Physiological and Yield Responses of Selected Mungbean Genotypes to Terminal Drought

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Extreme drought conditions have been a major limiting factor in legume production worldwide. The development of drought-resilient crops should help mitigate adverse effects of unfavorable environmental conditions such as drought; however, initial characterization is necessary to ensure that the germplasm has the essential genetic variation for drought tolerance. In this study, a field trial was conducted to characterize drought response and potential drought tolerance of 100 mungbean genotypes through the evaluation of several physiological parameters and seed yield. The genotypes were subjected to terminal drought stress before flowering (~ 30 d after planting), reducing soil moisture content (SMC) from 25.0–30.0% to 13.0–15.0% during treatment imposition. Significant differences were observed between mungbean genotypes for total chlorophyll content (Chl), total scavenging activity (SA), electrolyte leakage (EC), and chlorophyll fluorescence (Fv/Fm) during drought. Furthermore, by transforming each physiological parameter into principal components (PCs) via principal component analysis (PCA), five distinct clusters were generated. Based on their physiological response and seed yield, Clusters I and II were identified as potential drought susceptible group, Cluster III was moderately susceptible, and Clusters IV and V were identified as the potential drought-tolerant group. Correlation analysis and PCA also demonstrated the significant relationship among several key traits for drought tolerance such as chlorophyll content, electrolyte leakage, and antioxidant scavenging to chlorophyll fluorescence – suggesting the suitability of fluorescence parameter in mungbean mass screening for drought tolerance. Although weak correlation between physiological parameters to seed yield was present (0.3 < |r| < 0.5), correlations among each physiological parameter were moderately strong (0.5 < |r| < 0.7). The findings suggest that a physiological characterization during initial screening for drought tolerance was an effective approach in identifying possible sources of drought tolerance traits complementary with yield-related traits. The evaluation of chlorophyll fluorescence, scavenging activity, electrolyte leakage, and chlorophyll content was enough to differentiate and characterize the drought response of several mungbean genotypes and will be vital in the development of climate-resilient crops.

Keywords: antioxidant, drought, electrolyte leakage, Fv/Fm, mungbean, PCA

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INTRODUCTION

Mungbean [V. radiata (L.) R. Wilczek], locally known as “mongo” or “balatong,” is among the cheapest sources of protein and has been regarded as one of the most important leguminous crops in South and Southeast Asia by the World Vegetable Center. It plays a significant role in both food and agriculture due to its nutritional value, as well as its nitrogen-fixing capabilities. An increase in available nitrogen in the soil makes mungbean an excellent rotation and intercrop in most cereal-based agricultural systems. Previous studies have reported a significant increase in yield and nitrogen uptake in both rice and maize when cultivating mungbean between planting seasons (Sharma and Prasad 1999; Timsina et al. 2010). The role of mungbean as an integral part of any agricultural system can be demonstrated by its wide range of uses. However, mungbean is still tagged as one of the under-utilized crops even with at least 5.7–6.0 million ha allotted as areas of production worldwide (Ebert 2014). The potential to further enhance mungbean production depends on both yield improvement and its cultivation under a wide range of growing conditions. The development of a drought-resilient mungbean variety will broaden possible areas of production and potentially increase its productivity.

Drought has been identified as one of the most important abiotic stresses that negatively influence crop growth and development. It is described as a major climatic event from both an agricultural and biological perspective that alters crop productivity and survivability (Passioura 2007). Recent data suggest that the reduction of crop productivity due to extreme drought conditions is likely to occur based on crop and climatic models in the coming years (Leng and Hall 2019). The unpredictability of the weather at both regional and global levels could pose a threat to crop productivity, and the number of areas susceptible to drought could increase. Hence, varietal improvement towards climate resiliency can be a viable and long-term solution for food security and sustainability.

Plant response to drought is a complex process involving different morpho-physiological, biochemical, and molecular activities (Rambabu et al. 2016). Therefore, breeding for drought tolerance has been proven to be similarly complex. It is imperative to properly characterize the response of different mungbean genotypes to determine whether the initial collection has the suitable genetic base for drought tolerance breeding. Evaluation of physiological responses of different mungbean accessions is a good approach for detecting genotypes with traits that infer drought tolerance, as well as the overall diversity of the population. Raina et al. (2016) observed the variation in a mungbean germplasm collection using physiological traits related to drought. Identification and evaluation of key drought-tolerant traits for mungbean are vital steps to recognize potential genotypes for the development of new crop varieties efficiently (Singh et al. 2021). Physiological parameters such as chlorophyll fluorescence are relatively easy to gather yet provide significant information on the relative photosynthetic efficiency of the plant. Additionally, Afzal et al. (2014) suggest that effective antioxidant systems could improve photosynthetic capacity and reduce oxidative damages of mungbean under drought, resulting in higher crop productivity. Analysis of these parameters and their relationship with yield could identify characters that mostly affect productivity and offer criteria for drought tolerance selection.

The importance of mungbean in both agricultural and food sustainability suggests that mungbean should be further cultivated and improved in terms of stress tolerance. With limited recommendations for drought-tolerant varieties in the country, farmers and plant breeders alike will both benefit from the development of new climate-resilient mungbean lines. The early stage in variety development will be to screen possible mungbean genotypes that will be utilized as parentals for drought tolerance breeding. The indirect selection of genotypes through these physiological parameters has been reported to be vital for complementing yield-based selection breeding (Larkunthod et al. 2018). This study evaluated the physiological response of different mungbean genotypes under terminal drought to detect potential drought-tolerant mungbean genotypes that can be used in the development of drought-resilient varieties. Additionally, the study assessed the relationship between the different drought-related parameters to detect complementary traits for better characterization and selection of mungbean genotypes.

MATERIALS AND METHODS

Planting Materials

A total of 100 mungbean genotypes were used for the characterization of drought response. The evaluated genotypes consisted of both Pag-asa mungbean varieties, and germplasm, which were collected from the National Plant Genetic Resources Laboratory–Institute of Plant Breeding, University of the Philippines Los Baños. Pag-asa 7 was used as a check variety since it was recommended by the National Seed Industry Council–Bureau of Plant Industry–Department of Agriculture as drought resistant.

Field Condition and Experimental Setup

The field drought screening was conducted from January–March 2020 at the Central Experimental
Station, Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines at Los Baños (UPLB), Laguna, Philippines (14°8′ N, 238°44′ W, 36 masl). The experimental field has a textural grade equivalent to loam with a 67% water holding capacity (37% sand, 44% silt, and 19% clay). Meteorological data such as cumulative rainfall and the average daily temperature were also gathered from the UPLB–National Agromet Station (14°19′ N, 238°44′ W, 28 m above sea level) to assess the growing conditions for the whole duration of the experiment. Cumulative rainfall during drought imposition was significantly low with only a total of 27.3 mm. The average temperature in the field ranged from 22.9–31.8 ºC from January–April 2020.

The trial followed a randomized complete block design with four replications. Each genotype was planted in a 3-m row plot with 0.5-m spacing between rows. Drought was imposed by discontinuing irrigation water in the drought treatment at 30 d after planting (or during the early flowering stage). Soil moisture was determined weekly at a soil depth of 25–30 cm using an HH2 soil moisture meter (Delta-T Devices, United Kingdom). SMC before discontinuation of irrigation ranged from 25–30% then SMC was reduced to 13.0–15.0% during drought imposition. After terminal drought stress was imposed, no recovery phase or rewatering occurred after irrigation was withheld. Basal field fertilization was applied during land preparation as following 30-30-30 kg N: P: K fertilization rate. Insect and disease control was only utilized when pest incidence was evident.

### Physiological Response Characterization

The physiological traits and seed yield were evaluated to determine the potential drought tolerance of each mungbean genotype under terminal field drought stress.

**Total chlorophyll content.** Leaf samples were collected from the second youngest leaf of five randomly selected plants in each plot for each mungbean accession, 30 d after drought imposition. The total chlorophyll content of dried plant tissue was evaluated using a modified procedure by Yoshida et al. (1976). The leaf samples were oven-dried for 48 h at 65 ºC. Dried samples were powdered using an ED-5 Wiley mill. Powdered samples (50 mg) and 4 mL of 80% aqueous acetonitrile (v/v) were mixed in 13 × 100 mm test tubes. The mixture was shaken for 5 min and then centrifuged for another 5 min. The extracts were transferred into a new test tube and volumes were adjusted to 15.0 mL by adding 80% aqueous acetonitrile. The total chlorophyll content was obtained using the protocol by MacKinney (1941). Absorbance readings of the sample extracts were determined at 645 nm (OD645) and 663 nm (OD663) using a Shimadzu UV-Vis spectrometer (Model UV-mini 1480). Total chlorophyll content was computed using the formula:

\[
\text{Total chlorophyll content} = 20.2 \times OD_{645} + (8.02 \times OD_{663})
\]  

**Scavenging activity.** Leaf samples were collected from the second youngest leaf of five randomly selected plants in each plot for each mungbean accession, 30 d after drought imposition. The total antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Zlatev and Lidon 2012). Following the protocol by Chiappero et al. (2019) with slight modification, 200-mg dried leaf samples were used to obtain 1.0-mL leaf extract. Five (5.0) ml 80% methanol was added to the leaf samples and the mixture was incubated for 30 min with mixing every 10 min. After incubation, the samples were centrifuged, and the resulting supernatant was filtered. One (1.0) mL of the supernatant was added to 2.9 mL DPPH and was incubated in the dark for another 30 min at room temperature. Absorbance was measured at 517 nm and the DPPH solution was used as a blank. Scavenging activity was computed using the formula:

\[
\% \text{scavenging activity} = \frac{1 - (OD_{\text{blank}} - OD_{517})}{OD_{\text{blank}}} \times 100
\]  

**Electrolyte leakage.** Leaf disks were collected from the second fully expanded leaf from five random plants from each plot for each mungbean accession, 30 d after drought imposition. Leaf discs were washed with distilled water three times to remove dirt. The samples were placed in test tubes with 10-mL distilled water and incubated for 24 h at room temperature. The initial electrolyte (EC_{initial}) reading was measured after incubation. Following a modified procedure by Gargouri et al. (2019), samples were placed in a hot water bath at 90 ºC for 1 h. Another electrolyte reading (EC_{final}) was measured after samples were cool at room temperature. All readings were measured using the Ionix EC1 Conductivity pocket tester (Ionix Instruments, Singapore).

\[
EC_{\text{leakage}} = \frac{EC_{\text{initial}}}{EC_{\text{final}}} \times 100
\]  

**Chlorophyll fluorescence.** Most studies evaluate drought stress damage by measuring the chlorophyll fluorescence (Fm/Fv) of photosystem II (PSII), which describes the maximum quantum efficiency of PSII during stressed conditions. Fm/Fv was measured using a Handy PEA+ leaf chlorophyll fluorescence meter (Hansatech Instruments, United Kingdom). Measurements were done in the second fully expanded leaf from the top, 30 d after drought imposition. Three Fv/Fm readings were collected from five randomly selected plants in each plot.
Statistical Analysis  
Data were analyzed using SAS OnDemand for Academics (version 3.8 – Enterprise Edition) for the ANOVA and Tukey’s honest significant difference at a 5% level of significance. Pearson correlation analysis was also used to evaluate the relationship of all parameters to each other. A PCA was done using R version 4.1.1 and was visualized using ggbiplot function under the ggbiplot package (Vu 2015) to evaluate the genotype by trait relationship in the experiment as well as distinguish potential drought-tolerant and drought-susceptible mungbean genotypes. PCA has been described as one of the most widely used multivariate analyses to assess differences, and similarities in a data set was used to describe the overall variation of a multivariate data set by transforming each original variable – in this case, the physiological responses – into a new set of specific linear combinations of PCs.

RESULTS  

Analysis of Variance and Descriptive Summary Statistics  
Results from the analysis of variance revealed that significant differences ($P < 0.05$) were present in total chlorophyll content, scavenging activity, electrolyte leakage, chlorophyll fluorescence (Fv/Fm), and seed yield of the tested mungbean genotypes grown under terminal drought (Table 1). The total chlorophyll values from the mungbean genotypes ranged from 6.33–31.77 mg/g with an average of 15.40 mg/g. Scavenging activity values from the DPPH scavenging assay varied from 14.05–98.30% with an average of 43.55%. EC values varied from 20.7–88.3 % from across the tested genotypes with an average equal to 68.1%. Chlorophyll fluorescence values during the peak drought stress differed from 0.69–0.83 with an average of 0.763. Lastly, estimated seed yield from the tested mungbean genotypes varied greatly from as low as no yield to 0.76 t ha$^{-1}$.

<table>
<thead>
<tr>
<th>Source of error</th>
<th>Chl (mg/g)</th>
<th>SA (%)</th>
<th>EC (%)</th>
<th>Fv/Fm</th>
<th>SY (t ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>33.0**</td>
<td>67.63**</td>
<td>24.75**</td>
<td>14.97**</td>
<td>77.55**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>9.713573</td>
<td>7.546040</td>
<td>12.48211</td>
<td>1.938003</td>
<td>8.605508</td>
</tr>
<tr>
<td>Min</td>
<td>6.33</td>
<td>14.05</td>
<td>20.74</td>
<td>0.685</td>
<td>0.0</td>
</tr>
<tr>
<td>Max</td>
<td>31.77</td>
<td>98.30</td>
<td>88.33</td>
<td>0.834</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean</td>
<td>15.40</td>
<td>43.55</td>
<td>68.10</td>
<td>0.763</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*aChl – total chlorophyll content; SA – scavenging activity; EC – electrolyte leakage; Fv/Fm – chlorophyll fluorescence; SY – estimated seed yield

Physiological Response to Drought  
The chlorophyll content of the tested mungbean genotypes indicated variation among each other under terminal drought stress. Among all the genotypes, PHL 12630 showed the highest chlorophyll content (31.77 mg/g), which was at least two-fold higher than the check. Other mungbean genotypes maintained higher chlorophyll content than the check, *i.e.* Pag-asa 7 (10.61 mg/g) during the drought stress, with the top 10 having values ranging from 20.95–31.77 mg. It was worth noting that Pag-asa 5 (20.96 mg/g) was able to maintain significantly higher chlorophyll values than Pag-asa 7 (Figure 1).

The DPPH scavenging assay revealed significant variation among mungbean genotypes in terms of scavenging activity. Top mungbean genotypes generated scavenging activity values ranging from 54.20–92.10%, with PHL 12635 (92.01%) being the highest. The check, Pag-asa 7 (10.61 %) belonged to the bottom 25%, while the Pag-asa 3 and Pag-asa 5 were in the top 40% in terms of scavenging activity with 16.69 and 20.96%, respectively (Figure 1).

Another indicator of plant resilience under drought conditions is membrane stability (Blum and Ebercon 1981). Results indicated that 54 out of 100 genotypes have electrolyte leakage values higher than the average equal to 51.98%. Pag-asa 7 exhibited significantly high electrolyte leakage values equal to 68.16% as compared to most of the genotypes, the second-highest in the Pag-asa varieties next to Pag-asa 19 with 68.10%. Genotypes such as PHL 14613, PHL 15274, and Pag-asa 5 maintained their membrane stability under terminal drought conditions, with leakage values ranging only from 20.74–26.13% (Figure 1).

The last physiological parameter tested in the study was the chlorophyll fluorescence or the quantum efficiency (Fv/Fm). Healthy plants usually showed values ranging from 0.800 and above, and the values lower than 0.800 are considered plants under stress indicating some degree of photoinhibition (Maxwell and Johnson 2000). Most of the genotypes (80 out of 100) had Fv/Fm values lower
Figure 1. Distribution and top mungbean performer for each physiological parameter evaluated under terminal drought.
than 0.80. Pag-asa 7, whose value was equal to 0.75, was significantly lower than at least 48.0% of the total genotypes tested. In comparison, Pag-asa 3 and Pag-asa 5 were able to maintain Fv/Fm values equal to 0.80 during the peak stress. Furthermore, these values were comparable with those of the top mungbean genotypes having Fv/Fm values greater than 0.80, with PHL 12957 having the highest value equal to 0.84 (Figure 1).

Estimated Seed Yield under Drought
The seed yield for each mungbean genotype was significantly different from each other under drought conditions. It should be noted that all the genotypes tested only produced estimated seed yield lower than 1.0 t ha\(^{-1}\), and the highest estimated seed yield under drought was observed in PHL 14613 (0.76 t ha\(^{-1}\)). The check variety, Pag-asa 7, produced an estimated seed yield of 0.22 t ha\(^{-1}\), which was 71.1% lower compared to the highest yielding accession. The highest yielding variety was Pag-asa 5 with 0.32 t ha\(^{-1}\) estimated yield under terminal drought (Figure 1).

Trait Correlations
Pearson correlation analysis was done to evaluate the relationships among the physiological traits, as well as their association with seed yield. Significant linear relationships were observed between the physiological parameters. Results showed that positive linear correlations were present between Chl x SA (r-value = 0.52), Chl x Fv/Fm (r-value = 0.51), and SA x Fv/Fm (r-value = 0.59) – suggesting a moderately strong relationship between the parameters (0.5 < |r| < 0.7). Moreover, negative linear correlation was observed between EC x Chl (r-value = −0.43), EC x Fv/Fm (r-value = −0.55), and EC x SA (r-value = −0.84). Furthermore, the relationship between the physiological parameters and seed yield, despite being significant, were all low in magnitude. Results suggest a moderately weak (0.3 < |r| < 0.5) association of seed yield with Chl (r-value = 0.40), SA (r-value = 0.44), EC (r-value = −0.37), and Fv/Fm (r-value = 0.40).

Principal Component Analysis
Results from PCA revealed that 88.03% of the total variation of the data was associated with the first PCs. All the physiological parameters were positively correlated with the first PC except for electrolyte leakage. The PC1 accounted for more than half of the total variation, which was equal to 53.43%. For PC2, only the total chlorophyll content and scavenging activity showed a direct correlation, while the opposite was observed with electrolyte leakage, chlorophyll fluorescence, and seed yield. The PC2 contributed 18.81% of the total variation of the data set (Table 2).

Specific contributions or loadings of each parameter for each PC were obtained from the PCA. The PC1 has relatively balanced contribution values for each of the physiological parameters – CHL (13.45), ASA (27.65), EC (26.77), and Fv/Fm (24.42) – while the PC2 was mainly affected by seed yield (76.93) (Figure 2A). The parameters used in the study were all well represented in the selected PCs (PC1 and PC2) and could be identified as significant and major contributors in the present drought tolerance study.

Table 2. PCA indicating the relationship of each physiological parameter and seed yield to each PC and the proportion of variance contributed by each PC to the overall variation.

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHL</td>
<td>0.3668</td>
<td>0.0408</td>
<td>−0.7218</td>
<td>−0.4199</td>
<td>0.0412</td>
</tr>
<tr>
<td>SA</td>
<td>0.5258</td>
<td>0.07274</td>
<td>0.4388</td>
<td>−0.1547</td>
<td>0.7084</td>
</tr>
<tr>
<td>EC</td>
<td>−0.5174</td>
<td>−0.2150</td>
<td>−0.4239</td>
<td>0.1364</td>
<td>0.6985</td>
</tr>
<tr>
<td>Fv/Fm</td>
<td>0.4942</td>
<td>−0.1126</td>
<td>−0.2856</td>
<td>0.8133</td>
<td>−0.0007</td>
</tr>
<tr>
<td>S. Yield</td>
<td>0.2776</td>
<td>−0.8771</td>
<td>−0.1588</td>
<td>−0.3460</td>
<td>−0.0932</td>
</tr>
<tr>
<td>Proportion of variance</td>
<td>0.5343</td>
<td>0.1881</td>
<td>0.1579</td>
<td>0.08195</td>
<td>0.0377</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.6345</td>
<td>0.9698</td>
<td>0.8887</td>
<td>0.64012</td>
<td>0.4342</td>
</tr>
</tbody>
</table>

*Chl – total chlorophyll content; SA – scavenging activity; EC – electrolyte leakage; Fv/Fm – chlorophyll fluorescence; S. Yield – estimated seed yield; PC – principal component
parameters and estimated seed yield (Table 3). Cluster I (red) and II (blue) were considered drought susceptible groups because of their significantly low values across all the physiological parameters. On average, both clusters had the lowest physiological values among all the groups – Cluster I had Chl (12.77 ± 4.13 mg/g), SA (41.84 ± 13.19%), EC (59.34 ± 16.41%), and Fv/Fm (0.74 ± 0.029) whereas Cluster II had Chl (14.57 ± 4.37 mg/g), SA (43.39 ± 14.40%), EC (59.50 ± 16.69%), and Fv/Fm (0.75 ± 0.031). The estimated seed yield from these groups was also the lowest with 0.23 ± 0.080 and 0.24 ± 0.122 t ha$^{-1}$, respectively.

In comparison with the first two clusters, Cluster III (yellow) can be described as moderate drought susceptible, as suggested by slightly higher physiological values and estimated seed yield (Table 3). Cluster I (red) and II (blue) were considered drought susceptible groups because of their significantly low values across all the physiological parameters. On average, both clusters had the lowest physiological values among all the groups – Cluster I had Chl (12.77 ± 4.13 mg/g), SA (41.84 ± 13.19%), EC (59.34 ± 16.41%), and Fv/Fm (0.74 ± 0.029) whereas Cluster II had Chl (14.57 ± 4.37 mg/g),
estimated seed yield obtained in the group: Chl (15.84 ± 4.17 mg/g), SA (54.59 ± 17.46%), EC (50.16 ± 14.64%), Fv/Fm (0.77 ± 0.029), and estimated seed yield (0.25 ± 0.073 t ha⁻¹).

The next two clusters can be classified as potential drought-tolerant groups but with distinct differences. Both Cluster IV (purple) and Cluster V (green) can be characterized by having significantly better physiological values than the rest. For Cluster IV, Chl (16.74 ± 5.45 mg/g), SA (62.48 ± 14.35%), Fv/Fm (0.78 ± 0.023), and lowest EC (40.42 ± 14.38%) values were obtained. Values for Cluster V were Chl (19.03 ± 4.29 mg/g), SA (61.82 ± 21.69%), EC (44.38 ± 22.33%), and Fv/Fm (0.78 ± 0.033). The main difference between the two clusters was the higher estimated seed yield obtained from Cluster V with an average of 0.37 ± 0.156 t ha⁻¹ as compared to Cluster IV with only 0.23 ± 0.07 t ha⁻¹. Interestingly, all Pag-asa varieties (Pag-asa 3, 5, 7, and 19) were contained within Cluster IV.

**DISCUSSION**

Drought stress has been considered as one of the most significant factors affecting crop productivity and yield. Singh and Singh (2011) reiterated that susceptibility of mungbean to various abiotic stresses such as drought during various critical growth stages such as flowering and pod setting contributes mainly to yield loss. The differences in seed yield among the mungbean genotypes could be attributed to the inherent genetic potential of each genotype (Belay et al. 2019). The complex processes involving the drought response in plants would play an important role in maintaining crop productivity under unfavorable conditions. Improvement of yield is possible through the selection of genotypes using these yield-related characteristics (Nainu and Murugan 2020). The modifications in physiological processes such as chlorophyll synthesis, antioxidant activity, oxidative stress mitigation, and photosynthetic efficiency could dictate the overall productivity and growth of the crop (Dutta et al. 2018). The proper identification of traits related to drought tolerance is important in variety development, and these physiological parameters have shown to be a significant indicator of crop resilience during adverse conditions such as drought (Nadeem et al. 2019).

Several genotypes in the current study maintained a higher concentration of chlorophyll even under severe drought stress as compared to the check variety. Chlorophyll content has been widely used as a parameter for drought stress evaluation and is considered as one of the most noticeable responses of the crop to water deficit conditions. High chlorophyll content in plants usually relates to the stay-green trait or the ability of plants to delay senescence during drought extending the plant carbon assimilation (Rambabu et al. 2016). The reduction in chlorophyll content has been attributed mainly to the disruption of chloroplast caused by the up-regulation of reactive oxygen species (ROS) during stressful conditions such as drought (Sofi et al. 2021). Studies showed that most pulse crops tend to have significantly lower chlorophyll content when subjected to water stress with reduction levels ranging from 24% to as high as 88% (Arefian and Shafaroudi 2015). As an integral part of the photosynthetic process, the chlorophyll content could provide important information on the drought tolerance potential of each mungbean genotype tested.

The increased production of ROS during drought stress can be managed through an effective ROS scavenging mechanism. ROS are highly reactive compounds that are responsible for oxidative stress damages in the plant cell, ultimately leading to cell death (Hsiao 1973). Minimizing the damages due to these compounds requires an efficient scavenging system to reduce or remove the concentration of these free radicals. This also includes an effective signaling system to ensure induction of gene expression of different antioxidant enzymes related to drought tolerance (Zlatev and Lidon 2012). Genotypes with better antioxidant defense activities could be considered stress-tolerant and could perform well under stressful environments (Correia et al. 2020). Some mungbean genotypes have shown high scavenging activity at peak stress, suggesting the difference in the ROS scavenging capability among the tested genotypes. An increase in concentrations of several antioxidants (i.e. catalases, superoxide dismutase, and ascorbate peroxidase) during drought stress has been observed in mungbean and other leguminous crops, which led to better crop performance (Ali et al. 2018). The utilization of antioxidant assays to evaluate differences in the level of adaptability of the genotypes have yielded significant results for variety development in other pulses (Dutta et al. 2018).

In addition to ROS scavenging, another indicator of plant resilience under drought conditions is membrane stability (Blum and Ebercon 1981). Structural damages due to oxidative stress in the plant cells can be evaluated by measuring the electrolyte leakage of the samples (Blum and Ebercon 1981). Since cell membranes are one of the first components to be affected during the onset of abiotic stresses, it was expected that most of the mungbean genotypes in this study had high leakage values (Kaur et al. 2015). Plant membrane integrity is compromised during drought stress, resulting in increased membrane permeability, allowing plant solutes/ions to easily permeate and be measured. (Rehman et al. 2016). Measuring the electrical conductivity could help assess
the relative membrane injury of the leaf samples. Studies with mungbean plants by Nazran et al. (2019) reported the increasing electrolyte leakage values as the moisture in the soil decreases. Most of the check varieties, as well as some of the mungbean accessions in the present study, have significantly high leakage values, suggesting the differences in the susceptibility of the majority of the mungbean genotypes to drought.

Moreover, changes in chlorophyll fluorescence can be a reliable early estimate of the photosynthetic efficiency of a plant (Zhou et al. 2015). Evaluating the ratio between the variable fluorescence (Fv) and the maximum fluorescence (Fm) of the chlorophyll reveals the ability of the photosystem II (PSII) to maintain its function under stress efficiently (Mathur et al. 2011). The reduction in the Fm/Fv can be easily measured with chlorophyll fluorescence meters. The Fv/Fm ratio readings can provide information on the degree of damage incurred by the PSII during drought conditions (Zhou et al. 2015). Only a small number of mungbean genotypes (~20) maintained a healthy PSII fluorescence reading (≥0.80), which suggests stable photosynthetic capability during drought (Narina et al. 2014). Drought-tolerant mungbean genotypes were more likely to maintain higher chlorophyll fluorescence during drought conditions due to their ability to sustain the PSII activity as compared to drought-sensitive genotypes (Raina et al. 2019). The combination of these adaptive traits based on the physiological responses of the mungbean genotypes could identify potential drought-tolerant mungbean lines.

The relationship between traits was analyzed to determine the positive and negative relationship of traits evaluated – in this case, physiological traits and seed yield. Likewise, these analyses determine the traits most likely to be selected together during the selection of parentals for drought tolerance breeding, thereby increasing the efficiency in crop development and minimizing trait selection redundancy (Yan and Fregeau-Reid 2008). The results in the current study indicated the significant correlation among several key traits for drought tolerance such as chlorophyll content, electrolyte leakage (membrane injury), and antioxidant scavenging to chlorophyll fluorescence, which suggests the suitability of fluorescence parameter in mungbean mass screening for drought tolerance (Singh et al. 2021). Several drought studies in other pulse crops also reported significant relationships between seed yield and physiological parameters similar to those found in other studies (Seyahjani et al. 2020; Bano et al. 2021). Drought has been known to alter different physiological processes resulting in a reduction of yield (Singh and Singh 2011). Recognizing physiological traits that correlate with seed yield will be crucial in enhancing genetic gains during variety development, as well as optimizing screening and characterization of potential drought tolerant genotypes.

Variability in the population is vital to crop variety development (Singh et al. 2021). The utilization of physiological parameters to characterize the performance of each mungbean genotype was effective in detecting variation among the selection, which was evident in the results of the present study. Additionally, physiological breeding has resulted in significant genetic gains in different crops (Reynolds and Langridge 2016). PCA revealed that the parameters used were effective in differentiating the performance of each genotype. The PCA grouped the mungbean genotypes into five clusters, two of which could potentially be identified as drought-tolerant groups. Clusters IV and V were identified as potential drought-tolerant groups because both have superior physiological responses as compared to the other clusters. The main difference between the two was the estimated seed yield, wherein Cluster V has higher average values than Cluster IV. The results can be attributed to the possible adaptation mechanism each plant follows during terminal drought stress, where some plants tend to focus the majority of assimilates for plant maintenance and survival instead of seed yield (Ludlow and Munchow 1990). The source-sink relationship under drought stress has also been observed to be altered, resulting in reduced yield due to the weakening of source strength during filling stages of the crop (Basu et al. 2016). The differences in the performance of each grouping suggest the dissimilarity of the adaptive mechanisms followed by genotypes in each of the groups (Osakabe et al. 2014). These natural variations are vital resources for developing drought-tolerant mungbean lines and should be considered before and during the breeding process. PCA has been used in different legumes and other crops to facilitate the selection of genotypes with characteristics related to drought tolerance and could produce better yield (Cortés et al. 2013; Sousa et al. 2015; AbdElgawad et al. 2015; Paramesh et al. 2016). This physiological breeding approach to develop climate change-ready varieties can only be achieved with the presence of superior germplasm having wide genetic variations (Reynolds and Langridge 2016). The selection of adaptive traits complementing yield is a well-documented strategy to improve crop production in water deficit environments (Singh and Singh 2011).

CONCLUSION

Drought is an extreme environmental condition highly affecting crop development and productivity through a complex series of biochemical-physiological processes.
In this study, selected physiological parameters were used to evaluate the response of one hundred mungbean accessions, including several Pag-asa varieties under terminal drought stress to detect potential drought-tolerant lines that can be used for crop variety development. The evaluation of chlorophyll fluorescence, scavenging activity, electrolyte leakage, and chlorophyll content in the mungbean population was effective in identifying potential sources of drought tolerance. The PCA and clustering analysis were able to categorize the evaluated mungbean genotypes into five distinct clusters (I, II, III, IV, and V) based on PCs generated from physiological parameters and estimated seed yield. The first two PCs, having the highest cumulative variance, contributed at 72.2% and were used in the clustering analysis. PC1 has relatively balanced contribution values from each of the physiological parameters at Chl (13.45), SA (27.65), EC (26.77), and Fv/Fm (24.42) whereas PC2 was mainly affected by seed yield (76.93). Clusters I and II were identified as potential drought susceptible groups due to their weak physiological response and low seed yield, Cluster III was moderately susceptible since it has better physiological values than Cluster I and II, and Clusters IV and V were identified as the potential drought-tolerant group for having the best physiological performance among the rest. One difference between Clusters IV and V was higher seed yield was observed in Cluster IV, which can be attributed to possible differences in the adaptation mechanism present plants follow to counter the adverse effects of drought. Furthermore, the results in the current study indicated the significant correlation among several key traits for drought tolerance such as chlorophyll content, electrolyte leakage, and antioxidant scavenging to chlorophyll fluorescence – suggesting the suitability of fluorescence parameter in mungbean mass screening for drought tolerance. Although weak correlation to seed yield was present (0.3 < │r│ < 0.5), correlations among each physiological parameter were moderately strong (0.5 < │r│ < 0.7). The findings exhibited the value of physiological parameters in discriminating mungbean germplasm for drought tolerance screening. The mungbean genotypes in the potential drought-tolerant groups could be further evaluated especially for trait heritability to assess their usefulness as sources of drought tolerance for climate-resilient mungbean breeding, as well as multilocation trial to determine genotype x environment interaction.

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