resulting in a reduction in root length [19].

Seed vigour is adversely associated with water potential, and acute osmotic stress that occurred during treatment had a negative impact on seed vigour. Seed vigour is reduced with increased water potential in rice, according to [20], who used a PEG concentration of 5- 20%. Seed vigour is reduced as a result of the osmotic stress caused by PEG treatment, which causes a reduction in cellular activity [21].

The root length stress index was shown to be positively linked with the degree of water potential. [20] found that increasing the PEG concentration level raised the root length stress index, which was similar to our findings. Because of the significant stress put on the root during osmotic stress, the root length stress index rose [22].

The present study indicates that drought stress at the seedling stage affects the germination rate, root length, shoot length, seed vigour, and RLSI of rice. The variation in germination and seedling growth characteristics was specific for genotypes under reduced water potential [23]. The difference in germination rate and growth characteristics of genotypes under moisture stress conditions would be helpful to identify the drought-tolerant genotype [24, 15].

Among the 11 genotypes landraces Sengalpattu sirumani, Iluppaipoo sambha, Poongar, and Karuppukavuni w showed higher germination percentage, root length, shoot length, seed vigour, and Root length Stress Index (RLSI) than the other genotypes under seedling stage drought condition.

Effect of seedling Stage Drought on Biochemical Parameters

Chlorophyll is one of the most important components for photosynthesis in the chloroplast [25]. The Photosynthetic pigment and total chlorophyll content were analyzed and found that there was an increase of chlorophyll content from the vegetative to the

reproductive stage but rapidly declined thereafter due to water stress-induced degradation. The chlorophyll content of the plant tissue represents the photosynthetic capacity of the plant. Among the rice landraces, Sengalpattu sirumani recorded the lowest reduction in chlorophyll content due to drought over its control (16.94 %) followed by Poongar (23.53 %) and Karuppukavuni (29.32 %) under seedling stage drought condition.

Sengalpattu sirumani registered higher total chlorophyll content under drought (3.83 mg g^{-1}) compared to other rice genotypes. Drought-induced chlorophyll loss is mostly due to damage to chloroplasts caused by active oxygen species [26], which are formed more often under abiotic stress conditions. The increased activity of the chlorophyll degrading enzyme chlorophyllase may also contribute to the decrease in chlorophyll content during drought stress. Chlorophyllase and peroxidase enzymes increased in response to extreme drought stress, lowering chlorophyll concentration [27]. Furthermore, the decline in chlorophyll content during drought stress is influenced by the length and intensity of the drought [28]. Drought-induced chlorophyll depletion has long been thought to be a sign of pigment photooxidation and chlorophyll degradation. This was in corroboration with the results of the present study that recorded higher total chlorophyll content in landrace Sengalpattu sirumani. During water stress, [29] discovered that mesophyll cells were the primary cause of chlorophyll loss. When the chloroplast membrane was strained, it lost its intensity [30]. Drought stress caused structural changes in the chloroplast, such as excessive swelling, lamellae distortion, vesiculation, and the development of lipid droplets, which all led to structural and functional changes in the chloroplast [31].

Proline, a heterocyclic amino acid, accumulated under drought stress conditions due to hydrolysis of protein [32]. Proline is believed to protect plant tissues against stress by acting as nitrogen storage, an osmoregulator, and a protectant for enzymes and cellular structure. Analysis of proline content revealed that there was an increase due to the water stress observed in all the genotypes and a significant difference was observed across all the genotypes used in the present study. Irrespective of the genotypes, the mean increase in proline content was 58.20 %. The accumulation of proline could have provided the plants with an osmotic mechanism to maintain a favorable water potential gradient for water entry through the roots [33]. [34] reported that proline was synthesized to depress the internal osmotic potential to maintain a positive gradient for water uptake under water stress conditions. The enormous increase in proline content was encountered as the stress advanced. Among the genotypes, Sengalpattu sirumani recorded the highest proline content of 11.56 mg

g^{-1} in the reproductive stages. Drought-induced proline accumulation has been observed in many plant species, namely rice, maize, and finger millet and has been associated with adaptation to drought stress [35].

Many authors have observed proline increases in various plant species as a result of dehydration. Proline build-up has always been accompanied by a decrease in tissue water potential throughout time, according to all known evidence [37]. This was supported by other findings in the current investigation. [38], found that proline build-up was significant at panicle emergence in genotypes with a high susceptibility to water stress in sorghum. Drought-resistant genotypes, on the other hand, collected more proline at maturity than drought-tolerant genotypes. In the current investigation, a similar pattern was seen.

Conclusion

Drought tolerance is a complex trait, which is a combined function of various morphological, biochemical, and molecular traits which ultimately contribute to higher tolerance under drought conditions. Selection for drought tolerance can be performed by measuring yield under stress conditions and/or measuring secondary traits such as morphological, physiological, and biochemical parameters correlated with yield under stress conditions. A better understanding of the physiological basis of yield under drought will probably help in screening large germplasm for drought tolerance.

The present study was conducted using a set of 11 rice genotypes to investigate the physiological and molecular responses of rice genotypes to drought stress during the seedling stage to identify the drought-tolerant rice landraces.

The conclusions from the present study are summarized below.

- The most significant finding from this research is the identification of tolerant landraces of rice *viz.*,Sengalpattu sirumani, Poongar, Karuppukavuni, Iluppaipoo sambha which performed better under drought stress from the pool of 11 rice genotypes which includes 9 traditional landraces and two check varieties.
- In the laboratory experiment, the level of osmotic stress was standardized using PEG 6000 and found that 50% mortality was observed at -0.8 MPa and the rice genotypes were screened for drought tolerance using various germination-associated traits. The rice landraces Sengalpattu sirumani, Poongar, Karuppukavuni, and Iluppaipoo

sambha recorded higher germination percentage, seedling length, seed vigor, and root length stress index.

- The physiological traits, such as chlorophyll content and proline content were significantly higher in Sengalpattu sirumani, Poongar, Karuppukavuni, and Iluppaipoo sambha whereas it was lower in Mappillai sambha compared to the tolerant check (N22).

It was observed that the rice landraces Sengalpattu sirumani, Poongar, Karuppukavuni, and Iluppaipoo sambha could withstand drought stress during the seedling stage and under stressful situations.

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Table 2. Germination percentage (%)of different rice genotypes under control and – 0.8 MPa PEG 6000

Genotypes	Germination percentage (%)			
	Control	- 0.5 Mpa	- 0.7 Mpa	- 0.8 Mpa
Athurkichilisambha	100	71.80	60.11	60.00
Iluppai poo sambha	100	82.54	79.24	65.00

Karuppukavuni	100	86.12	80.23	66.00
Kitchidisambha	100	67.45	53.12	55.00
Kullakar	100	80.14	75.69	63.00
Mappilaisambha	100	55.03	46.21	35.50
Poongkar	100	87.45	82.12	68.00
Sengalpattusirumani	100	90.20	88.26	72.00
Thangasambha	100	69.15	54.24	59.00
N 22	100	71.15	59.41	48.41
IR 64	100	58.46	47.15	40.45
Mean	**100**	**74.49**	**65.95**	**57.48**
	G	**T**	**GxT**	
SEd	1.67	1.89	3.25	
CD (0.05)	3.22	3.67	7.65	

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388 **Table 3. Root length (cm) of different rice genotypes under control and – 0.8 MPa PEG**
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Genotypes	**Root length (cm)**			
	Control	**- 0.5 Mpa**	**- 0.7 Mpa**	**- 0.8 Mpa**
Athurkichilisambha	22.50	21.08	19.97	17.51
Iluppai poo sambha	26.28	24.78	23.67	23.38
Karuppukavuni	27.12	25.68	24.57	23.52

Kitchidisambha	17.34	15.88	14.77	15.39
Kullakar	23.22	21.78	20.67	16.12
Mappilaisambha	16.50	15.08	13.97	13.43
Poongkar	29.00	27.58	26.47	24.90
Sengalpattusirumani	29.41	27.98	26.87	25.40
Thangasambha	18.60	17.18	16.07	16.21
N 22	23.56	20.01	18.25	16.85
IR 64	22.56	17.85	15.95	15.87
Mean	**23.27**	**21.35**	**21.92**	**18.93**
	G	**T**	**GxT**	
SEd	0.45	0.54	1.03	
CD (0.05)	1.00	1.08	2.31	

Table 4. Shoot length (cm) of different rice genotypes under control and – 0.8 MPa PEG 6000

Genotypes	**Shoot length (cm)**			
	Control	**- 0.5 Mpa**	**- 0.7 Mpa**	**- 0.8 Mpa**
Athurkichilisambha	16.00	15.49	14.90	10.35
Iluppai poo sambha	16.20	15.69	15.35	12.35
Karuppukavuni	16.90	16.39	16.05	12.85
Kitchidisambha	15.60	15.09	14.50	9.80

Kullakar	16.15	15.64	15.05	11.65
Mappilaisambha	15.00	14.49	13.90	8.50
Poongkar	17.10	16.59	16.25	14.25
Sengalpattusirumani	18.12	17.59	17.25	15.25
Thangasambha	15.60	15.09	14.50	10.20
N 22	13.56	12.45	10.45	8.90
IR 64	13.45	10.98	10.75	9.30
Mean	**15.69**	**15.04**	**14.45**	**10.44**
	G	**T**	**GxT**	
SEd	0.23	2.28	0.57	
CD (0.05)	0.54	0.59	1.08	

Table 5. Seedling length (cm) of different rice genotypes under control and – 0.8 MPa PEG 6000

Genotypes	Seedling length (cm)			
	Control	**- 0.5 Mpa**	**- 0.7 Mpa**	**- 0.8 Mpa**
Athurkichilisambha	38.50	36.57	34.87	26.55
Iluppai poo sambha	42.40	40.47	39.02	35.65
Karuppukavuni	44.00	42.07	40.62	36.35
Kitchidisambha	32.90	30.97	29.27	25.10

Kullakar	39.35	37.42	35.72	29.15
Mappilaisambha	31.50	29.57	27.87	21.90
Poongkar	46.10	44.17	42.72	39.15
Sengalpattusirumani	47.52	45.57	44.12	40.65
Thangasambha	34.20	32.27	30.57	26.30
N 22	37.12	32.46	28.70	27.15
IR 64	36.01	28.83	26.70	25.17
Mean	**39.04**	**36.40**	**34.56**	**30.28**
	G	**T**	**GxT**	
SEd	0.45	0.54	1.03	
CD (0.05)	1.00	1.08	2.31	

Table 6. Seed vigour of different rice genotypes under control and – 0.8 MPa PEG 6000

Genotypes	Seed vigour	
	Control	**- 0.8 Mpa**
Athurkichilisambha	3845	663
Iluppai poo sambha	3992	319
Karuppukavuni	4255	547
Kitchidisambha	3744	585
Kullakar	3892	531

Mappilaisambha	3619		648
Poongkar	4275		1983
Sengalpattusirumani	4560		2297
Thangasambha	3780		411
N 22	3572		621
IR 64	3448		448
Mean	**3907**		**823**
	G	**T**	**GxT**
SEd	49.02	10.96	69.32
CD (0.05)	96.90	21.67	137.04

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421 **Table 7. Total chlorophyll content (mg g^{-1}) of different rice genotypes under control and**
422 **– 0.8 MPa PEG 6000**

Genotypes	Total chlorophyll content (mg g^{-1})	
	Control	**- 0.8 Mpa**
Athurkichilisambha	2.65	1.93
Iluppai poo sambha	2.79	2.04
Karuppukavuni	2.87	2.11
Kitchidisambha	2.54	1.74
Kullakar	2.76	1.94
Mappilaisambha	2.54	1.51

Poongkar	3.00		2.27
Sengalpattusirumani	3.01		3.83
Thangasambha	2.61		2.50
N 22	2.76		1.94
IR 64	2.50		1.29
Mean	**2.73**		**2.09**
	G	**T**	**GxT**
SEd	0.06	0.01	0.08
CD (0.05)	0.11	0.03	0.16

Table 8. Proline (mg g^{-1}) content of different rice genotypes under control and – 0.8 MPa PEG 6000

Genotypes	Proline (mg g^{-1}) content	
	Control	**- 0.8 Mpa**
Athurkichilisambha	11.31	8.81
Iluppai poo sambha	11.51	10.81
Karuppukavuni	12.02	10.80
Kitchidisambha	10.64	7.79
Kullakar	11.35	9.16
Mappilaisambha	10.61	7.54
Poongkar	12.40	11.09

Sengalpattusirumani	12.64		11.56
Thangasambha	10.94		8.77
N 22	10.61		8.77
IR 64	11.35		7.79
Mean	**11.39**		**8.37**
	G	**T**	**GxT**
SEd	0.29	0.07	0.42
CD (0.05)	0.58	0.13	0.82