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Research Article

Physiological and Biochemical Responses of two Ground nut (*Arachis hypogaea L.*) cultivars under Drought stress

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Article Info

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Abstract

A drought stress is a significant abiotic factor restricts the groundnut productivity especially in semi-arid and rainfed agro-ecosystems. In order to identify important characteristics linked to drought resistance, the current study set out to assess the physiological and biochemical changes of two distinct groundnut cultivars, TMV-2, and GPBD-4, under drought stress condition. Changes in relative water content (RWC), cell membrane integrity, proline accumulation total chlorophyll content, lipid peroxidation and epicuticular wax accumulation were measured when plants were exposed to drought stress. Both cultivars RWC and Chlorophyll content significantly decreased under drought stress, however TMV-2 drop was more noticeable under stressful circumstances, the relatively tolerant cultivar GPBD-4 preserved greater tissue hydration and membrane stability. In both genotypes, proline accumulation improved in response to drought, however GPBD-4 showed relatively greater levels, suggesting a better correlation with osmotic adjustment. Malondialdehyde (MDA) content, a marker of lipid peroxidation was considerably higher in TMV-2 than GPBD-4. This difference in MDA levels indicates less oxidative damage and greater membrane integrity maintenance. Furthermore, epicuticular wax content increased during drought stress and was grater in GPBD-4, indicating a function in mechanisms for avoiding drought. These results provide a coordinated collection of physiological and biochemical characteristics that give groundnuts drought resistance. They also offer useful markers for screening germplasm and directing breeding tactics meant to increase yield stability in drought conditions.

Keywords: Drought stress, Epicuticular wax, Groundnut, Malondialdehyde content. Physiological and Biochemical changes. Proline.

Introduction

Ground nut (*Arachis hypogaes* L.) is an essential oilseed crop that provides 44-56% edible oil and 22-28% protein produced on 26.71 million hectares of land area with a yield of 1.68 t per hectare generating 44.86 million metric tons in 82 countries [1]. Asia is the greatest groundnut-growing region in the world, producing 65.1% of the world's total. In india, with the production of 6.70 million metric tons per annum [2], it is cultivated on 5.34 million hectares of land with yield of 1.25 t per hectares. Global groundnut output will be limited in the future due to severe crop irrigation water constraints in major groundnut producing nations like China, India, Nigeria and the United States[3]. Both natural environmental conditions and unrefined agriculture techniques frequently cause abiotic stress to plants. More than 50% of the production area is in arid and semi-arid areas, where drought stress often changes in duration and intensity[4].

Drought is one of the main environmental factors that significantly lowers crop productivity, because of the increasing global population and the necessity to expand initiatives for optimal growth, food crop production is quite desirable. Most plants are able to withstand drought stress, though the degree of this ability varies depending on the species. When plants are under drought stress, the reproductive stage is more susceptible than the pre and post reproductive stages [5]. In both dry land and irrigated crops, drought stress limits plant development and productivity and has significant effects on plant morphology, physiology and biochemistry [6]. Routine breeding initiatives to increase crop drought resistance have shown limited success due to qualities. Both avoidance and stress tolerance are components of a plant's drought resistance function.

Recently physiological features such as relative water content and electrolyte leakage have been associated to drought resistance in groundnut [7]. The buildup of osmolyte proline is one of the physiological factors that help plants combat the negative consequences of water deficiencies. According to Blokhina and Fagerstedt [8] oxidative stress can result in protein oxidation, lipid peroxidation and DNA damage in plants. All living things undergo the extremely harmful process of lipid peroxidation (LPOX) [9]. Increased cellular disruption increases the chances of membrane LPOX which leads to the buildup of oxygen in plant tissue. Thereby, under stress, LPOX is a measure of oxidative damage.

Recurrent drought conditions, which are predicted to worsen with climate change, pose a danger to groundnut production worldwide in the primary production regions. Groundnut cultivars that are resistant to drought are the ideal solution to protect the crop from the negative impacts of drought. Groundnut play a significant role in oil production and improving ground genotype yields under drought stress and agroclimatic conditions is a crucial job for researchers. Therefore, identifying and choosing genotypes with heightened resilience to drought stress is a primary goal of groundnut breeding projects.

Materials and methods

Plant material and stress imposition

In this study we used two groundnut (*Arachis hypogaes* L.) cultivars. Plants were cultivated in pots containing a consistent soil combination under regulated environmental conditions. Drought stress was produced and maintained using the gravimetric approach, which involves controlling soil moisture content based on pot weight. Pots containing soil and sand were initially soaked with water and let to drain before being measured for field capacity. After that seeds are sowed in a pot and allow to grow upto 21 days with well-watered, then pots were divided into different groups. For Control plants maintain a 100% soil moisture level, for stress plants maintained different soil moisture level i.e., 75%, 50%, and 25% soil moisture level. All pots are allowed to grow upto 7 days after stress imposition. At the end of the stress period, fully expanded young leaves were collected to conduct

physiological and biochemical studies. All measurements were carried out with three independent biological replicates.

Relative water content

The relative water content was determined using Barrs and Weatherley's technique [10]. Fresh leaf samples were weighed immediately to determine their fresh weight (FW). The leaves were then floated in distilled water for 4-6 hours at room temperature and dim light to acquire turgid weight (TW). After blotting off excess surface water, the leaves were dried in a hot air oven at 70˚C for 48 hours to estimate dry weight (DW). RWC was determined using the following formula:

$$\text{Relative water content} = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

Cell membrane integrity:

Cork borer was used to prepare 1cm diameter leaf discs from control and stressed plants. These discs were then incubated in 10ml of water for 2 hours. The solution was filtered and the OD was measured at 273nm (initial OD). Leaf discs were then cooked in the same solution for 30 minutes, cooled, filtered and OD measured at 273nm (final OD). The percentage leakage was computed using the formula. [11].

$$\text{Cell membrane intigrity} = \frac{Initial\ OD}{Final\ OD} \times 100$$

Free proline content

Bates et al technique was used to measure free proline concentration. Fresh leaf tissue was homogenized in 3% sulfosalicylic acid and then filtered. An aliquot of the filtrate was treated with acid ninhydrin and glacial acetic acid and incubation in a boiling water bath for 1 hour. The reaction was stopped by immersing in the tubes in an ice bath, and the chromophore was extracted using toluene. The absorbance of the toluene phase was measured at 520nm with a spectrophotometer. Proline concentration was calculated using a standard curve containing L-proline and expressed as $\mu g g^{-1}$ fresh weight [12]

Total Chlorophyll content

The chlorophyll content was estimated using Arnons [13] approach. Fresh leaf tissue was homogenized in 80% acetone before centrifugation to produce a clear supernatant. A spectrophotometer was used to measure the absorbance at 645 and 663nm. The total chlorophyll content was estimated using the formula:

TCC = 20.2 X O.D. (at 645) + 8.02 X O.D. (at 663nm).

Lipid peroxidation

The thiobarbituric acid (TBA) method was used to determine malondialdehyde (MDA) level and hence estimate lipid peroxidation. Fresh leaf tissue was homogenized in 0.1% trichloroacetic acid (TCA) before centrifugation. An aliquot of the supernatant was combined with 0.5% TBA dissolved in 20% TCA and cooked in a boiling water bath for 30 min. after quick cooling the mixture was centrifuged and the supernatants absorbance was measured at 532nm and corrected for nonspecific turbidity at 600nm. MDA concentration was estimated with an extinction value of $155 mM^{-1} cm^{-1}$ and reported as $molg^{-1}$ fresh weight [14,15].

Wax estimation

By holding a petiole, a freshly picked leaf was submerged in 20 to 30 seconds of redistilled chloroform for 15 seconds. The extract was filtered and the chloroform wax evaporated to dryness at 70˚C in a fume hood. To the extracted waxes, add 5ml of acidic $K_2Cr_2O_7$ reagent. After chilling, 12ml of deionized water was added to each sample to allow for color development. The optical density of the samples was measured at 590nm using a spectrophotometer. Carnauba wax (Sigma, USA) served as a standard for surface wax quantification[16].

Statistical analysis

All trials used a completely randomized design with three biological replicates. The data were presented as mean ± standard error (SE). ANOVA was used to establish statistical significance between treatments

and cultivars. Mean comparisons were performed at a p value of $p \leq 0.05$.

Result

Stress treatments caused significant and genotype dependent alterations in water status, membrane stability, osmolyte accumulation, pigment content and oxidative damage among the ground nut types investigated. The GPBD-4 cultivar continuously maintained a much greater relative water content (RWC) than the TMV-2 (Fig 1), indicating superior cellular hydration and turgor maintenance under both drought stressors. However, a steady reduction in RWC was noted as stress severity increased. TMV-2, on the other hand, showed a marked decrease in RWC, indicating poor water retention and heightened vulnerability to dehydration stress. Changes in cell membrane stability as indicated be electrolyte leakage, provided additional proof of the loss of cellular integrity under stress. Across all stress levels, TMV-2 showed much higher membrane leakage, indicating increased membrane permeability and damage. In contrast, the GPBD-4 cultivar showed improved membrane protection (Fig 2) and structural integrity under stress by maintaining lower leakage values. Changes in cell membrane stability as indicated by electrolyte electrolyte leakage, provided additional proof of the loss of cellular integrity under stress. Across all stress levels, TMV-2 showed much higher membrane leakage, indicating increased membrane permeability and damage. In contrast, the GPBD-4 cultivar showed improved membrane protection and structural integrity under stress by maintaining lower leakage values. In both cultivars, free proline levels gradually increased with stress severity suggesting that osmotic compensation mechanisms were activated. Interestingly, under extreme stress, TMV-2 collected more proline a sign of a metabolic change linked to damage but responsive to stress. Proline build-up was minimal in the GPBD-4 cultivar (Fig 3), indicating a more balanced osmoprotective response that maintains cellular function without causing undue metabolic disturbance. With increasing stress intensity, TMV-2 showed a substantial decrease in total chlorophyll concentration, suggesting increased pigment degradation and photosynthetic system damage. on the other hand, The GPBD-4 genotype (Fig 4), maintained larger quantities of chlorophyll during stress treatments, indicating improved maintenance of photosynthetic efficiency and defence of chloroplast integrity in challenging circumstances. Lipid peroxidation (malondialdehyde or MDA) content, which measures oxidative damage, revealed a pronounced genotype specific pattern. Under stress, TMV-2 showed a significant rise in MDA build-up (Fig 5), suggesting increased oxidative load and ROS-mediated membrane lipid oxidation. Significantly lower MDA levels were maintained by the GPBD-4 cultivars, indicating a more effective antioxidative defence system and less lipid peroxidation chain reaction propagation. Both the cultivars showed a gradual increase in wax content under stress. GPBD-4 had a greater wax content under control circumstances than TMV-2 (Fig 6). Wax accumulation increased in both types as stress levels increased. Under stress condition GPBD-4 continuously maintained a larger wax content than TMV-2 suggesting a more robust defence mechanism and increased stress tolerance.

Discussion

Tissue relative water content (RWC), which reflects the combined impacts of soil moisture availability, plant water absorption and transpiration water loss, is widely accepted as an effective and less erratic measure of plant water status. Barss and Weatherly [10] suggested using leaf RWC as a crucial measure for evaluating drought tolerance and highlighted it as a physiological parameter that determines as plants capacity to sustain hydration under moisture stress. Both groundnut cultivars in the current study saw a considerable decrease in leaf RWC as a result of drought imposition however the degree of this fall varied considerably between the relatively drought tolerant cultivar GPBD-4 and the drought sensitive cultivar TMV-2. Leaf water potential and

osmotic potential clearly decreased under continuous water shortage, indicating growing cellular dryness and compromised water relations. TMV-2 showed a more noticeable a reduced ability to keep tissues hydrated under stress. On the other hand, during drought exposure, GPBD-4 maintained noticeably higher RWC values, indicating a better capacity to maintain cellular water balance (Fig 1). The physiological underpinnings of drought tolerance in GPBD-4 are highlighted by this differential response, which may include improved osmotic adjustment mechanisms decreased transpiration losses or more effective water intake. These results are in accordance with previous studies showing that stressed leaves of groundnut, mulberry and solanum plants had significantly lower RWC than well-watered controls [16-18]. The current study's observation of cultivar specific variation in RWC lends additional credence to the parameter's usage as an acceptable screening method for locating genotypes that are drought tolerant in groundnut breeding initiatives.

Membrane compatible solutes like sugars and amino acids typically shield the plasma membrane from damage caused by desiccation. Thus, there might be as connection between the degree of membrane protection against the effects of dehydration and the ability to alter osmotic pressure. A common indicator is the preservation of membrane integrity and function under a specific degree of dehydration stress [19,20]. As a result, the increased membrane integrity seen in GPBD-4 under drought stress suggests a higher ability to adjust osmotic pressure and shield the plasma membrane from damage brought on by dehydration. on the other hand, TMV-2, showed a higher loss of membrane integrity, indicating a decreased capacity to sustain membrane stability under similar stressors.

Proline accumulation is seen as an early physiological reaction to drought stress[21], and it is a crucial metric for evaluating plants ability to withstand stress [22]. The current study found that both groundnut cultivars had proline accumulation in response to drought stress however the degree of accumulation varied between relatively drought tolerant cultivar GPBD-2 and the drought sensitive cultivar TMV-2 (Fig 3). In transgenic tobacco overexpression of pyrroline-5-carboxylate synthase led to higher proline levels and greater resistance to osmotic stress [23]. Given the current data, the increased proline accumulation in GPBD-4 during drought stress points to a stronger correlation with osmotic adjustment than in TMV-2.

Chlorophyllase, peroxidase and phenolic chemicals, which aid in the decomposition, to chlorophyll, seem to be linked to the drop-in chlorophyll content [24]. A drop-in chlorophyll concentration during dehydration has been proposed as a non-stomatal limiting factor. An increase in chlorophyllase activity is one explanation for the decrease in chlorophyll content during drought stress. Stress triggers the production of this enzyme's gene, which speeds up the degradation of chlorophyll. Comparatively GPBD-4, TMV-2 showed a more noticeable drop in chlorophyll concentration, suggesting a higher vulnerability to stress-induced chlorophyll degradation (Fig 4).

When plants are under extreme stress, more Active oxygen species (AOS) may be produced than the antioxidant defence system can scavenge [25]. As a result, AOS can build up and harm cells by oxidizing phospholipids and other unsaturated lipids as well as causing lipid peroxidation. Through loss of fluidity, lipid crosslinking and membrane enzyme inactivation, peroxidation leads to the disintegration of lipids and membrane function [26]. Malondialdehyde (MDA) concentration a secondary breakdown product of lipid peroxidation was measured in the current investigation to determine the degree of lipid peroxidation [27] maintaining low MDA levels has been linked to increased drought stress resistance in a number of plant species [28-31]. MDA content is a frequently used indicator for assessing oxidative damage in plant tissues. The drought sensitive cultivar TMV-2 in this study showed increased MDA accumulation under stress suggesting under

stress suggesting a higher degree of membrane damage and lipid peroxidation. On the other hand, GPBD-4 maintained lower MDA levels (Fig 5), indicating a lesser level of oxidative damage and improved membrane integrity maintenance under dehydration stress.

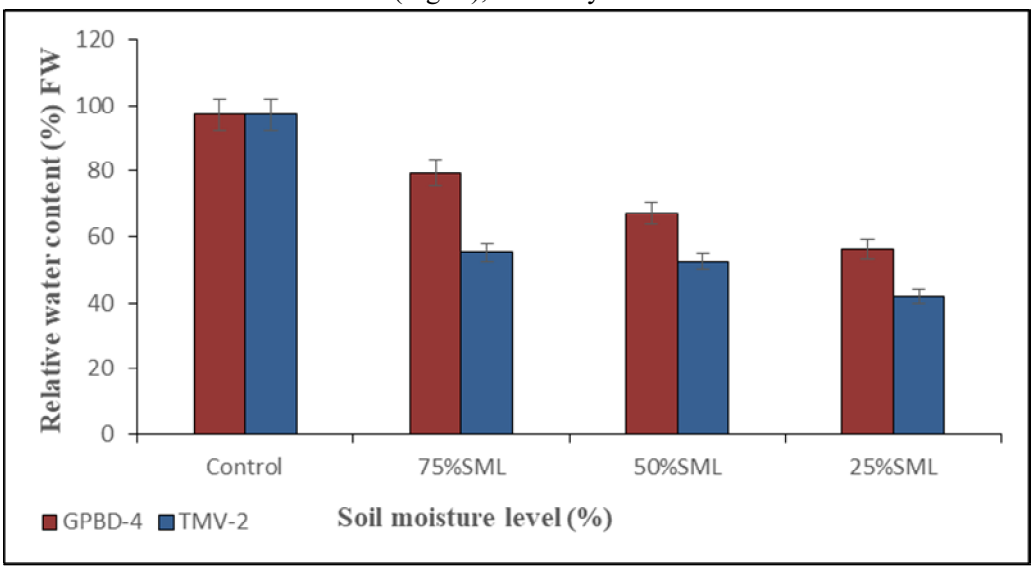

Figure 1: Relative water content of ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

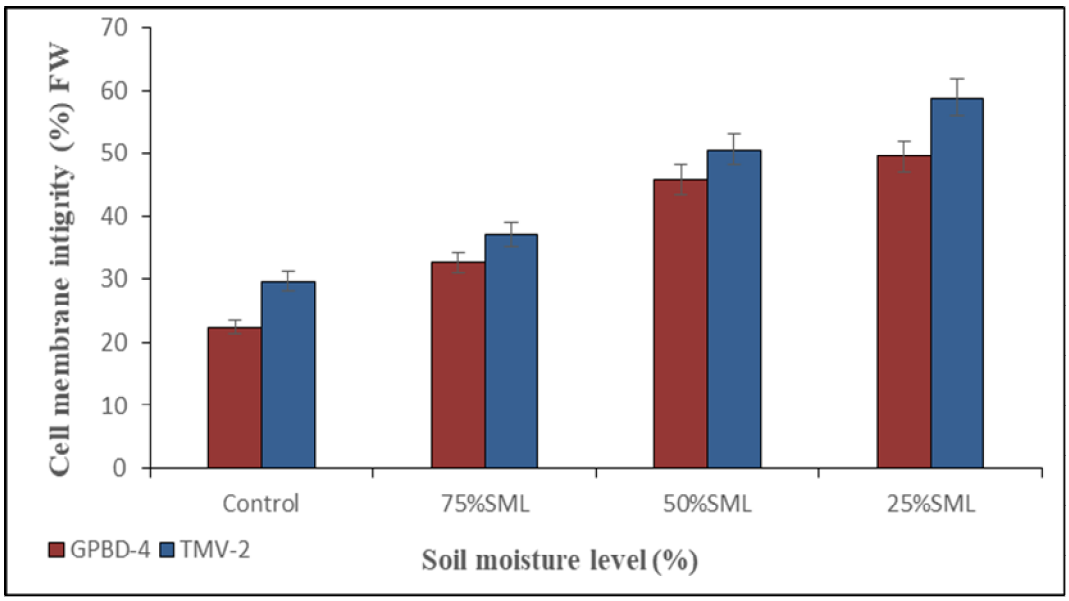

Figure 2: Cell membrane integrity of Ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

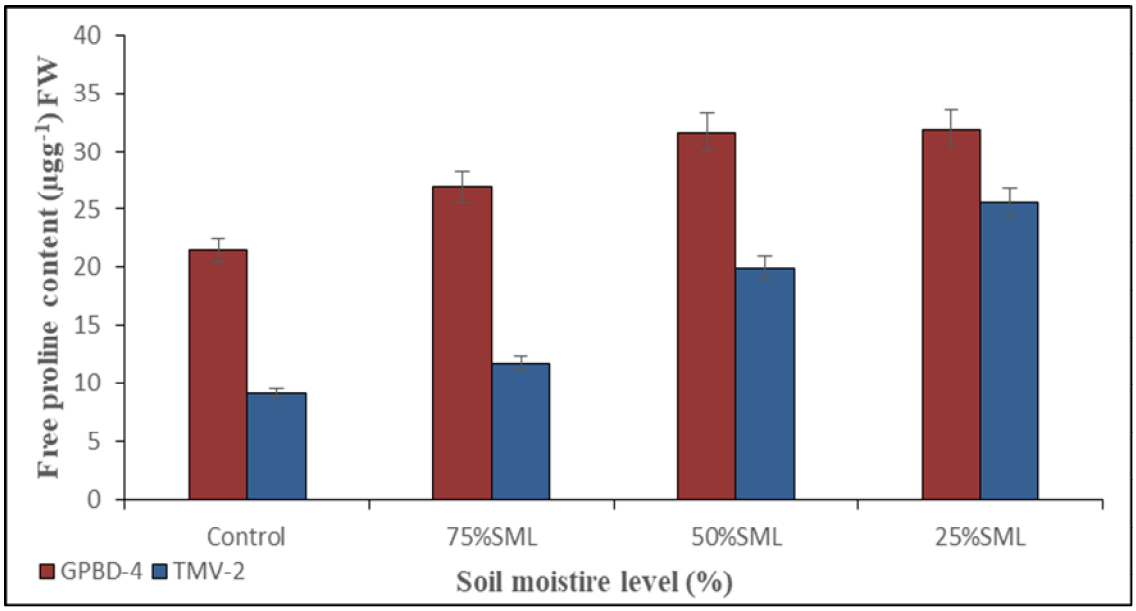

Figure 3: Free proline content of Ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

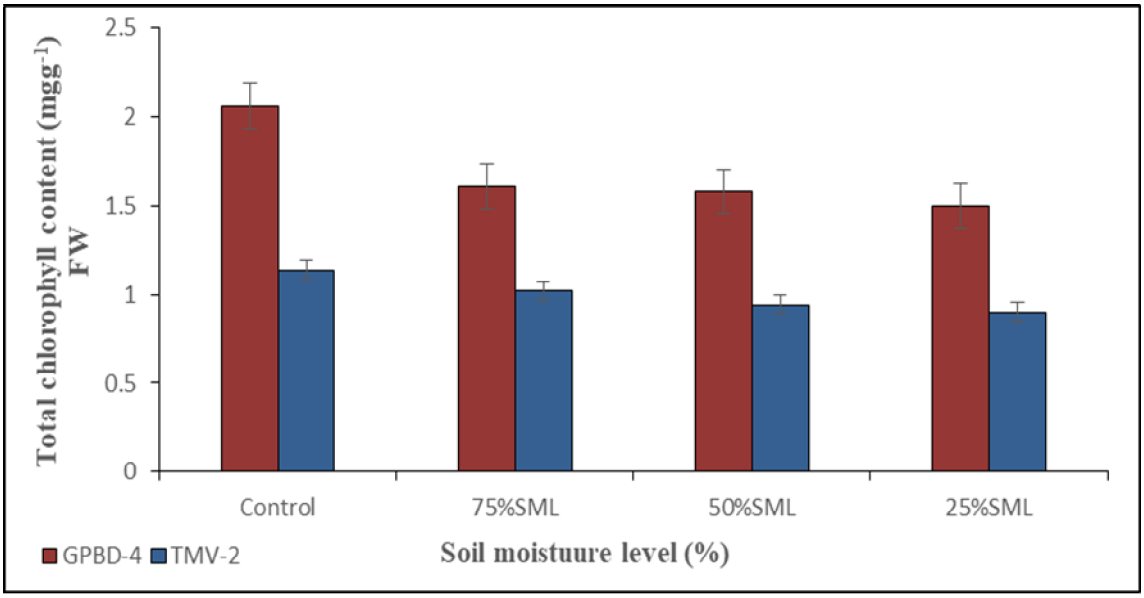

Figure 4: Total chlorophyll content of Ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

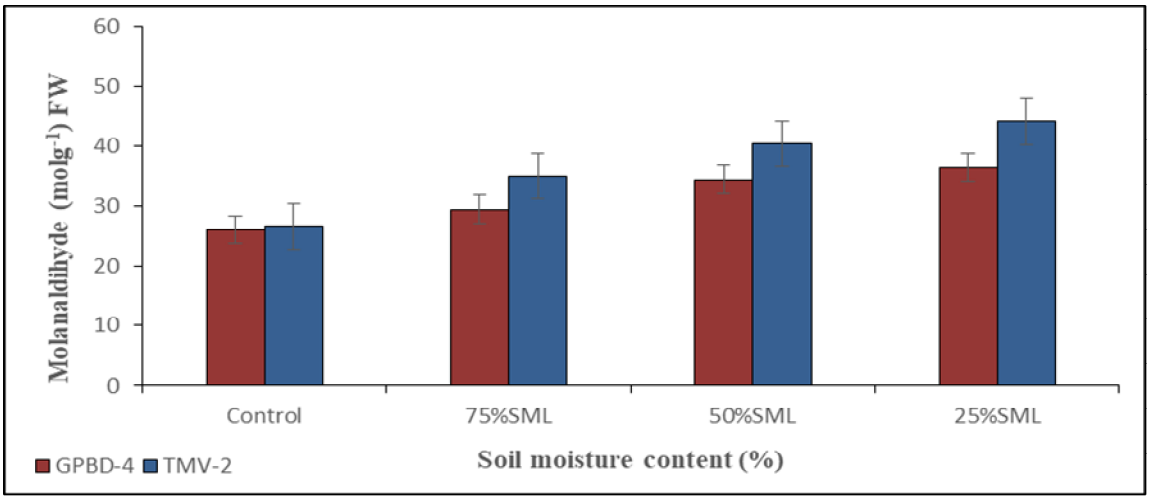

Figure 5: Lipid peroxidation of Ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

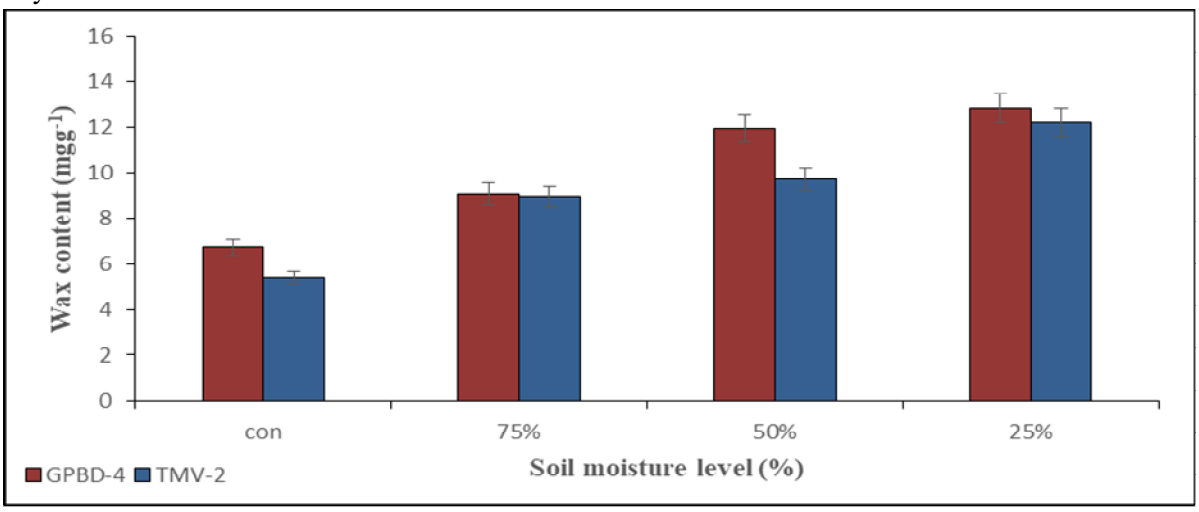

Figure 6: Wax content of Ground nut cultivars were subjected to control, 75%, 50%, and 25% soil moisture levels maintained by the gravimetric method. Values represent mean ± SE (n = 3). Data were analyzed using two-way ANOVA to assess the effects of soil moisture level and cultivar. The effects of drought stress and cultivar were statistically significant ($p \leq 0.05$). Mean comparisons were performed using Tukey's HSD test.

According to Ni et al [32], wax concentration increased in one genotype of alfalfa during drought stress, while it stayed the same in several other genotypes, suggesting that wax response to dehydration varies by genotype. Sorghum genotypes that are drought tolerant have been shown to have high levels of epicuticular wax and it has been demonstrated that an increase in wax under drought stress enhance the plant's capacity to withstand dehydration by means of an avoidance mechanism [33]. Compared to the TMV-2 cultivar, GPBD-4 showed greater wax accumulation under stress in the current investigation (Fig 6), suggesting a stronger correlation between drought tolerance and higher leaf wax content.

Conclusion

The present study demonstrates that physiological and biochemical differences between the two ground nut cultivars GPBD-4 and TMV-2. The tolerant cultivar GPBD-4 showed a better capacity to sustain tissue hydration and membrane stability during dehydration stress by continuously maintaining grater relative water content and cell membrane integrity. In addition, GPBD-4 shows increased proline accumulation points to a greater ability to respond to osmotic changes than TMV-2. Malondialdehyde (MDA) content, a marker of lipid peroxidation, was considerably higher in TMV-2 than in GPBD-4 suggesting less oxidative damage. In addition to offering useful markers for screening germplasm and directing breeding methods targeted at enhancing yield stability under water-scare conditions, these findings highlight a coordinated collection of physiological and biochemical features that confer drought tolerance in groundnuts.

Declarations:

Acknowledgement: None stated.

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Conflict of Interest statement

The authors declare that no commercial or financial relationships exist that could be construed as a potential conflict of interest.

Declaration of Non-Use of AI: The authors confirm that no artificial intelligence tools were used in this study.

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