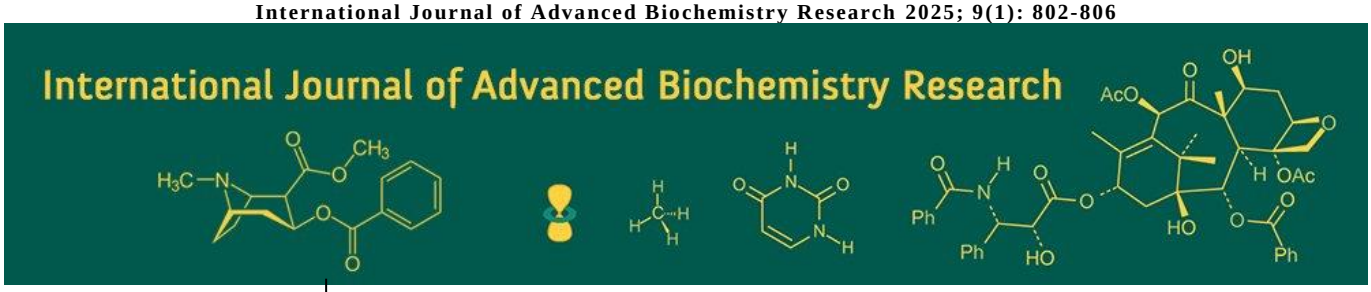

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Comparative analysis of chickpea genotypes under drought stress conditions: The role of seed protein content in drought adaptation

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Abstract
In this study, we selected two chickpea genotypes with distinct differences in seed protein content (SPC): ICC8397 (High Protein Content, HPC) and FG212 (Low Protein Content, LPC). These genotypes were subjected to terminal drought stress conditions and compared with ICC4948, a drought-tolerant variety, and ICC1882, a drought-sensitive variety. Our comparative analysis revealed that the LPC genotype, FG212, exhibited phenotypic and physiological traits similar to the drought-tolerant genotype ICC4948. In contrast, the HPC genotype, ICC8397, displayed traits more akin to the drought-sensitive genotype ICC1882. Based on these observations, we hypothesize that genotypes with lower protein content may adapt to drought stress by utilizing key amino acids, thereby reducing protein synthesis as a strategy to conserve resources during water deficit conditions.

Keywords: Physiological, during, conditions

1. Introduction
Chickpeas are one of the main sources of dietary protein for the Indian subcontinent vegetarian population. Thus, the availability of chickpea cultivars with improved nutritional profiles is crucial to ensuring food and nutritional security in an era of constantly shifting global climatic conditions [1]. Finding the key genes that control the seed protein content (SPC) and the pertinent post-transcriptional gene regulatory mechanisms is essential to achieving this aim. Accordingly, efforts have been undertaken to comprehend the molecular underpinnings of the control of gene expression that governs the production of seed proteins [2, 3].

A significant leguminous crop, chickpeas (*Cicer arietinum* L.) are cultivated for their high protein content, making them a mainstay in many diets, especially in dry areas. However, chickpea development and output are greatly impacted by drought stress, with genotype-specific heterogeneity noted. A crucial characteristic for increasing chickpea yield, particularly in areas vulnerable to water constraint, is the capacity to withstand drought stress.

Additionally, the acquisition of organic N is significantly influenced by the transport of amino acids in plants [4]. In order to reduce plant damage under water stress, it has been revealed that a large number of AAT (Amino acid transporter) genes are engaged in the transfer of stress-related compounds and suitable solutes [5]. Numerous physiological characteristics, including osmotic adjustment, stomatal conductance, and leaf water potential, have been connected in earlier research to chickpea drought resistance. However, not much research has been done on how seed protein content (SPC) affects drought adaptation. With the hypothesis that a reduced protein content may improve drought tolerance by lowering the energy required for protein synthesis and so conserving resources for stress adaption, this study aims to examine how chickpea genotypes with varying SPC react to drought stress. The main causes of terminal drought-induced loss in grain yield include a decrease in the rate of net photosynthesis as well as inadequate grain set and development. Understanding the effects of terminal drought and its tolerance mechanisms is necessary for successful crop development initiatives that seek to increase grain legume resistance to terminal drought [5].

It is well established that plants exhibit photorespiration activity in stressful situations in order to defend themselves, i.e., to maintain the redox state and remove dangerous compounds caused by light. Therefore, it was thought that sulfur-containing amino acids, such as cysteine and methionine, are synthesised by photorespiration in plants (6,7,8,9). Hence, to check the responses of contrasting SPC genotypes in this study we studied the phenotypic and physiological changes under drought stress.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The chickpea genotypes ICC8397 (High Protein Content, HPC) and FG212 (Low Protein Content, LPC) were selected because of their differing seed protein contents (10). For comparison, two well-characterized genotypes that are drought-tolerant (ICC4948) and drought-susceptible (ICC1882) were also included. Every genotype was cultivated in a Plant Phenomics facility under carefully monitored conditions using two treatments: well-watered control and drought stress. The drought stress was imposed at later stage of flower development, to evaluate the effect of terminal drought.

2.2. Phenotypic Analysis

Visual observations were taken in order to assess each genotype phenotypic response to drought stress. Symptoms included leaf withering, chlorosis, and growth retardation. The height and leaf area of the plant were measured at the start and end of the drought treatment.

2.3. Physiological Analysis

Using standard techniques, physiological markers suggestive of water stress were measured:

A porometer was used to measure the stomatal conductivity.

Using a SPAD meter, the amount of chlorophyll was measured.

The ratio of fresh weight to turgid weight was used to determine the relative water content, or RWC. A cryoscopic osmometer was used to measure the osmotic potential.

3. Results

3.1. Phenotypic Response to Drought Stress

In contrast to ICC8397 (HPC) and ICC1882 (drought-sensitive), ICC4948 (drought-tolerant) and FG212 (LPC) showed fewer symptoms of stress, including leaf wilting and chlorosis, under drought conditions. Compared to ICC8397 and ICC1882, FG212 shown a moderate decrease in size but kept a larger total leaf area and a healthier look, whilst ICC4948 maintained its growth and leaf turgidity. Similar to the drought-sensitive genotype ICC1882, ICC8397 showed noticeable wilting, decreased leaf area, and a marked decline in plant height (Fig. 1 &2).

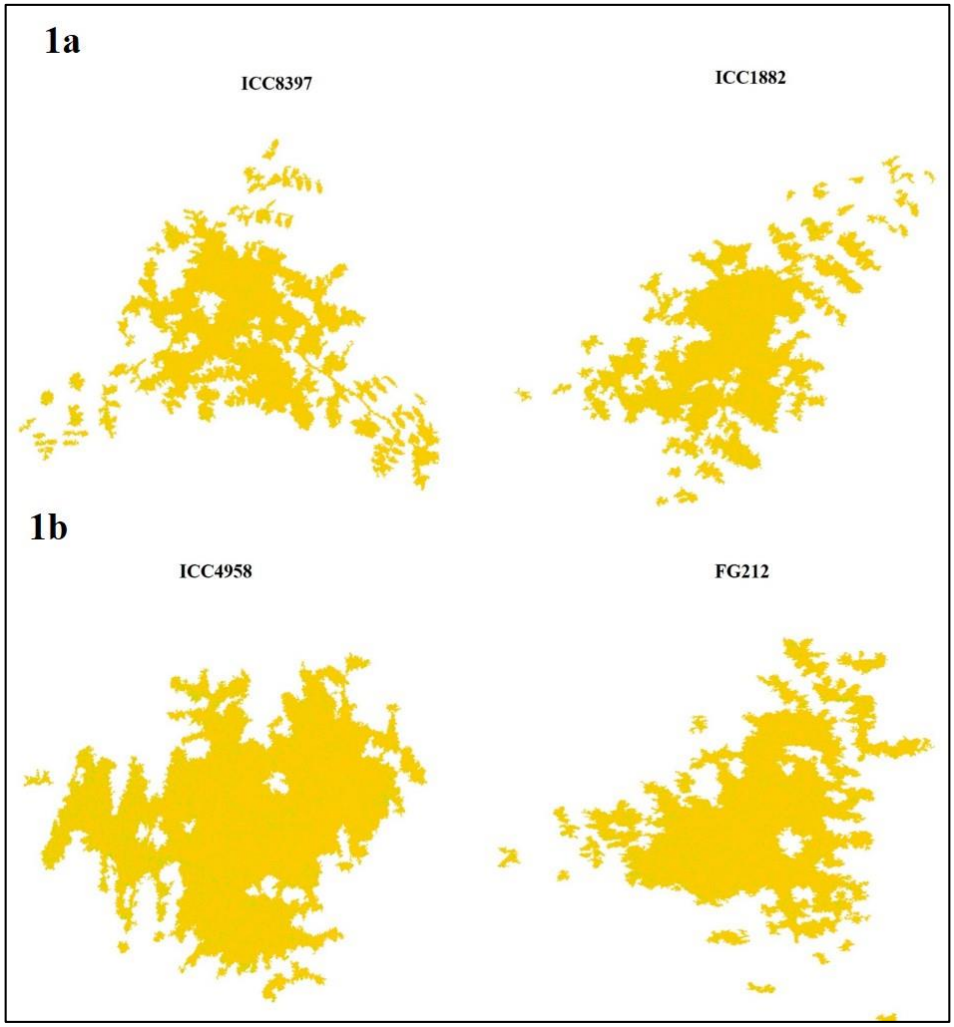

Fig 1a and 1b: Detailed comparative phenotypic analysis (Using PhenoVation Fluorescence Detector, Crop Reporter Version 1.0) of drought sensitive, FG212 and tolerant genotype, ICC4958, with genotypes with varied seed protein content (ICC8397, HPC) & FG212, LPC).

3.2. Physiological Response to Drought Stress

A comparative study of the physiological reactions of four plant genotypes (ICC4948, FG212, ICC8397, and ICC1882) to drought stress is studied. In order to provide light on their drought tolerance and photosynthetic efficiency, the study primarily examines their capacity to maintain critical physiological markers in water-limited environments.

During drought stress, ICC4948 and FG212 showed the ability to sustain comparatively high levels of stomatal conductance, relative water content (RWC), and chlorophyll content. The efficiency with which plants control the flow of gases such as CO_2 and water vapour through their stomata were measured by stomatal conductance. Despite having little water available, plants with high stomatal conductance may still be able to absorb CO2 for photosynthesis and preserve water balance. A high RWC shows that the plant is successfully retaining water, which is essential for preserving cellular function under drought. RWC is a measure of the plant's level of hydration. Since chlorophyll is necessary for absorbing light energy for photosynthesis, its concentration is a crucial indicator of photosynthetic activity. The capacity of ICC4948 and FG212 to maintain these parameters suggests that these genotypes are more drought-tolerant than others because they are better at controlling water and sustaining photosynthetic activity under drought stress.

In contrast, when exposed to drought stress, ICC8397 and ICC1882 demonstrated notable reductions in stomatal conductance, RWC, and chlorophyll content. These decreases point to a decline in photosynthetic capability, which means that during drought, these plants were less effective at absorbing sunlight and turning it into chemical energy. Additionally, a drop in RWC suggests that these plants were less able to hold onto water, which probably resulted in dehydration and diminished physiological function. Moreover, the reduction in chlorophyll concentration indicates a drop in photosynthetic efficiency, indicating that ICC8397 and ICC1882 have less effective water utilisation under drought.

One particularly fascinating observation was that, in spite of having less protein, FG212 maintained an osmotic potential comparable to ICC4948. The ability of plant cells to absorb water is known as osmotic potential, and it is essential for preserving turgor pressure, which is the internal pressure that keeps cells structurally stable. The ability of a plant to maintain its osmotic potential under drought stress is essential because it keeps cells from withering and guarantees that they keep their shape and function. The fact that FG212 can sustain osmotic potential in spite of reduced protein levels indicates that it possesses a special capacity to preserve cellular turgor in the face of water stress. As a result, it is more capable of handling drought than ICC8397, which shown a notable decline in osmotic potential. This decrease in osmotic potential in ICC8397 was linked to its increased susceptibility to drought stress, suggesting that it is less able to preserve cellular structure and function in the face of water scarcity.

The study included metrics including canopy temperature, mean green area, NDVI (Normalised Difference Vegetation Index), and FV/FM (the ratio of variable to maximum fluorescence) to do a more thorough investigation of photosynthetic efficiency in addition to the fundamental physiological markers. A crucial component of photosynthesis, photosystem II efficiency is gauged by its FV/FM ratio (Fig. 2a and b, Supplementary Table 1).

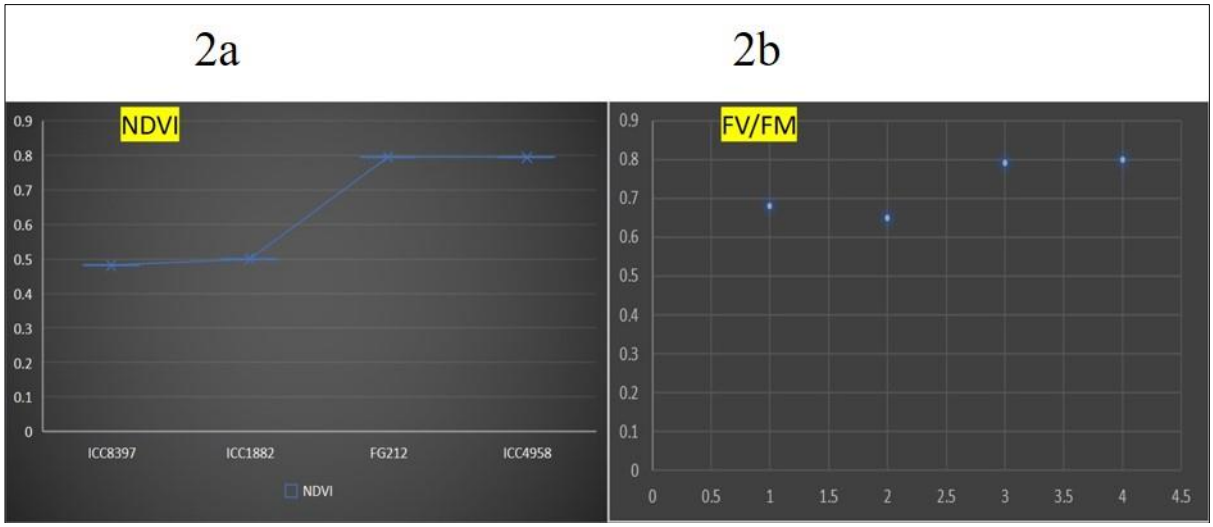

Fig 2a and b: We have analysed the data obtained from the phenotyping, genotype having low protein content FG212, is showing most of the parameters such as NDVI, total plant area, canopy temperature and FV/FM ratio (Using PhenoVation Fluorescence Detector, Crop Reporter Version 1.0, at Phenomics Facility IARI)

Because stressed plants frequently have higher canopy temperatures because of decreased transpiration, canopy temperature is a good indicator of the plant's general health and water status. NDVI is a remote sensing technology used to evaluate the health and vigour of plants, and mean green area gives an estimate of the area of the plant photosynthetically active leaves. When combined, these evaluations provide a thorough assessment of each genotype's capacity to maintain photosynthesis and general plant health during drought.

In conclusion, the study emphasises how the four genotypes differ in their ability to withstand drought. ICC8397 and ICC1882 were more sensitive to drought, whereas ICC4948 and FG212 demonstrated robust drought resilience. The findings highlight how crucial it is to preserve essential physiological processes including photosynthesis, stomatal

conductance, and osmotic potential in order to survive drought conditions.

3.3. Seed Protein Content and Amino Acid Profile

According to amino acid analysis, FG212 made use of particular amino acids, including proline and cysteine, which are known to be important for osmotic adjustment and stress response. On the other hand, ICC8397 displayed a less noticeable change in amino acid profiles, which might be because maintaining protein synthesis under stress requires a lot of energy.

4. Discussion

In order to evaluate photosynthetic efficiency, the study additionally used sophisticated measurements including mean green area, canopy temperature, FV/FM (the ratio of variable to maximum fluorescence), and NDVI (Normalised Difference Vegetation Index). The efficiency of photosystem II, a vital part of photosynthesis, can be inferred from the FV/FM ratio. Better photosynthetic efficiency under drought stress was indicated by the greater FV/FM ratios maintained by ICC4948 and FG212. This implies that these genotypes-maintained photosynthesis in spite of decreased water supply, which is essential for growth and energy production during drought. On the other hand, ICC8397 and ICC1882 displayed reduced FV/FM ratios, which further confirmed their vulnerability to dryness and indicated a drop in photosynthetic efficiency.

Another critical indicator of plant health is canopy temperature, since stressed plants typically have higher canopy temperatures because they transpire less. Lower canopy temperatures were maintained by ICC4948 and FG212, indicating that these genotypes were better at controlling water loss and preserving hydration. Additionally, ICC4948 and FG212 performed higher on the NDVI, a measure of plant health and vigour, suggesting that these plants were healthier and better equipped to survive drought stress. Increased stress and compromised physiological function during drought are seen in the lower NDVI values and higher canopy temperature in ICC8397 and ICC1882. In comparison to ICC8397 and ICC1882, the mean green area, which quantifies the photosynthetically active leaf area, was higher in ICC4948 and FG212, indicating that these genotypes retained a higher potential for photosynthesis **(**Supplementary Table 1). The fact that ICC4948 and FG212 were able to maintain photosynthetically active tissue under stress is further evidence of their superior drought resistance.

The examination of the amino acid profile and seed protein content showed that FG212 made use of particular amino acids, like proline and cysteine, which are known to be important for stress response and osmotic adjustment. Under water stress, these amino acids support cellular activity. The high energy expenditure of sustaining protein synthesis during drought circumstances may be the reason why ICC8397 displayed fewer noticeable alterations in its amino acid profile. This finding implies that FG212 may be better using these substances to regulate osmotic pressure and reduce water loss, underscoring the possible significance of amino acid metabolism in drought resistance.

5. Conclusion

This thorough investigation clearly demonstrates that FG212 and ICC4948 are more drought-tolerant than ICC1882 and ICC8397. The robustness of ICC4948 and FG212 is demonstrated by their capacity to sustain essential physiological processes during drought stress, including stomatal conductance, chlorophyll content, RWC, and osmotic potential. These physiological markers, however, showed notable decreases in ICC8397 and ICC1882, underscoring their sensitivity to drought. The findings imply that improving drought tolerance in crops can be achieved by screening for characteristics such as effective stomatal regulation, osmotic adjustment, and photosynthetic maintenance.

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