

EFFECT OF PEG-INDUCED DROUGHT STRESS ON MUNGBEAN PLANTS REVEALED RESISTANT VARIETIES BASED ON LEAF WILTING INDEX AND BIOCHEMICAL MOLECULES

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Abstract: At the early vegetative growth stage, mungbean are mostly affected by drought, and it is also one of the most promising stages that can be used to screen for drought stress tolerance traits in multiple varieties. Therefore, this study utilized polyethylene glycol (PEG-6000) to induce drought stress towards selection of drought tolerance mungbean varieties in their early vegetative growth stage using both hydroponics and soil based systems. In this study, leaf wilting index and responses of biochemical molecules were used as the basic factors to determine the effect of PEG-induced drought stress among the mungbean varieties. Prior to the imposition of drought stress, germination potentials of the varieties were evaluated and all had germination $\geq 60\%$. Except for Tvr29 and Tvr44, hydroponic system revealed that $\geq 80\%$ of the varieties had ≥ 1 of their leaves significantly ($P \leq 0.05$) wilted. The highest LWI were recorded for Tvr49 and Tvr79. Re-evaluation of Tvr29, Tvr44, Tvr49 and Tvr79 using soil, shows that Tvr29 and Tvr44 resisted drought stress. The hydrogen peroxide, superoxide radical and malondialdehyde contents decreased in Tvr29 and Tvr44, and increased in Tvr49 and Tvr79 in comparison to the control. Tvr29 and Tvr44 had high proline content than Tvr49 and Tvr79. Based on LWI and biochemical molecules, this study revealed that Tvr29 and Tvr44 should be utilized where water deficit is a challenge to mungbean globally.

Keywords: legumes, plant stress, seed germination, stress tolerance, water stress.

Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is a short-duration grain legume cultivated across Asia and rapidly spreading to other parts of the world which include Africa and Latin America [KARUPPANAPANDIAN & al. 2006]. The high content of digestible protein, fiber, antioxidants, and phytonutrients [ITOH & al. 2006] has demand mungbean to be in high demand [GHOSH & al. 2015]. However, mungbean's growing environment has become increasingly barren, and drought is the major problem towards mungbean's growth [YIN & al. 2015]. Like any other crop, it responds to a decrease in available soil moisture by reducing its growth and hence productivity [CHAUHAN & al. 2010; SINGH & SINGH, 2011; HANUMANTHARAO & al. 2016]. Yield loss of 31-57% used to occur in mungbean at flowering and 26% at post flowering/podding stages [NADEEM & al. 2019] during drought stress [FATHY & al. 2018]. According to SADASIVAN & al. (1988), drought stress during vegetative phase reduces grain yield through restricted plant size leaf area, root growth, dry matter accumulation, number of pods per plant and low harvest index. Technically, the effects of drought on mungbean begin with osmotic imbalance which gradually develops into metabolic and physiological disorders. These consequently affects photosynthesis

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[SANCHEZ & al. 2012] which is the most important physiological processes that regulate developmental stages in mungbean [ATHAR & ASHRAF, 2005].

Studies have shown variability in morpho-physiological traits for drought tolerance among mungbean varieties during different developmental stages of growth [NARESH & al. 2013; UDDIN & al. 2013]. Apart from the fact that specific changes can occur in plant tissues throughout their life cycle due to drought stress, developing criteria for selection of the best character may be itself a difficult option due to complexity of environment by genotype interactions [MURILLO-AMADOR & al. 2002]. On this note, a better understanding of the responses of mungbean varieties under drought stress condition is required [ABENAVOLI & al. 2016]. Thus, assessment of specific traits and their correlation under drought conditions would be helpful in selecting diverse valuable varieties with defined growth traits [SARKAR & al. 2013; ABRAHA & al. 2015; MISHRA & PANDA, 2017; TIWARI & al. 2018].

Among the various traits, seed germination, seed emergence to seedling stage, leaf damage, chlorosis and genotypic differences within species and leaf wilting have been established for screening drought tolerance traits [RANAWAKE & al. 2012; ALDERFASI & al. 2017; SWATHI & al. 2017] in any crop. Specifically, leaf wilting still remains a fundamental indicator for drought response; and it reduces the complexities associated with drought evaluation in crops. In fact, it was proposed that leaf wilting index (LWI) is the best indicator for crops in their early vegetative growth stage under drought stress [PUNGULANI & al. 2013]. The appearance of leaf wilting can impede photosynthesis as a result of overproduction of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) and reactive carbonyl species such as malondialdehyde (MDA) [GUO & al. 2012; SUI & al. 2015; HASANUZZAMAN & al. 2017]. Mostly often, under drought stress when ROS level exceeds the defense mechanisms, production and accumulation of H_2O_2 and $O_2^{\cdot-}$ normally enhance MDA which can damage macromolecules, cell structures [FARNESE & al. 2016] and alteration of intrinsic properties of biomolecules and eventually cell death [KURUTAS, 2016]. On the other hand, ROS are tactically exploited as a messenger to activate defense biochemical molecules in plants. Among the biochemical molecules, proline have been established [KAUSHAL & al. 2016] and can be used as criteria to screen mungbean varieties due to the fact proline accumulation is always more than that of amino acids [FAHRAMAND & al. 2014] under drought stress. Based on the above facts on leaf wilting index and plant biochemical molecules, we examined the LWI as the first indicator of drought stress as well as the H_2O_2 , $O_2^{\cdot-}$, MDA and proline contents in mungbean varieties in-view to add to reservoir of knowledge on drought stress tolerance mungbean globally.

Materials and methods

Seed germination potential of mungbean varieties

Prior to evaluation of mungbean varieties using Polyethyleneglycol (PEG)-6000, the germination potential of each varieties was determined. Exactly 18 varieties differing in seed morphology and colours were used. The varieties were obtained from Germplasm unit of International Institute of Tropical Agriculture, Ibadan, Nigeria. The seeds were surface sterilized with 0.5% NaOCl for 2 min, followed by 30 sec in 70% ethanol and thoroughly rinsed three times with sterile distilled water. Thereafter, the surface sterilized seeds were allowed to air dry under laminar air flow for 1hr. Exactly 10 seeds were placed at equidistant position in already prepared Petri dish (9 cm – diameter) moistened with two layers of filter papers (Whatman

No.1). This was placed in the dark for 2 days (temperature - 25 ± 2 °C, relative humidity - $65\pm 5\%$) and later in the light for another 5 long-days photoperiod (16 hr light / 8 hr dark) and maintained under $420 \mu\text{molm}^{-2} \text{s}^{-1}$ of photosynthetically active radiation in growth chamber. This experiment was done in three replicates for each varieties. At day 7, seeds were considered to have germinated once the radicle protruded at least 2 mm from testa. The germination percentage was calculated as described by Kader's (2005), (Germination [%] = (number of germinated seeds/total number of seeds) x 100.

Effect of PEG - induced drought stress on mungbean using hydroponics system

Pre-surface sterilized and already germinated seedlings of each varieties were separately and carefully arranged in sizeable netted holes rubber bound to a container (6 cm x 6 cm x 7 cm). Exactly 5 seedlings were positioned to maintain contact with the ¼-strength Hoagland's nutrient solution in the container. Three replicates of each varieties were set up, arranged in randomized complete design and maintained in growth chamber (photoperiod – 16 hr light / 8 hr dark) under the mixture of fluorescent light (about $420 \mu\text{molm}^{-2} \text{s}^{-1}$ of photosynthetically active radiation) and incandescent lamps. At 2 days interval, the ¼-strength nutrient solution were regularly changed to prevent algae growth. After emergence of 2 leaves per seedling, the ¼-strength nutrient was supplemented with 20% PEG – 6000 solution. Control seedlings were maintained in ¼-strength nutrients solution (without PEG). The whole experiment (both treatments and control) were allowed to stay for 10 days so that each varieties can maintain interaction with the PEG. A day to termination of the experiment, the LWI was determined using the method described by PUNGULANI & al. (2013). At termination of the experiment, the treated seedlings were again changed into ¼-strength nutrients solution (without PEG) for another 7 days to give room for recovery and the recovery percentage was calculated as the ratio of non-wilted leaves per seedling to that of total number of leaves per seedling.

Effect of PEG - induced drought stress on mungbean using soil

From hydroponics experiment, the two most tolerant (Tvr29 and TVr44) and the two most susceptible (Tvr49 and Tvr79) varieties were selected and re-evaluated in soil using PEG-6000 specifically to re-ascertain their response to drought stress. Pre-sterilized healthy seeds were sown in potted (6 cm – height and 7 cm – diameter) soil. Four seeds were sown into each pot, watered regularly with 20 ml of distilled water and maintained in a growth chamber with temperature of 24 ± 3 °C and relative humidity of $65\pm 5\%$ (photoperiod – 16 hr light / 8 hr dark) under the mixture of fluorescent light (about $420 \mu\text{molm}^{-2} \text{s}^{-1}$ of photosynthetically active radiation) and incandescent lamps. At 3-leaf stage, non-uniform seedlings were removed to maintain one seedling per pot. At 4-leaf stage, 20% PEG – 6000 solution was sequentially added on daily basis as 5 ml, 10 ml, 15 ml and 20 ml, and 20 ml was maintained till day 10 while nutrient solution was gradually withdrawn on daily basis as 20 ml, 15 ml, 10 ml, 5 ml and 0 ml was maintained till day10). A day before termination of the experiment, total chlorophyll content [ARNON, 1949], LWI [PUNGULANI & al. 2013] and biochemical molecules were determined from the leaf samples. In addition to the control (without PEG – 6000), the experiment was carried out in three replicates in randomized complete block design. That is, changing the pot positions to reduce environmental factors on the plants. At termination of the experiment, the treatments were re-watered for 7 days and the recovery percentage was calculated as the ratio of non-wilted leaves per plant to that of total number of leaves per plant.

Biochemical assays in mungbean

The proline contents of Tvr29, Tvr44, Tvr49 and Tvr79 were quantified with slight modification in the method described by BATES & al. (1973). Exactly 0.5 g of three replicated leaf samples were homogenized with 5 ml of 3% (w/v) sulfosalicylic acid. Homogenate was obtained by centrifugation using centrifuge (5000 g, 23 °C); 2 ml of the supernatant incubated with 2 ml glacial acetic acid and 2 ml ninhydrin reagent at a ratio of 1:1:1 in a boiling water bath at 100 °C incubated for 30 min. The reaction mixture was allowed to cool down to room temperature, the proline content was assayed through the absorbance of 520 nm. The amount of MDA was determined by the thiobarbituric acid (TBA) reaction in respect to lipid peroxidation according to HEATH & PACKER (1968). Fresh leaf samples (0.2 g) of each mungbean varieties in three replicates were homogenized with 5 ml of 0.25% TBA containing 10% TCA (trichloroacetic acid). The homogenate was subjected to boiling for 30 min at 95 °C and centrifuged at 10,000 g for 10 min. Specific absorbance values at 532 nm was subtracted from values corresponding to non-specific absorption at 600 nm. The MDA content was calculated according to the molar extinction coefficient of MDA ($155 \text{ mM}^{-1} \text{ cm}^{-1}$). H_2O_2 was extracted by homogenizing 200 mg of each varieties tissue separately in three replicates with 5% TCA. The homogenate was centrifuged at 12,500 g for 10 min. To 0.4 ml of 50% TCA, 0.2 ml of (2.5 M) potassium thiocyanate and 0.4 ml of 10 mM of ferrous ammonium sulphate were added with 1.6 ml supernatant to determine H_2O_2 level at the absorbance of 480 nm [SAGISAKA, 1976]. O_2^- was determined based on total O_2 content as described by ELSTNER & HEUPEL (1976). Leaf tissue of 200 mg of each varieties (in three replicates) was homogenized with 5 ml of 65 mM phosphate buffer (pH 7.8). After homogenate was centrifuged at 10000 g for 10 min, 1 ml supernatant, 0.9 ml of phosphate buffer 65 mM (pH 7.8) and 0.1 ml of 10 mM hydroxylamine were added and subjected to 25 °C for 30 min. After incubation, 1 ml of 17 mM sulphinalamide and 1 ml of 7 mM α – naphthyl were added for appropriate reaction at 25 °C. To the reaction mixture, 1 ml of diethyl ether was added and centrifuged at 15,000 g for 5 min and the absorbance was measured at 530 nm. Determination of H_2O_2 , O_2^- , MDA and proline were independently repeated to ascertain values.

Statistical analysis

Experimental treatments were compared using SAS software, version 9.1 (SAS Institute, Cary, NC, USA). For each experiment, three replicated data sets were subjected to the analysis of variance (ANOVA) technique according to the experimental design to find out the significance of the treatments. ANOVA was also used to determine the effect of treatments and error associated with the experiment. Mean comparison of traits was used and carried out by protected LSD ($P = 0.05$; Students-Newman-Keuls Test) where the error mean square served as the standard error of differences between mean.

Results**Seed germination potentials of mungbean varieties**

Germination potential of each mungbean varieties was determined in-vitro under control conditions. All the varieties had $\geq 60\%$ germination. Out of the 18 varieties, 9 (50%) which include Tvr21, Tvr42, Tvr43, Tvr44, Tvr47, Tvr48, Tvr62, Tvr79 and Tvr97 had 100% germination while Tvr29 and Tvr82 had the least germination. Tvr17, Tvr19, Tvr32, Tvr40 and Tvr49 were not significantly ($P \leq 0.05$) different from each other in comparison to Tvr14 and Tvr46 (Figure 1).

Effect of PEG-induced drought stress on mungbean varieties based on leaf wilting index

Under hydroponics system using LWI as indicator of drought stress, observation shows that $\geq 80\%$ of the varieties had ≥ 1 of their leaves wilted. Specifically, Tvr21, Tvr42, Tvr43, Tvr47 and Tvr82 had 50% of their leaves wilted in comparison to their controls. Tvr49 had the highest LWI, followed by Tvr79. On the contrary, Tvr29, followed by Tvr44 exhibited resistance to the PEG-induced drought stress with no evidence of leaf wilting. Although, Tvr46 and Tvr48 demonstrated moderate resistance in comparison to Tvr29 (Figure 2A). After the treatments, we re-engaged the treated plants to ascertain their recovery level. Recovery was maintained for 7 days and percentage recovery was recorded. Tvr29 demonstrated remarkable recovery of 100%, followed by Tvr44 (80%) and Tvr48 (65%) while Tvr49 and Tvr79 were unable to recover. More than 60% of the mungbean varieties had $\leq 40\%$ recovery chances in comparison to Tvr29, Tvr44, Tvr46 and Tvr48 (Figure 2B).

Next, the two most resistant varieties (Tvr29 and Tvr44) and sensitive varieties (Tvr49 and Tvr79) were selected and re-evaluated using soil. Based on observations from soil, the 20% of PEG-induced the highest LWI of 55% and 33% on Tvr49 and Tvr79 respectively. The LWI of Tvr49 was 48% higher than that of Tvr29. Both Tvr29 and Tvr44 had the lowest LWI of 7% and 15% respectively (Figure 2C). The recovery of Tvr29 and Tvr44 in soil was similar to that of hydroponics. Unfortunately, Tvr49 and Tvr79 were unable to recover. Obviously, all the controls recovered irrespective of the resistant or sensitive varieties (Figure 2D). In addition, the effect of the leaf wilting as a result of PEG – induced drought stress also reflected negatively on the chlorophyll of the treated leaves. Specifically, the chlorophyll contents of the controls were relatively insignificant ($P \leq 0.05$) while that of Tvr29 and Tvr44 were outstanding in comparison to the low chlorophyll contents of Tvr49 and Tvr79 (Figure 3).

Phenotypically, apart from the fact that Tvr29 was not showing any sign of leaf wilting, even when compared with the control (without PEG), Tvr29 had a unique leaf rolling morphology pattern. Interestingly, both the PEG treated and the control maintained similar leaf rolling morphology pattern throughout the experiment (Figure 4).

Influence of PEG-induced drought stress on biochemical molecules of mungbean varieties

Biochemical responses of the selected resistant (Tvr29 and Tvr44) and sensitive varieties (Tvr49 and Tvr79) were examined. Under PEG-induced drought stress, the H_2O_2 contents were on the high side in comparison to the control (without PEG). The H_2O_2 content of Tvr49 increased, followed by that of Tvr79 and Tvr29 had the least H_2O_2 content (Figure 5A). Similar observation was recorded for O_2^- content with respect to the examined varieties. All the controls maintain similar trend of significance in comparison to the PEG treated varieties. Tvr49 varieties had a unique O_2^- content in comparison to Tvr79, while Tvr29 and Tvr44 had the least (Figure 5B). Both the resistant and sensitive varieties produced proline. However, Tvr49 and Tvr79 had low proline content while Tvr29 had the highest, followed by Tvr44 (Figure 5C). In comparison with the control, the MDA content of the Tvr49 was the highest, followed by Tvr79. Tvr29 and Tvr44 had low MDA content (Figure 5D). Generally, H_2O_2 , O_2^- and MDA followed similar response pattern where $Tvr49 > Tvr79 > Tvr44 > Tvr29$. Only proline content followed opposite pattern of $Tvr29 > Tvr44 > Tvr79 > Tvr49$.

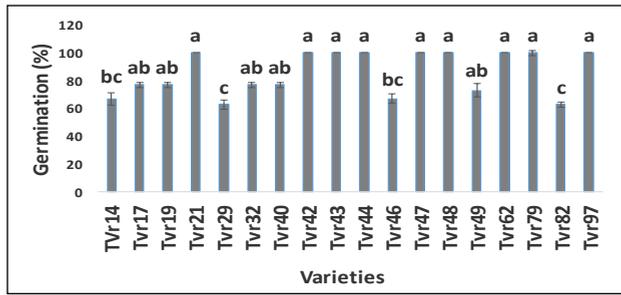


Figure 1. Seed germination potential of each mungbean variety. All the mungbean varieties germinated well ($\geq 60\%$). Means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls Test. The results shown are means \pm standard error (n=3).

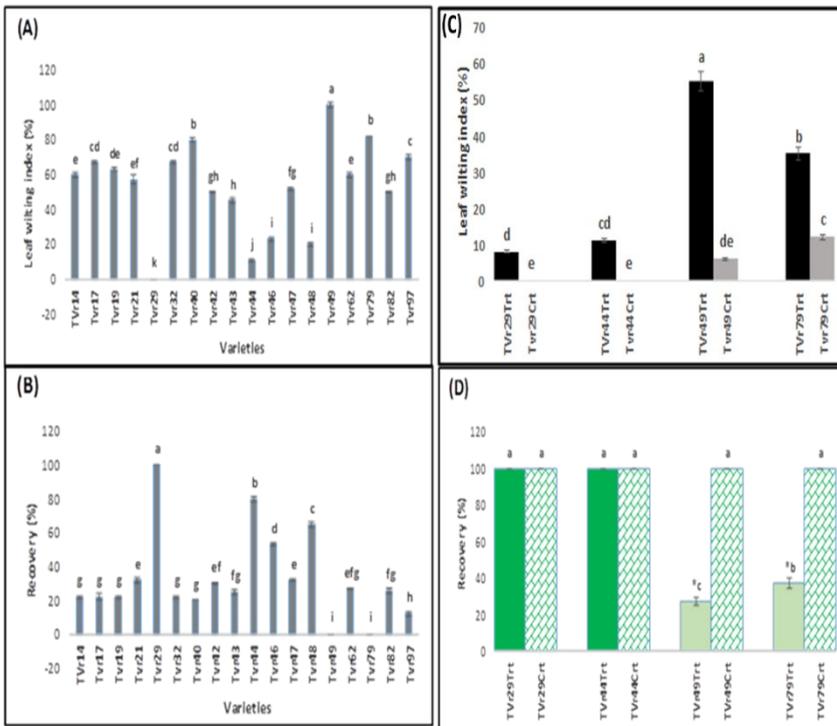


Figure 2. (A) Under hydroponics system, effect of 20% PEG-6000 was determined on leaf wilting and calculated based on leaf wilting index (B) Plant recovery (%) under hydroponics system, (C) Under soil system, effect of 20% PEG-6000 was determined on leaf wilting and calculated based on leaf wilting index, (D) Plant recovery (%) in soil based on effect of PEG-induced drought stress. Tvr29Trt (Tvr29 treated with PEG), Tvr29CrT (Tvr29 without PEG), Tvr44Trt (Tvr44 treated with PEG), Tvr44CrT (Tvr44 without PEG), Tvr49Trt (Tvr49 treated with PEG), Tvr49CrT (Tvr49 without PEG) and Tvr79Trt (Tvr79 treated with PEG), Tvr79CrT (Tvr79 without PEG). (*) indicates significantly ($P \leq 0.05$) sensitive and unable to recover. Means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls Test. The results shown are means \pm standard error (n=3).

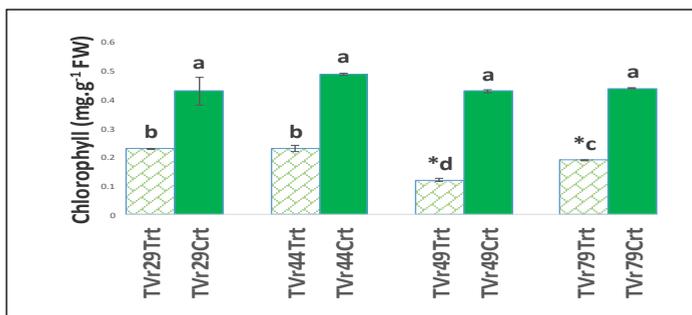


Figure 3. Effect of 20% PEG-6000 on chlorophyll content of selected mungbean varieties (Tvr29, Tvr44, Tvr49 and Tvr79). Tvr29Trt (Tvr29 treated with PEG), Tvr29CrT (Tvr29 without PEG), Tvr44Trt (Tvr44 treated with PEG), Tvr44CrT (Tvr44 without PEG), Tvr49Trt (Tvr49 treated with PEG), Tvr49CrT (Tvr49 without PEG) and Tvr79Trt (Tvr79 treated with PEG), Tvr79CrT (Tvr79 without PEG). (*) indicates significantly ($P \leq 0.05$) sensitive with very low chlorophyll content. Means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls Test. The results shown are means \pm standard error (n=3).

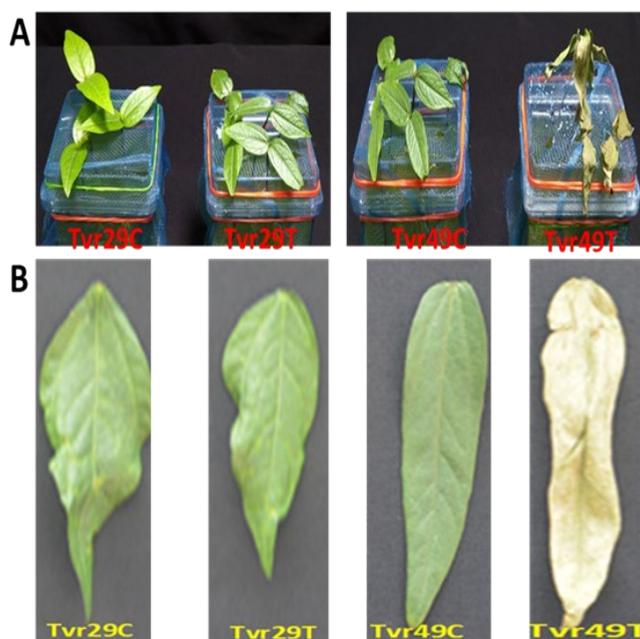


Figure 4. The leaves of Tvr29 exhibited resistance to drought stress in comparison to Tvr49 that had extreme leaf wilting using (A) hydroponics system and (B) soil system. Tvr29Trt (Tvr29 treated with PEG), Tvr29CrT (Tvr29 without PEG), Tvr44Trt (Tvr44 treated with PEG), Tvr44CrT (Tvr44 without PEG), Tvr49Trt (Tvr49 treated with PEG), Tvr49CrT (Tvr49 without PEG) and Tvr79Trt (Tvr79 treated with PEG), Tvr79CrT (Tvr79 without PEG). Means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls Test. The results shown are means \pm standard error (n=3).

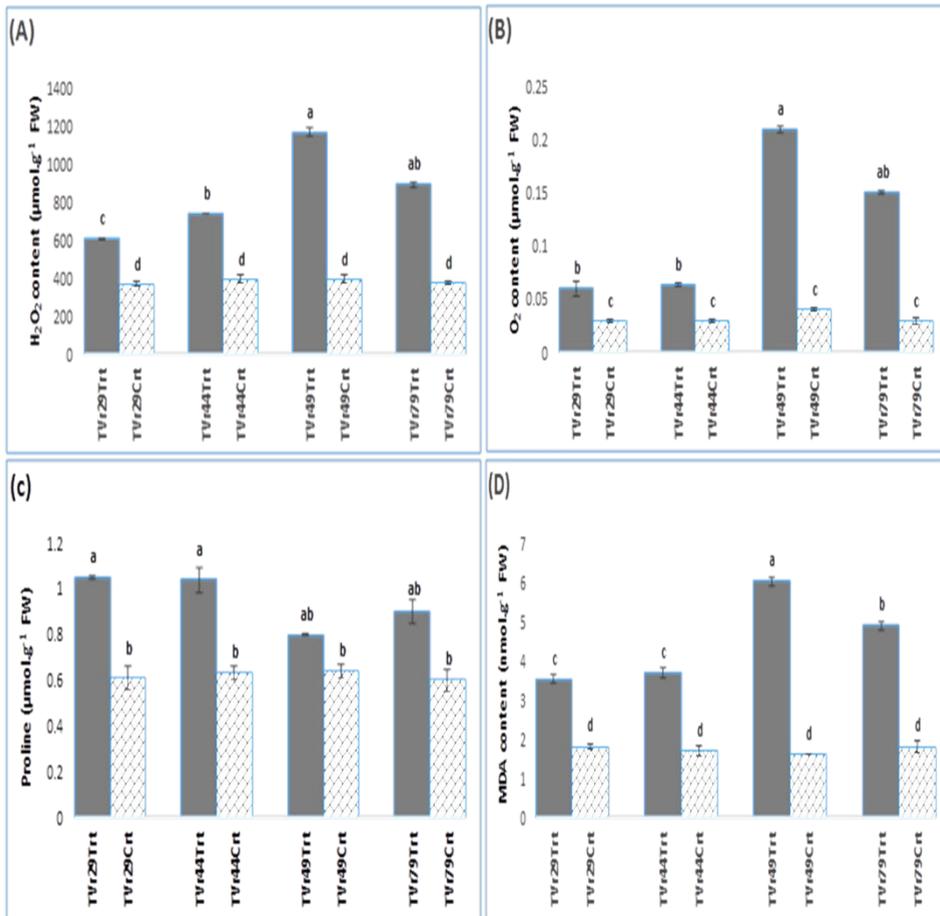


Figure 5. The effect of PEG-induced drought stress on (A) H_2O_2 content, (B) O_2^- content, (C) Proline content, (D) Lipid peroxidation-MDA content, in leaves of mungbean cultivars (Tvr29, Tvr44, Tvr49 and Tvr79) evaluated in experiment carried out in soil system. Tvr29Trt (Tvr29 treated with PEG), Tvr29Crt (Tvr29 without PEG), Tvr44Trt (Tvr44 treated with PEG), Tvr44Crt (Tvr44 without PEG), Tvr49Trt (Tvr49 treated with PEG), Tvr49Crt (Tvr49 without PEG) and Tvr79Trt (Tvr79 treated with PEG), Tvr79Crt (Tvr79 without PEG). Means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls Test. The results shown are means \pm standard error ($n = 3$).

Discussion

Seed germination is an important stage in plant development playing crucial roles in seedling emergence and adaptation to environmental factors. Prior to PEG-induced drought stress, seed germination of all the 18 mungbean varieties were evaluated to ascertain the germination potential of each varieties. Their seed germination potential is important, basically, not to misinterpret poor germination for the effect of PEG-induced drought stress. Exactly, 9 out of the 18 varieties had 100% germination, and the remaining 9 had $\geq 60\%$ germination. Although, none of the varieties had poor germination. The potential of a seed to germinate

depends on the ability to utilize the nutritional reserves which was demonstrated among the mungbean varieties [RAO & SINHA, 1993].

Mungbean have the potentials to withstand drought stress [NAHAR & al. 2015; NAIR & al. 2019]. However, extreme drought stress has continued to threaten mungbean production in many parts of the world [ITOH & al. 2006; NAIR & al. 2019]. Both hydroponics and soil based system were conducted using LWI and biochemical molecules such as H_2O_2 , O_2^- , MDA and proline as response to drought stress on mungbean. For LWI, $\geq 80\%$ of the mungbean varieties had ≥ 1 of their leaves wilted. This implies that, drought can affect any stage of mungbean growth including the early vegetative growth stage [ITOH & al. 2006; BANGAR & al. 2019; NAIR & al. 2019]. Similar observation was reported for early vegetative growth of soybean under PEG-induced drought stress, the soybean varieties at their early vegetative growth had ≥ 1 of their leaves wilted [WANG & al. 2021]. Moderate wilting exhibited by Tvr21, Tvr42, Tvr43, Tvr47 and Tvr82 were relatively important traits in evaluating drought tolerance [PATHAN & al. 2014] but were not considered for mungbean varieties in this study knowing fully well that we are interested in significant demarcation between resistant or sensitive varieties. This is due to the fact that leaf wilting removes complexities and doubt associated with drought tolerance, it is also a fundamental factor that cannot be relegated in phenotyping drought response in crops [PUNGULANI & al. 2013]. In the past, many different leaf wilting indicators have been successfully used [HUANG & al. 1998; OBER & al. 2005; CHARLSON & al. 2009]. However, not without bias as several limitations have been uncovered due to, not only visual and qualitative assessment [PUNGULANI & al. 2013] but also imprecise demarcation between tolerance and sensitive varieties. That was why in our study, Tvr46 and Tvr48 were completely removed and were not either categorized as tolerance or sensitive because they do not have close match with Tvr29 and Tvr44 regarded as tolerant varieties or Tvr49 and Tvr79 identified as sensitive.

In the hydroponics system, wilting was evident and highly pronounced in Tvr49 and Tvr79 at day 7 out of 10 days. This findings is not against the observation of PUNGULANI & al. (2013) on cowpea. PUNGULANI & al. (2013) stated that LWI is a better approach for leaf wilting, especially for crops in which wilting is a good indicator for response to drought stress. This was evidence in Tvr49 and Tvr79 at the initial stage of drought stress. The early wilting in Tvr49 and Tvr79 revealed alteration in their physiological characteristics, thus, enhance water loss from the leaf tissues. Previous study have established that early wilting varieties can keep their stomata open immediately they sensed drought [AGBICODO & al. 2009]. This suggest that Tvr49 and Tvr79 could no longer withstand the drought stress imposed by PEG which eventually aggravated extreme wilting for them. The leaves of Tvr49 and Tvr79 lost rigidity, leading to a flaccid state due to increase turgor pressure [TAIZ & ZEIGNER, 2010]. In addition, absence of appropriate drought stress tolerance physiological traits such as stomatal conductance, leaf water potential and osmotic adjustment [SHARMA & KUMAR, 2008] may have trigger the high level of LWI experienced by Tvr49 and Tvr79. Apart from Tvr29 that was highly resistant to PEG-induced drought stress in both hydroponics and soil based systems, insignificant LWI was also recorded for Tvr44. This suggest that both Tvr29 and Tvr44 may have closed their stomata during the initial drought stress. As the PEG-induced stress continue to advance, a unique aperture in stomata opening, high level of water potential and accumulation of osmolytes defense mechanisms could be considered as parts of attributes that maintained Tvr29 and Tvr44.

The recovery followed reverse pattern of the LWI across the varieties. The ability to survive drought stress is an important evolutionary component of plant life. That is, recovery is a crucial component of crop adaptation to drought condition [BLUM, 2011; BLUM &

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TUBEROSA, 2018]. The variation observed across the varieties with respect to recovery could be associated with differences in their physiological and biochemical responses [MANE & al. 2008; VASQUEZ-ROBINET & al. 2008; EVERS & al. 2010] of each varieties after drought stress. Notably, the complete wilting in Tvr49 and Tvr79 corroborated with the report of TAIZ & ZEIGNER (1998) that most plants are interrupted in their physiological process when the leaf water potentials extremely falls below normal and could results to either low recovery or death of plants. Thus, it is pertinent to note that the morphological and physiological responses of leaves to drought stress are crucial to reduce water loss and promote water use efficiency. When plants sense severe water deficiency, their leaves droop or roll because of loss of cell turgor pressure [POORTER & MARKESTEIJN, 2008]. Leaf rolling as one of the common defense mechanisms in plants against drought stress. Specifically, leaf rolling is a unique mechanism and a drought-adaptation trait induced by turgor pressure [HSIAO & al. 1984] to reduce leaf surface temperature and protects plants from excessive water loss [FANG & XIONG, 2015]. In addition to leave rolling, Tvr29 had smaller and thicker leaves. ESAU (1960) corroborated that among the attributes of drought resistant plants are smaller and thicker leaves expected to have more epidermal trichomes, smaller and denser stomata. This implies that these attributes may have contributed to drought resistant ability of Tvr29. In addition to the fact that plants have developed protective mechanisms to recognize signals allowing them to sense and respond to drought stress, the level of tolerance vary from species to species [HOSSAIN & al. 2015]. In our study, Tvr49 and Tvr79 were extremely sensitive to drought stress, while Tvr29 and Tvr44 exhibited high level of resistant to drought stress [ZHU, 2002]. Most importantly, responses that were expressed on plant growth could be survival or death. However, all plants struggled to adapt by utilizing their adaptive mechanism. Adaptation of plants to drought can be avoidance of tissue water deficits or tolerance of tissue water deficits. Based on our findings, Tvr29 tolerated tissue water deficits due to its small, thicker leaves and maintenance of turgor pressure against drought stress. This observation is in-line with the report of MORGAN (1984) that tolerance of tissue water deficits most commonly involves maintenance of turgor, rigid cell walls or decreased cell size.

The demarcation between Tvr29 and Tvr49 signifies the crucial roles of LWI and recovery to ascertain the status of mungbean under drought stress. This suggest that both LWI and recovery could be useful to mungbean breeders since it can easily demarcate between resistant and sensitive varieties using quantitative index [PUNGULANI & al. 2013]. Also, an early response to drying during which leaf colour changes indicates photosynthetic shutdown. A late response to drying during which leaves fold adaxially and exposed surfaces suggest when respiration ceases and tissues eventually reach an air-dry state [FARRANT & al. 2015] could be responsible for the extreme demarcation in response to drought stress between Tvr29 and Tvr49. Available data have shown that drought stress has the potentials to influence the process of photosynthesis in most plants by adjusting the cell organelles and pigments [MAXWELL & JOHNSON, 2000]. The induced drought stress by PEG significantly reduced the chlorophyll content of Tvr49 and Tvr79. As the drought stress advances, photosynthesis gradually reduced until finally shutdown. As a result of this, the Tvr49 and Tvr79 may have lost their chlorophyll. This agreed with that of FANG & XIONG (2015) of which the decrease in total chlorophyll content can affect growth of mungbean. This could as well be attributed to destruction of chloroplasts and / or instability of the pigment protein complex [KAUR & al. 2016]. Similarly, the chlorophyllase may have increased with direct impact on Tvr49 and Tvr79 varieties [REDDY & VORA, 1986; REDDY & al. 2004]. Tvr29 and Tvr44 retain their chlorophyll content which implies that both of them may have evolved a protective mechanism against the

ROS-induced damage to cellular components which include synthesis of protective pigments. Protection on the integrity of chloroplast membrane is very crucial for the maintenance of the photosynthetic activity of mungbean under drought stress [EFEÖĞLU & al. 2009]. Furthermore, the photosynthetic system of Tvr29 and Tvr44 were not destroyed, they were just reversibly inactivated which enables them to recover fast after rehydration [STRASSER & al. 2010]. Therefore, Tvr29 and Tvr44 can be considered to have utilized high energy more efficiently, thus, enhanced water holding ability to avoid damage on exposure to drought stress.

Drought has the potentials to enhance disruption of osmotic balance and over production of ROS like H_2O_2 and $O_2^{\cdot-}$ which used to cause oxidative stress and damage cells [FAIZE & al. 2011; NAHAR & al. 2015]. In wilted leaves, the level of ROS is expected to rise and can lead to permanent metabolic dysfunction and death as observed for Tvr49 [ANJUM & al. 2015]. The redox imbalance due to drought stress increases the rate of metabolism and directly upregulated H_2O_2 and $O_2^{\cdot-}$ production in Tvr49 and Tvr79 [GECHEV & HILLE, 2005; BHATTACHARJEE, 2012]. Further observation suggest that exposure of Tvr49 and Tvr79 to drought stress may have broken the metabolites equilibrium which could have led to oxidative deterioration and eventually cell death [CRUZ DE CARVALHO, 2008]. As a result of this, the membrane phospholipids and fatty acids which are sensitive to overproduction of ROS would have damaged, and resulted to peroxidation of membrane lipids in Tvr49 and Tvr79. H_2O_2 , $O_2^{\cdot-}$ and MDA were relatively insignificant in Tvr29 and Tvr44. This indicates that the lower concentrations of H_2O_2 , $O_2^{\cdot-}$ [GECHEV & HILLE, 2005; BHATTACHARJEE, 2012; NAHAR & al. 2018] and MDA [MOLLER & al. 2007] were needed for cell signaling and adaptation mechanism [JONES, 2014; OBIDIEGWU & al. 2015]. It was evidence in our study, that Tvr29 and Tvr44 exhibited resistance against drought stress. Apart from other morphological and physiological defense mechanisms, Tvr29 and Tvr44 may have enjoyed bioprotective mechanisms of proline which was significantly ($P \leq 0.05$) expressed in their tissues. Presence of proline in mungbean have been considered as an adaptive strategies to withstand drought stress [BANGAR & al. 2019]. On a more specific note, interaction with scavenging free radicals and buffering cellular redox [TRIPATHI & GAUR, 2004; BANGAR & al. 2019] are parts of the activities of proline in plants under drought stress and this may have been expressed in Tvr29 and Tvr44 due to the high content of proline.

Conclusion

Among the varieties, Tvr29 and Tvr44 exhibited high level of resistance to drought stress in comparison to Tvr49 and Tvr79 that had very low resistance based on LWI and production of H_2O_2 , $O_2^{\cdot-}$, MDA and proline. Under PEG-induced drought stress, high level of proline content was remarkably produced by Tvr29, followed by Tvr44. Most importantly, the high proline content and unique leaf rolling morphology were parts of the factors that may have facilitated adaptation of Tvr29 to drought stress in comparison to other varieties. Therefore, Tvr29 and Tvr44 should be evaluated and utilized by breeders and farmers where drought is a challenge on mungbean globally.

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