

Original Research Article

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Screening of Sunflower (*Helianthus annuus* L) Genotypes for Moisture Stress Tolerance using PEG-6000

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ABSTRACT

An *in-vitro* experiment was conducted using Polyethylene glycol-6000 (PEG-6000) at MARS, UAS, Raichur, to identify genotypes which overcome moisture stress condition at germination and seedling establishment stage of Sunflower based on stress tolerance index. A total of 160 sunflower genotypes in two replications were screened in petri-dishes at six water stress levels (0, 10, 15, 20, 25 and 30% of PEG-6000) induced by PEG-6000 in two factorial CRD fashion. The germination percentage, shoot length, root length and seedling length were recorded on seven days after of sowing, later these observations are converted into the germination stress tolerance index (GTI), root length stress tolerance index (RLTI), plant height stress tolerance index (PHTI) and seedling length stress tolerance index (SLTI). The results of analysis of variance showed that, all treatments, genotypes and G X T interactions were significant for all the studied traits. The treatment means indicated that GTI, RLTI, PHTI and SLTI are drastically reduced with the increased percentage of PEG. The genotype x treatment interactions at 20 and 25% PEG few genotypes exhibited significant differences for studied traits, hence, these concentrations are considered as critical doses to isolate the real moisture tolerant genotypes. Based on *per se* mean performance for GTI, RLTI, PHTI and SLTI, there are 34 promising genotypes were identified as a moisture stress tolerant genotypes.

Keywords

PEG-6000,
GTI,
RLTI,
PHTI and
SLTI

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Introduction

The sunflower (*Helianthus annuus*) is an annual oilseed crop in the family *Asteraceae* and it is valued and healthy vegetable oil. Sunflower plant growth and productivity is limited by several biotic and abiotic stresses. Among the abiotic stresses, moisture stress or drought is most important stress, which affecting germination, seedling establishment, flowering and seed filling stages of sunflower. Seed germination is the most sensitive stage

to moisture stress in the plant life cycle (Ashraf and Mehmood, 1990) and it is foremost stage to maintain adequate population of the crop for obtaining prominent yield, in order to overcome such situation. Hence, an experiment undertook to identify genotypes which are suitable to grow under moisture stress conditions through germination test under laboratory condition using PEG-6000.

PEG-6000 is moisture stress inducing agent having a molecular weight of 6000. Using PEG-6000 Ahmad *et al.*, (2009) imposed drought stress at germination and seedling growth stages at five water stress levels and identified the two best stress tolerant sunflower genotypes viz., G-101 and 64-A-93 from *in vitro* response of PEG-6000. PEG based screening of genotypes for moisture stress tolerance is a complementary method to field screening (Ahmad, 2009 and Geetha *et al.*, 2012) because it overcome the difficulties of field trails like uncontrolled climatic conditions, heterogeneity of soil, large number of germplasm and labor consumption. Turhan and Baser (2004) also opined that *in vitro* approach could be useful in screening and selecting for drought response prior to field trial. Hence, in the present investigation PEG-6000 used to develop moisture stress under *in vitro* condition mainly to study the response of genotypes to moisture stress condition.

Materials and Methods

In vitro screening of genotypes is an alternate and simple method to screen large number of genotypes by way of limited space and in this experiment, a total 160 genotypes (12 maintainers, 12 restorers and 136 germplasm

lines) collected from Indian Institute of Oilseed Research, Hyderabad were tested for moisture stress response at MARS, UAS, Raichur, Karnataka. PEG-6000 is a water stress stimulator and there are six moisture stress treatments of 0 (control), -0.6 (10 %), -0.9 (15 %), -1.2 (20 %), -1.5 (25 %) and -1.8 (30 %) M Pa was imposed by dissolving 0, 10, 15, 20, 25 and 30 g of PEG-6000 in 100 ml of distilled water. Two replications were maintained for each osmotic potential and the experiment was laid out in two factorial-CRD fashion. Twenty seeds of each genotype were rinsed and soaked separately for ten minutes using distilled water. The seeds were kept on the germinating paper in each petri plate, and then treated ten ml of designated treatment solution was applied daily in each Petri plate after washing out the previous solution, separately.

The germination percentage, root length, shoot length and seedling length were recorded on five randomly selected plants seven days after sowing and from these measurements, germination stress tolerance index, root length stress tolerance index, plant height stress tolerance index and seedling length stress tolerance index were calculated using the following formulae given by Ashraf *et al.*, (2006).

$$\text{Germination stress tolerance index (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

$$\text{Root length stress tolerance index} = \frac{\text{Root length stressed plants}}{\text{Root length of control plants}} \times 100$$

$$\text{Plant height stress tolerance index} = \frac{\text{Plant height of stressed plants}}{\text{Plant height of control plants}} \times 100$$

$$\text{Seedling length stress tolerance index} = \frac{\text{Seedling length of stressed plants}}{\text{Seedling length of control plants}} \times 100$$

Results and Discussion

The analysis of variance for the data on 160 sunflower genotypes under laboratory screening using PEG-6000 is presented in the Table 1. The mean sum of squares of analysis of variance exhibited that, all the treatments, genotypes and genotypes x treatment interactions are found to be highly significant for all the studied characters under laboratory condition, this indicates that sunflower germplasm differed significantly for their responses in used in the study. Highly Significant differences were reported by Ahmad *et al.*, (2009) for GSI, PHSI and RLSI in the ANOVA.

The obtained treatment mean effects for characters are presented in Table 2. The significant differences were noticed for most of the studied characters at 10 and 15 per cent concentration of PEG-6000. At 20 per cent of PEG-6000 concentration, significant differences observed for GSI and RLSI. At 25 and at 30 per cent PEG-6000 concentration, none of the character found significant. The overall treatment means effects indicated that GSI, RLSI, PHSI and SLSI drastically reduced with increased concentrations of PEG-6000. These results are accordance with Ahmad *et al.*, (2009) and Saensee (2011) for GSI, PHSI and RLSI.

The results of the genotype x treatment interaction of 160 sunflower genotypes under laboratory evaluation are presented in Table 3. The *per se* performance of interaction effect of 160 sunflower genotypes are screened at 0, 10, 15, 20, 25 and 30 per cent concentration of PEG-6000. The results revealed that at 10 and 15 per cent concentrations (data not shown) majority of the genotypes showed significant mean *per se* performance. At 30 per cent PEG-6000 concentration (data not shown) majority of the genotypes could not survive and could not

exhibit significant *per se* performance for many of the characters and this concentration acted as lethal doses. However, few genotypes could express significant difference for studied traits at 20 and 25 per cent of PEG-6000, and are presented in Table 4.

The effect of water stress created by PEG-6000 indicated that germination stress tolerance index is varied among 160 genotypes and it is ranged from 0 to 100. At 20 per cent concentration 106 out of 160 genotypes showed significant *per se* performance, while, at 25 per cent concentration 28 genotypes were found to be significant and highest GSI were recorded for GP₆-2255 and GP₆-305 (95.00 %). Among CMS lines, CMS 857B, among restorer lines, R-12-2, R-78 and among germplasm lines GP₆-11, GP₆-54, GP₆-118, GP₆-211, GP₆-305, GP₆-310, GP₆-326, GP₆-366, GP₆-370, GP₆-371, GP₆-424, GP₆-442, GP₆-578, GP₆-614, GP₆-714, GP₆-863, GP₆-912, GP₆-967, GP₆-969, GP₆-1060, GP₆-1072, GP₆-1102, GP₆-1228, GP₆-1576 and GP₆-2255 exhibited significant *per se* performance.

The RLSI under all PEG concentrations worked as per the formula given in the materials and methods and it is ranged from 0 to 744.41. At 20 per cent concentration indicated 43 out of 160 genotypes were showed significant *per se* performance, while, at 25 per cent concentration six genotypes found to be significant and the highest was recorded for CMS 857B (417.58 %). Among CMS lines, CMS 104B and CMS 857B, among germplasm lines, GP₆-325, GP₆-586, GP₆-714 and GP₆-589 exhibited significant *per se* performance. And among restorer lines, none of the lines exhibited significant effects. The increased root length stress index with increased level of PEG-6000 was observed upto 15 % concentrations but at higher concentration (20-30 %) observed the decreased performance, these results

accordance with reports of Ahmad *et al.*, (2009) and Saense *et al.*, (2012).

The PHSI is also varied among 160 sunflower genotypes studied and it was ranged from 0 to 93.72. The plant height stress tolerance index at 20 per cent concentration indicated 11 out of 160 genotypes showed significant *per se* performance, while at 25 per cent concentration none of the genotypes are found to be significant because the shoot length most affected apart from germination and root length. The PHTI decreased with increased PEG-6000 concentrations, these results accordance with reports of Ahmad *et al.*, (2009) and Saense *et al.*, (2012).

The Seedling length stress tolerance index (SLTI) at 20 and 25 per cent concentration of PEG-6000 was also worked out and it is ranged from 0.00-282.85. At 20 per cent concentration indicated 43 out of 160 genotypes were showed significant *per se* performance, while, at 25 per cent concentration six genotypes found to be significant and top performing genotype is CMS 857B (137.45%). Among CMS lines, CMS 104B and CMS 857B, among germplasm lines, GP6-325, GP6-586, GP6-589 and GP6-714 exhibited significant *per se* performance. And among restorer lines, none of the lines exhibited significant effects.

Table.1 Analysis of variance for eight different characters in sunflower under laboratory evaluation using PEG-6000

| Source | DF | GTI | RLTI | PHTI | SLTI |
|----------------------|-----|-------------|--------------|-------------|-------------|
| Treatment | 4 | 422295.32** | 1220734.18** | 133502.02** | 476047.95** |
| Genotypes | 159 | 1765.31** | 22119.52** | 564.30** | 3923.39** |
| Treatment x Genotype | 636 | 382.65** | 2830.30** | 129.076** | 573.75** |
| Error | 800 | 18.92 | 101.60 | 10.60 | 14.88 |

* - significant at 5% probability; ** - significant at 1% probability

Table.2 Treatment effect means of different concentrations of PEG-6000 for characters in sunflower

| Treatment | GTI | RLTI | PHTI | SLTI |
|-----------|---------|----------|---------|---------|
| 10 % | 95.15** | 152.02** | 52.48** | 97.33** |
| 15 % | 89.52** | 133.06** | 30.24** | 76.35** |
| 20 % | 74.49** | 84.54* | 14.99 | 46.46 |
| 25 % | 41.49 | 37.54 | 7.01 | 20.29 |
| 30 % | 8.65 | 6.05 | 1.99 | 3.88 |
| Mean | 61.86 | 82.64 | 21.34 | 48.86 |
| S.Em+/- | 0.24 | 0.56 | 0.18 | 0.21 |
| CD 5% | 0.67 | 1.56 | 0.50 | 0.59 |
| CD 1% | 0.88 | 2.05 | 0.66 | 0.78 |
| CV | 7.03 | 12.19 | 15.25 | 7.89 |

* - significant at 5% probability

** - significant at 1% probability

Table.3 Genotype x treatment interaction effects of GTI, RLTI, PHTI and SLTI at 20 and 25 % of PEG-6000

| Sl. No. | Genotypes | GTI | | RLTI | | PHTI | | SLTI | |
|---------|-----------------------|-------------------------|---------|-------------------------|----------|-------------------------|-------|-------------------------|----------|
| | | PEG 6000 concentrations | | PEG 6000 concentrations | | PEG 6000 concentrations | | PEG 6000 concentrations | |
| | | 20 % | 25 % | 20 % | 25 % | 20 % | 25 % | 20 % | 25 % |
| 1 | CMS-17B | 48.57 | 0.00 | 56.42 | 0.00 | 14.40 | 0.00 | 35.37 | 0.00 |
| 2 | CMS-103B | 55.56 | 38.89 | 76.41 | 55.64 | 25.57 | 18.53 | 50.84 | 37.07 |
| 3 | CMS-104B | 74.34** | 33.29 | 296.54** | 140.60** | 25.94 | 13.00 | 137.90** | 65.82** |
| 4 | CMS-148B | 100.00** | 70.00 | 177.06** | 59.41 | 16.99 | 9.44 | 66.64** | 25.12 |
| 5 | CMS-335B | 90.00** | 65.00 | 60.68 | 44.07 | 16.86 | 9.53 | 38.04 | 26.25 |
| 6 | CMS-338B | 90.00** | 45.00 | 57.00 | 20.30 | 26.53 | 9.11 | 40.57 | 14.29 |
| 7 | CMS-351B | 85.00** | 40.00 | 72.51 | 2.16 | 20.58 | 6.67 | 53.78 | 3.79 |
| 8 | CMS-378B | 75.00** | 45.00 | 97.46 | 36.54 | 9.15 | 3.63 | 43.46 | 16.46 |
| 9 | CMS-607B | 70.47* | 0.00 | 28.08 | 0.00 | 14.97 | 0.00 | 21.54 | 0.00 |
| 10 | CMS-850B | 95.00** | 45.00 | 54.51 | 17.90 | 16.80 | 5.63 | 33.40 | 11.09 |
| 11 | CMS-852B | 95.00** | 65.00 | 49.44 | 14.29 | 14.48 | 6.19 | 31.83 | 10.20 |
| 12 | CMS-857B | 95.00** | 90.00** | 522.92** | 417.58** | 19.97 | 16.11 | 172.01** | 137.45** |
| 13 | R-12-2 | 100.00** | 80.00** | 128.01** | 55.78 | 37.78** | 14.12 | 86.13** | 36.42 |
| 14 | R 127-1 | 94.74** | 29.82 | 159.64** | 68.31 | 16.80 | 3.70 | 40.40 | 14.44 |
| 15 | R 64NB | 82.50** | 60.00 | 96.04 | 40.15 | 23.14 | 11.15 | 59.20** | 25.58 |
| 16 | R-78 | 95.00** | 87.50** | 95.74 | 80.02 | 21.68 | 16.94 | 61.37** | 50.72 |
| 17 | R-630 | 87.50** | 37.50 | 90.74 | 53.89 | 20.99 | 10.75 | 58.35* | 33.87 |
| 18 | GM-27R | 77.50** | 48.33 | 36.24 | 25.37 | 23.55 | 16.62 | 30.16 | 21.19 |
| 19 | GM-37R | 81.29** | 8.19 | 46.17 | 26.42 | 27.77* | 13.39 | 37.61 | 20.37 |
| 20 | GM-41R | 95.00** | 50.00 | 81.49 | 58.82 | 23.92 | 11.25 | 50.26 | 33.01 |
| 21 | GM-44R | 69.85 | 66.54 | 77.73 | 63.67 | 25.80 | 18.16 | 49.68 | 39.09 |
| 22 | GM-56R | 61.11 | 27.78 | 64.88 | 4.01 | 40.70** | 10.64 | 56.86* | 6.21 |
| 23 | GM-59R | 55.56 | 33.33 | 46.36 | 34.57 | 37.20** | 14.32 | 42.53 | 26.07 |
| 24 | GM-71R | 77.78** | 66.67 | 51.17 | 37.40 | 16.50 | 4.50 | 36.97 | 23.93 |
| 25 | GP ₆ -11 | 90.00** | 90.00** | 53.28 | 35.20 | 15.27 | 11.19 | 34.19 | 23.14 |
| 26 | GP ₆ -18 | 85.00** | 15.00 | 53.00 | 29.76 | 11.95 | 6.43 | 32.59 | 18.19 |
| 27 | GP ₆ -18-1 | 94.74** | 21.05 | 24.84 | 11.52 | 6.32 | 4.85 | 14.80 | 7.91 |
| 28 | GP ₆ -54 | 87.50** | 75.00** | 91.46 | 34.73 | 14.70 | 10.46 | 59.22** | 24.54 |
| 29 | GP ₆ -63 | 63.16 | 23.68 | 64.07 | 19.35 | 7.83 | 4.83 | 34.09 | 11.60 |
| 30 | GP ₆ -83 | 81.58** | 28.95 | 103.79* | 51.71 | 12.69 | 12.07 | 52.12 | 29.22 |
| 31 | GP ₆ -109 | 60.00 | 10.00 | 36.00 | 7.24 | 6.85 | 3.72 | 19.64 | 5.24 |
| 32 | GP ₆ -118 | 90.00** | 85.00** | 53.54 | 38.18 | 13.64 | 4.46 | 37.03 | 24.22 |
| 33 | GP ₆ -127 | 76.32** | 31.58 | 73.12 | 15.35 | 27.72* | 7.22 | 48.52 | 10.94 |
| 34 | GP ₆ -135 | 57.89 | 15.79 | 56.16 | 15.69 | 14.91 | 5.66 | 37.24 | 11.09 |
| 35 | GP ₆ -139 | 47.22 | 25.00 | 76.04 | 21.71 | 4.80 | 1.80 | 36.65 | 10.74 |
| 36 | GP ₆ -160 | 42.11 | 0.00 | 43.18 | 0.00 | 14.85 | 0.00 | 25.00 | 0.00 |
| 37 | GP ₆ -173 | 82.50** | 65.00 | 71.94 | 26.57 | 16.22 | 8.18 | 47.03 | 18.34 |
| 38 | GP ₆ -175 | 67.50 | 22.50 | 40.79 | 50.88 | 10.92 | 8.05 | 25.42 | 28.80 |

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|----|----------------------|----------|---------|----------|---------|---------|-------|----------|---------|
| 39 | GP ₆ -176 | 69.85 | 57.54 | 96.34 | 80.08 | 13.95 | 7.50 | 45.09 | 34.94 |
| 40 | GP ₆ -181 | 50.00 | 0.00 | 103.57* | 0.00 | 5.15 | 0.00 | 53.81 | 0.00 |
| 41 | GP ₆ -211 | 100.00** | 85.00** | 94.29 | 44.48 | 13.33 | 7.48 | 55.51 | 26.76 |
| 42 | GP ₆ -217 | 90.00** | 7.50 | 90.44 | 11.45 | 7.43 | 6.64 | 57.16* | 9.53 |
| 43 | GP ₆ -219 | 40.00 | 5.00 | 43.50 | 18.45 | 4.54 | 1.71 | 23.20 | 9.73 |
| 44 | GP ₆ -226 | 85.00** | 32.50 | 39.41 | 23.68 | 10.02 | 4.28 | 23.36 | 13.08 |
| 45 | GP ₆ -236 | 67.50 | 55.00 | 37.33 | 12.93 | 13.75 | 9.12 | 29.14 | 11.61 |
| 46 | GP ₆ -250 | 52.63 | 15.79 | 85.12 | 39.81 | 16.84 | 8.38 | 49.14 | 23.26 |
| 47 | GP ₆ -263 | 63.16 | 7.89 | 69.04 | 13.64 | 15.78 | 6.72 | 45.89 | 10.62 |
| 48 | GP ₆ -271 | 90.00** | 65.00 | 194.94** | 72.43 | 14.39 | 9.61 | 102.88** | 40.40 |
| 49 | GP ₆ -276 | 89.47** | 60.53 | 57.35 | 40.00 | 14.77 | 10.54 | 30.15 | 21.20 |
| 50 | GP ₆ -282 | 39.47 | 0.00 | 54.60 | 0.00 | 6.51 | 0.00 | 25.00 | 0.00 |
| 51 | GP ₆ -286 | 72.95* | 43.27 | 156.91** | 58.61 | 18.30 | 10.66 | 87.16** | 34.49 |
| 52 | GP ₆ -297 | 82.50** | 27.50 | 94.49 | 6.73 | 13.68 | 3.57 | 51.52 | 5.02 |
| 53 | GP ₆ -303 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 54 | GP ₆ -305 | 100.00** | 95.00** | 141.48** | 60.62 | 9.77 | 3.89 | 66.24** | 28.21 |
| 55 | GP ₆ -310 | 92.50** | 87.50** | 36.31 | 22.99 | 10.88 | 6.52 | 21.54 | 13.42 |
| 56 | GP ₆ -312 | 52.50 | 27.50 | 72.14 | 19.28 | 8.30 | 5.19 | 38.87 | 11.94 |
| 57 | GP ₆ -313 | 25.00 | 0.00 | 14.35 | 0.00 | 3.98 | 0.00 | 8.73 | 0.00 |
| 58 | GP ₆ -317 | 27.50 | 0.00 | 36.62 | 0.00 | 10.34 | 0.00 | 22.99 | 0.00 |
| 59 | GP ₆ -324 | 84.21** | 50.00 | 89.73 | 18.84 | 11.47 | 5.64 | 51.12 | 12.34 |
| 60 | GP ₆ -325 | 87.50** | 62.50 | 195.38** | 105.23* | 9.69 | 5.29 | 113.23** | 60.97** |
| 61 | GP ₆ -326 | 100.00** | 80.00** | 159.36** | 70.34 | 34.00** | 11.04 | 74.85** | 30.40 |
| 62 | GP ₆ -327 | 77.50** | 52.50 | 97.74 | 35.74 | 21.07 | 7.13 | 52.18 | 18.74 |
| 63 | GP ₆ -331 | 82.50** | 57.50 | 128.91** | 45.13 | 23.53 | 11.44 | 81.83** | 30.09 |
| 64 | GP ₆ -332 | 84.21** | 50.00 | 49.00 | 20.69 | 10.33 | 5.30 | 36.50 | 15.72 |
| 65 | GP ₆ -347 | 90.00** | 30.00 | 31.97 | 9.27 | 9.08 | 6.63 | 20.27 | 7.94 |
| 66 | GP ₆ -358 | 34.21 | 13.16 | 69.10 | 42.10 | 14.36 | 13.40 | 44.62 | 29.27 |
| 67 | GP ₆ -366 | 86.84** | 81.58** | 123.35** | 80.34 | 27.67 | 16.05 | 74.62** | 47.59 |
| 68 | GP ₆ -370 | 100.00** | 75.00** | 44.47 | 15.95 | 16.81 | 9.17 | 35.64 | 13.78 |
| 69 | GP ₆ -371 | 95.00** | 92.50** | 120.03** | 64.50 | 14.76 | 4.39 | 61.14** | 30.87 |
| 70 | GP ₆ -374 | 90.63** | 65.63 | 72.03 | 33.86 | 26.08 | 15.55 | 50.96 | 25.49 |
| 71 | GP ₆ -384 | 72.50* | 52.50 | 43.48 | 17.39 | 8.03 | 4.31 | 29.65 | 12.29 |
| 72 | GP ₆ -387 | 90.00** | 25.00 | 68.59 | 39.43 | 11.65 | 6.72 | 39.38 | 22.65 |
| 73 | GP ₆ -400 | 86.11** | 47.22 | 61.23 | 31.65 | 9.74 | 7.75 | 32.45 | 18.32 |
| 74 | GP ₆ -420 | 70.00 | 55.00 | 84.09 | 42.18 | 7.55 | 3.40 | 42.32 | 21.01 |
| 75 | GP ₆ -424 | 95.00** | 92.50** | 187.31** | 66.76 | 16.80 | 6.53 | 69.74** | 25.16 |
| 76 | GP ₆ -442 | 100.00** | 92.50** | 186.67** | 70.14 | 20.39 | 9.93 | 83.66** | 32.77 |
| 77 | GP ₆ -451 | 81.58** | 39.47 | 46.96 | 20.93 | 13.60 | 8.34 | 29.90 | 14.49 |
| 78 | GP ₆ -459 | 85.00** | 37.50 | 73.71 | 16.60 | 13.12 | 6.17 | 41.70 | 11.11 |
| 79 | GP ₆ -511 | 60.00 | 10.00 | 76.88 | 15.76 | 11.18 | 7.00 | 46.45 | 11.70 |
| 80 | GP ₆ -517 | 65.00 | 60.00 | 59.93 | 56.85 | 27.51 | 2.95 | 39.15 | 22.31 |
| 81 | GP ₆ -534 | 23.68 | 0.00 | 40.69 | 0.00 | 2.09 | 0.00 | 14.46 | 0.00 |
| 82 | GP ₆ -561 | 88.89** | 58.33 | 62.80 | 29.09 | 22.38 | 8.56 | 45.44 | 20.28 |
| 83 | GP ₆ -570 | 75.00** | 55.00 | 86.08 | 39.92 | 14.96 | 6.92 | 45.79 | 21.17 |

| | | | | | | | | | |
|-----|-----------------------|---------------------|---------------------|----------------------|----------------------|---------------------|-------|----------------------|---------------------|
| 84 | GP ₆ -578 | 92.11 ^{**} | 76.32 ^{**} | 141.86 ^{**} | 59.55 | 13.95 | 5.10 | 49.15 | 20.08 |
| 85 | GP ₆ -579 | 90.00 ^{**} | 50.00 | 36.44 | 13.88 | 5.63 | 3.77 | 22.55 | 9.32 |
| 86 | GP ₆ -586 | 65.00 | 65.00 | 149.59 ^{**} | 109.83 ^{**} | 26.56 | 17.05 | 85.22 ^{**} | 61.31 ^{**} |
| 87 | GP ₆ -589 | 19.44 | 22.22 | 133.33 ^{**} | 181.94 ^{**} | 31.47 ^{**} | 12.18 | 58.64 [*] | 56.88 [*] |
| 88 | GP ₆ -614 | 97.50 ^{**} | 77.50 ^{**} | 104.31 [*] | 68.43 | 16.64 | 9.39 | 53.29 | 34.09 |
| 89 | GP ₆ -615 | 67.50 | 5.00 | 45.05 | 8.78 | 8.98 | 1.79 | 25.06 | 4.92 |
| 90 | GP ₆ -656 | 92.50 ^{**} | 22.50 | 78.88 | 31.18 | 11.65 | 8.29 | 45.35 | 19.77 |
| 91 | GP ₆ -657 | 77.50 ^{**} | 0.00 | 148.68 ^{**} | 0.00 | 8.54 | 0.00 | 68.97 ^{**} | 0.00 |
| 92 | GP ₆ -699 | 77.50 ^{**} | 67.50 | 69.07 | 19.40 | 11.21 | 5.56 | 44.73 | 13.58 |
| 93 | GP ₆ -714 | 85.00 ^{**} | 80.00 ^{**} | 204.95 ^{**} | 176.94 ^{**} | 41.19 ^{**} | 9.65 | 107.39 ^{**} | 77.31 ^{**} |
| 94 | GP ₆ -734 | 92.50 ^{**} | 55.00 | 130.33 ^{**} | 72.48 | 16.06 | 5.24 | 69.27 ^{**} | 36.53 |
| 95 | GP ₆ -764 | 72.22 [*] | 22.22 | 56.83 | 27.70 | 6.66 | 3.23 | 29.87 | 14.55 |
| 96 | GP ₆ -792 | 85.00 ^{**} | 37.50 | 85.49 | 21.23 | 14.50 | 5.54 | 52.15 | 13.93 |
| 97 | GP ₆ -794 | 75.00 ^{**} | 52.50 | 64.99 | 22.77 | 3.30 | 2.92 | 34.27 | 12.89 |
| 98 | GP ₆ -819 | 37.50 | 5.00 | 12.25 | 9.88 | 2.47 | 1.72 | 7.16 | 5.63 |
| 99 | GP ₆ -847 | 52.63 | 13.16 | 47.01 | 9.56 | 16.01 | 11.32 | 32.97 | 10.32 |
| 100 | GP ₆ -854 | 92.50 ^{**} | 67.50 | 108.11 [*] | 61.16 | 21.37 | 11.93 | 71.06 ^{**} | 40.14 |
| 101 | GP ₆ -861 | 33.33 | 0.00 | 26.66 | 0.00 | 2.65 | 0.00 | 16.41 | 0.00 |
| 102 | GP ₆ -863 | 92.50 ^{**} | 92.50 ^{**} | 122.28 ^{**} | 41.00 | 14.83 | 9.79 | 70.25 ^{**} | 25.89 |
| 103 | GP ₆ -872 | 58.82 | 0.00 | 62.12 | 0.00 | 17.91 | 0.00 | 39.07 | 0.00 |
| 104 | GP ₆ -875 | 40.00 | 7.50 | 62.99 | 9.02 | 4.88 | 3.25 | 27.92 | 5.60 |
| 105 | GP ₆ -883 | 72.50 [*] | 12.50 | 43.63 | 12.77 | 12.67 | 4.68 | 29.40 | 9.05 |
| 106 | GP ₆ -887 | 70.00 | 0.00 | 142.31 ^{**} | 0.00 | 17.46 | 0.00 | 81.43 ^{**} | 0.00 |
| 107 | GP ₆ -891 | 52.50 | 52.50 | 23.02 | 22.00 | 16.29 | 10.80 | 19.68 | 16.49 |
| 108 | GP ₆ -899 | 68.42 | 10.53 | 43.25 | 3.56 | 5.36 | 3.07 | 24.58 | 3.31 |
| 109 | GP ₆ -906 | 40.00 | 40.00 | 24.57 | 21.80 | 6.78 | 7.21 | 16.00 | 14.80 |
| 110 | GP ₆ -912 | 87.50 ^{**} | 72.50 [*] | 140.49 ^{**} | 68.15 | 18.77 | 5.59 | 78.41 ^{**} | 36.24 |
| 111 | GP ₆ -917 | 88.89 ^{**} | 38.89 | 74.95 | 16.52 | 24.16 | 6.08 | 49.61 | 11.30 |
| 112 | GP ₆ -951 | 31.25 | 18.75 | 58.52 | 25.80 | 7.34 | 3.74 | 33.10 | 14.84 |
| 113 | GP ₆ -952 | 65.00 | 10.00 | 40.21 | 20.38 | 7.28 | 4.27 | 23.78 | 12.33 |
| 114 | GP ₆ -953 | 70.00 | 20.00 | 50.38 | 11.41 | 7.11 | 3.87 | 33.93 | 8.55 |
| 115 | GP ₆ -961 | 53.13 | 46.88 | 44.85 | 25.94 | 18.89 | 7.33 | 34.83 | 18.76 |
| 116 | GP ₆ -965 | 97.50 ^{**} | 65.00 | 61.33 | 42.92 | 6.61 | 4.34 | 32.19 | 22.37 |
| 117 | GP ₆ -967 | 90.00 ^{**} | 80.00 ^{**} | 142.37 ^{**} | 80.86 | 15.83 | 6.96 | 58.36 [*] | 31.86 |
| 118 | GP ₆ -969 | 90.00 ^{**} | 90.00 ^{**} | 120.76 ^{**} | 51.12 | 20.95 | 7.05 | 55.43 | 22.33 |
| 119 | GP ₆ -990 | 45.00 | 15.00 | 32.53 | 19.28 | 8.45 | 5.43 | 17.73 | 10.76 |
| 120 | GP ₆ -1001 | 81.58 ^{**} | 47.37 | 38.60 | 16.86 | 8.64 | 6.34 | 25.63 | 12.30 |
| 121 | GP ₆ -1020 | 82.50 ^{**} | 17.50 | 45.60 | 33.04 | 6.86 | 5.06 | 28.80 | 20.90 |
| 122 | GP ₆ -1023 | 89.47 ^{**} | 57.89 | 130.53 ^{**} | 30.20 | 14.14 | 4.20 | 58.21 [*] | 14.05 |
| 123 | GP ₆ -1026 | 75.00 ^{**} | 0.00 | 32.66 | 0.00 | 7.62 | 0.00 | 17.50 | 0.00 |
| 124 | GP ₆ -1037 | 81.58 ^{**} | 34.21 | 154.44 ^{**} | 48.16 | 9.44 | 4.08 | 62.97 ^{**} | 20.34 |
| 125 | GP ₆ -1047 | 90.00 ^{**} | 52.50 | 87.96 | 11.42 | 11.19 | 4.79 | 37.06 | 7.05 |
| 126 | GP ₆ -1060 | 95.00 ^{**} | 80.00 ^{**} | 166.48 ^{**} | 74.60 | 13.47 | 9.44 | 69.68 ^{**} | 33.30 |
| 127 | GP ₆ -1063 | 90.00 ^{**} | 20.00 | 62.68 | 26.97 | 5.03 | 3.41 | 26.43 | 12.15 |
| 128 | GP ₆ -1072 | 80.00 ^{**} | 75.00 ^{**} | 60.95 | 61.78 | 7.59 | 4.54 | 31.83 | 30.57 |

| | | | | | | | | | |
|---------------------|-----------------------|----------------------|---------------------|----------------------|-------|---------------------|-------|----------------------|-------|
| 129 | GP ₆ -1075 | 90.00 ^{**} | 57.50 | 154.14 ^{**} | 77.26 | 17.36 | 5.56 | 65.27 ^{**} | 30.68 |
| 130 | GP ₆ -1089 | 12.50 | 0.00 | 7.12 | 0.00 | 7.47 | 0.00 | 7.28 | 0.00 |
| 131 | GP ₆ -1101 | 82.50 ^{**} | 40.00 | 142.31 ^{**} | 56.87 | 22.76 | 13.20 | 84.35 ^{**} | 35.57 |
| 132 | GP ₆ -1102 | 82.50 ^{**} | 75.00 ^{**} | 153.13 ^{**} | 92.62 | 33.59 ^{**} | 13.70 | 90.35 ^{**} | 51.18 |
| 133 | GP ₆ -1114 | 76.84 ^{**} | 18.03 | 66.59 | 9.60 | 18.28 | 9.62 | 51.65 | 9.58 |
| 134 | GP ₆ -1117 | 87.50 ^{**} | 35.00 | 153.47 ^{**} | 32.15 | 30.72 ^{**} | 8.97 | 87.29 ^{**} | 19.65 |
| 135 | GP ₆ -1127 | 57.50 | 7.50 | 61.24 | 6.17 | 8.79 | 9.34 | 35.71 | 7.71 |
| 136 | GP ₆ -1135 | 57.50 | 0.00 | 23.71 | 0.00 | 6.28 | 0.00 | 16.61 | 0.00 |
| 137 | GP ₆ -1150 | 55.56 | 19.44 | 54.37 | 24.77 | 8.13 | 6.94 | 29.70 | 15.26 |
| 138 | GP ₆ -1207 | 90.00 ^{**} | 20.00 | 52.31 | 8.18 | 8.96 | 3.25 | 32.86 | 5.97 |
| 139 | GP ₆ -1217 | 92.11 ^{**} | 10.53 | 39.18 | 1.53 | 9.76 | 1.88 | 23.32 | 1.72 |
| 140 | GP ₆ -1227 | 36.11 | 13.89 | 52.20 | 2.29 | 15.92 | 7.10 | 37.97 | 4.18 |
| 141 | GP ₆ -1228 | 78.95 ^{**} | 71.05 [*] | 59.34 | 22.62 | 18.41 | 3.70 | 43.57 | 15.33 |
| 142 | GP ₆ -1254 | 88.89 ^{**} | 69.44 | 95.93 | 60.09 | 13.77 | 7.29 | 55.44 | 34.07 |
| 143 | GP ₆ 1350 | 32.08 | 16.25 | 35.31 | 32.55 | 17.87 | 9.95 | 26.14 | 20.70 |
| 144 | GP ₆ 1436 | 61.46 | 32.50 | 69.39 | 13.87 | 10.02 | 5.24 | 43.94 | 10.18 |
| 145 | GP ₆ 1450 | 15.00 | 0.00 | 10.01 | 0.00 | 3.58 | 0.00 | 6.12 | 0.00 |
| 146 | GP ₆ -1468 | 87.50 ^{**} | 32.50 | 247.51 ^{**} | 77.62 | 17.48 | 9.21 | 112.88 ^{**} | 37.63 |
| 147 | GP ₆ -1477 | 80.00 ^{**} | 32.50 | 106.95 [*] | 66.86 | 19.38 | 9.85 | 52.70 | 31.52 |
| 148 | GP ₆ 1482 | 80.56 ^{**} | 33.33 | 119.51 ^{**} | 64.71 | 29.58 [*] | 20.49 | 63.94 ^{**} | 37.34 |
| 149 | GP ₆ -1509 | 69.44 | 38.89 | 86.56 | 30.55 | 15.62 | 6.27 | 51.13 | 18.45 |
| 150 | GP ₆ -1518 | 87.50 ^{**} | 35.00 | 53.93 | 8.53 | 10.75 | 5.61 | 36.28 | 7.34 |
| 151 | GP ₆ -1533 | 83.33 ^{**} | 63.89 | 120.34 ^{**} | 58.30 | 14.38 | 7.94 | 63.86 ^{**} | 31.47 |
| 152 | GP ₆ -1561 | 82.50 ^{**} | 35.00 | 76.12 | 19.27 | 14.58 | 5.55 | 46.05 | 12.57 |
| 153 | GP ₆ -1573 | 95.00 ^{**} | 45.00 | 95.79 | 54.84 | 11.68 | 7.11 | 54.60 | 31.48 |
| 154 | GP ₆ -1576 | 95.00 ^{**} | 85.00 ^{**} | 120.45 ^{**} | 50.22 | 22.48 | 12.50 | 72.75 ^{**} | 31.98 |
| 155 | GP ₆ -1588 | 55.26 | 7.89 | 54.18 | 18.96 | 8.74 | 7.90 | 27.73 | 12.52 |
| 156 | GP ₆ -1595 | 75.00 ^{**} | 65.00 | 51.48 | 40.75 | 9.83 | 8.59 | 27.10 | 21.90 |
| 157 | GP ₆ -1616 | 95.00 ^{**} | 70.00 | 84.91 | 66.48 | 19.90 | 5.31 | 49.02 | 32.71 |
| 158 | GP ₆ -1665 | 95.00 ^{**} | 27.50 | 34.95 | 6.83 | 8.75 | 8.58 | 22.82 | 7.63 |
| 159 | GP ₆ -1725 | 85.00 ^{**} | 60.00 | 97.09 | 60.59 | 22.29 | 15.26 | 65.88 ^{**} | 41.60 |
| 160 | GP ₆ -2255 | 100.00 ^{**} | 95.00 ^{**} | 91.09 | 42.44 | 20.84 | 12.01 | 59.75 ^{**} | 28.86 |
| Range | | 0.00-100.00 | | 0.00-744.41 | | 0.00-93.72 | | 0.00-282.85 | |
| Overall Mean | | 61.86 | | 82.64 | | 21.34 | | 48.86 | |
| S.Em+/- | | 3.08 | | 7.13 | | 2.30 | | 2.73 | |
| CD 5% | | 8.54 | | 19.76 | | 6.38 | | 7.57 | |
| CD 1% | | 11.23 | | 26.00 | | 8.40 | | 9.96 | |
| CV | | 7.03 | | 12.20 | | 15.26 | | 7.90 | |

* - significant at 5% probability; ** - significant at 1% probability

The investigation strongly agreed to use PEG-6000 for inducing as well as selection of genotypes for moisture stress tolerance under in vitro condition. Based on overall mean performance of stress tolerance indices like,

GTI, RLTI, PHTI and SLTI, there are 34 promising genotypes were identified *viz.*, CMS 857B, CMS 104B, CMS 148B, R 78, R-12-2, GP₆-11, GP₆-54, GP₆-118, GP₆-211, GP₆-305, GP₆-310, GP₆-325, GP₆-326, GP₆-

366, GP₆-370, GP₆-371, GP₆-424, GP₆-442, GP₆-578, GP₆-586, GP₆-589, GP₆-614, GP₆-714, GP₆-734, GP₆-863, GP₆-912, GP₆-967, GP₆-969, GP₆-1060, GP₆-1072, GP₆-1102, GP₆-1228, GP₆-1576 and GP₆-2255. These were found to be most tolerant lines can be utilized for further breeding programs to improve the genotypes to overcome drought stress at germination stage.

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