

# PHYSIOLOGICAL CHARACTERIZATION OF TRADITIONAL RICE LANDRACES UNDER DROUGHT STRESS INDUCED BY POLYETHYLENE GLYCOL

TAMILSELVI, C.<sup>1</sup> – YOGAMEENAKSHI, P.<sup>2\*</sup> – ARULSELVI, S.<sup>3\*</sup> – VIJAYASHANTHI, V. A.<sup>1</sup> – SATHISH, G.<sup>4</sup> – PUNITHA, A.<sup>2</sup> – PARAMESWARI, K.<sup>5</sup> – ANANTHI, K.<sup>6</sup> – SIVAGAMY, K.<sup>1</sup> – PREETHI, T. L.<sup>1</sup>

<sup>1</sup>ICAR - Krishi Vigyan Kendra, Tamil Nadu Agricultural University,  
Thirur 602025, Tamil Nadu, India

<sup>2</sup>Rice Research Station, Tamil Nadu Agricultural University, Thirur 602025, Tamil Nadu, India

<sup>3</sup>ICAR - Krishi Vigyan Kendra, Tamil Nadu Agricultural University,  
Thiruvavur 614404, Tamil Nadu, India

<sup>4</sup>Regional Research Station, Tamil Nadu Agricultural University,  
Vridhachalam, Tamil Nadu, India

<sup>5</sup>ICAR - Krishi Vigyan Kendra, Tamil Nadu Agricultural University,  
Vamban 622303, Tamil Nadu, India

<sup>6</sup>Agricultural College and Research Institute, Thiruvannamalai 606753, Tamil Nadu, India

\*Corresponding authors  
e-mail: pyogameenakshi@tnau.ac.in; arulselvi.s@tnau.ac.in

(Received 11<sup>th</sup> Sep 2024; accepted 7<sup>th</sup> Jan 2025)

**Abstract.** An investigation was carried out to study the drought tolerance potential of fifteen traditional rice landraces using PEG 6000. Fifteen traditional landraces were subjected to drought stress at five concentrations such as -2, -4, -6, -8 and -10 bars of water potential. Under this study, growth parameters including germination per cent, shoot length, root length, seedling vigor index, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight were recorded. Further, physiological parameters including soluble protein, nitrate reductase, and proline content were also recorded. Out of 15 genotypes studied, two genotypes viz., Mettumaranelu and Chengalpattu sirumani recorded significant germination per cent (above 40%) over the mean. The maximal root length observed at these bars, was only 3.5 cm in -8 bar and 2.3 cm in -10 bar (Chengalpattu sirugamani). Chengalpattu sirumani maintained significant vigor index even at -6 bars making it the best candidate for drought tolerance. Based on the data, it was found that the variety Chengalpattu sirumani had the maximum root fresh weight (145.55 mg) under -8.0 bar drought circumstances. Chengalpattu sirumani showed higher accumulation of soluble protein (12.15 mg g<sup>-1</sup>), nitrate reductase activity (21.25 µg NO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) and proline content (11.26 mg g<sup>-1</sup>) than other traditional landraces. According to the findings, Chengalpattu sirumani, Pisini, Mapillai samba, and Karungkuruvai exhibited a high degree of drought tolerance, as evidenced by their notable germination rate, high seedling vigour index and root length compared to the general mean. The traditional landrace Mettumaranelu exhibited a higher germination per cent across all levels of water potential but failed to show a significant vigor index.

**Keywords:** abiotic stress, PEG, traditional rice, seedling, performance

## Introduction

Over 50% of the global population consume rice (*Oryza sativa* L.) as a staple food. 154 million hectares of land were planted to rice, with 476 million tons produced globally and productivity rate of 2948.82 kg ha<sup>-1</sup>. Since Asia is the primary source of

the world's population growth, there will be a reliance will continue to increase on rice as a staple food. In 2022, the FAO estimates that 519.5 million tons of rice was produced worldwide. However, there was a 2.5 million tons shortage in the 522 million tons demand for milled rice. In rainfed habitats, minimizing yield losses is critical to increasing rice production. Drought stress is one of the abiotic factors influencing crop output in rainfed habitats; when stress is eased, cultivars cannot recover, leading to significant yield losses.

Major abiotic stress like drought lower crop yield and threaten global food security, particularly in light of the ongoing and expanding effects of climate change as well as rising rates of both stressors. Drought hinders membrane transport, disrupts cell metabolism, reduces respiration and ATP production, and has a negative impact on maintaining internal water balance. These factors all contribute to bad seed germination. According to Mishra and Panda (2017), Farooq et al. (2018), and others, water stress may result in a reduction in plant height, leaf area, and biomass, which subsequently lowers yield. Numerous variables, including soil moisture, evaporation, and rainfall frequency, affect the intensity and/or severity of drought (Panda et al., 2021). However, a significant factor in drought tolerance and the ensuing production loss is also the genotypes' innate potential. Therefore, a sustainable and commercially feasible way to increase rice yield is to develop rice types that are resistant to drought stress. Because they have naturally developed to tolerate harsh climates, traditional landraces always have an advantage over enhanced modern cultivars that merely aim for increased yield potential.

Plants have “developed dynamic responses at the” morphological, physiological, and biochemical “levels allowing them to escape and/or adapt to unfavorable environmental” situations (Gagendra Singh et al., 2022)). The complex process of drought resistance entails the interplay of several genes, pathways, and characteristics. Drought tolerance, drought escape, and drought avoidance are three categories of drought resistance mechanisms that crop plants use to fend off drought stress (Levitt, 1980). Plants that are able to withstand drought are those that have low osmotic potential, cellular flexibility, and improved membrane resistance. In order to minimize drought stress, plants must regulate their vegetative and reproductive growth. This can be done by finishing the life cycle before the drought occurs, growing slowly during the dry season, or growing rapidly during the wet season. Since traditional landraces within species have different drought adaptation strategies and can offer greater insights to improve yield and resilience under drought conditions, it is necessary to take advantage of the natural variation of agricultural plants in drought adaptation.

Significant potential has arisen to speed up the production of drought-tolerant variants through plant breeding and the creation of transgenic plants through gene manipulation of genes related to drought. Thus, one of the most important methods for raising crop productivity is the development and breeding of genotypes resistant to drought. Somaclonal variation provides a way to produce drought-tolerant plants using PEG-induced in vitro culturing in areas with low water resources. Previous research has shown that a sharp drop in the moisture content of tissues coincided with an increase in drought stress caused by PEG (Adkins et al., 1995; El-Tayeb and Hassanein, 2000). Water is removed from the cell wall as well as the cell itself since PEG does not penetrate the apoplast. Thus, PEG replicates soil drying more closely than other low molecular weight osmotica. As a result, it has been employed to simulate drought stress in plants and the selection of resistant cell lines (Nepomuceno et al., 1998). Therefore, the goal of the current study is to determine whether fifteen numbers of South Indian

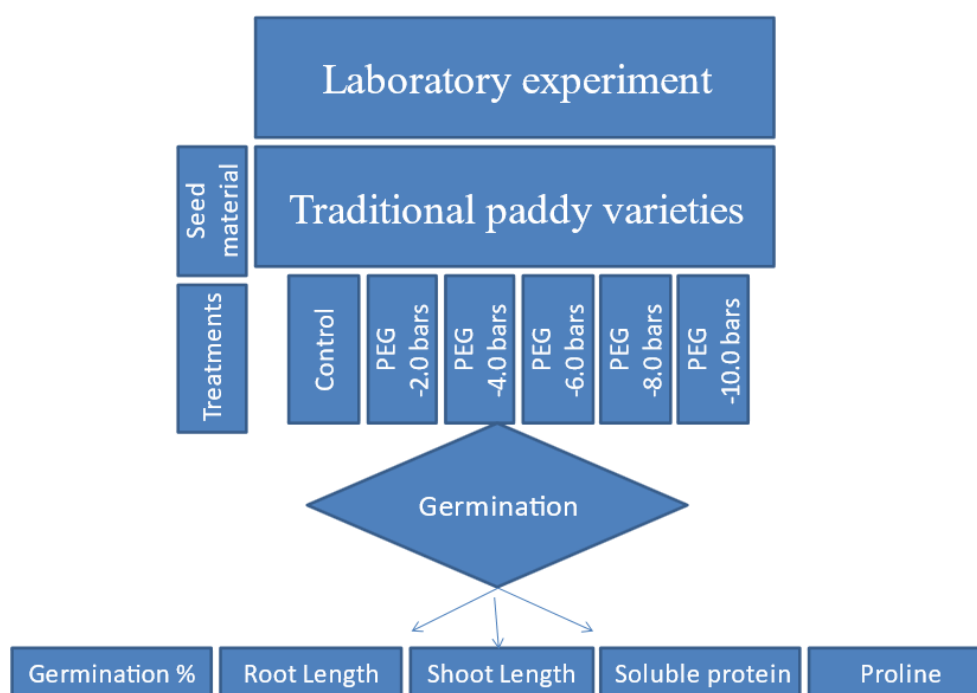
landraces have the potential to withstand drought by screening them in vitro for drought stress using PEG 6000 at different concentrations.

## Materials and methods

### Experimental details

The present study was carried out at the Agricultural College and Research Institute, Eachangkottai during 2021 & 2022. Experimental material comprised fifteen traditional landraces viz., Kasalath, Poongkar, Thooyamalli, Illuppai poo samba, Karupukavuni, Karudan samba, Rakthashali, Mapillai samba, Pisini, Chengalpattu sirugamani, Mani samba, Kaatuyaanam and Kalabaththat were collected from local farmers of Thanjavur district, India. In vitro screening for drought tolerance was carried out using Polyethylene Glycol (PEG 6000 MW) for creating different osmotic potentials. A horizontal line with 25 points spaced one centimeter apart was drawn in the germination paper, with the line drawn three centimeters from the top. In order to prevent the seeds from touching one another, twenty-five identical genotype seeds were positioned at the designated spot on the moistened germination sheet. After covering the seeds with a strip of moistened germination paper, the paper towels and a polythene sheet underneath were loosely wrapped into a tube and secured with a rubber band. The rolls were arranged in trays with varying PEG concentrations. The five concentrations of drought stress that were reproduced were -2.0, -4.0, -6.0, -8.0, and -10.0 bars of water potential. These were achieved by dissolving 118, 175, 212, 254, and 302 grams of PEG 6000, respectively, in 1000 mL of distilled water. Water deficiency stress is intentionally created during screening by using certain strengths of polyethylene glycol 6000 (PEG-6000; Sigma Chemicals). Water stress effects in plants have been simulated using polyethylene glycol (Swapna and Shylaraj, 2017).

## METHODOLOGY



### Parameters recorded

The experiment was laid out in a complete randomized design (CRD) with five levels of drought stress and three replications. Distilled water was used as a control (0 bars). At 14<sup>th</sup> day, data were recorded on seed germination, shoot length, root length, seedling vigor index in all the PEG concentrations, and root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight were recorded at -4 bars of water potential. The mean data in each replication was subject to statistical analysis and a test of significance was calculated by two factor ANOVA using the software OPSTAT. Physiological attributes viz., soluble protein content (mg g<sup>-1</sup>), nitrate reductase activity (μmole NO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) and proline content (μg g<sup>-1</sup>) also analyzed from the fresh leaf sample at -4 bars because most of the genotypes survive only up to -4.0 bars.

### Si analysis using scanning electron microscope

Chengalpattu sirumani was selected for Si analysis to confirm the drought tolerance capacity using scanning electron microscope by the following procedure.

### Sample preparation

The rice husk was washed with water to remove dirt and other contaminants present in them and then dried in an oven at 100°C for 24 h. An adequate process of acid leaching was carried out with 3% HCl by boiling for 2 h, at a ratio of 50 g husk/L. The solution was filtered and the husk was washed with distilled water several times until the filtrate was free from acid. The acid leached husk was dried at 100°C for 12 h to remove moisture. These samples were burnt at temperature 500 for 12 h with the heating rate of 10°C per minute.

### Result

The analysis of variance revealed considerable variation among the genotypes, osmotic potential and their interaction (*Table 1*). Significant differences were observed among the genotypes concerning germination, shoot, root length, and seedling. Vigour index under different PEG concentrations (-2, -4, -6, -8 & -10 bars) under this study.

**Table 1.** ANOVA of genotypes for various characters under different levels of stress

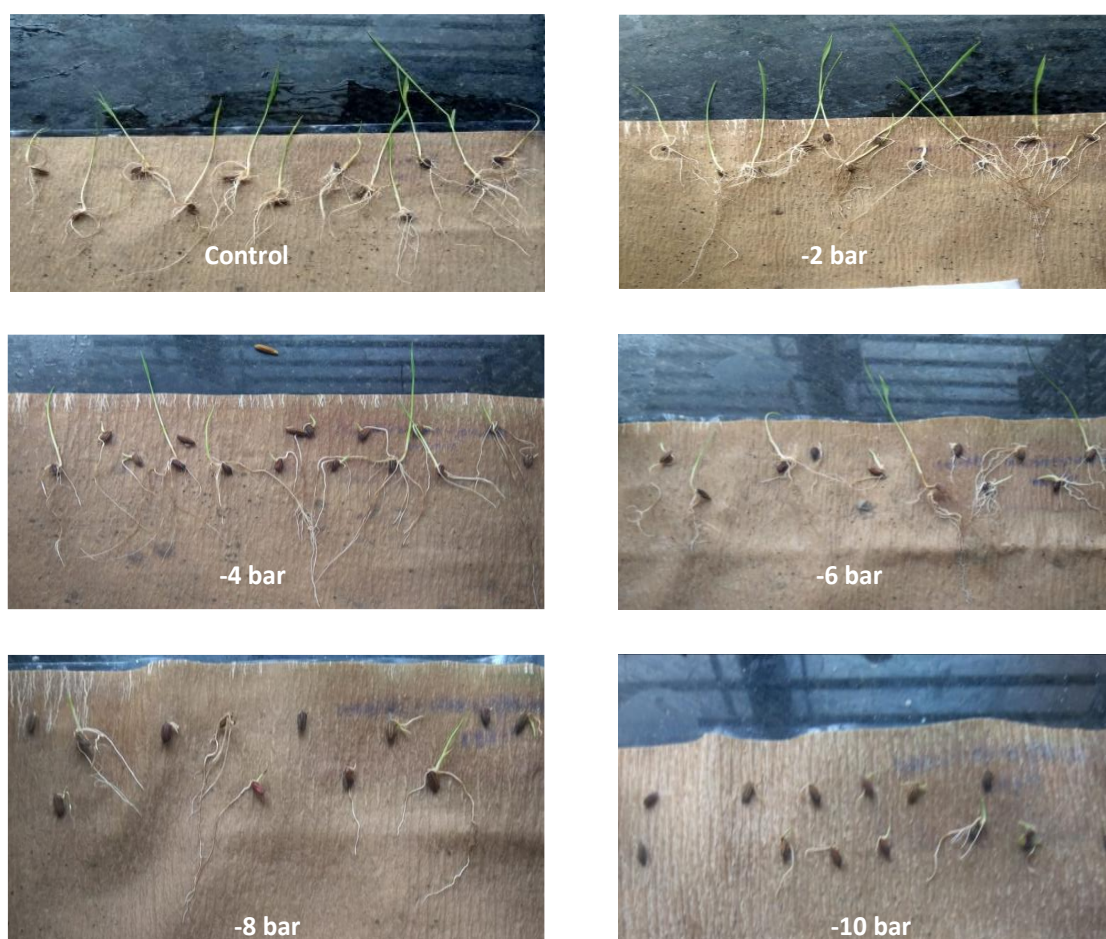
| Parameters                   | D <sub>f</sub> | Mean sum of squares |                   |                  |                      |
|------------------------------|----------------|---------------------|-------------------|------------------|----------------------|
|                              |                | Germination percent | Shoot length (cm) | Root length (cm) | Seedling vigor index |
| Genotypes (Factor A)         | 14             | 2,447.752*          | 12.822*           | 31.097*          | 47,864.121*          |
| Stress Treatments (Factor B) | 5              | 13,096.561*         | 156.240*          | 145.803*         | 1,077,354.320*       |
| Genotypes × Treatments       | 70             | 142.486*            | 3.053*            | 4.086*           | 18,333.289*          |
| Error                        | 90             | 4.159               | 0.167             | 0.178            | 1,086.626            |
| Total                        | 179            |                     |                   |                  |                      |

### Seed germination and root length

Drought stress was induced on different levels by PEG 6000 which showed a significant effect on seed germination per cent (*Table 2*; *Fig. 1*). The germination per cent ranged from (93.5% in control to 0% in -10 bars) between various landraces. Out of 15 genotypes studied, two genotypes viz., Mettumaranelu and Chengalpattu sirumani

recorded significant germination per cent (above 40%) over the mean. Most of the varieties were severely affected by the drought stress at -10 bars. Some genotypes viz., Mettumaranelu, Chengalpattu sirumani, Pisini, Karungkuruvai show gradual reduction in germination per cent with the decrease in water potential. Genotypes viz., Kattuyanam, Kalabath, Karudan samba, Mappillai samba show a severe reduction in germination per cent which could not sustain even under -4 to -6 bars of water potential.

The root is the most essential part of water absorption from the soil and makes the plant sustain transpiration. The root length is considerably declined with increasing PEG concentration (*Table 2*). In control, five genotypes showed a significant root length (above 8 cm) over the mean root length of 5.9 cm. At -2 bar, significant root length was recorded in 7 landraces, while in -4 bar and -6 bar, 5 landraces each with significant root length was observed. At -8 bar and -10 bar, significant root length was recorded in Chengalpattu sirugamani and Karungkuruvi, respectively. In case of -8 and -10 bars, the root growth was highly affected and the landraces do not show significant root length. The maximal root length observed at these bars, was only 3.5 cm in -8 bar and 2.3 cm in -10 bar (Chengalpattu sirugamani). In most of the varieties, roots were not at all emerged from -8 bar onwards. Among the landraces, Pisini recorded the maximum root length of 12.05 cm in control and 5.425 cm on an average. However, Pisini recorded significant reduction in root growth from -6 bars onwards.



**Figure 1.** Germination status of *Chengalpattu sirumani* at different stress induced by PEG concentrations

**Table 2.** Shoot length and seedling vigor index of landraces in different water potential (pooled data of two years 2021 & 2022)

| S. No | Genotypes               | Germination percent |        |        |        |        |         | Root length (cm) |        |        |        |        |         |
|-------|-------------------------|---------------------|--------|--------|--------|--------|---------|------------------|--------|--------|--------|--------|---------|
|       |                         | Treatments          |        |        |        |        |         | Treatments       |        |        |        |        |         |
|       |                         | Control             | -2 bar | -4 bar | -6 bar | -8 bar | -10 bar | Control          | -2 bar | -4 bar | -6 bar | -8 bar | -10 bar |
| 1.    | Karunguruvai            | 83.50               | 41.50  | 32.50  | 26.0*  | 19.50  | 13.00   | 8.25*            | 6.20*  | 4.55*  | 3.95*  | 1.60*  | 0.45    |
| 2.    | Mettumaranellu          | 93.50*              | 86.50* | 78.50* | 73.50* | 67.00* | 67.00*  | 3.60             | 2.15   | 2.10   | 0.95   | 0.45   | 0.00    |
| 3.    | Kasalath                | 84.50               | 13.50  | 7.50   | 7.50   | 0.00   | 0.00    | 5.85             | 1.80   | 2.10   | 0.10   | 0.00   | 0.00    |
| 4.    | Poongkar                | 83.50               | 27.00  | 21.00  | 7.00   | 7.00   | 0.00    | 2.20             | 1.85   | 1.15   | 0.90   | 0.15   | 0.00    |
| 5.    | Thooyamalli             | 82.00               | 62.00* | 43.00* | 8.00   | 8.00   | 0.00    | 3.25             | 2.75   | 3.30   | 2.70*  | 0.10   | 0.00    |
| 6.    | Illuppai poo samba      | 91.50*              | 31.00  | 20.00  | 20.00  | 7.00   | 0.00    | 7.20*            | 5.30*  | 1.65   | 0.25   | 0.10   | 0.00    |
| 7.    | Karupukavuni            | 87.50               | 28.00  | 8.00   | 3.50   | 0.00   | 0.00    | 8.05*            | 8.00*  | 6.70*  | 5.45*  | 0.00   | 0.000   |
| 8.    | Karudan samba           | 85.50               | 36.50  | 0.00   | 0.00   | 0.00   | 0.00    | 3.45             | 0.60   | 0.00   | 0.00   | 0.000  | 0.00    |
| 9.    | Rakthashali             | 83.50               | 54.00* | 34.00* | 14.00  | 7.00   | 0.00    | 6.75             | 5.90*  | 3.50   | 2.65   | 1.55*  | 0.00    |
| 10.   | Mapillai samba          | 80.50               | 6.50   | 6.50   | 0.00   | 0.00   | 0.00    | 3.85             | 2.05   | 0.25   | 0.00   | 0.00   | 0.00    |
| 11.   | Pisini                  | 86.50               | 53.00* | 46.00* | 40.00* | 33.00* | 13.50   | 12.05*           | 10.55* | 6.75*  | 2.60   | 0.45   | 0.15    |
| 12.   | Chengalpattu sirugamani | 86.00               | 81.00* | 81.00* | 59.50* | 55.00* | 40.50*  | 8.45*            | 6.25*  | 5.40*  | 4.15*  | 3.50*  | 2.30*   |
| 13.   | Mani samba              | 83.50               | 46.00* | 20.50  | 20.50  | 14.00  | 0.00    | 5.50             | 4.75   | 5.60*  | 4.00*  | 2.20*  | 0.00    |
| 14.   | Kaatuyaanam             | 82.00               | 18.50  | 0.00   | 0.00   | 0.00   | 0.00    | 6.50             | 5.65*  | 0.00   | 0.00   | 0.00   | 0.00    |
| 15.   | Kalabath                | 81.50               | 18.50  | 6.00   | 0.00   | 0.00   | 0.00    | 3.60             | 2.25   | 0.20   | 0.00   | 0.00   | 0.00    |
|       | SE (d) Factor A         | 1.147               |        |        |        |        |         | 0.172            |        |        |        |        |         |
|       | Factor B                | 0.725               |        |        |        |        |         | 0.109            |        |        |        |        |         |
|       | Factor AxB              | 2.809               |        |        |        |        |         | 0.422            |        |        |        |        |         |
|       | CD Factor A             | 2.282               |        |        |        |        |         | 0.342            |        |        |        |        |         |
|       | Factor B                | 1.443               |        |        |        |        |         | 0.217            |        |        |        |        |         |
|       | Factor AxB              | 5.589               |        |        |        |        |         | 0.839            |        |        |        |        |         |

\*Denotes significant at 5% level

### Shoot length and seedling vigor index

Out of 15 genotypes, 7 genotypes each recorded significant shoot length in control and -2 bars ranging from 10.0 cm in control to 4.35 cm in -2 bar (Table 3). Shoot length shows gradual reduction in entries viz., Karungkuruvai, Mani Samba, Chengalpattu Sirugamani and Pisini, but none of the genotypes could put forth significant shoot growth in -8 and -10 bars. Karudan Samba, Kattuyanam could not withstand even -4 bars and failed to produce shoot growth. The entries Mettumaranelu, Kasalath, Poongkar and Kalabath could not perform well even in control. These genotypes also exhibited lower root length. This may be attributed to their slow growth potential in the initial days of germination.

The present studies show that, we can easily study how the increase in PEG concentration inversely affects the seedling vigor (Table 3). In control, Pisini (865.40) recorded the highest vigor index followed by Illuppai poo Samba (735.15) and Mappillai Samba (697.45) out of the 6 genotypes that showed significant vigor index in control. However, Chengalpattu sirumani (112.60) managed to maintain significant vigor index compared to mean + 2 SE even at -6 bars and hence can be selected as the best genotype for drought tolerance. None other genotypes expressed significant vigor index even at -4 bars. The genotypes, Pisini, Mani Samba, Karungkuruvai, Illuppai poo Samba and Rathasali however recorded significant vigor index on an average and can also be considered for drought tolerance.

**Table 3.** Shoot length and seedling vigor index of landraces in different water potential (pooled data of two years 2021 & 2022)

| S. No | Genotypes               | Shoot length (cm) |        |        |        |        |         | Seedling vigor index |         |         |         |        |         |
|-------|-------------------------|-------------------|--------|--------|--------|--------|---------|----------------------|---------|---------|---------|--------|---------|
|       |                         | Treatments        |        |        |        |        |         | Treatments           |         |         |         |        |         |
|       |                         | Control           | -2 bar | -4 bar | -6 bar | -8 bar | -10 bar | Control              | -2 bar  | -4 bar  | -6 bar  | -8 bar | -10 bar |
| 1.    | Karunguruvai            | 6.55*             | 6.30*  | 2.35*  | 2.20*  | 0.75   | 0.70    | 546.55*              | 259.20* | 76.00   | 56.90   | 14.55  | 9.10    |
| 2.    | Mettumaranelu           | 1.95              | 0.30   | 0.15   | 0.13   | 0.10   | 0.10    | 182.40               | 25.95   | 11.90   | 9.22    | 6.70   | 6.70    |
| 3.    | Kasalath                | 2.55              | 0.50   | 0.15   | 0.15   | 0.00   | 0.00    | 217.40               | 6.60    | 1.10    | 1.10    | 0.00   | 0.00    |
| 4.    | Poongkar                | 2.30              | 0.65   | 0.40   | 0.10   | 0.10   | 0.00    | 192.25               | 17.70   | 8.50    | 0.70    | 0.70   | 0.00    |
| 5.    | Thooyamalli             | 5.45              | 2.55   | 1.20   | 0.15   | 0.15   | 0.00    | 448.20               | 160.00  | 51.80   | 1.25    | 1.25   | 0.00    |
| 6.    | Illuppai poo samba      | 8.00*             | 5.30*  | 0.75   | 0.70   | 0.10   | 0.00    | 735.15*              | 164.60  | 15.15   | 14.00   | 0.70   | 0.00    |
| 7.    | Karupukavuni            | 6.55*             | 6.10*  | 0.55   | 0.35   | 0.00   | 0.00    | 573.00*              | 170.20  | 4.35    | 1.20    | 0.00   | 0.00    |
| 8.    | Karudan samba           | 4.00              | 0.95   | 0.00   | 0.00   | 0.00   | 0.00    | 342.50               | 35.50   | 0.00    | 0.00    | 0.00   | 0.00    |
| 9.    | Rakthashali             | 7.55*             | 4.35*  | 1.23   | 0.75   | 0.15   | 0.00    | 630.60*              | 236.25* | 41.88   | 10.65   | 1.15   | 0.00    |
| 10.   | Mapillai samba          | 8.65*             | 3.00   | 0.15   | 0.00   | 0.00   | 0.00    | 697.45*              | 19.65   | 1.05    | 0.00    | 0.00   | 0.00    |
| 11.   | Pisini                  | 10.00*            | 6.10*  | 2.10*  | 1.10   | 0.30   | 0.20    | 865.40*              | 322.70* | 96.20   | 43.80   | 9.70   | 2.70    |
| 12.   | Chengalpattu sirugamani | 5.70              | 4.45*  | 2.95*  | 1.90*  | 0.25   | 0.15    | 488.20               | 359.40* | 238.50* | 112.60* | 13.50  | 5.90    |
| 13.   | Mani samba              | 7.95*             | 7.35*  | 2.60*  | 1.40*  | 0.80   | 0.00    | 662.55*              | 335.90* | 52.80   | 28.20   | 11.00  | 0.00    |
| 14.   | Kaatayaanam             | 6.45              | 3.35   | 0.00   | 0.00   | 0.00   | 0.00    | 528.20               | 61.85   | 0.00    | 0.00    | 0.00   | 0.00    |
| 15.   | Kalabath                | 2.40              | 1.30   | 0.70   | 0.00   | 0.00   | 0.00    | 197.60               | 23.95   | 3.90    | 0.00    | 0.00   | 0.00    |
|       | SE (d) Factor A         | 0.167             |        |        |        |        |         | 13.46                |         |         |         |        |         |
|       | Factor B                | 0.105             |        |        |        |        |         | 8.51                 |         |         |         |        |         |
|       | Factor AxB              | 0.408             |        |        |        |        |         | 32.96                |         |         |         |        |         |
|       | CD Factor A             | 0.332             |        |        |        |        |         | 26.78                |         |         |         |        |         |
|       | Factor B                | 0.210             |        |        |        |        |         | 16.94                |         |         |         |        |         |
|       | Factor AxB              | 0.813             |        |        |        |        |         | 65.60                |         |         |         |        |         |

\*Denotes significant at 5% level

### Root and shoot fresh weight

Drought stress adversely affected the fresh weights of the roots and shoots of several rice types. Table 4 shows that the increased PEG concentration caused a decrease in fresh weight of roots in all cultivars. Based on the data, it was found that the variety Chengalpattu sirugamani had the maximum root fresh weight (145.55 mg) under -8.0 bar drought circumstances, whereas Kasalath had the lowest root fresh weight (0.25 mg). Yet, at the maximum level of drought stress, Chengalpattu sirugamani (145.55 mg), Manisamba (104.7 mg), and Karunguruvai (61.3 mg) likewise fared better (Table 4). The findings presented in Table 4 showed that the lowest SFW of 0.95 was recorded in Kasalath and the highest SFW of 99.8 mg was recorded in Chengalpattu sirugamani under -8 bar drought conditions.

### Physiological parameters

The accumulation of soluble protein, nitrate reductase activity and proline in PEG treated traditional landraces was presented in Table 5. The PEG concentration affected the accumulation of soluble protein, nitrate reductase and proline in rice varieties. Chengalpattu sirugamani showed higher accumulation of soluble protein (12.15 mg g<sup>-1</sup>), nitrate reductase activity (21.25 µg NO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) and proline content (11.26 mg g<sup>-1</sup>) than other traditional landraces.

**Table 4.** Root fresh weight (mg), root dry weight (mg), shoot dry weight (mg) and shoot dry weight of landraces in -4.0 bars PEG 6000 (pooled data of two years 2021 & 2022)

| Sl. No | Genotypes               | Root                        |                           | Shoot                       |                           |
|--------|-------------------------|-----------------------------|---------------------------|-----------------------------|---------------------------|
|        |                         | Fresh weight (mg/10 plants) | Dry weight (mg/10 plants) | Fresh weight (mg/10 plants) | Dry weight (mg/10 plants) |
| 1.     | Karunguruvai            | 61.3*                       | 9.48                      | 45.15*                      | 11.6*                     |
| 2.     | Mettumaranellu          | 37.8*                       | 5.1                       | 27.65                       | 6.9                       |
| 3.     | Kasalath                | 0.25                        | 0.06                      | 0.95                        | 0.04                      |
| 4.     | Poongkar                | 1.4                         | 0.25                      | 10.6                        | 5.65                      |
| 5.     | Thooyamalli             | 2.4                         | 0.88                      | 9.8                         | 2.8                       |
| 6.     | Illuppai poo samba      | 2.85                        | 2.74                      | 8.8                         | 3.8                       |
| 7.     | Karupukavuni            | 23.6                        | 6.4                       | 15.9                        | 5.1                       |
| 8.     | Karudan samba           | 5.0                         | 1.15                      | 8.7                         | 4.85                      |
| 9.     | Rakthashali             | 0.78                        | 0.01                      | 6.7                         | 2.2                       |
| 10.    | Mapillai samba          | 0.25                        | 0.02                      | 14.55                       | 2.9                       |
| 11.    | Pisini                  | 25.85                       | 8.54                      | 47.2*                       | 7.95                      |
| 12.    | Chengalpattu sirugamani | 145.55*                     | 50.45*                    | 99.8*                       | 38.45*                    |
| 13.    | Mani samba              | 104.7*                      | 38.75*                    | 54.95*                      | 24.55*                    |
| 14.    | Kaatuyaanam             | 34.55                       | 15.6                      | 21.85                       | 6.83                      |
| 15.    | Kalabath                | 40.7*                       | 17.65*                    | 38.45*                      | 7.8                       |
|        | <b>Mean</b>             | <b>32.46</b>                | <b>10.47</b>              | <b>27.4</b>                 | <b>8.76</b>               |
|        | SEd                     | 2.11                        | 0.82                      | 1.52                        | 0.68                      |
|        | CD                      | 4.35                        | 1.69                      | 3.14                        | 1.39                      |
|        | CV                      | 7.98                        | 9.61                      | 6.81                        | 9.45                      |

\*Indicates significant at 5% level of significance

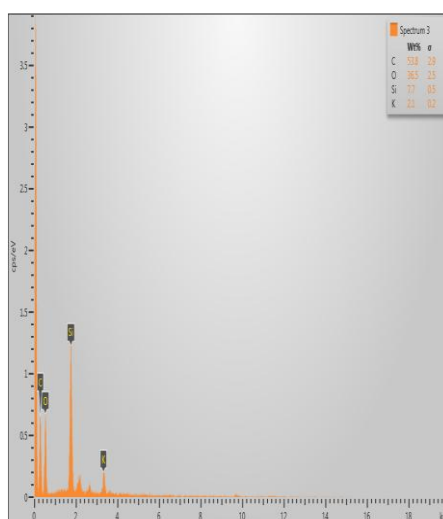
**Table 5.** Impact of drought stress on soluble protein content ( $\text{mg g}^{-1}$ ), nitrate reductase activity ( $\mu\text{g NO}_2\text{g}^{-1}\text{h}^{-1}$ ) and proline content ( $\text{mg g}^{-1}$ ) in rice landraces in -4.0 bars PEG 6000 (pooled data of two years 2021 & 2022)

| Sl. No | Rice variety            | Soluble protein ( $\text{mg g}^{-1}$ ) | Nitrate reductase activity ( $\mu\text{g NO}_2\text{g}^{-1}\text{h}^{-1}$ ) | Proline ( $\text{mg g}^{-1}$ ) |
|--------|-------------------------|----------------------------------------|-----------------------------------------------------------------------------|--------------------------------|
| 1.     | Karunguruvai            | 10.05*                                 | 17.58*                                                                      | 11.2*                          |
| 2.     | Mettumaranellu          | 8.73*                                  | 11.8                                                                        | 6.35                           |
| 3.     | Kasalath                | 4.64                                   | 11.38                                                                       | 6.32                           |
| 4.     | Poongkar                | 4.03                                   | 10.55                                                                       | 6.08                           |
| 5.     | Thooyamalli             | 3.8                                    | 10.21                                                                       | 4.56                           |
| 6.     | Illuppai poo samba      | 4.9                                    | 11.04                                                                       | 5.78                           |
| 7.     | Karupukavuni            | 3.27                                   | 9.25                                                                        | 2.4                            |
| 8.     | Karudan samba           | 2.11                                   | 8.31                                                                        | 2.71                           |
| 9.     | Rakthashali             | 2.07                                   | 7.6                                                                         | 2.29                           |
| 10.    | Mapillai samba          | 3.81                                   | 9.39                                                                        | 3.59                           |
| 11.    | Pisini                  | 9.58*                                  | 15.8*                                                                       | 9.78*                          |
| 12.    | Chengalpattu sirugamani | 12.64*                                 | 20.38*                                                                      | 11.95*                         |
| 13.    | Mani samba              | 11.03*                                 | 19.47*                                                                      | 9.98*                          |
| 14.    | Kaatuyaanam             | 8.36*                                  | 13.68                                                                       | 8.52*                          |
| 15.    | Kalabath                | 4.05                                   | 9.58                                                                        | 3.82                           |
|        | <b>Mean</b>             | <b>6.21</b>                            | <b>12.4</b>                                                                 | <b>6.36</b>                    |
|        | SEd                     | 0.40                                   | 0.87                                                                        | 0.49                           |
|        | CD                      | 0.86                                   | 1.88                                                                        | 1.06                           |
|        | CV                      | 6.4                                    | 7.01                                                                        | 7.72                           |

\*Denotes significant at 5% level

### Si analysis using SEM

Twenty percent of SiO<sub>2</sub> in hydrated amorphous form (Si–OH) is found in rice husk ash (RHA) of Chengalpattu sirumani. Thermal processing of SiO<sub>2</sub> results in the crystalline, non-reactive form known as cristobalite. On the other hand, high reactivity amorphous SiO<sub>2</sub> is created in regulated settings. X-ray diffraction was used to compare the amorphous and crystalline forms of SiO<sub>2</sub>, and the results showed that SiO<sub>2</sub> is completely amorphous. The comparative morphological characteristics of the rice husk ash of Chengalpattu sirugamani were shown in the scanning electron microscope (SEM) images (Fig. 2). The per cent of SiO<sub>2</sub> in RHA as well as its presence on the upper surface of rice leaves were ascertained using energy dispersive spectroscopy analysis. Under a scanning electron microscope, it was discovered that the aggregated SiO<sub>2</sub> particles were micron-sized.



**Figure 2.** SEM image and EDS of silica obtained by burning rice husk at 500°C for 12 h

### Discussion

Water potential was declining, which may have been caused by the environment's low hydraulic conductivity. This resulted in a decrease in the germination per cent. Because PEG blocks the seeds' access to water, it affects how much of it the seeds absorb. The higher viscosity, which decreased oxygen solubility and diffusion as compared to the control, may be the cause of the significant decrease in germination % with increasing PEG solution concentration. Seeds begin to undergo germination when their metabolism begins, and one of the most critical things to help that process along is water. The metabolic process in the seeds is disturbed and may even cease if there is insufficient water. Water is not the only factor that affects the germination rate; seed variety and shelf life also play major roles. Additional differences between seed kinds include germination times and metabolisms. As PEG concentration rose, seedling vigor declined. Tolerant seeds flourish under suboptimal land conditions due to high seedling vigor, creating an environment unsuitable for seed germination and growth. As per Khodarahmpour (2011), seeds with low vigor values tend to require less energy compared to those with high values. PEG 6000-treated rice landraces have decreased water potential, which lowers the amount of water that can be absorbed by the roots and

inhibits the sprouts' ability to produce roots. Pavli et al. (2020) reported that when stress intensity rose, seed water absorption trended downward. This disorder distracted the metabolism of seeds from their growth. One sign that rice can withstand water scarcity is the extension of plant roots in an attempt to locate water. When seeds germinate, roots known as seminal roots emerge from the radicles and are transient in nature. With the increase of PEG-6000, water potential dropped, making it more difficult for plants to absorb water. Insufficient water during the vegetative stage can slow down the rate at which leaves spread and elongate, which can impede the growth of shoots as seen by a decrease in shoot height. Due to the fact that drought stress alters growth morphology, anatomy, and physiology, PEG treatment reduces shoot length.

Rice's photosynthetic rate and biochemical processes are eventually lowered by reductions in the weights and lengths of fresh roots and shoots (Usman et al., 2013). Water stress may have an adverse effect on the development of root cells, which in turn affects the intake of nutrients and finally has an adverse effect on photosynthesis, which is necessary for the accumulation of biomass and, consequently, on the elongation of the shoots and roots. In a drought, plants cannot maintain normal development because of the massive reduction in their ability to absorb and utilize water. According to Xu et al. (2006), a plant's dry weight is an excellent indicator of its health and can be used to determine how exposed it is to various stimuli. The dry weight of the roots and shoots of various traditional landraces was affected by drought stress. A higher PEG concentration resulted in a lower root dry weight (RDW). Under controlled settings, the RDW was at its maximum, and at the highest level of drought stress, it was at its minimum. The highest root dry weight (RDW) at the highest drought level (-8.0 BAR) was observed in Chengalpattu sirugamani (50.45 mg), whereas the lowest RDW was observed in Kasalath and Mapillai samba (0.6 and 0.2 mg, respectively). Under PEG concentration settings of -8.0 bars, Chengalpattu sirugamani had the highest SDW (38.45 mg), whereas Kasalath had the lowest (0.04 mg). The capacity of a plant to absorb water is indicated by its root dry weight; plants that have a high root dry weight have larger roots and are more drought-tolerant than those that have a low root dry weight. The results of the shoot dry weight treatment with PEG 6000 at concentrations of -8.0 and -10.0 bars were found to differ in weight from the treatment without PEG 6000 (control). This can happen when there is a drought since there is less water available. A dry leaf cuticle can impair water permeability, which can slow down metabolism, protein synthesis, ion transport, and inhibit leaf cell division. Osmotic stress reduces the amount of water available to plants, which inhibits cell growth and division by decreasing turgor pressure. This results in a loss in dry mass and biomass (Farooq et al., 2015; Sagar, 2017; Roy et al., 2018). The rice seedlings' fresh and dry weights showed a drop when they were subjected to settings with higher PEG concentrations.

According to the findings, Chengalpattu sirugamani may be more resistant to drought if it has a larger proline accumulation during periods of stress. Concurrent with these findings, further researchers have confirmed the role of PEG in proline accumulation in rice leaves (Lum et al., 2014; Kadhimi et al., 2016). The proline content rose in drought-stressed conditions, which is consistent with the findings of Tatar and Gevrek (2008) and Mohammadkhani and Heidari (2008). According to Farooq et al. (2009), proline serves as an osmotic substitute for water, stores carbon and nitrogen after recovery from water stress, and stabilizes macromolecules, proteins, and cell

membranes in plant tissues. It is also the primary means by which plants fend off the damaging effects of drought.

According to Zhu and Gong's (2014) research, the Si effect varies with crop progress and becomes more prominent over time. Moreover, the number of polysaccharides in the cell wall was greater in leaves with Si than in leaves without Si.

## Conclusion

From the study, the following conclusion were drawn for selecting suitable rice landraces grown under drought condition and noticed that PEG 6000 can be used to effectively induce drought stress in order to study the effect of water stress on seed germination capacity and seedling growth parameters. For studying the drought tolerance potential of the genotypes, we have to select the genotypes that show significant germination, root length and seedling vigor index and at the same time shows sustainability/gradual reduction with the decrease in water potential. Chengalpattu sirumani expressed significant germination, seedling vigor index, root length, shoot and root fresh and dry weight, soluble protein, nitrate reductase activity and proline content with gradual reduction in decreasing water potential followed by Karunkuruvai and Pisini. Mettumaranelu topped the traditional landraces in germination per cent but failed to put forth significant shoot or root growth and hence showed a poor vigor index. This method can be used to screen large numbers of varieties within short span of time. The results of the study revealed that PEG applied drought stress reduces the seed germination per cent, shoot and root length, seedling vigor index in different landraces of rice. Hence could not be considered as drought tolerant. In order to study this topic deeply, undertaking molecular techniques is encouraged to know their genomic composition which confers tolerance to drought stress.

## REFERENCES

- [1] Adkins, S. W., Kumanuvatchaidach, R., Godwin, I. D. (1995): Somaclonal variation in rice-2 drought tolerance and other agronomic characters. – Australian Journal of Botany 43(2): 201-209.
- [2] El-Tayeb, M. A., Hassanein, A. (2000): Germination, seedling growth, some organic solutes and peroxidase expression of different *Vicia faba* lines as influenced by water stress. – Acta Agronomica Hungarica 48(1): 11-20.
- [3] Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. M. A. (2009): Plant drought stress: effects, mechanisms and management. – Agronomy for Sustainable Development 29: 185-212.
- [4] Farooq, M., Hussain, M., Usman, M., Farooq, S., Alghamdi, S. S., Siddique, K. H. M. (2018): Impact of abiotic stresses on grain composition and quality in food legumes. – J Agric Food Chemistry 66: 8887-8897.
- [5] Gagendra Singh, R., Kuruwanshi, V. B., Arti, G. (2022): Polyethylene glycol induced screening for drought tolerance of different rice genotype. – The Pharma Innovation Journal 11(9): 996-1000.
- [6] Kadhim, A. A., Zain, C. R. C. M., Alhasnawi, A. N., Isahak, A., Ashraf, M. F., Mohamad, A., Doni, F., Yusoff, W. M. W. (2016): Effect of irradiation and polyethylene glycol on drought tolerance of MR269 genotype rice (*Oryza sativa* L.). – Asian Journal of Crop Science 8: 52-59.

- [7] Khodarahmpour, Z. (2011): Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrids. – African Journal of Biotechnology 10: 18222-18227.
- [8] Levitt, J. (1980): Responses of Plant to Environmental Stress: Water, Radiation, Salt and Other Stresses. – Academic Press, New York.
- [9] Lum, M., Hanafi, M., Rafii, Y., Akmar, A. (2014): Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. – Journal of Animal and Plant Sciences 24: 1487-1493.
- [10] Mishra, S. S., Panda, D. (2017): Leaf traits and antioxidant defense for drought tolerance during early growth stage in some popular traditional rice landraces from Koraput, India. – Rice Sci. 24(4): 207-217. DOI: 10.1016/j.rsci.2017.04.001.
- [11] Mohammadkhani, N., Heidari, R. (2008): Water stress induced by polyethylene glycol 6000 and sodium chloride in two maize cultivars. – Pakistan Journal of Biological Science 11(1): 9297.
- [12] Nepomuceno, A. L., Oosterhuis, D. M., Stewart, J. M. (1998): Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. – Environmental and Experimental Botany 40(1): 29-41.
- [13] Panda, D., Mishra, S. S., and Behera, P. K. (2021): Drought tolerance in rice: focus on recent mechanisms and approaches. – Rice Science 28(2): 119-132.
- [14] Pavli, O. I., Foti, C., Skoufogianni, G., Karastergiou, G., Panagou, A. M., Khah, E. (2020): PEG-induced drought stress during germination: effects on soybean germplasm. – Agricultural Research and Technology 23: n556250.
- [15] Roy, R. C., Sagar, A., Tajkia, J. E., Razzak, M. A., Hossain, A. K. M. Z. (2018): Effect of salt stress on growth of sorghum germplasms at vegetative stage. – Journal of Bangladesh Agricultural University 16: 67-72.
- [16] Sagar, A. (2017): Screening of sorghum germplasms for salinity tolerance based on morphophysiological and biochemical traits. – MSc. Thesis, Bangladesh Agricultural University, Bangladesh.
- [17] Tatar, O., Gevrek, M. N. (2008): Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. – Asian Journal of Plant Science 7: 409-412.
- [18] Usman, M., Raheem, Z., Ahsan, T., Iqbal, A., Sarfaraz, Z. N., Haq, Z. (2013): Morphological, physiological and biochemical attributes as indicators for drought tolerance in rice (*Oryza sativa* L.). – European Journal of Biological Sciences 5(1): 23-28.
- [19] Wapna, S., Shylaraj, K. S. (2017): Screening for osmotic stress responses in rice varieties under drought condition. – Science Direct Rice Science 24(5): 253-263.
- [20] Xu, S. G., Wang, J. H., Bao, L. J. (2006): Effect of water stress on seed germination and seedling growth of wheat. – Journal of Anhui Agricultural Science 34: 5784-5787.
- [21] Zhu, Y., Gong, H. (2014): Beneficial effects of silicon on salt and drought tolerance in plants. – Agronomy Substantiable Development 34: 455-472.