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Original Research Paper

# Evaluation of cucumber (*Cucumis sativus* L.) genotypes for drought tolerance using high throughput PEG induced drought indices

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## ABSTRACT

Cucumber is highly sensitive to drought due to its high-water requirement. Identifying genotypes that can tolerate early-stage moisture stress can reduce mortality and improve survival during drought. In this study, 16 genotypes were evaluated for seedling stage drought stress (SDS) tolerance using $PEG_{8000}$ (18%). Five traits namely germination percentage, germination index, germination energy, shoot length (85.26% reduction) and root length were recorded and analyzed using five selection indices, namely arithmetic mean productivity (AMP), geometric mean productivity (GMP), harmonic mean productivity (HMP), stress tolerance index (STI) and membership function value (MFV). The indices were applied to assess the variability among genotypes and identify those tolerant to drought stress. Among the indices MFV and AMP were determined to be most desirable due to the better discriminating ability and strong correlation with mean shoot length. Based on the indices, the genotypes G9, G2 and W4 were identified as the most tolerant to PEG induced drought stress at seedling stage. Thus, the identified genotypes can be used in further breeding program of cucumber improvement.

**Keywords:** Drought tolerance indices, germination traits, seedling stage drought stress

## INTRODUCTION

Cucumber is an important salad vegetable grown commercially all over the world. It being shallow-rooted is found to be sensitive to water deficiency (Li et al., 2011). Climate change has become evident, with drought being one of its major consequences. The osmotic effect under drought has been studied in many crops but there are only few reports in cucumber (Das et al., 2024). The cucumber is found to be sensitive to water fluctuations at the seedling stage compared to older plants (Liu et al., 2009; Li et al., 2011). Germination and seedling stages require less time and fewer resources compared to evaluating plants at later growth stages. Screening at these early stages allows researchers to test a large number of genotypes efficiently before selecting promising lines for further study (Farooq et al., 2009). Therefore, studying the effect of moisture stress at germination and seedling stage can be useful in selecting genotypes for drought stress tolerance. A few studies have shown that genotypes selected for moisture stress tolerance during *in vitro* germination test have also showed tolerance under field conditions (Khakwani et al., 2011; Agili et al., 2012). At the germination and seedling stage, drought tolerance screening involves assessing germination rate, seedling vigor, root-to-shoot ratio, and survival under water deficit. Physiological and biochemical markers like relative water content (RWC), proline accumulation, and electrolyte leakage are also used for evaluation.

Polyethylene glycol (PEG) is commonly used to induce osmotic stress in plants, simulating drought conditions (Das et al., 2022). Several studies have successfully identified drought tolerant cucumber genotypes using PEG as a selective medium (Klosinska et al., 2016; Das et al., 2024). Various selection indices, such as stress tolerance index (STI) (Fishcer & Maurer, 1978), arithmetic mean productivity (AMP) (Rosielle & Hamblin, 1981), geometric mean productivity (GMP) and hormonic mean productivity (HMP) (Fernandez, 1992) and membership function value (MFV) (Zadeh, 1965) have been widely used to quantify and select genotypes for drought stress tolerance (Bharathi et al., 2024; Hou et al., 2022; Kalpana et al., 2023).

**Table 1 : Genotypes used for screening for drought using PEG8000 (18%)**

| Code | Acc. No. | Code | Acc. No. | Code | Acc. No. |
|---|---|---|---|---|---|
| Green type: good yield, fruit size and fruit shape | | White type: fruits with white tinge | | Partial tolerance to Downy mildew | |
| G1 | IC 527431 | W1 | IC 523672 | DM1 | Pusa Uday |
| G2 | IC 523679 | W2 | IC 523673 | DM2 | IC 410682 |
| G3 | IC 354790 | W3 | IC 523670 | DM3 | IC 527397 |
| G4 | IC 523689 | W4 | IC 523681 | DM4 | Arka Veera |
| G5 | IC 572024 | W6 | IC 613463 | DM5 | IC 527431 |
| G6 | IC 613458 | W7 | EC 1041453 | DM6 | IC 572024 |
| G7 | IC 527400 | W9 | IC 354816 | DM7 | IC 527400 |
| G8 | EC 977504 | | | | |
| G9 | IC 523692 | | | | |
| G10 | Arka Veera | | | | |

The present study was envisaged to identify the most effective indices for discriminating cucumber genotypes based on their response to seedling stage drought stress (SDS) and also identify SDS tolerant genotypes using the combination of indices that exhibited highest power and discriminatory correlation with germination and seedling growth traits.

## MATERIALS AND METHODS

The experimental genetic material consisted of 16 accessions of advanced breeding lines having good fruit quality and some lines have partial tolerance to downy mildew (Table 1). The 16 cucumber accessions were screened for germination and seedling growth under 18% PEG (MW 8000) for identifying drought-tolerant genotype using a completely randomized design with three replications. The concentration of 18% PEG was chosen based on previous reports, indicating its effectiveness in differentiating the cucumber genotypes for germination and seedling growth (Kłosińska et al., 2016). For germination test, each of the accession 60 seeds in three replicates (10 seeds/replication/dish) were placed in petri dishes lined with layer of absorbent filter paper saturated with 5 mL of 18% $PEG_{8000}$ or 5 mL of double distilled water (control). Seed germination was observed and recorded for six days. The germinated seedlings were placed on filter paper saturated with 18% $PEG_{8000}$ and $ddH_2O$ for treatment and control conditions respectively for seedling growth test (Fig. 1).

A temperature of 24±1°C (room temperature) and 16/8 hours photoperiod was maintained during the experiment. The physiological traits such as germination percentage, germination energy, germination index (all three, respectively come under germination traits), shoot length and root length (the two come under growth traits) were calculated from the number of seeds germinated and the length

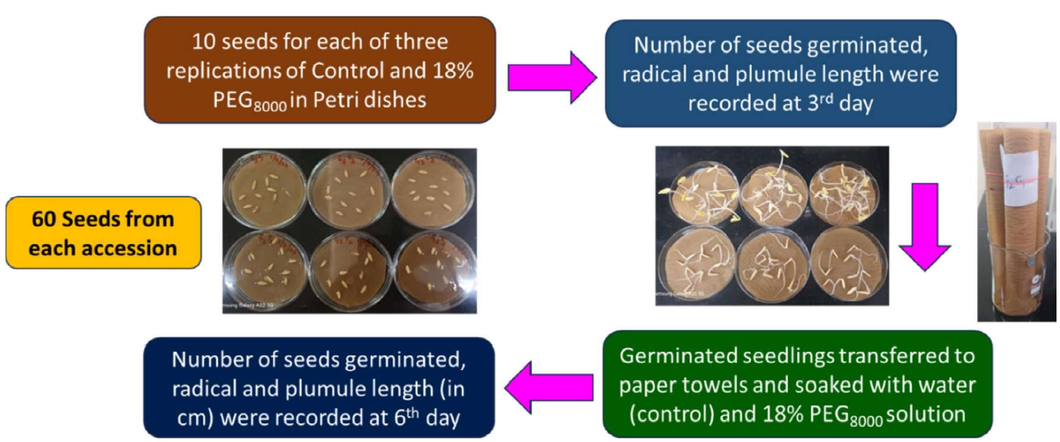


Fig. 1 : Flow chart showing the procedure followed during the seedling stage drought stress screening

**Table 2 : The indices and their formulae used for estimating the response of genotypes to drought stress**

| Indices | Code | Formula | Reference |
|---|---|---|---|
| Arithmetic mean productivity | AMP | $(Yp + Ys)/2$ | Rosielle & Hamblin (1981) |
| Geometric mean productivity | GMP | $\sqrt{(Ys \times Yp)}$ | Fernandez (1992) |
| Harmonic mean productivity | HMP | $2 * (Yp \times Ys)/(Yp + Ys)$ | Fernandez (1992) |
| Stress tolerance index | STI | $X = (Ys \times Yp)/(Yp)$ | Fischer & Maurer (1978) |
| Membership function value | MFV | $\frac{Xi - Xmin}{Xmax - Xmin} * 100$ | Zadeh (1965) |

Yp : traits under non-stress condition; Ys : traits under seedling stage drought stress condition

of radical and plumule, which was observed and recorded. The ANOVA test was performed using Microsoft excel version 2019 to determine the significance of differences among the genotypes for each of the traits tested under control and PEG treatment using. The pooled ANOVA results of genotypes for all the traits are represented in (Table 4).

The five indices were used to estimate the response of genotypes to moisture stress for germination and seedling growth traits (Table 2). The five indices were calculated based on the extent of reduction in trait values observed under stress (SDS) and non-stress (NS) conditions.

To assess the relationship of indices with the germination and growth traits, correlation coefficients were estimated for the five indices and the five traits under SDS and NS conditions (Table 3).

PEG tolerant genotypes were identified based on their higher AMP, GMP, HMP, STI and MFV/ their ability to better discriminate between genotypes in combination, and high correlation of such indices with the germination traits under SDS and NS conditions. Since the identified PEG tolerant genotypes varied among the indices, rank sum (RS) method (Farshadfar et al., 2012) was employed to integrate all indices into single composite index. According to this method, a lower rank-sum value corresponds to higher drought tolerance (Table 6).

The genotypes were further classified to four distinct classes (A, B, C and D) (Fernandez et al., 1992). Class ‘A’ response genotypes are those which express superior performance in both control and PEG stress conditions. Class ‘B; response genotypes are those which perform better only in control conditions. Class ‘C’ response is where the genotypes perform well only under stress and class ‘D’ response genotypes are those which perform poorly in both control and PEG stress.

## RESULTS AND DISCUSSION

Genotypes differed significantly for majority of germination parameters except the germination percentage and root length under NS conditions. These results show that the genotypes selected for the study are appropriate for the study as three of the five parameters exhibited significant differences among the genotypes (Table 3).

**Table 3 : Correlation values between SDS and NS conditions for different germination traits**

| Trait | r (S vs NS) |
|---|---|
| Germination % | 0.02 |
| Germination index | 0.45 |
| Germination energy | 0.59 |
| Shoot length | 0.61 |
| Root length | -0.07 |

SDS has shown considerable effects on the germination traits such as germination percentage, germination index and germination energy, shoot length and root length. The genotypes varied for most of these traits under PEG stress conditions. The mean per cent reduction in these traits varied considerably (Fig. 2).

Among the five traits, significant correlations were observed for germination index, germination energy and mean shoot length among SDS and NS conditions (Table 3). However, germination percentage showed

**Table 4: Pooled analysis of variance of cucumber genotypes evaluated for germination traits under SDS and NS conditions**

| Source | Degrees of freedom | Mean sum of squares | 'F' statistics | Significance |
|---|---|---|---|---|
| **Germination %** | | | | |
| **Control** | | | | |
| Genotypes | 15 | 448.8889 | 2.071795 | * |
| Error | 32 | 216.6667 | | |
| **Treatment** | | | | |
| Genotypes | 15 | 333.3333 | 1.46789 | NS |
| Error | 32 | 227.0833 | | |
| **Germination index** | | | | |
| **Control** | | | | |
| Genotypes | 15 | 0.64321 | 2.710894 | ** |
| Error | 32 | 0.237269 | | |
| **Treatment** | | | | |
| Genotypes | 15 | 1.035802 | 2.351993 | * |
| Error | 32 | 0.440394 | | |
| **Germination energy** | | | | |
| **Control** | | | | |
| Genotypes | 15 | 244.4444 | 3.259259 | ** |
| Error | 32 | 75 | | |
| **Treatment** | | | | |
| Genotypes | 15 | 520 | 2.773333 | ** |
| Error | 32 | 187.5 | | |
| **Shoot length** | | | | |
| **Control** | | | | |
| Genotypes | 15 | 9.557368 | 4.797519 | ** |
| Error | 32 | 1.992148 | | |
| **Treatment** | | | | |
| Genotypes | 15 | 1.452597 | 9.558031 | ** |
| Error | 32 | 0.151977 | | |
| **Root length** | | | | |
| **Control** | | | | |
| Genotypes | 15 | 5.909771 | 1.270368 | NS |
| Error | 32 | 4.652017 | | |
| **Treatment** | | | | |
| Genotypes | 15 | 8.20756 | 5.941399 | ** |
| Error | 32 | 1.381419 | | |

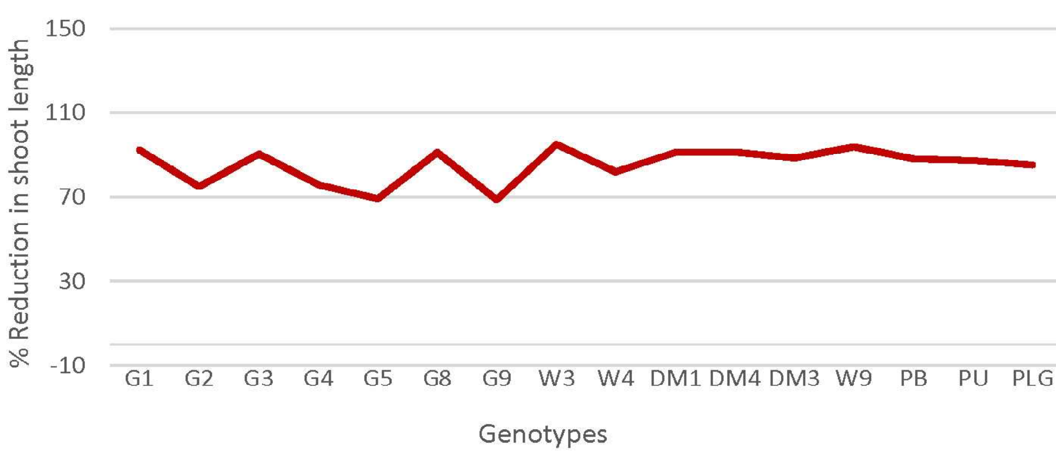


Fig. 2 : Per cent reduction in shoot length of genotypes evaluated under SDS and NS conditions

a rather low phenotypic correlation coefficient (0.02) between SDS and NS conditions.

Indices capable of efficiently distinguishing desirable genotypes for PEG tolerance are highly valuable. In this study MFV was most effective in discriminating the genotypes for PEG stress as indicated by high SR and PCV estimates (Table 5).

**Table 5 : Estimates of descriptive statistics for drought tolerance indices based on shoot length of genotypes evaluated under SDS and NS conditions**

| Index | Standardised range (SR) | PCV (%) |
|---|---|---|
| AMP | 1.28 | 34.19 |
| GMP | 1.98 | 49.77 |
| HMP | 2.72 | 70.1 |
| STI | 1.79 | 58.46 |
| MFV | 2.71 | 88.46 |

Based on findings discussed, the PEG stress tolerant genotypes were identified using all five indices and mean shoot length under SDS and NS conditions via the rank sum method (Table 6). According to these criterions, three genotypes (with class' A' response) G9, G2 and W4 were classified as PEG stress tolerant. According to YREM (yield relative to environment maxima) prediction, the genotypes G9, G2 and W4 have shown higher shoot length under stress condition than other genotypes. The reduction in shoot length due to stress conditions is comparatively lesser than other genotypes (Table 7). This inference is based on the innate property of YREM.

Significant differences among the genotypes for majority of the traits was detected in the ANOVA test. However, considering genotypes that are significantly divergent than the genotypes chosen in the present study would be more precise. The present genotypes have been chosen based on the specific traits and not based on their pedigree. For instance, a study on wheat seedling traits under moisture stress found non-significant differences in certain traits, suggesting limited genetic variability among the genotypes evaluated (Khan et al., 2002). Hence, there has been non-significant difference among the genotypes for two traits in NS conditions. The variation observed in per cent reduction of the traits mediates that the applied PEG stress ($PEG_{8000}$ 18%) was sufficient to differentiate the genotypes based on their response to PEG induced drought stress (Fig. 3).

**Table 6 : Representation of rank sum method to identify drought tolerant genotypes based on hypothetical magnitudes of five indices**

| Genotype | AMP rank | GMP rank | HMP rank | STI rank | MFV rank | Rank mean (RM) | Standard deviation of ranks (SDR) | Rank sum (RS) (RM+SDR) |
|---|---|---|---|---|---|---|---|---|
| G1 | 5 | 9 | 9 | 14 | 14 | 10.2 | 3.83 | 14.03 |
| G2 | 2 | 2 | 2 | 3 | 3 | 2.4 | 0.55 | 2.95 |
| G3 | 4 | 5 | 6 | 10 | 10 | 7 | 2.83 | 9.83 |
| G4 | 11 | 6 | 5 | 4 | 4 | 6 | 2.92 | 8.92 |
| G5 | 9 | 4 | 4 | 2 | 2 | 4.2 | 2.86 | 7.06 |
| G8 | 7 | 7 | 8 | 11 | 11 | 8.8 | 2.05 | 10.85 |
| G9 | 1 | 1 | 1 | 1 | 1 | 1 | 0.00 | 1.00 |
| W3 | 15 | 16 | 16 | 16 | 16 | 15.8 | 0.45 | 16.25 |
| W4 | 3 | 3 | 3 | 5 | 5 | 3.8 | 1.10 | 4.90 |
| DM1 | 12 | 13 | 14 | 12 | 12 | 12.6 | 0.89 | 13.49 |
| DM4 | 10 | 11 | 13 | 13 | 13 | 12 | 1.41 | 13.41 |
| DM3 | 8 | 8 | 7 | 9 | 9 | 8.2 | 0.84 | 9.04 |
| W9 | 6 | 10 | 12 | 15 | 15 | 11.6 | 3.78 | 15.38 |
| PB | 16 | 15 | 15 | 8 | 8 | 12.4 | 4.04 | 16.44 |
| PU | 14 | 14 | 11 | 7 | 7 | 10.6 | 3.51 | 14.11 |
| PLG | 13 | 12 | 10 | 6 | 6 | 9.4 | 3.29 | 12.69 |

**Table 7 : Estimates of YREM of selected genotypes under non-stress and PEG stress conditions for shoot length**

| Genotype | Shoot length YREM (NS) | Shoot length YREM (S) |
|---|---|---|
| G1 | 0.8 | 0.2 |
| G2 | 0.84 | 0.67 |
| G3 | 0.8 | 0.25 |
| G4 | 0.49 | 0.37 |
| G5 | 0.49 | 0.48 |
| G8 | 0.76 | 0.21 |
| G9 | 1 | 1 |
| W3 | 0.4 | 0.06 |
| W4 | 0.78 | 0.45 |
| DM1 | 0.54 | 0.15 |
| DM4 | 0.57 | 0.16 |
| DM3 | 0.66 | 0.24 |
| W9 | 0.78 | 0.16 |
| PB | 0.34 | 0.13 |
| PU | 0.43 | 0.17 |
| PLG | 0.43 | 0.2 |

The low phenotypic correlation between SDS and NS suggests that genotype's performance for germination percentage under control conditions is not a good indication of their performance under SDS conditions and vice-versa. Germination under NS conditions is affected by availability of water and nutrients, seed vigor and basic metabolic processes. But under PEG-induced stress, polyethylene glycol (PEG) is used to simulate drought or osmotic stress by lowering the water potential. Germination under stress requires additional mechanisms such as osmotic adjustment, the production of osmoprotectants (e.g., proline, sugars), and stress hormone regulation (e.g., abscisic acid) (Bewley et al., 2013, Verslues et al., 2006). These extra layers of stress response mean that a seed's ability to germinate under normal conditions might not reflect its capacity to cope with water-limited conditions. Hence, considering only germination data from NS conditions could lead to the selection of genotypes that are not necessarily drought tolerant. It is important to include stress-specific screening (using PEG or actual drought conditions) in breeding programs aimed at improving drought tolerance (Blum, 2011).

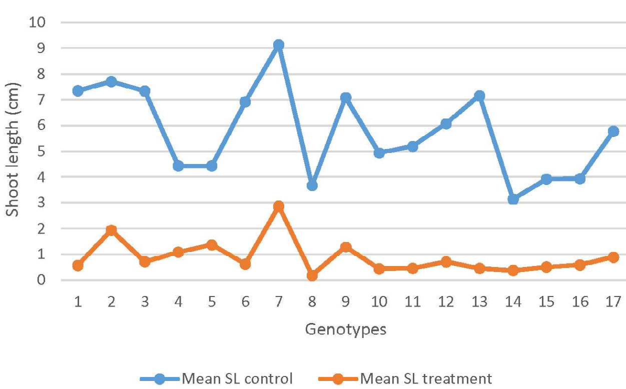


Fig. 3: Mean shoot length of genotypes in SDS and NS conditions

A negative correlation between root length under control and PEG stress conditions in cucumber indicates that genotypes with longer root perform well under NS conditions, further tend to have shorter roots under PEG-induced osmotic stress, and vice versa. In an optimal environment, some genotypes may invest heavily in rapid root elongation to maximize nutrient and water uptake. Energy and resources are directed toward elongation since water and nutrient availability are not limiting. Under PEG-Induced stress conditions, PEG reduces the water potential of the medium, creating a water deficit-like situation that limits cell expansion and elongation. Genotypes that are more stress-adaptive may redirect resources toward protective mechanisms (such as the synthesis of osmo-protectants, antioxidants, or stress hormones like abscisic acid) rather than growth. This can lead to a relatively reduced primary root length.

The high estimates of SR and PCV for MFV indicates that MFV can discriminate the genotypes which have high shoot length in both SDS and NS conditions than those which have lower shoot length. It is therefore desirable to use MFV to screen the genotypes for PEG stress in cucumber. MFV has been used in selecting genotypes for salt stress tolerance in sunflower (Li et al., 2020), drought tolerance in soybean (Yan et al., 2020) and sugarcane (Xu et al., 2023). Hence, our study also reports the use of MFV as a discriminable index for PEG stress tolerance among cucumber genotypes.

The depiction of significant positive and high magnitude of correlation between all five indices with mean shoot length under stress and non-stress

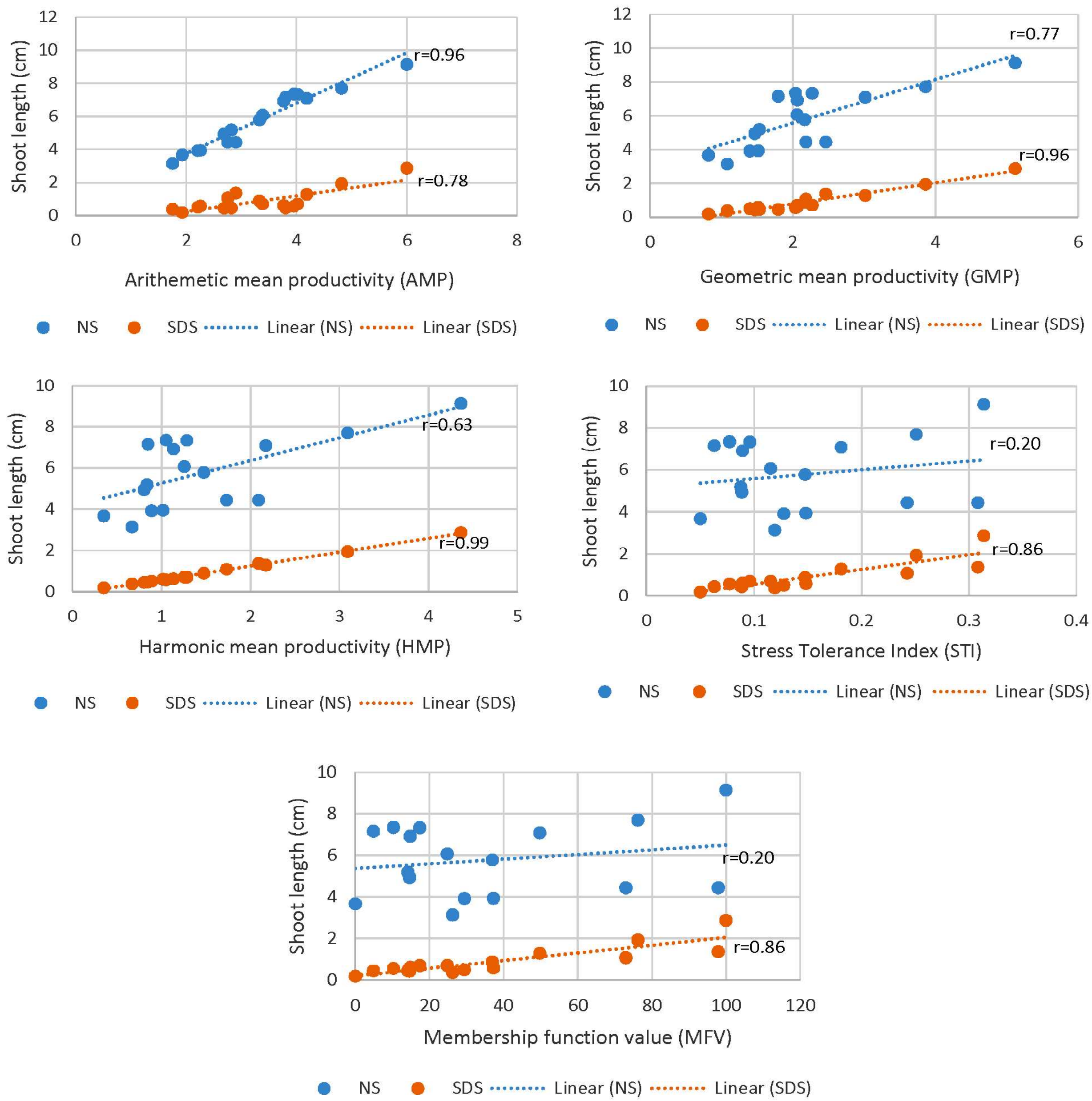


Fig. 4 : Relationship between arithmetic mean productivity (AMP), geometric mean productivity (GMP), harmonic mean productivity (HMP), stress tolerance index (STI) and membership function value (MFV) with shoot length of genotypes evaluated under NS and SDS conditions

conditions represents that any of the five indices could be used to select PEG stress tolerant genotypes. However, two indices i.e. STI and MFV with respect to non-stress conditions have shown a relatively lower correlation (Fig. 4). Moosavi et al. (2008), Bennani et al. (2017), Kalpana et al. (2023) and Bharathi et al. (2024) have also identified STI, AMP, GMP and HMP as most reliable indices for selecting drought tolerant genotypes based on the correlation analysis.

Three genotypes have shown drought tolerance based on all five indices. The accumulation of compatible solutes like proline in these genotypes may have contributed to maintaing cell turgor and support shoot growth under PEG induced water stress conditions. Some authors showed that genotypes that were tolerant to drought during *in vitro* germination tests like (PEG induced) were also tolerant under field conditions (Khakwani et al., 2011; Agili et al., 2012).

YREM is an intuitive, genotypes' attendance-independent dynamic statistics (Yan, 1999). The best genotype's performance is its potential shoot length yield attainable in a given environment. Hence, expected YREM of genotypes must be unity according to the test across diverse environments such as NS and SDS. Any deviation of genotype's YREM from unity is attributed to cross-over genotypes x MR (moisture regimes) interaction. The extent of reduction in attainable shoot length of a genotype depends on the extent of departure of its YREM from unity. YREM has been used to estimate reduction in yield in many studies such as Kalpana et al. (2023) and Bharathi et al. (2024) in dolichos and green gram, respectively.

## CONCLUSION

The present study identifies drought tolerant genotypes of cucumber based on PEG 8000- based screening by using different indices and ranksum method.

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