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# Rapid Screening of Small Millet Varieties for Seedling Stage Drought Stress Tolerance

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author NF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RR designed and supervised the study, proof reading and editing of manuscript. Author KI managed the statistical analyses of the study. Authors MR, TC, AS Inputs on designingthe study and interpretation of results. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** Drought is one of the most important abiotic stresses that affect the yield of crops globally. The present investigation was conducted to identify small millet genotypes tolerant to seedling stage drought stress.
**Study Design:** The experiment was laid out in Completely Randomized Design (CRD) with three replications with genotypes and stress treatments as factors.
**Place and Duration of Study:** It was carried out at Department of millets, Centre for plant breeding and genetics, Tamil Nadu Agricultural University, Coimbatore, during 2019.

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**Methodology:** Ten varieties of various small millets, CO 7 (foxtail millet), CO 4 and ATL 1 (little millet), CO 15 and CO 9 (finger millet), ATL 1 and CO (PV) 5 (proso millet), MDU 1 and CO 2 (barnyard millet) and CO 3 (kodo millet), were used for the study. *In vitro* screening of the seedlings in Polyethylene Glycol (PEG)-induced water stress at four levels (0, -3, -5 and -7 bars) were carried out based on germination percent, shoot and root length, plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and seedling vigour index (SVI).
**Results:** Analysis of variance of the genotypes and PEG treatments revealed significant variation for genotypes, treatments and genotype x treatment interactions at $P < 0.001$. A declining trend for germination percent, shoot length and root length was observed as the stress levels were increased. However, at mild and moderate stress root length was slightly increased. Under mild (-3 bars) and high stress (-7 bars), CO 7 (foxtail millet) recorded the highest SVI percent over control values (165% and 65% respectively). Under moderate stress CO 4 (little millet) recorded the highest SVI (191%). The lowest SVI values under high stress, 4% and 8%, were recorded for ATL 1 (little millet) and CO 3 (kodo millet) respectively.
**Conclusion:** Based on *invitro* screening of small millet varieties for seedling stage water stress**,** foxtail millet variety CO 7 and kodo millet variety CO 3 can be concluded as the tolerant and susceptible varieties respectively. Further a controlled field experiment may be carried out to understand the field level tolerance of the varieties and their growth stages to drought.

*Keywords: Small millets; drought; PEG; seedling vigour index.*

## 1. INTRODUCTION

Small millets are a group of small-grained cereals which are cultivated in arid and semi-arid regions as rainfed crops. They are unique due to their short duration, low water requirement, ability to grow on marginal lands and has comparable or even superior nutritional composition to common cereals like rice, wheat and maize. Small millet crops mainly cultivated in India are Finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinocloa* sp.), kodo millet (*Paspalum scrobiculatum*) and little millet (*Panicum sumatrense*) [1]. Though India is the largest producer of millets in the world, it produces only 2.4 million tonnes of small millets annually [2,3].The changing climate scenario with frequent occurrences of drought and low water availability for cultivation have increased the importance of small millets in agricultural production system.

Abiotic stresses that adversely impact plant growth is reported to decline the crop yields by 50% [4]. Drought is one of the most important abiotic stresses that affect the yield of crops globally [5].The detrimental effect of drought on growth and yield of the crops also varies with the growth stage of the crops. Identifying crops and genotypes that tolerate drought is necessary to meet the food demand. However, creating regulated field experiments is extremely difficult and expensive. Hence, *in vitro* screening methods are being widely used for drought screening [6]. Polyethylene Glycol (PEG) is a chemical that can mimic conditions of drought by reducing the water potential of the growing solution thereby decreasing the water uptake. The large molecular weight of PEG prevents it from getting absorbed by the plant cells and thus does not interfere with the experiment. Though millets are drought tolerant, identifying more tolerant crops among them and improving their genetic potential further is important. Hence, in the present study *invitro* screening of small millet varieties was done using Polyethylene Glycol (PEG-6000) induced drought stress to identify genotypes tolerant to seedling stage water stress.

## 2. MATERIALS AND METHODS

The experiment was conducted at Department of millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Ten small millet varieties popularly grown in Tamil Nadu were used for the present study. These ten varieties included two varieties each of finger millet, barnyard millet, proso millet, little millet and one variety each for kodo millet and foxtail millet. The seeds of the varieties were obtained from Department of millets, Coimbatore and Centre of Excellence in Millets, Athiyanthal of Tamil Nadu Agricultural University. Details of the varieties and stress treatments used are given in Table 1.

**Table 1. Details of genetic material and stress treatment levels imposed for drought screening**

| **Genotypes** | | **Treatments** |
|---|---|---|
| **Crop** | **Variety** | **Stress levels** |
| Foxtail millet | CO 7 | |
| Little Millet | CO 4 | |
| Little Millet | ATL 1 | Control (0 bar) |
| Finger Millet | CO 15 | Mild stress (-3 bars) |
| Finger Millet | CO 9 | Moderate stress (-5 bars) |
| Proso Millet | ATL 1 | High stress (-7 bars) |
| Proso Millet | CO (PV) 5 | |
| Barnyard Millet | MDU 1 | |
| Barnyard Millet | CO 2 | |
| Kodo Millet | CO 3 | |

The experiment was laid out in Completely Randomized Design (CRD) with three replications with genotype and PEG treatments as factors. PEG treatments were imposed at four different concentrations that gave four levels of osmotic stress i.e., 0 (control, only distilled water was used), -3, -5 and -7 bars. Seeds were surface sterilised using 0.1 % of sodium hypochlorite solution, washed thrice using sterile water and dried on tissue paper. Whatman no.1 filter papers cut as circular discs were placed in 90 mm petri plates. Five ml of distilled water (0 bar) or PEG solutions (-3, -5 and -7 bars) was added to each petri plate. Ten seeds per replication were placed and the plates were sealed using a cling film. Seeds with 0.5 mm growth of plumule and radicle were considered as germinated. Germination percentage, shoot length and root length were recorded on 10 DAS. These observations were used to calculate shoot to root ratio (R/S ratio), seedling vigour index (SVI), seedling vigour index (% over control), and drought stress tolerance indices like plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI). The formulas used are given below: [7,8]

1. Germination percentage, GP (%) = $\frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} x100$
2. Seedling length, SDL (cm) = $shoot\ length + root\ length$
3. Root shoot ratio, R/S ratio = $\frac{shoot\ length}{root\ length}$
4. Seedling Vigour Index, SVI = $germination\ \%\ x\ seedling\ length$ (cm)
5. Seedling Vigour Index (% Over Control) = $\frac{SVI\ of\ stressed\ plant}{SVI\ of\ control} x100$
6. Plant Height Stress Tolerance Index (PHSI) (%) = $\frac{shoot\ length\ of\ drought\ stressed\ plant}{shoot\ length\ of\ control\ plant} x100$
7. Root Length Stress Tolerance Index (RLSI) (%) = $\frac{root\ length\ of\ drought\ stressed\ plant}{root\ length\ of\ control\ plant} x100$

Analysis of variance (ANOVA) was carried out to find if significant difference exists between treatments, genotypes and genotype x treatment interactions for germination percent, shoot length, root length, seedling length, seedling vigour index and root to shoot ratio. Mean values were compared using Tukey's test for pair-wise mean comparison ($P$ = 0.05). All the statistical analysis was carried out using the analytical software SAS® OnDemand for Academics.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of PEG Induced Drought Stress on Germination and Growth of Seedlings

Drought being a major problem impacting crop growth and yield, understanding the variation in tolerance of genotypes is important. Analysis of variance (ANOVA) was carried out to understand the significance of variance for the seedling traits among small millet varieties under stress. Significant difference was observed for genotypes, treatments and genotype x treatment interactions for all the seedling parameters i.e., germination percentage, shoot length, root length, seedling length, root to shoot ratio and seedling vigour index (Table 2). This implies that there is considerable variation among the small millet crops and between their respective varieties. Similar genotypic variations for drought tolerance under PEG induced screening have been reported in pearl millet varieties and advanced breeding lines [9].

**Table 2. ANOVA for seedling traits under drought stress for small millet varieties**

| Source | df | GP | SL | RL | SDL | SVI | R/S ratio |
|---|---|---|---|---|---|---|---|
| Genotype | 9 | 746.09** | 33.62** | 61.01** | 119.44** | 1130536.46** | 3.30** |
| Treatment | 3 | 6571.09** | 56.74** | 78.77** | 264.20** | 3754808.43** | 0.30** |
| G x T | 27 | 550.62** | 7.69** | 8.52** | 14.95** | 149883.63** | 0.90** |
| Error | 72 | 4.43 | 0.033 | 0.03 | 0.06 | 873.26 | 0.01 |

******** *significant at P<0.001, GP; Germination Percent, SL; Shoot Length, RL; Root Length, SDL; Seedling Length, SVI; Seedling Vigour Index, R/S ratio; Root to Shoot Ratio*

**Table 3. Mean comparison of seedling traits of stress treatments among small millet varieties**

| Stress levels | GP | SL | RL | SDL | SVI | R/S ratio |
|---|---|---|---|---|---|---|
| Control | $100.00^{a}$ | $5.36^{a}$ | $5.63^{a}$ | $10.99^{a}$ | $1099.46^{a}$ | $1.27^{a}$ |
| Mild stress | $96.63^{b}$ | $5.41^{a}$ | $6.77^{b}$ | $12.17^{b}$ | $1180.61^{b}$ | $1.39^{b}$ |
| Moderate stress | $89.98^{c}$ | $5.46^{a}$ | $6.22^{c}$ | $11.64^{c}$ | $1055.10^{c}$ | $1.35^{b}$ |
| High stress | $67.13^{d}$ | $2.66^{b}$ | $3.10^{d}$ | $5.76^{d}$ | $411.84^{d}$ | $1.16^{ab}$ |

*Mean values followed by different letters indicate significant difference at P = 0.05, as per Tukey's test for pair-wise mean comparison. GP; Germination Percent, SL; Shoot Length, RL; Root Length, SDL; Seedling Length, SVI; Seedling Vigour Index, R/S ratio; Root to Shoot Ratio*

The results of the study showed an overall decline in the mean values of the traits as the PEG treatment levels was increased (Table 3). Germination percentage of all the genotypes declined with increase in levels of drought stress. In the present study, under mild stress maximum germination percentage (100 %) was recorded for CO 7 (foxtail millet), CO 4 (little millet), ATL 1(little millet) and CO 2 (Barnyard millet). The minimum germination (94 %) was observed in finger millet variety CO 9, proso millet varieties ATL 1 and CO (PV) 5 and barnyard millet variety MDU 1. Under moderate and high drought stress maximum germination (100 % and 94 % respectively) was observed in variety CO 4 (little millet). Minimum germination under moderate stress and high stress (79 % and 7 % respectively) was obtained in variety ATL 1 (little millet).

Shoot length exhibited a reducing trend with increase in levels of stress in seven out of ten varieties under all stress levels. However, in little millet varieties increase in shoot length over control was noted at mild and moderate stress levels. Similarly, for proso millet variety CO (PV) 5 shoot grew longer than control at mild stress. This might be due to the plants attempt to grow quickly and shorten the life cycle to escape drought. More studies into the drought response mechanism of these crops are required to understand further. For shoot length at mild stress, maximum (8.35 cm) and minimum (2.67 cm) values were observed in CO 2 (barnyard millet) and CO 9 (finger millet) respectively. Under moderate stress treatment maximum (13.25 cm) and minimum (2.17 cm) length of shoot was recorded in CO 4 (little millet) and CO 9 (finger millet) respectively. At high stress treatment maximum (4.52 cm) and minimum (1.24 cm) shoot length was exhibited by CO 2 (barnyard millet) and CO 3 (kodo millet) respectively.

Root length increased over control at mild stress in seven out of ten varieties. Similarly, six varieties exhibited increase in root length at moderate stress level. Increase in root length is an adaptation of plants in response to drought and helps them penetrate deeper to absorb water. Similar findings have been reported previously. PEG induced drought stress in pearl millet genotypes was found to reduce germination percent and shoot length but the root length has slightly increased [10]**.** Mukami et al. [11] also reported decrease in germination percent and shoot length but increase in root length in finger millet varieties evaluated under drought stress. For root length, under mild stress maximum (12.13 cm) and minimum (1.65 cm) values were recorded for MDU 1 (barnyard millet) and CO 3 (kodo millet) respectively. Maximum (11.87 cm) and minimum (1.56 cm) values for moderate stress was exhibited by CO 2 (barnyard millet) and CO 3 (kodo millet) respectively. Under high level of stress, maximum (7.33 cm) and minimum (0.72 cm) root length was obtained for MDU 1 (barnyard millet) and CO 9 (finger millet) respectively. In case of seedling length under mild and moderate stress levels, maximum (18.25 cm and 20.03 cm respectively) and minimum (7.78 cm and 6.4 cm respectively) seedling length was recorded for CO 2 (barnyard millet) and CO 3 (kodo millet).

Maximum (10.6 cm) and minimum (2.68 cm) length of seedlings under high stress level was recorded for MDU 1 (barnyard millet) and CO 3 (kodo millet) respectively. Root to shoot ratio indicates the length of root for corresponding length of shoot in each genotype. Higher the ratio indicates greater root length for the shoot length which is a beneficial adaption under drought. Under mild stress, the maximum (2.52) and minimum (0.27) R/S ratio was estimated for CO 15 (finger millet) and CO 3 (kodo millet) respectively. Under moderate stress maximum (2.20) and minimum (0.32) value for the trait was obtained for CO15 (finger millet) and CO 3 (kodo millet) respectively. High stress treatment gave maximum (2.24) and minimum (0.36)root to shoot ratio in MDU 1 (barnyard millet) and finger millet (CO 9) respectively. The results are substantiated by the findings of Zhang et al. [12]**.** Their study found correlation between root to shoot ratio and drought tolerance in foxtail millet and used the trait for identification of resistant varieties.

## 3.2 Stress Tolerance Index and Seedling Vigour Index

The effect of drought stress on the germination and growth of various small millet varieties were compared using stress tolerance indices like plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and seedling vigour index (SVI as percentage over control) (Table 4). Theses indices gives a clear understanding of the detrimental effect of drought on the genotypes in comparison to its non-stressed or control plant. The tolerance and vigour indices are better than direct comparison of the measured traits as they consider the inherent potential of the genotypes when grown under optimum conditions. They have been used in a similar study in maize and pearl millet genotypes to identify tolerant lines [7,13]. Plant Height Stress Tolerance Index shows the impact of drought treatments on the height of plant. Under mild stress maximum (170%) and minimum (70%) PHSI values were obtained for proso millet variety CO (Pv) 5and kodo millet variety CO 3respectively. Under moderate stress treatment most tolerant genotype for shoot growth was little millet variety CO 4 (263%) and most susceptible was Kodo millet variety CO 3 (55%). Under high stress treatment, most tolerant was finger millet variety CO 9 (73%) and most susceptible was Kodo millet variety CO 3 (14%). Root Length Stress Tolerance Index indicates the effect of drought on the root length of the plant. Root growth of foxtail millet variety CO 7 was found to be most tolerant under mild (297%), moderate (309%) and high (134%) levels of PEG treatments. Root growth of Proso millet variety ATL 1was found to be most impeded under mild (64%) and moderate (56%) stress treatments. Whereas under high stress, root growth of finger millet variety CO 9 (8%) was most inhibited. Similar findings of inhibition of root growth during seedling stage drought has been reported in proso millet by Yan et al. [14].

**Table 4. Stress tolerance and vigour indices of various small millet varieties under drought stress**

| Stress levels/ Genotypes | Foxtail Millet | Little Millet | | Finger Millet | | Proso Millet | | Barnyard Millet | | Kodo Millet |
|---|---|---|---|---|---|---|---|---|---|---|
| | **CO 7** | **CO 4** | **ATL 1** | **CO 15** | **CO 9** | **ATL 1** | **CO(PV)5** | **MDU 1** | **CO 2** | **CO 3** |
| **a) Plant Height Stress Tolerance Index (PHSI)** | | | | | | | | | | |
| Mild stress | 93 | 133 | 116 | 87 | 98 | 91 | 170 | 97 | 99 | 70 |
| Moderate stress | 89 | 263 | 106 | 82 | 79 | 88 | 82 | 96 | 97 | 55 |
| High stress | 52 | 64 | 70 | 63 | 73 | 34 | 57 | 57 | 54 | 14 |
| **b) Root Length Stress Tolerance Index (RLSI)** | | | | | | | | | | |
| Mild stress | 297 | 121 | 117 | 103 | 78 | 64 | 116 | 149 | 149 | 73 |
| Moderate stress | 309 | 114 | 117 | 85 | 57 | 56 | 98 | 112 | 179 | 68 |
| High stress | 134 | 39 | 55 | 33 | 8 | 44 | 36 | 90 | 82 | 64 |
| **c) Seedling Vigour Index (% over control)** | | | | | | | | | | |
| Mild stress | 165 | 127 | 117 | 94 | 78 | 69 | 130 | 120 | 121 | 66 |
| Moderate stress | 158 | 191 | 88 | 76 | 55 | 64 | 85 | 91 | 112 | 52 |
| High stress | 69 | 50 | 4 | 39 | 15 | 28 | 30 | 61 | 56 | 8 |

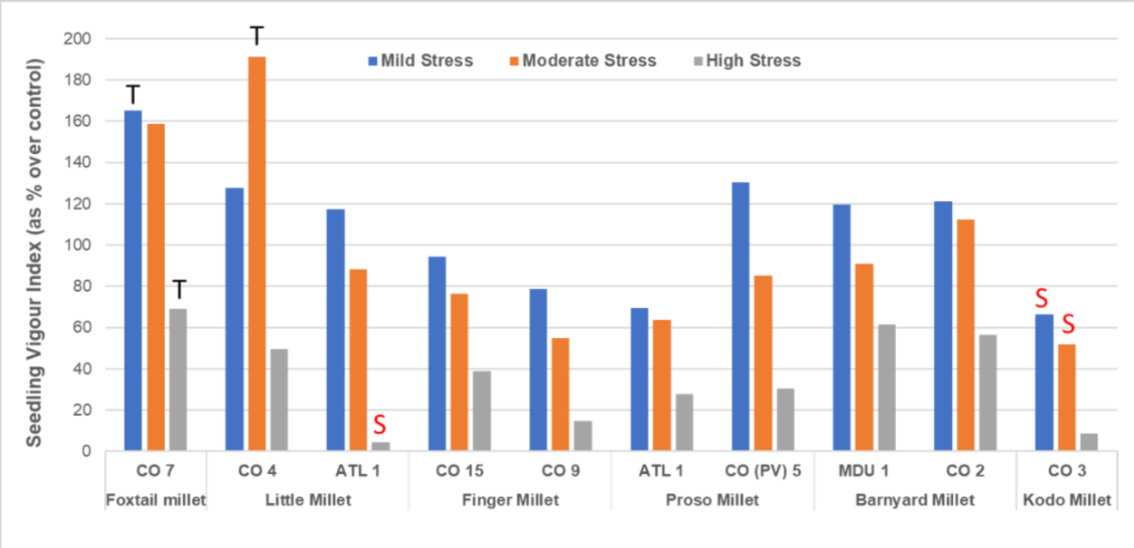


**Fig. 1. Seedling vigour index (as % over control) to identify tolerant and susceptible small millet varieties**

**T- tolerant S- susceptible, Microsoft® Excel® (Version 2110)*

Seedling vigour index calculation includes all the actual parameters observed in the present study i.e., shoot length, root length and germination percent. SVI as percentage over control values of the respective genotypes gives as fairer comparison of vigour of the seedling parameters under various levels of stress (Fig. 1). This index has thus been used to select the most tolerant and susceptible small millet varieties under stress. According the SVI (% over control) values, the most tolerant small millet variety under mild stress treatment is foxtail millet CO 7 (165 %) and most susceptible is kodo millet variety CO 3 (66). Under moderate stress level, little millet variety CO 4 (191 %)followed by foxtail millet variety CO 7 (158) is the most tolerant varieties and kodo millet variety CO 3 is the most susceptible (52 %). However, under high stress level maximum seedling vigour was recorded for foxtail millet variety CO 7 (69 %) and minimum seedling vigour was for little millet variety ATL 1 (4 %) very closely followed by kodo millet variety CO 3 (8 %).

## 4. CONCLUSION

The present study revealed that seedling stage drought stress reduced seed germination, seedling growth and vigour in the small millet varieties. The tolerance of genotypes varied with the stress level imposed and severe reduction of growth was observed at high stress. The findings of this *invitro* seedling stage screening, indicate varieties CO 7 (foxtail millet) and CO 3 (kodo millet) as tolerant and susceptible varieties respectively. Under moderate stress, variety CO 4 (little millet) showed highest tolerance. Under high stress, varieties ATL 1 (little millet) and CO 3 (kodo millet) exhibited drastic reduction in vigour. Further field level evaluations can throw more light into the tolerance potential of these genotypes and genetic mechanisms responsible. Though, small millets are relatively drought tolerant to common cereals like rice and wheat, variations exist among different small millets and between varieties of the same millet for their tolerance to drought. Identifying the critical stages of growth to drought and improving the tolerance of the small millet varieties can significantly increase the productivity of the crop under water stress.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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