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Phenotyping of Pulses for Enhanced Tolerance to Drought And Heat

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Phenotyping of Pulses for Enhanced Tolerance to Drought and Heat

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SUMMARY

1. **Identification of Promising Image-Based Traits for Assessing the Response of Plants to Abiotic Stresses**

   A total of 19 image parameters were assessed for their effectiveness in explaining the variation in the area of the images at different growth stages in different crops. Convex hull area, absolute z rotation second moment, and minimum rectangle area was found to be important in identifying differential response of crops to various stresses

2. **Optimization of High Throughput Phenotyping Protocol for Drought Tolerance on Chickpea**

   Parameters such as digital area, convex hull area (CHA) and boundary point count (BPC) could differentiate the responses of chickpea genotypes more efficiently under depleting soil moisture and Cultivar, JG-16 was found to be promising for moisture stress tolerance.

3. **Optimization of High Throughput Phenotyping for Drought Tolerance in Pigeonpea**

   Parameters such as digital area, convex hull area (CHA), as well as NIR intensity, could differentiate the responses of pigeonpea genotypes under depleting soil moisture. Genotype SMVT-II-1834 was found to be a promising genotype, which can be used as a donor for drought tolerance as it had high biomass and low NIR Intensity relative to other genotypes.

4. **Optimisation of Phenomics Protocol for Identification of High Growth Rates Per Unit of Water**

   The partial least square (PLS) method was identified as the best method to predict biomass. The predicted biomass was used to compute the plant growth rates and water-use indices, which were found to be highly promising surrogate traits as they could differentiate the response of genotypes to soil moisture stress more effectively in mungbean genetic resources

5. **Phenomics to Elucidate the Influence of Rootstocks on Drought Response of Tomato**

   Among the rootstocks, an interspecific (*Solanum lycopersicum* × *S. pennellii*) derivative RF4A was highly efficient in terms of productive use of water. The RF4A rootstock-grafted plants were more conservative in water use with higher plant water status through relatively better stomatal regulation and hence were more efficient in generating greater biomass under water stress conditions

6. **Optimization of High Throughput Phenotyping Protocol for Heat Tolerance in Chickpea**

   Parameters such as digital area and boundary point count (BPC) could differentiate the responses of chickpea genotypes more efficiently under heat stress. Genotype JG 16 showed the highest digital area pixel and boundary point count under heat stress conditions.

7. **Optimization of Affordable Phenotyping Protocol for Drought Tolerance in Different Legumes**

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   In vitro culture using Murashige & Skoog Basal medium and osmotic stress induced by 5% PEG 6000 (Polyethylene glycol 6000) are the best combination to study PEG-induced RSA in chickpea using digital images.

9. **Cooler Canopy Leverages Sorghum Adaptation to Drought and Heat Stress**

   Genotypes having cooler canopy under individual and combined effects of drought and heat stress can overcome the stress efficiently compared to genotypes with high canopy temperatures.

10. **Physiological Traits Identified in Drought Tolerant Mungbean Genotypes**

    Cooler canopy genotypes (VC-6173-C, IC-325770, and ML-2082) and a genotype (DMG-1050) with novel trait combinations like high SPAD and better PSII health despite high canopy temperature found tolerant to drought which can be used as donors in mungbean breeding programs

11. **Evaluation of Pod Pedicel as a Component Trait to Facilitate Photosynthate Supply to Developing Grains During Soil Moisture Depletion in Chickpea**

    The study revealed genetic variation in pedicel parameters under both water-stressed and well-watered conditions. Pedicel parameter and seed weight were found to be correlated indicating that the pedicel can play a crucial role in determining the yield of chickpea.

12. **Identification of Drought Tolerant, Ascorbic Acid-Rich Chickpea Genetic Resources**

    A total of 106 germplasm and 6 checks were screened for terminal moisture stress tolerance for three years (2020, 2021, and 2022) under field and control conditions. A total of 8 germplasms were (BDNG-2018-15, PG-1201-20, C-19315, C-19186, BDNG-2017, PG-1012-15, C-19190, C-19291) identified as tolerant to moisture stress and, they had high ascorbic acid in leaf and seed as well.

13. **Phenotyping of Mungbean Genotypes for Waterlogging Tolerance**

    A study was conducted to establish a relationship between grain yield and non-invasive physiological parameters such as NDVI and Qmax under stress. Correlation and regression analysis revealed there was a high and significant positive association between grain yield and Qmax and NDVI. Hence, these traits can be used as surrogate traits to identify waterlogging tolerant mungbean genetic resources.

14. **Deciphering Endurance Capacity of Mango Tree to Desiccation Stress Based on Efficiency of PSII**

    Studies revealed that the mango tree can maintain its carboxylation efficiency over some time. IR studies confirmed that the mango tree maintained its canopy coolness during the dry season. In addition, the chlorophyll fluorescence study revealed that mango leaves retained 50% of initial PSII efficiency for as many as 4 days after desiccation and chlorophyll fluorogram also depicted the observations. Phenomics studies concluded that mango twigs retained tissue water content even up to 164 h of desiccation with a gradual decrease in the canopy area.

15. **Desiccation Tolerance of Photosystem II in Dryland Fruit Crops**

    The PS-II tolerance to tissue dehydration observed in karonda (*Carissa carandas* L) and sweet orange (*Citrus sinensis*), was higher than that of mango (*Mangifera indica* L) and grape (*Vitis vinifera* L). This study reveals the method to assess the sensitivity of fruit crops to desiccation, which can be useful in water management, and in assessing the efficacy of novel chemicals for alleviating abiotic stresses.

16. **Relative Tolerance of Photosystem II in Spike, Leaf, and Stem of Bread and Durum Wheat Under Desiccation**

    Durum wheat had higher quantum efficiency and lower photoinhibition of PSII relative to bread wheat across spike, stem, and leaf. The rate of decline in maximum photochemical efficiency of PSII with increased desiccation was seen higher in bread wheat spikes as compared to durum wheat. ChlF imaging could be effectively deployed as a phenotyping tool to differentiate wheat genotypes for their photosynthetic performance under desiccation.

17. **Canopy Temperature Depression (CTD) and Canopy Greenness Associated with Variation in Seed Yield of Soybean Genotypes Under Soil Moisture Stress**

    Studies revealed that CTD along with canopy greenness can be used as a key trait of leaves in the selection of soybean genotypes for higher adaptability to low soil moisture stress conditions, a common feature that exists under semi-arid regions.

18. **Responses of Chickpea Genotypes to Soil Moisture Deficit Imposed Under Field Condition**

    Genotypes Such as D24, D5, D15, IPC06-11, and ICE15654B had high seed yield and could maintain their canopy cooler in stress conditions induced by soil moisture deficit.

19. **Responses of Pigeonpea Genotypes to Soil Moisture Deficit Imposed Under Field Condition**

    Genotype RVK 285 was identified as tolerant to seedling stage moisture stress by adopting lower digital biomass as an adaptive strategy under depleting soil moisture conditions and hence had better recovery.

20. **Screening and Identification of Waterlogging Tolerant Pigeonpea Genetic Resources**

    A total of 106 germplasm along with checks were screened for waterlogging tolerance for two years (8 days stress) at the seedling stage. Based on the morpho-physiological traits and tolerance indices, genotypes *viz*., ICP-10397, ICP-7507, ICP-7869, ICP-7148, ICP-4903 ICP-16309, ICP-7375, ICP-6815, ICP-7507, and ICP-6128 were identified as tolerant to waterlogging.

21. **Non-Destructive Assessment of Seed Phenotypes in Mungbean Mini-Core Collection (MMC) by Image Analysis**

    Seeds of 296 accessions of mungbean were phenotyped using low-cost mobile-based phenotyping. The results revealed the possibility to employ low-cost phenotyping tools to study variation in seed morphology of a large number of germplasms of different crops.

22. **Identification of Trait-Specific Germplasms for High-Temperature Stress Tolerance in Cowpea**

    A total of 250 germplasm and five checks were screened for high-temperature stress tolerance under field conditions during the summer month of 2022. Among them 50 germplasm found better than checks for the traits such as canopy temperature, leaf and pod florescence, grain yield, and other physiological traits.

PLANT PHENOTYPING: NEEDS AND SCOPE

I. Introduction

Pulses, including pigeonpea, chickpeas, cowpea, and mungbean hold immense importance as a staple food source for millions of people worldwide. These nutrient-dense crops provide plant-based protein, dietary fibre, vitamins, minerals, and phytochemicals necessary for a healthy and balanced diet, especially in regions where access to animal protein is limited. Pulses contribute to food security by offering an affordable and sustainable source of nutrition, improving the dietary diversity and quality of populations. Moreover, pulse crops have agronomic benefits, as they fix atmospheric nitrogen, reduce the need for synthetic fertilizers, and require less water, making them suitable for cultivation in water-scarce areas. However, pulses are highly vulnerable to drought and heat stress. Their shallow root systems and limited water-holding capacity make them sensitive to drought, leading to reduced crop productivity and economic losses. High temperatures associated with heat stress affect various physiological processes, ultimately decreasing photosynthesis, flower and pod formation, and seed quality. With climate change exacerbating these challenges, developing pulse varieties with enhanced tolerance to drought and heat stress becomes imperative. Phenotyping techniques play a vital role in identifying and selecting tolerant genotypes, paving the way for the development of climate-resilient pulse crops, and ensuring food security in the face of changing climatic conditions.

As global temperatures rise and extreme weather events become more frequent, it is crucial to identify and develop pulse varieties that can withstand these challenging conditions. By screening genotypes for tolerance, researchers and breeders can identify traits and characteristics that contribute to resilience. This knowledge can then be utilized to breed and select genotypes that are better equipped to handle drought and heat stress, ensuring sustained productivity and food security. Moreover, screening for tolerance allows for the conservation of valuable resources such as water, as more efficient genotypes can be identified that require less irrigation. By developing pulse varieties with enhanced tolerance to drought and heat stress, we can create more sustainable farming systems that promote environmental stewardship and long-term agricultural viability. Ultimately, the screening process plays a critical role in adapting pulse crops to the changing climate, safeguarding food production, and ensuring the resilience of farming communities.

Phenotyping plays a significant role in identifying and selecting tolerant genotypes with enhanced resilience to drought and heat stress. Through phenotyping, researchers can evaluate and quantify the phenotypic variation among different genotypes, allowing for the characterization of traits associated with tolerance. Traits such as plant height, leaf area, biomass accumulation, root characteristics, physiological parameters, and stress-related biochemical markers can be assessed to determine the performance of genotypes under stress conditions. The efficient screening enabled by phenotyping technologies and methodologies allows for the evaluation of large populations, expediting the identification of tolerant individuals. Furthermore, phenotyping provides valuable insights into the genetic basis and heritability of tolerance traits, aiding in the selection of genotypes with stable and heritable characteristics. The data obtained from phenotyping guide breeding strategies and the development of improved varieties through informed selection, crossbreeding, and targeted genetic transformation.

II. High Throughput Plant Phenomics Facility under NICRA

Under the National Initiative on Climate Resilient Agriculture (NICRA) component, a plant phenomics facility was established at NIASM, Baramati to facilitate the high throughput phenotyping of plant germplasm for tolerance to various abiotic stresses. The greenhouse covers an area of 432 m^2 with a gable front length of 27 m and a gutter front length of 16 m. Zinc-coated pre-galvanized steel pipes and double polycarbonate sheets (8 mm thick) are being used to construct the greenhouse. Inside the greenhouse, there are four zones (A, D, B, and C) to create different climatic conditions. The greenhouse has concrete flooring and electrically operated roof vents with thermal and shading screens.

Under National Innovations for Climate Resilient Agriculture (NICRA) project the installation of the phenomics facility by LemnaTec, GMBH, Germany was completed on 1st September 2015. The facilities allow screening of 216 plants at a time and it is possible to screen thousands of lines for responses of plants to a particular phase of crop growth with staggered planting and growth initially under natural conditions. The facility is equipped with cameras for acquiring images in visual (VIS), infrared (IR), and near-infrared range (NIR) for morpho-physiological traits, surface temperature, and plant water relations respectively. It has programmable and automated irrigation and weighing system to create and monitor soil moisture stress. Automated temperature regulation can allow screening for high-temperature tolerance. Robust software and hardware allow the acquisition, storage, and analysis of huge sets of images. Research projects facilitated by this technology vary from large-scale screening of early growth and tolerance to abiotic factors like soil moisture stress, salinity, and nutrient imbalances.

III. Objectives

- To develop plant phenomic protocol for the characterization of responses of crops to abiotic stresses mainly drought, high temperature, and salinity.
- To identify alternative traits to accelerate the characterization of plants' responses to complex and difficult-to-measure traits associated with stress tolerance
- To identify promising genotypes that have attributes contributing to stress tolerance
- To identify traits and genes associated with tolerance to drought, high temperature, and salinity
- To complement the efforts of plant breeders and molecular biologists involved in the investigation of genes associated with tolerance to abiotic stresses in field and horticultural crops
- To develop a plant phenome database by employing common methods and comparable procedures
- To facilitate the development of low-cost indigenous plant phenotyping tools for controlled and field experiments by validation of results in high throughput (HTP) phenotyping

RESEARCH METHODOLOGY

1. Planning an Experiment

This includes identifying the objectives, selecting appropriate treatments or conditions, determining the sample size, and defining the experimental setup.

2. Imposition of Stress

Stress imposition refers to intentionally subjecting the genotypes to specific environmental conditions to evaluate their performance.

3. Planning Water Job

This step ensures regular and optimal water management to maintain the desired control over the duckweed population.

4. Imaging

4.1 Visible Imaging

In plant science, visible light imaging has been broadly adopted due to its low cost and simplicity. Using this imaging system, with a similar wavelength (ranging from 4 00 to 700 nm) perception as the human eye, two-dimensional (2D) images can be used to analyze numerous phenotypic characteristics and to record the changes in plants. To spread the spatial and volumetric information of phenotype images, three-dimensional (3D) imaging approaches have been developed, which could provide more accurate estimations of the morphological features. Therefore, during the integration of 2D and 3D image analysis, visible light imaging techniques are popular components for the integrated plant phenotyping platform. It represents raw data of a phenotype image in spatial matrices based on the intensity values relating to photon fluxes (red~600 nm, green~550 nm, blue~450 nm) of the visible light spectral band.

4.2 Infrared Imaging

Infrared imaging technologies are used for screening objects of internal molecular movements which emit infrared radiation. Two popular infrared imaging devices- a near-infrared (NIR) and a far-infrared (Far-IR, also called IR thermal) - can be used to screen radiation images. Many studies have combined visible and NIR imaging to detect vegetative indices because healthy plants reflect a large proportion of NIR light (800–1400 nm), whereas soil reflects little NIR light.

4.3 Fluorescence Imaging

Fluorescence imaging is used from laboratory to field. This imaging technique describes the information about the plant metabolic status that can be obtained by the artificial excitation of the plant photo systems and observation of the relevant responses (Li et al., 2014). It is based on charge-couple device (CCD) cameras with sensitive fluorescence signals, where the signals occur by illuminating samples with visible or ultraviolet light. There are two types of fluorescence (red to the far-red region and the blue to green region) generated by the ultraviolet illumination ranging from 340 to 360 nm and is expressed as a principle of underlying multi-colour fluorescence imaging. This technique offers the simultaneous capture of fluorescence emission and provides a quick way to probe photosystem II status in vivo (Maxwell and Johnson, 2000).

5. Image Analysis

Once the images are captured, image analysis techniques are utilized to extract quantitative information from the images. This involves processing the images using software or algorithms to measure parameters such as leaf coverage, density, or biomass. Image analysis helps in obtaining objective and consistent data for further analysis (Figure 1).

6. Data Analysis

After collecting the relevant data, statistical methods and data analysis techniques are applied to analyse and interpret the findings. This step involves performing calculations, statistical tests, and modelling to determine the impact of stress on genotype performance, identify patterns, and draw meaningful conclusions.

7. Data Interpretation

Data interpretation involves making sense of the analyzed results and drawing conclusions based on the findings. Interpretation often involves comparing the experimental results with existing literature, discussing limitations, and suggesting future research directions.

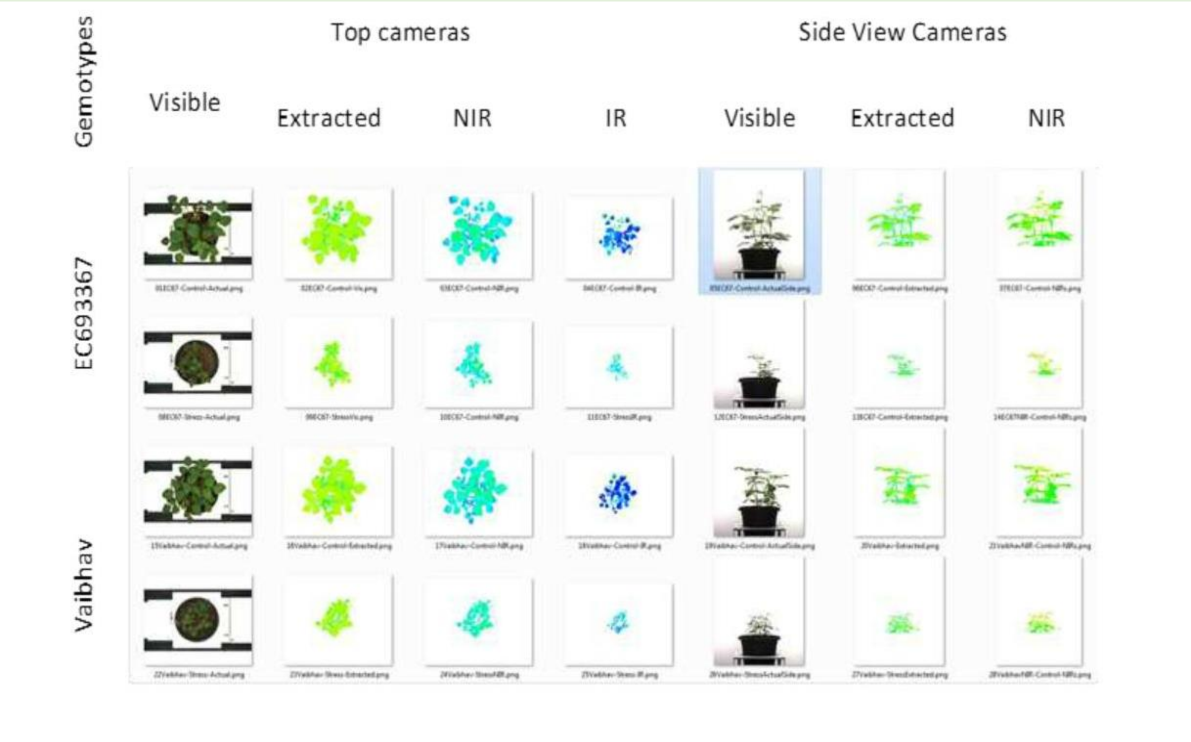


Fig. 1: Extraction of images from original images (A case study in mungbean)

RESEARCH ACHIEVEMENTS

1. Optimization of Screening Protocols for Different Pulse Crops

Several IUCs (Imaging Unit Configuration) and IACs (Image Analysis Configuration) were prepared for different crops including mungbean, chickpea, and pigeonpea which can be now used for various experiments involving the above-mentioned crop plants. IUCs and IACs were also prepared for assessing water use efficiency, leaf water loss, and responses of chickpea genotypes to different microbial consortiums. With project objectives in focus, the experiments were conducted with mungbean, chickpea, pigeonpea, and lentil.

2. Optimizing Methods for Imposing Stress

Initial experiments at the plant phenomics facility of NIASM included soil moisture stress imposed by creating two levels of soil moisture *viz.*, 60-80% of field capacity as control and 40-50% field capacity as stress. However, this method could not create sufficient soil moisture stress for differentiating the genotypes for their tolerance to stress. Hence, it was decided to assess the responses of plants to depleting soil moisture by withholding irrigation at particular growth stages of crop plants. Depletion of soil moisture was recorded almost every day after withholding the irrigation till wilting symptoms appeared in plants. In some experiments, recovery from the soil moisture stress was assessed by replenishing the soil moisture to field capacity and then terminating the experiments after 3 to 5 days.

3. Protocols Optimized for Water Use Efficiency, Leaf Senescence, Canopy Architecture

The phenomics facility allows the estimation of water consumed by plants during their growth or during the imposition of stress to assess the genetic variation in the plant responses. Water consumed included both evaporation and transpiration though dummy pots were used as blanks to get an idea about the total evaporation in the absence of plants in the pots. Data generated on water consumption and the biomass produced by plants were used in differentiating the plants that produce more biomass with less water as compared to locally adapted cultivars included as checks. Image analysis configuration tools were used to quantify green, red, and blue pixels, which could provide fare idea about the leaf senescence as well as the rate at which it occurs in pulse crops like chickpea.

Image analysis configurations designed at NIASM provide more than a dozen of image parameters that can explain the responses of genotypes of different pulse crops to managed soil moisture stress under controlled environmental conditions. It is well established that digital volume derived from image area captured from the top view and two sides can explain variation in actual biomass. We observed that this works particularly when sufficient tissue moisture exists in plants. Hence this can be utilized for assessing the rate of change in plant biomass in response to depleting soil moisture stress. Conditions under which this parameter fails to explain the biomass are being assessed. Among other parameters, convex hull area and compactness could differentiate the plant responses to depleting soil moisture stress.

4. Optimization of high throughput phenotyping protocol for drought tolerance in chickpea

An experiment was conducted to optimize image-based phenotyping methods for assessing plant responses to depleting soil moisture stress and to identify promising traits and potential genotype (s) for drought tolerance with 22 genotypes of chickpea including local check Digvijay (Fig 2). Plants were initially grown under natural conditions in pots, and then were shifted to a plant phenomics facility 25 days after sowing (DAS) for phenotyping. Water stress treatment was imposed at 30 DAS by withholding water whereas optimum moisture was maintained in the control. Soil moisture trends and NIR intensity during the experiment differentiated control and drought stress treatment. High-resolution visible (400-700 nm) and

NIR (700-1700 nm) sensors were used to capture images of shoot image parameters in different pots. Parameters such as digital area, convex hull area (CHA), and boundary point count (BPC) could differentiate the responses of chickpea genotypes more efficiently under depleting soil moisture. Genotype JG16 showed the highest digital area pixel, boundary point count, and boundary point roundness during the initial phase in response to two levels in well-watered (C) and water-stressed (S) plants.

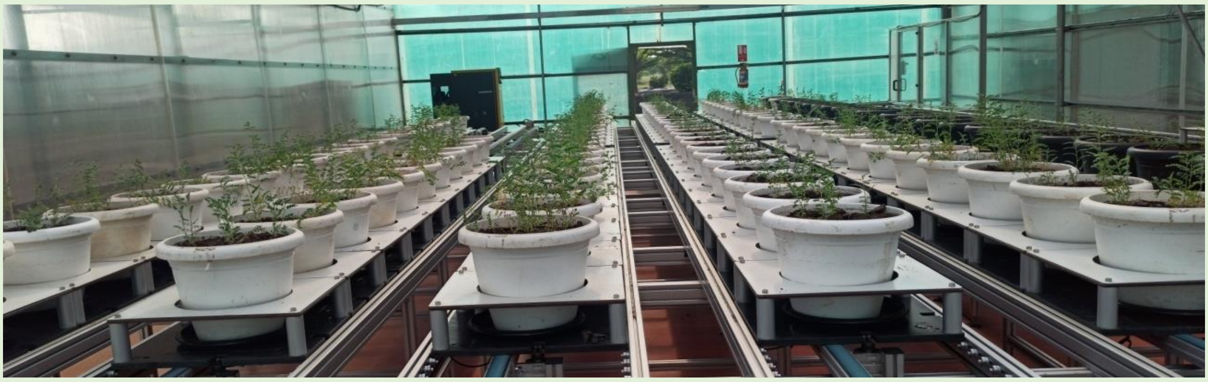


Fig. 2: Experimental setup in plant phenotyping facility at NIASM Baramati.

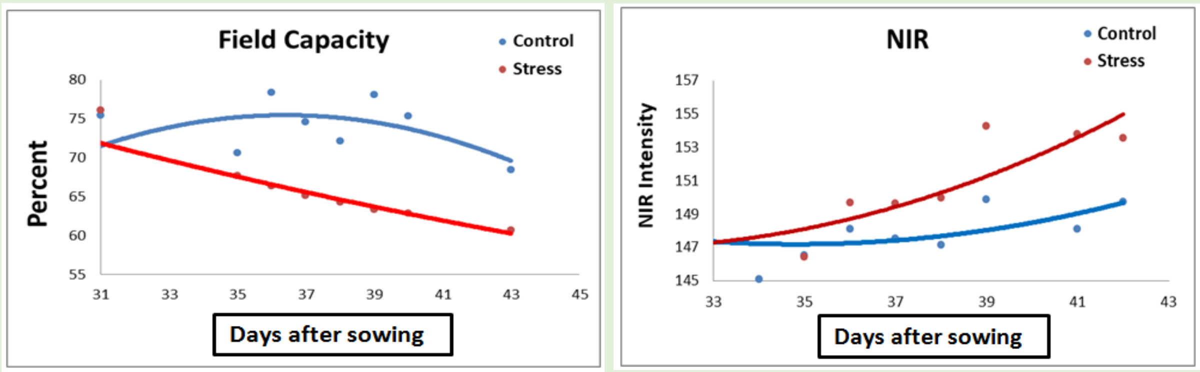


Fig. 3: Soil moisture trends and NIR intensity in control and drought stress treatment

Fig. 4: Heat Map: Image parameters differentiating plant responses to water stress and well-watered condition from top and side view

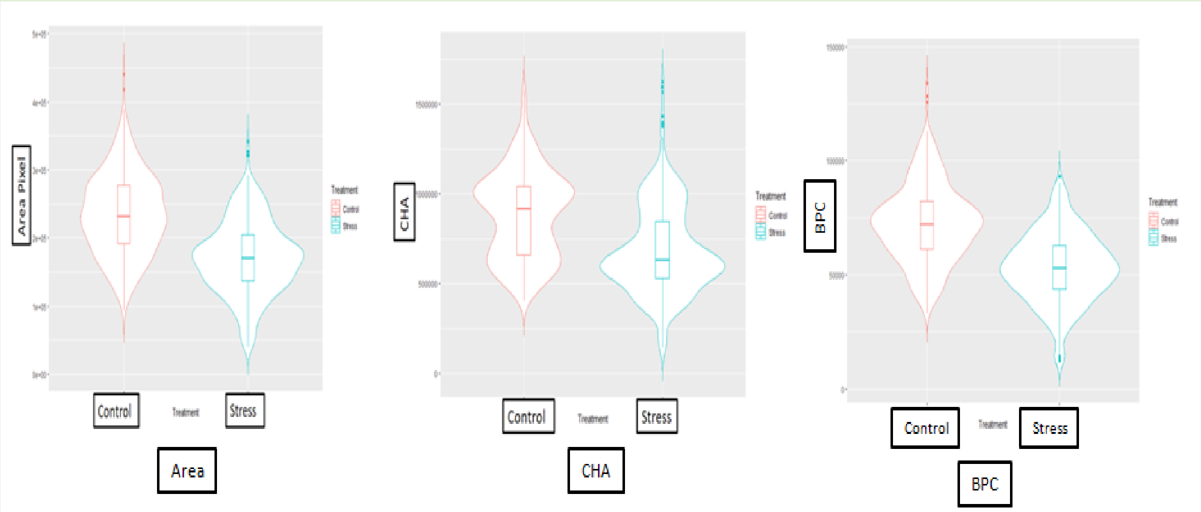


Fig. 5: Mean values of digital area (a), Convex hull area (b), Boundary Point Count (c) of shoots of chickpea plants in drought and control conditions.

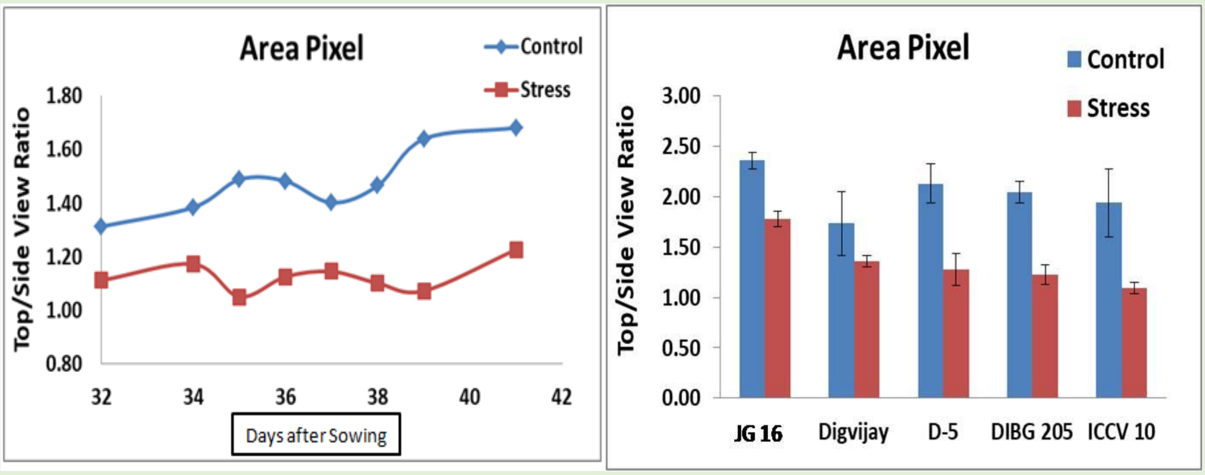


Fig. 6: Top and side view ratio of digital area pixel (a) Genotypic variation under control and drought condition (40 DAS) (b).

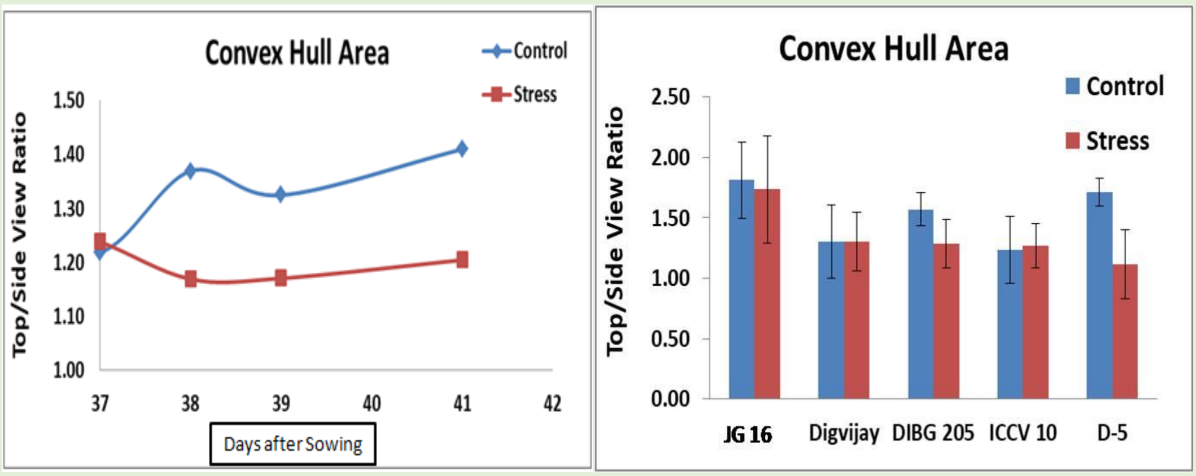


Fig. 7: Top and side view ratio of convex hull rea (a) Genotypic variation under control and drought conditions (40 DAS).

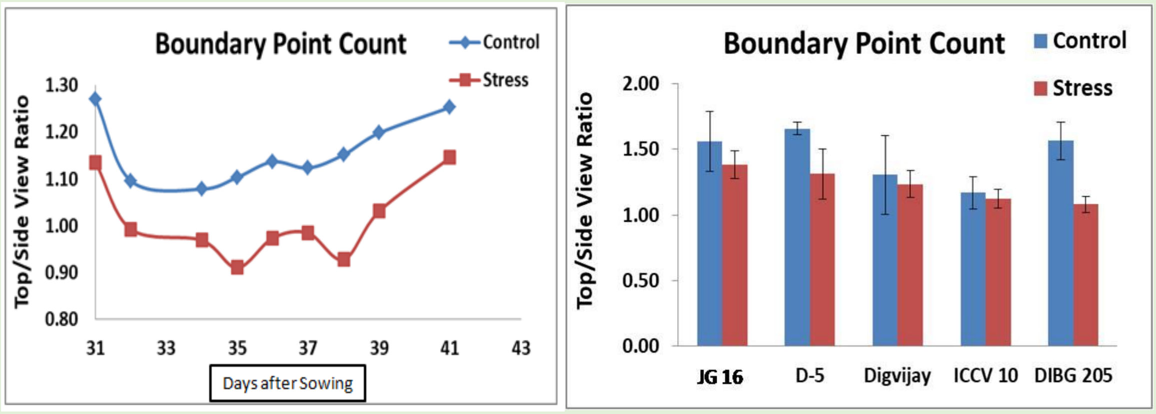


Fig. 8: Top and side view ratio of boundary point count (a) Genotypic variation under control and drought condition (40 DAS) (b).

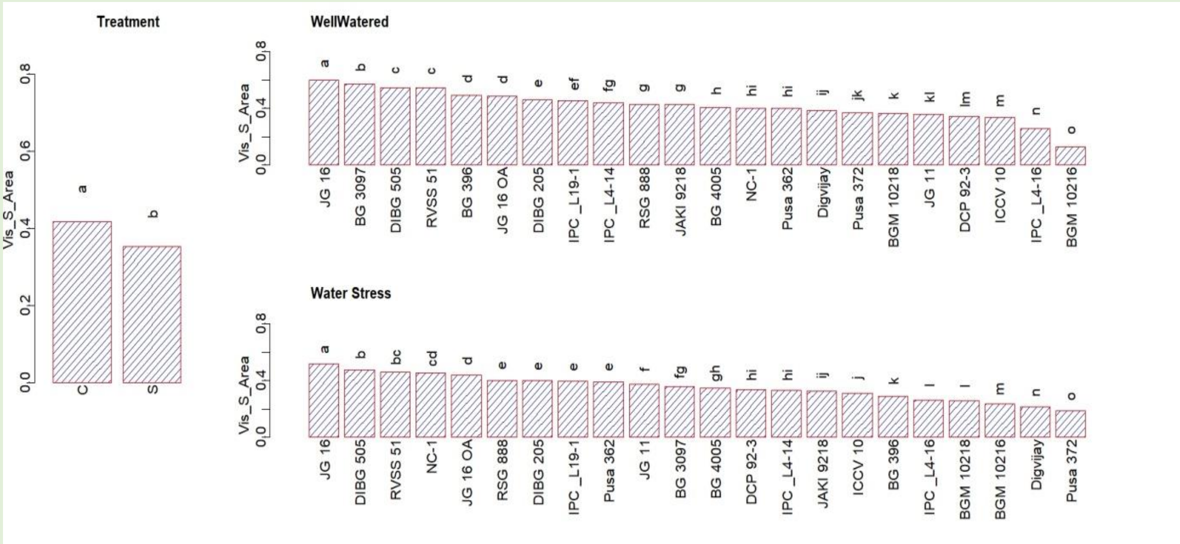


Fig. 9: Genetic variation in digital area pixel during the initial phase in response to two levels in well-watered (C) and water-stressed (S) plants.

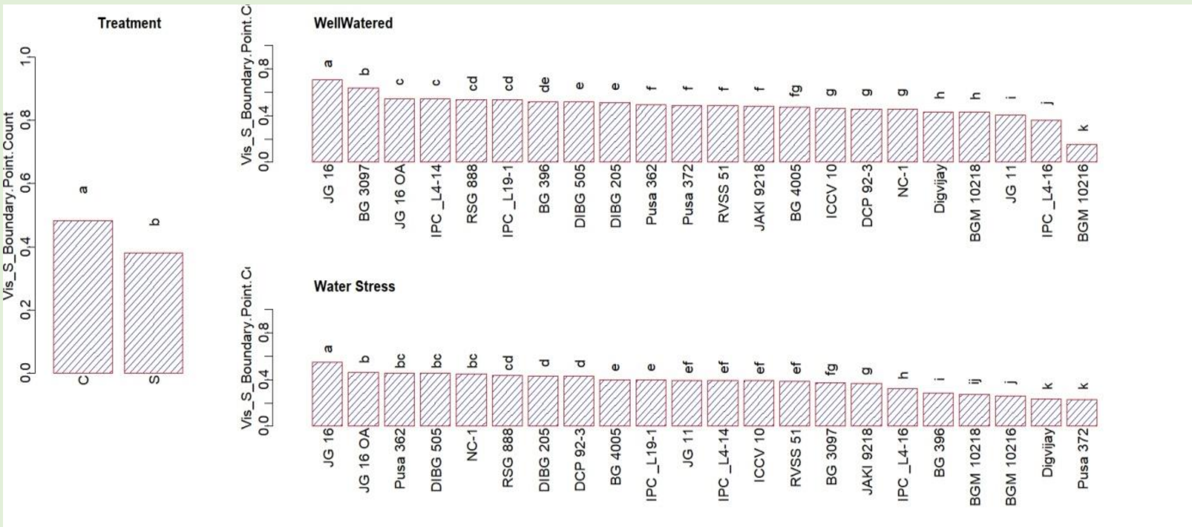


Fig. 10: Genetic variation in boundary point count during the initial phase in response to two levels in well-watered(C) and water-stressed(S) plants.

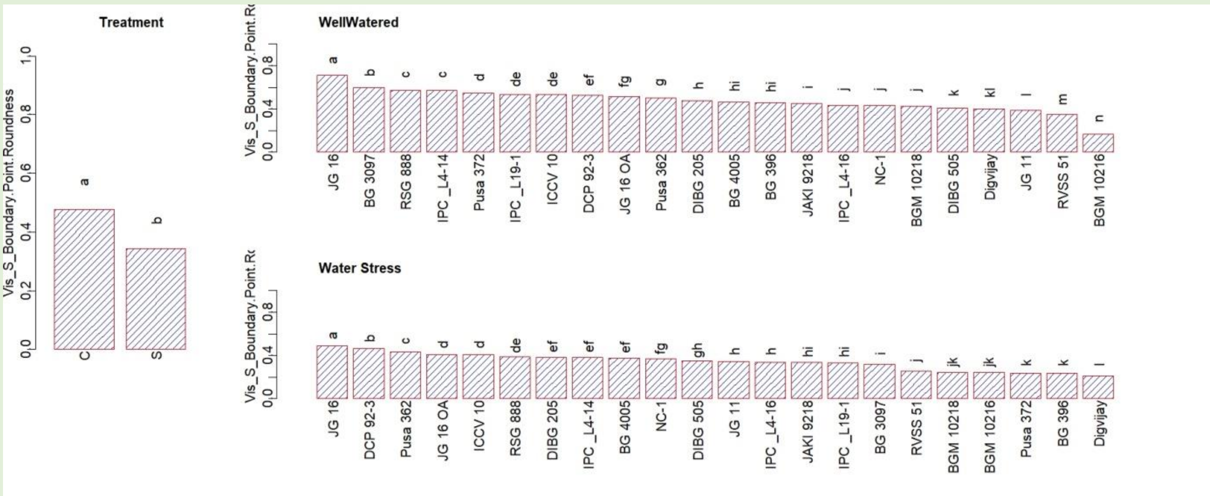


Fig. 11: Genetic variation in boundary point roundness during the initial phase in response to two levels in well-watered(C) and water-stressed(S) plants.

Conclusion: Parameters such as digital area, convex hull area (CHA), boundary point count (BPC), as well as NIR intensity, could differentiate the responses of chickpea genotypes more efficiently under depleting soil moisture. Genotype JG-16 was found to be a promising genotype, which can be used as a donor for drought tolerance as it had high biomass relative to Digvijay.

i. Optimization of high throughput phenotyping for drought tolerance in pigeonpea

An experiment was conducted to optimize image-based phenotyping methods for assessing plant responses to depleting soil moisture stress and to identify promising traits and potential genotypes for drought tolerance of pigeonpea. Soil moisture stress treatment was imposed 25 days after sowing. Visible (400-700 nm) and NIR (700-1700 nm) sensors were used to capture images at various angles for soil water status and shoot image parameters. Image parameters such as digital Area (a), Convex Hull Area (b), and NIR Intensity (c) of shoots of pigionpea differentiating plant responses to soil moisture stress conditions. Genotype SMVT-II-1834 showed the highest digital biomass accumulation and convex hull area (CHA) during the experiment under the stress condition. Genotypic variation of Low NIR intensity showed by genotypes SMVT-II-1822, and 1825 followed by SMVT-II-1834 under drought stress conditions indicating higher tissue water accumulation by these genotypes.

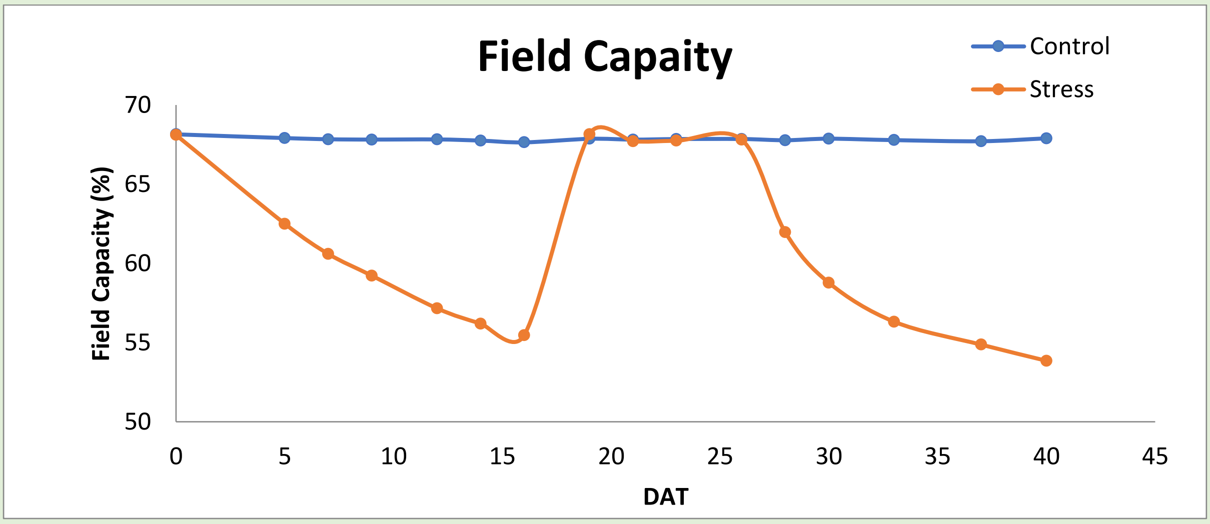


Fig. 12: Soil moisture trends during the experiment in control and drought stress treatment.

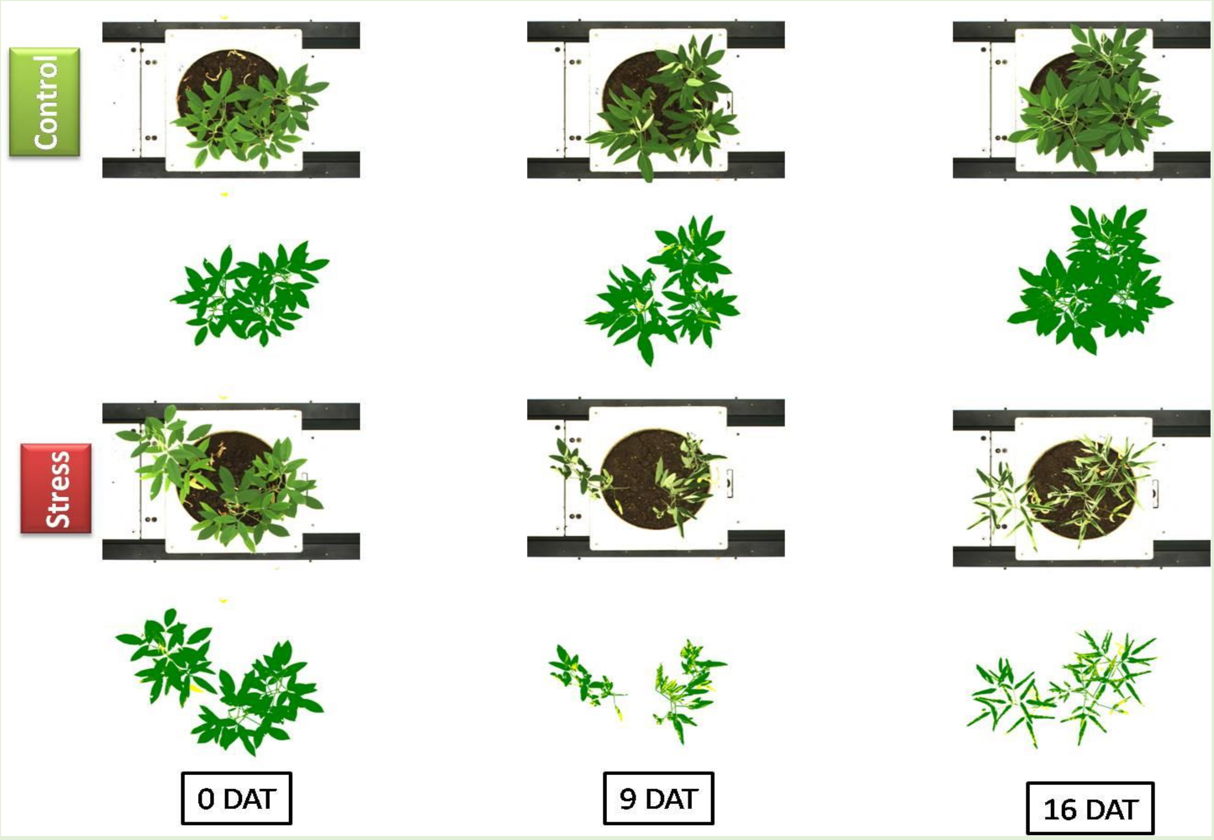


Fig. 13: RGB image of a pigeonpeagenotypeSMVT-II-1834the top view represents the same plant from 0 to 16 days of control and water stress treatments.

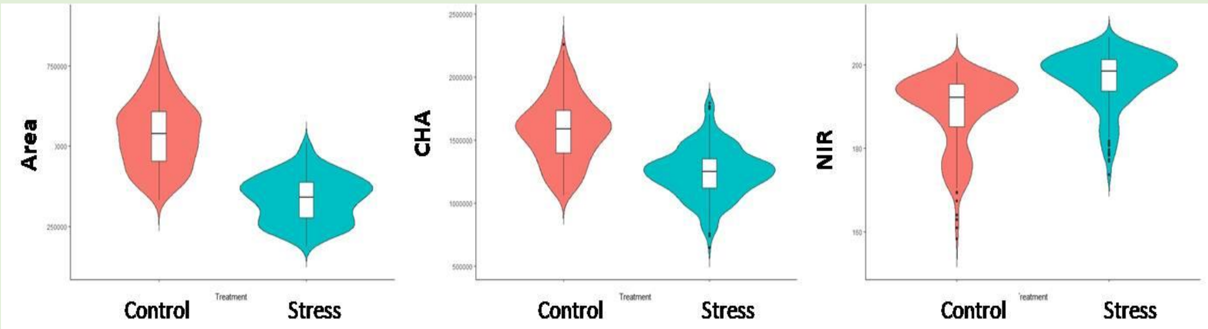


Fig. 14: Mean values of digital area (a), Convex Hull Area (b) NIR Intensity (c) of shoots of pigionpea plants in drought and control condition

Conclusion: Parameters such as digital area, convex hull area (CHA), as well as NIR intensity, could differentiate the responses of pigeonpea genotypes more efficiently under depleting soil moisture. Genotype SMVT-II-1834 was found to be a promising genotype, which can be used as a donor for drought tolerance as it had high biomass and low NIR Intensity relative to other genotypes.

i. Optimization of phenomics protocol for identifying high growth rates per unit of water applied

Twenty-four elite mungbean genotypes were grown with and without water stress for 25 days in a controlled environment. Top view and side view (two) images of all genotypes captured by a high-resolution camera installed in the high-throughput phenomics were analyzed to extract the pertinent parameters associated with plant features. We tested eight different multivariate models employing machine learning algorithms to predict fresh biomass from different features extracted from the images of diverse genotypes in the presence and absence of soil moisture stress. Based on the mean absolute error (MAE), root mean square error (RMSE), and R squared (R^2) values, which are used to assess the precision of a model, the partial least square (PLS) method among the eight models was selected for the prediction of biomass. The predicted biomass was used to compute the plant growth rates and water-use indices, which were found to be highly promising surrogate traits as they could differentiate the response of genotypes to soil moisture stress more effectively.

Fig. 16: Red-green-blue (RGB) image of a mungbean genotype IC-415144 on the top view (A) and side view (B) represents the same plant from 0 to 37 days of control and water stress treatments.

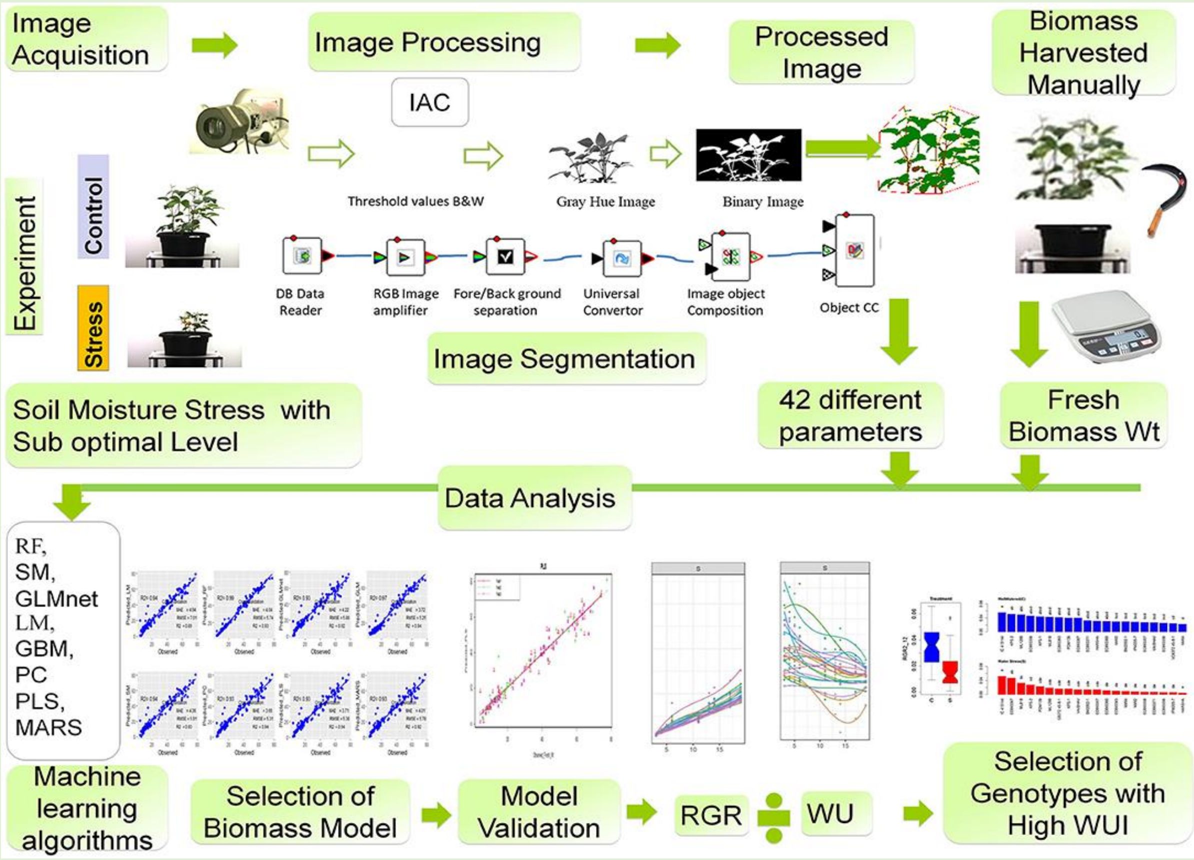


Fig. 17: A suggested phenotyping protocol for mungbean genotypes. Workflow for image-derived biomass model construction consists of the following steps: (1) image acquisition, (2) image processing, (3) different geometric and colour-related phenotypic traits observed, (4) biomass harvested manually at the last day of

imaging and fresh weight and dry weight measured, (5) different machine learning algorithms RF, SM, GLMnet, LM, GBM, PC, PLS, MARS models were used to predict plant biomass, (6) model validation, (7) model selection, evaluation, and result interpretation, and (8) selection of genotypes with high water-use index (WUI).

ii. Phenomics to Elucidate the Influence of Rootstocks on Drought Response of Tomato

In this study, we employed a high throughput phenomics facility to assess the efficiency of tomatoes, grafted on the rootstocks of different genetic backgrounds, at different levels of moisture in the soil. Rootstocks included tomato cultivars and hybrids, derived from the crosses involving wild relatives as donor parents. Among the rootstocks, an interspecific (*Solanum lycopersicum*×*S. pennellii*) derivative RF4A was highly efficient in terms of productive use of water. The RF4A rootstock-grafted plants were more conservative in water use with higher plant water status through relatively better stomatal regulation and hence were more efficient in generating greater biomass under water stress conditions. These plants could maintain a higher level of PSII efficiency, signifying better photosynthetic efficiency even under water stress. The distinct response of interspecific rootstock, RF4A, to water stress can be ascribed to the effective root system acquired from a wild parent (*S. pennellii*), and hence efficient water uptake. Overall, the study demonstrated the efficient use of a phenomics platform and developed a protocol to identify promising rootstock–scion combinations of tomato for optimization of water use.

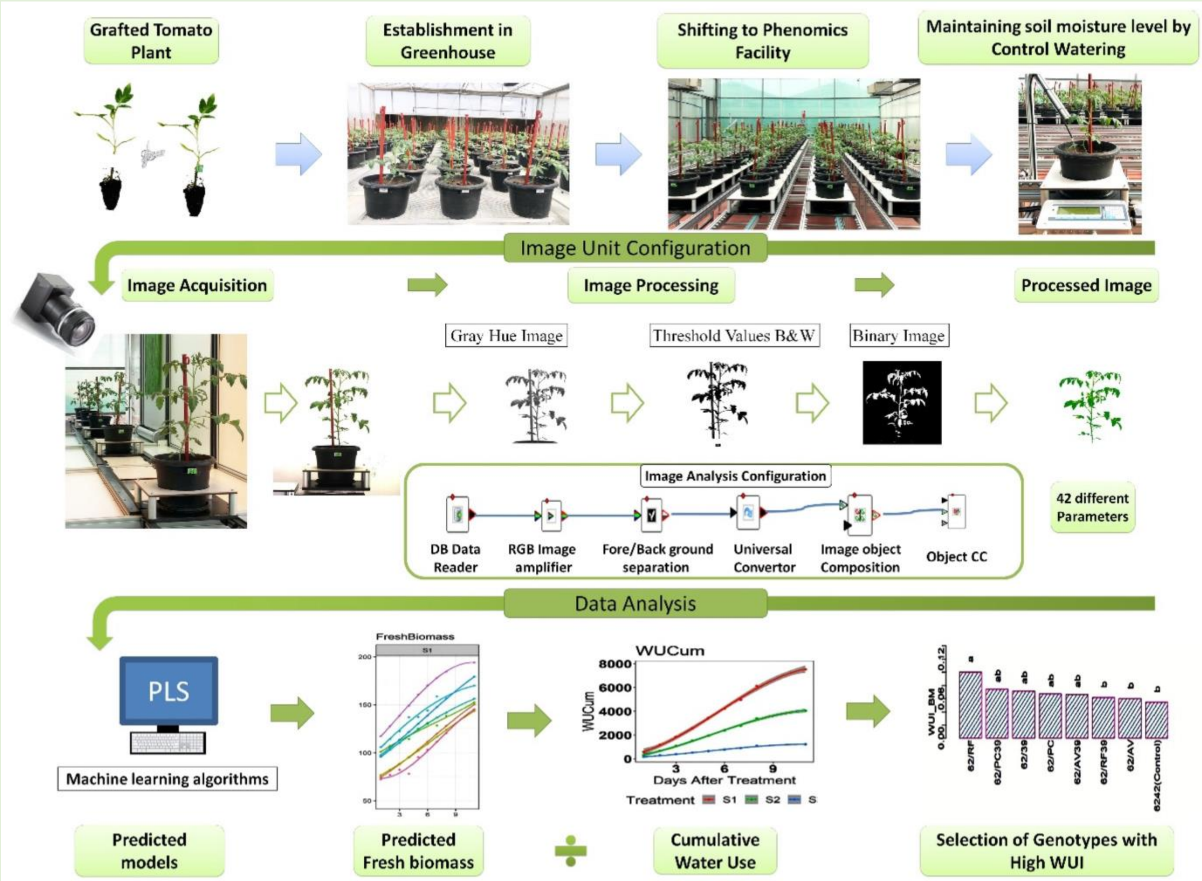


Fig. 18: Protocol for phenotyping of grafted tomato plants under different soil moisture regimes in phonemics facility.

iii. Phenomics for differentiating responses of chickpea plants to the microbial consortium

Beneficial microbes individually or in a consortium can be applied to improve the growth and productivity of plants, with specifically to be used for drought tolerance. A solid understanding of the physiological and biochemical changes in plants induced by these beneficial microbes is required. The present study was carried out to investigate the effect of microbial consortium on the phenotypic changes in chickpea grown under drought stress conditions and their association with drought tolerance by using high throughput phenomics platform facility. The phenotype data from plants treated or not treated with microbial consortium were captured in a non-destructive manner for quantitative studies of complex traits, such as growth, tolerance, architecture, and physiology. The experiment was conducted with 8 treatments including the uninoculated control. Chickpea seedlings were grown in a shade net with uncontrolled conditions in a pot containing black soil fertilized with N:P 20:40 till 43 days in 12 replications. Moisture content was maintained in all pots till the drought treatment started. All the pots were then shifted to the greenhouse in the phenomics platform with controlled conditions and irrigation was terminated in half of the pots of each treatment. Imaging (Visible/NIR/IR spectral range) was carried out until the 16th day of stress with a total of 11 observations. The image data was then exported for analysis using Lemna miner and saved the file in CSV format. A table lay out was made for the fast processing of each data set. Reduction in moisture content during the stress period was recorded using the weight data from the phenomics platform. Digital volume estimated from the visible images exhibited the positive effect of microbial consortium under drought conditions.

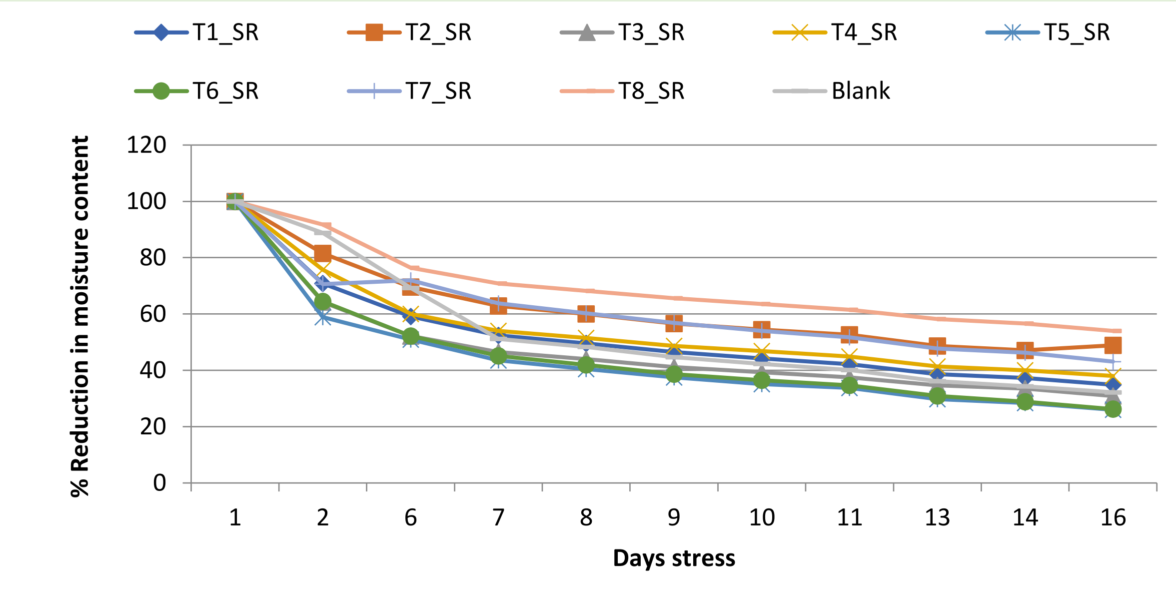


Fig. 19: Reduction in moisture content during the stress period

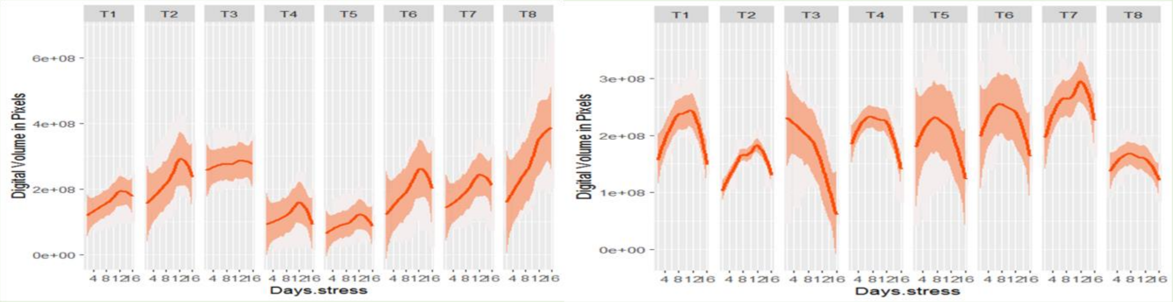


Fig. 20: Effect of Microbial consortium on the digital volume of chickpea plants a) under well-watered conditions and b) under drought conditions. T1-T7 plants treated with the microbial consortium, T8 is uninoculated control

Shoot architecture as revealed by the convex hull area was higher in most of the treatments as compared to uninoculated control (T8) under drought conditions. Significantly higher convex hull area was recorded in T1, T5, and T7 than in T8. Likewise, the digital area (top) also was higher in the chickpea plants treated with the microbial consortium. Compactness extracted by visible images was found significantly higher in T5 and T7 treatment than in uninoculated control (T8) under stress conditions. Canopy temperature was also found lower in T5, T6, and T7 treatments compared to T8 under stress conditions as revealed by IR image analysis. The results revealed that under well-watered conditions no significant difference between the treatments but under stress conditionT1, T5, T6, and T7 were better than T8.

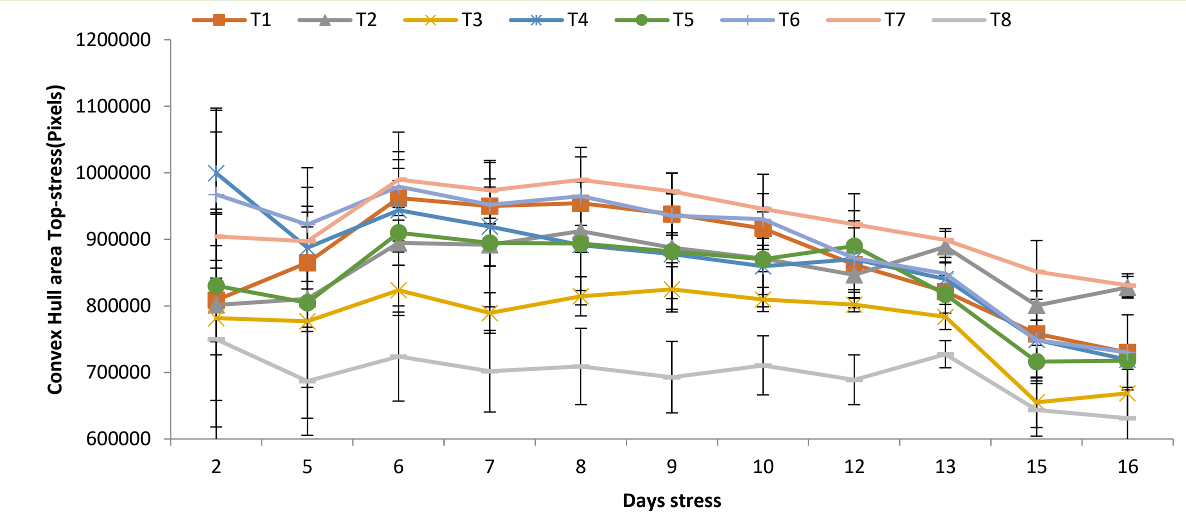


Fig. 21: Effect of water stress on shoot architecture as revealed by top convex hull area

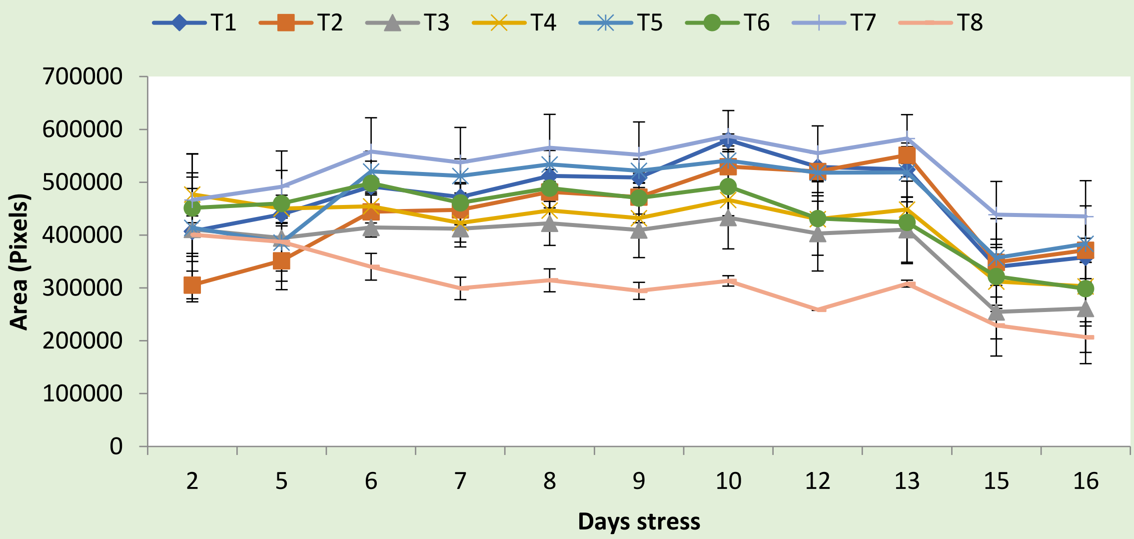


Fig. 22: Effect of water stress on the top area of chickpea plants

iv. Optimization of high throughput phenotyping protocol for heat tolerance in chickpea

The experiment was conducted to optimize the image-based phenotyping method for assessing plant responses to heat stress with 14 genotypes of chickpeas including local check Digvijay. Plants initially grown under natural conditions in pots were shifted to a plant phenomics facility 30 days after sowing (DAS) for phenotyping. Heat stress treatment was imposed at 30 DAS by shifting one set of genotypes to a heat chamber where an average 35 °C temperature was maintained and the other set was kept in normal condition maintained as control. High-resolution visible (400-700 nm), NIR (700-1700 nm), and IR camera sensors were used to capture images of shoot image parameters in different pots. Image parameters significantly differentiate plant responses to heat stress on top and side views. Parameters such as digital area and boundary point count (BPC) could differentiate the responses of chickpea genotypes more efficiently under heat stress. Genotype JG 16 showed the highest digital area pixel and boundary point count under heat stress conditions.

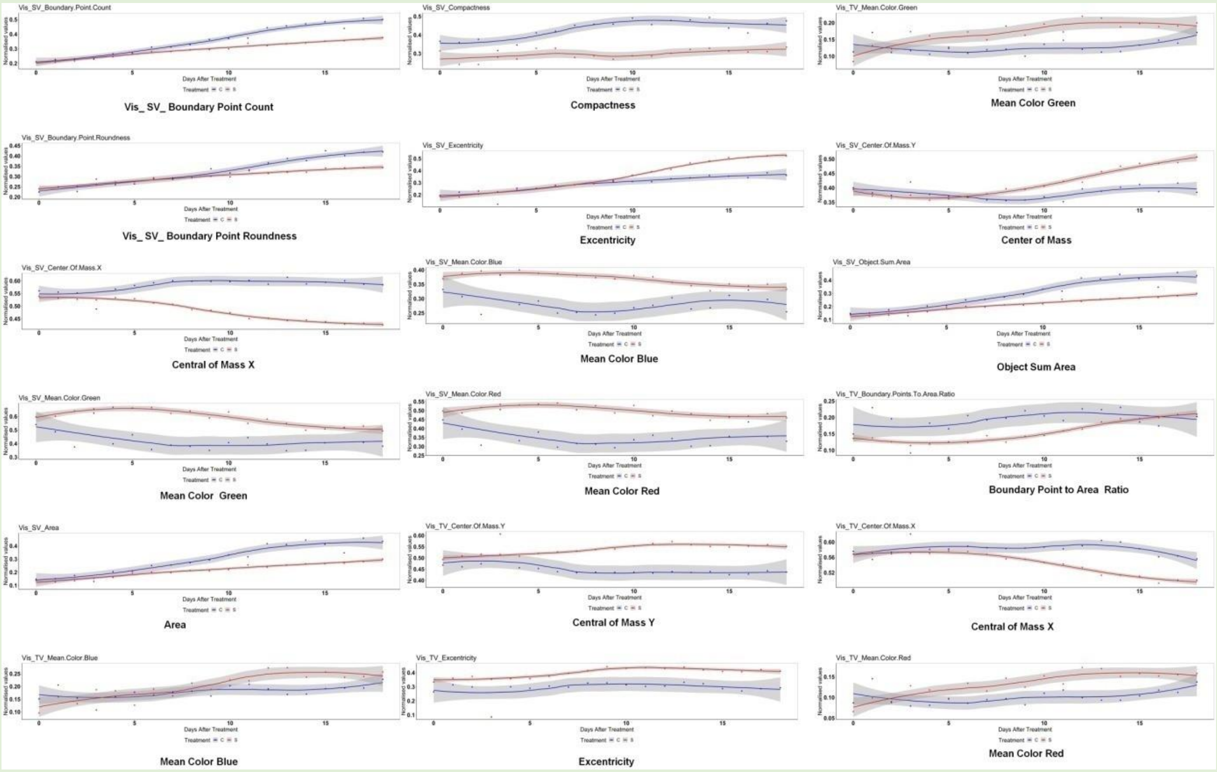


Fig. 24: Image-derived phenotypic parameters differentiating plant responses to heat stress on top and side view

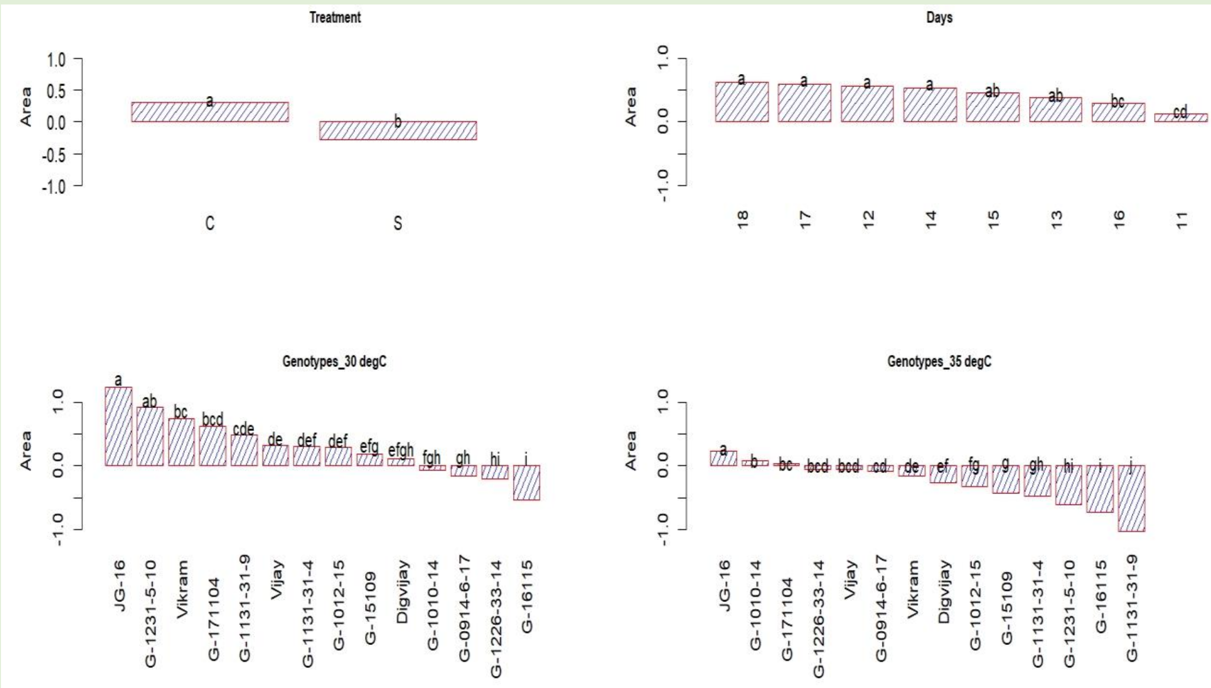


Fig. 26: Treatment, day-wise, and genotypic differences in digital area in chickpea genotype under control and heat stress.

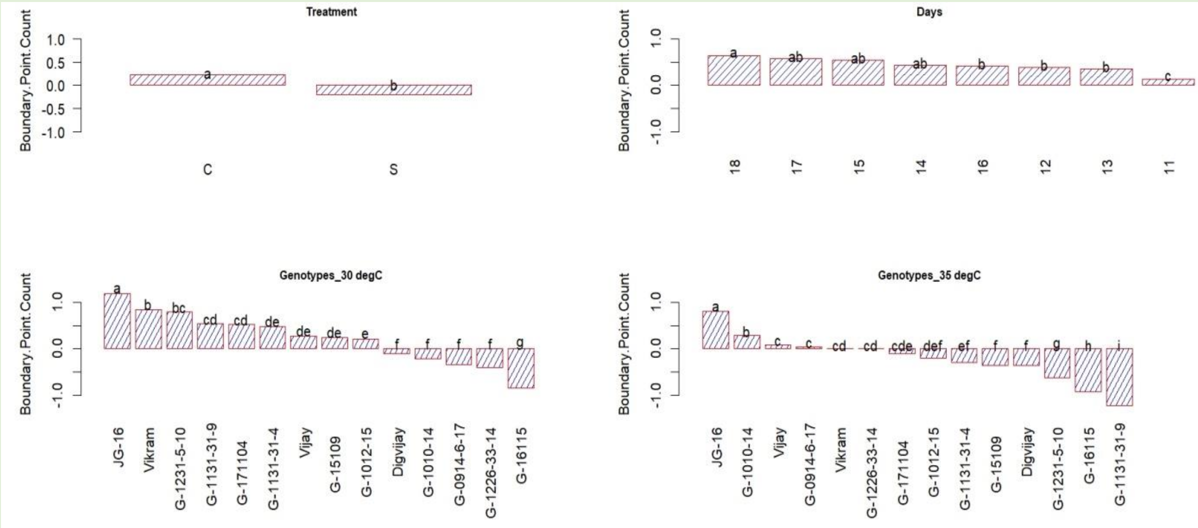


Fig. 27: Treatment, day wise, and genotypic differences in boundary point count in chickpea genotype under control and heat stress.

Salient findings: Various Parameters such as digital area, boundary point count, compactness, excentricity, and various colour-based parameters could differentiate the responses of pigeonpea genotypes more efficiently. Genotype JG 16 showed higher area pixel and boundary point count under 35 °C, which can be tolerance to heat stress.

v. Leaf senescence pattern based phenomics approach for differentiating field performance of chickpea genotypes

An experiment was conducted with 22 genotypes of chickpea with sufficient and depleting soil moisture conditions in the Plant Phenomics facility. The responses of plant shoots were captured regularly with high-resolution cameras during soil moisture depletion. Leaf colour and RGB pixel values derived from images of plants were used to classify genotypes for interpretation of their senescence pattern by K-mean clustering under drought stress. Variations in the senescence pattern of the different groups were classified as G1 (Late), G2 (Early) and G3 (Moderate) senescing groups under water stress conditions. Genotypes were grouped by two different methods based on analysed image. green and yellow leaf colour (Method-I) and R, G and B Pixels of Images (Method-II), both methods could able to differentiate the senescence pattern of chickpea genotypes under stress conditions. The leaf senescence pattern was able to differentiate the actual field performances of chickpea genotypes in terms of grain yield under soil moisture stress conditions. The phenotyping protocol for senescence patterns may be useful in predicting the field performance of breeding lines. This study can help in screening a large number of genotypes non-invasively to identify potential genotypes of chickpea that can perform better under drought conditions rapidly. Selected ones can be further validated before using them as donors of drought tolerance in chickpea cultivars in breeding programs for drought-prone areas.

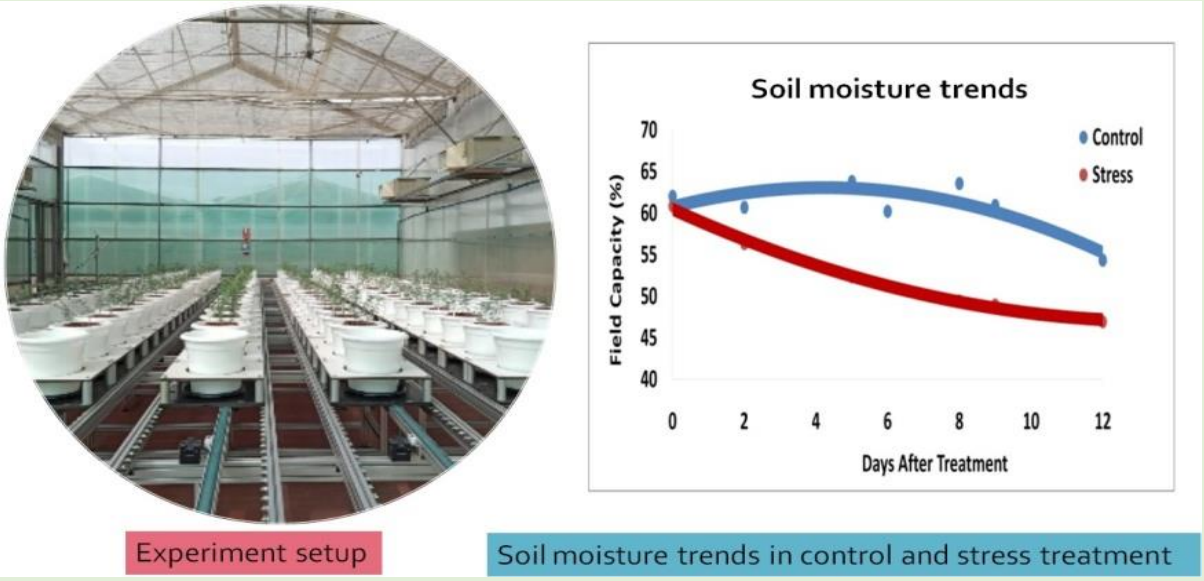


Fig. 28: Experimental setup in Plant Phenomics Facility and Soil moisture trends in control and stress treatment

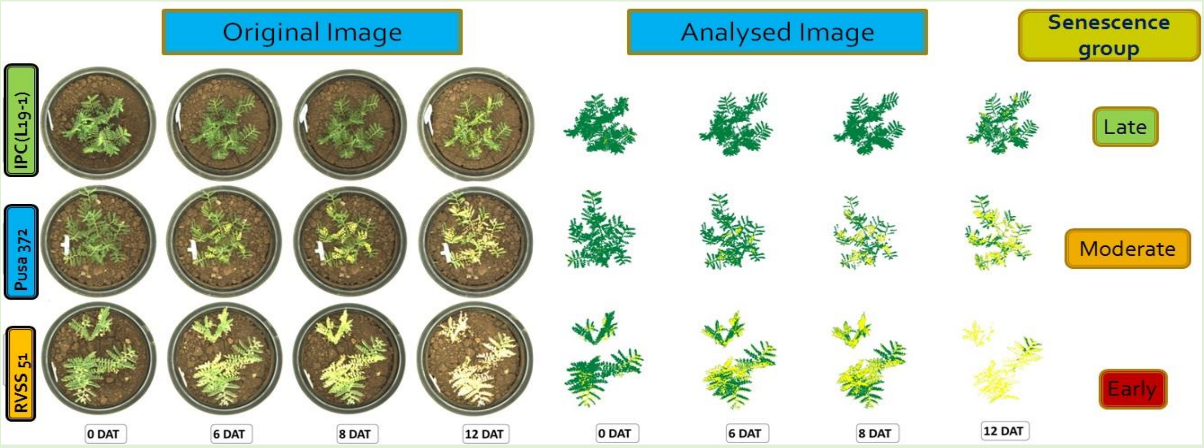


Fig. 29: Variation in Senescence pattern of different group G1(Late), G2 (Moderate) and G3(Early) senescing Group under water stress condition (Exp 01)

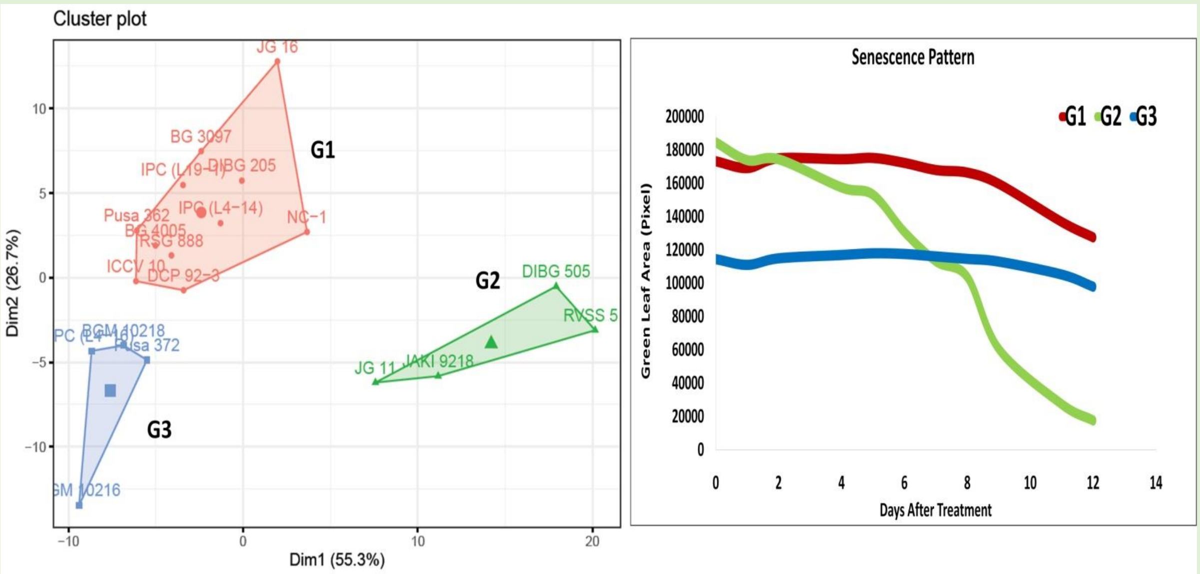


Figure 30 a. Method-I: Green & Yellow Leaf Area Based Cluster and Leaf Senescence pattern

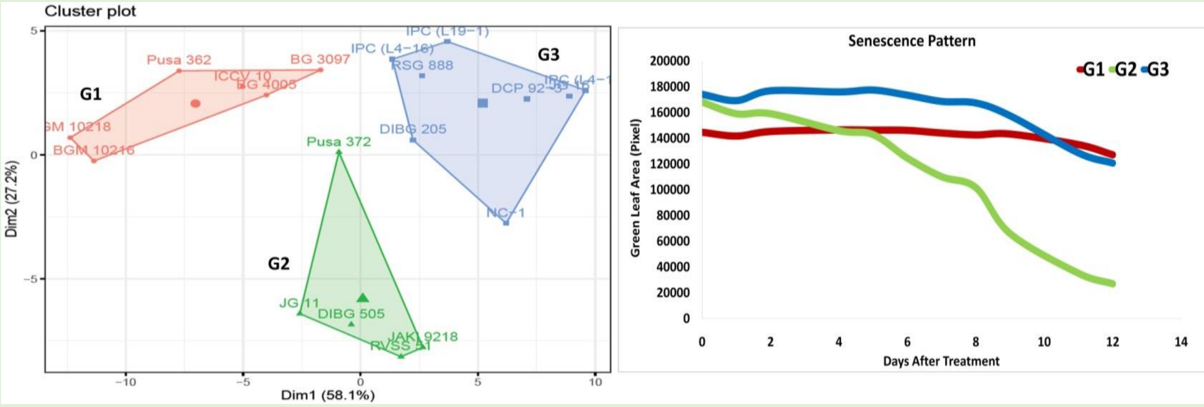


Fig. 30b. Method-II: RGB pixel top-based cluster and leaf senescence pattern

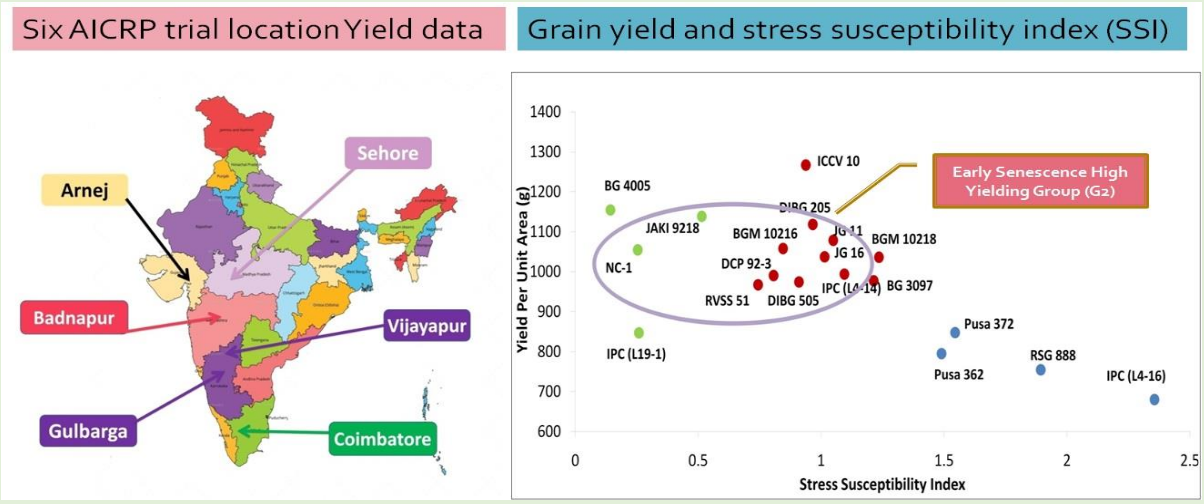


Fig. 31: Six AICRP trial locations yield data and establish a relationship between senescence patterns and yield potential of genotypes

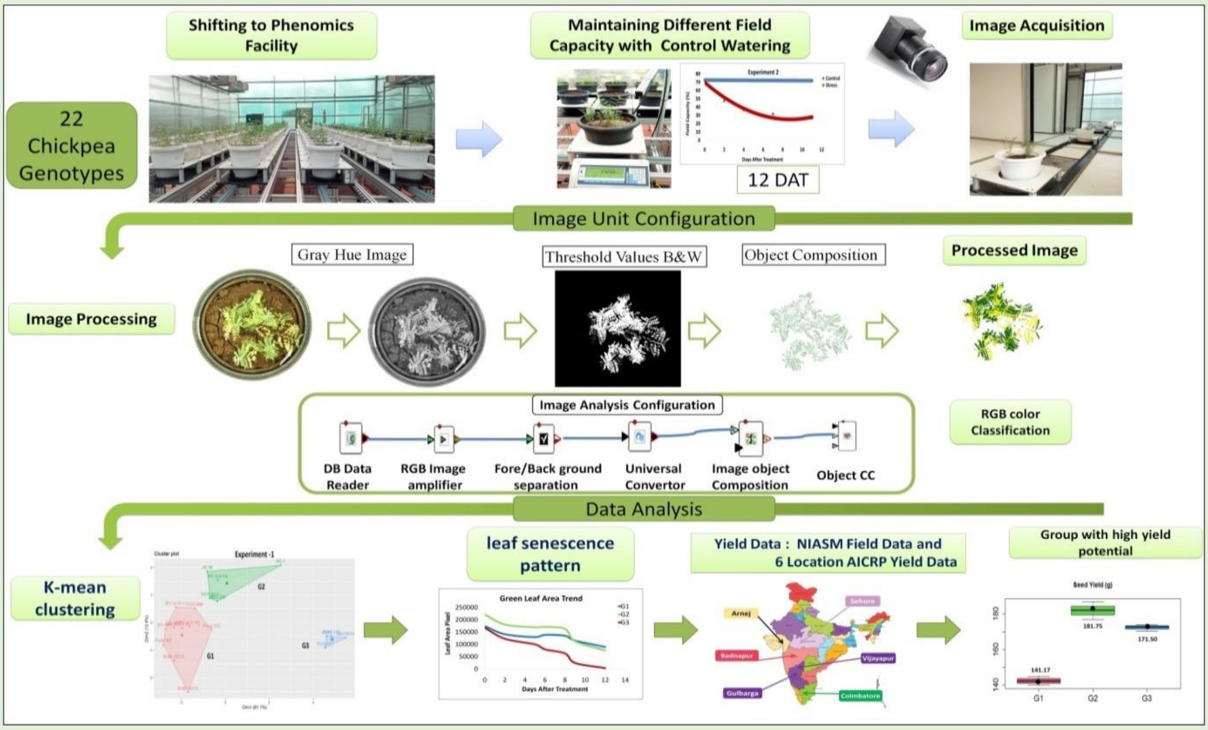


Fig. 32: Suggested phenotyping protocol for establishing the relationship between senescence patterns and yield potential of genotypes at different environmental conditions.

vi. Optimization of affordable phenotyping protocol for drought tolerance in different legumes

An experiment was conducted to assess plant responses to depleting soil moisture stress in 4 different legume species with 3 different genotypes in each by using an image-based phenotyping method. Plants were initially grown under natural conditions in pots and then were shifted to a plant phenomics facility 14 days of sowing (DAS) for phenotyping. Water stress treatment was imposed at 15 DAS by withholding water whereas optimum moisture was maintained in the control. Soil moisture trends and digital biomass during the experiment differentiated control and drought stress treatment. High-resolution visible (400-700 nm) and NIR (700-1700 nm) sensors were used to capture images of shoot image parameters in different pots. It was demonstrated that it is possible to use image parameters to differentiate drought responses of legume crops.

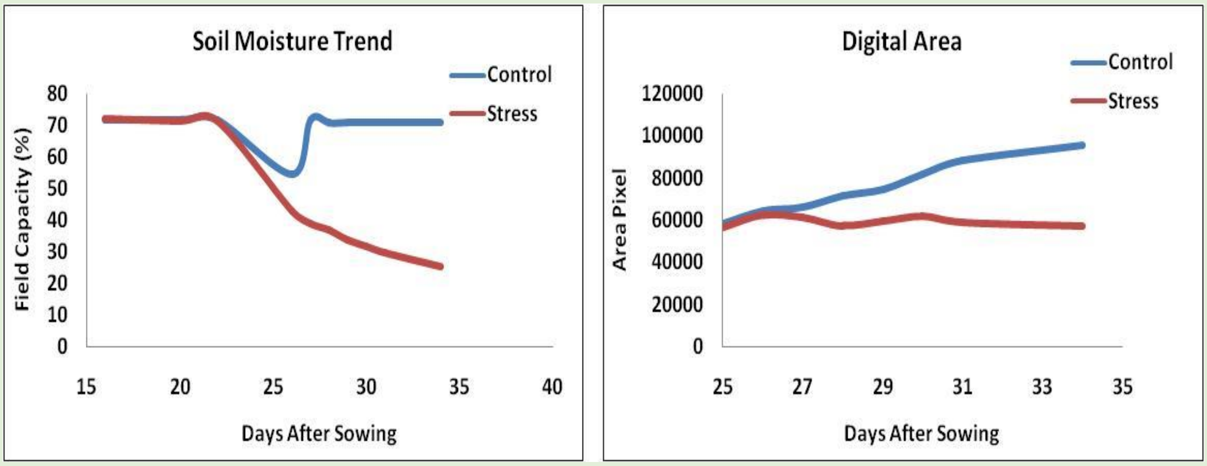


Fig. 33: Difference in Soil moisture trends and digital biomass during the experiment in control and drought stress treatment

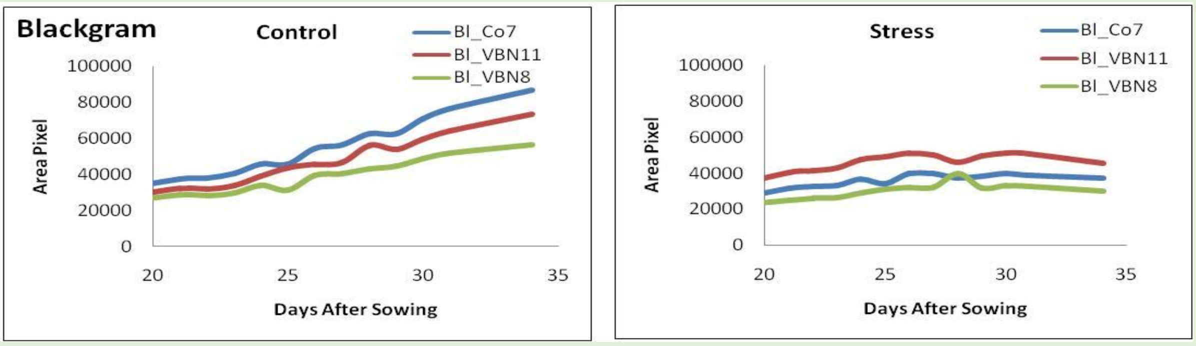


Fig. 34: Digital area (Pixels)differentiating plant responses to water stress and control condition in Blackgram.

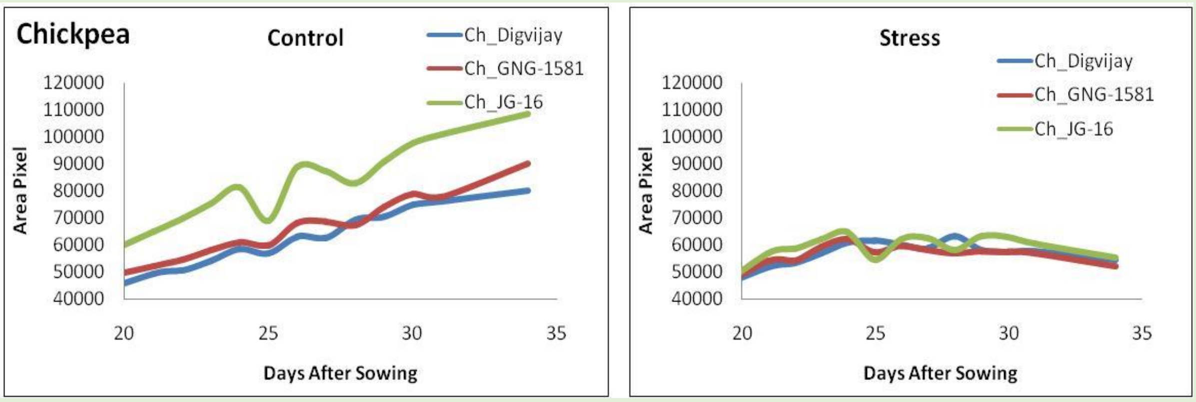


Fig. 35: Digital area (Pixels)differentiating plant responses to water stress and control condition in Chickpea.

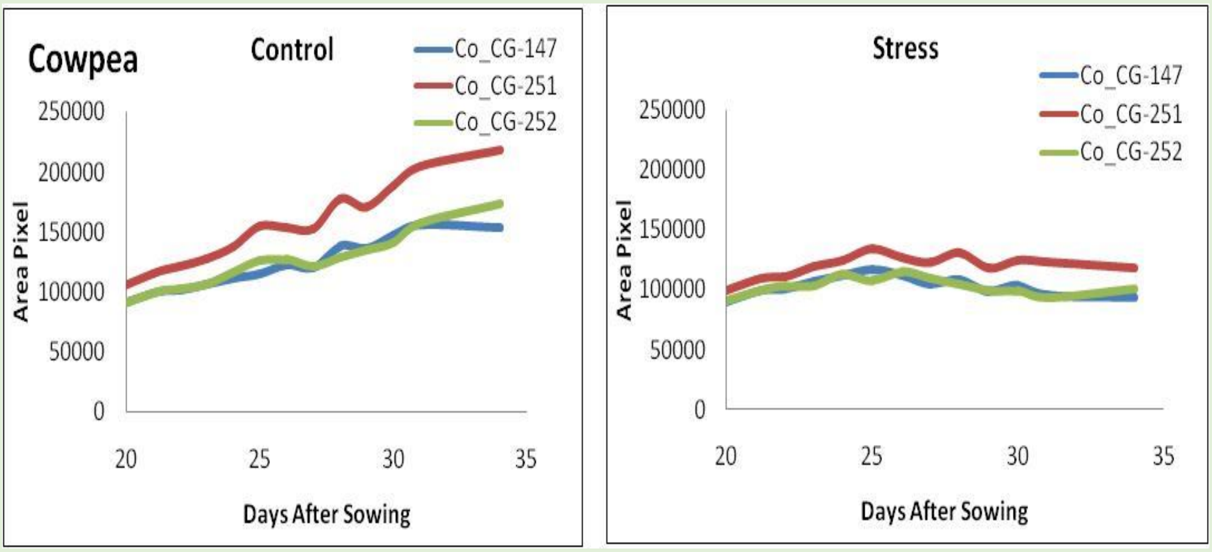


Fig. 36: Digital area (Pixels)differentiating plant responses to water stress and control conditions in Cowpea

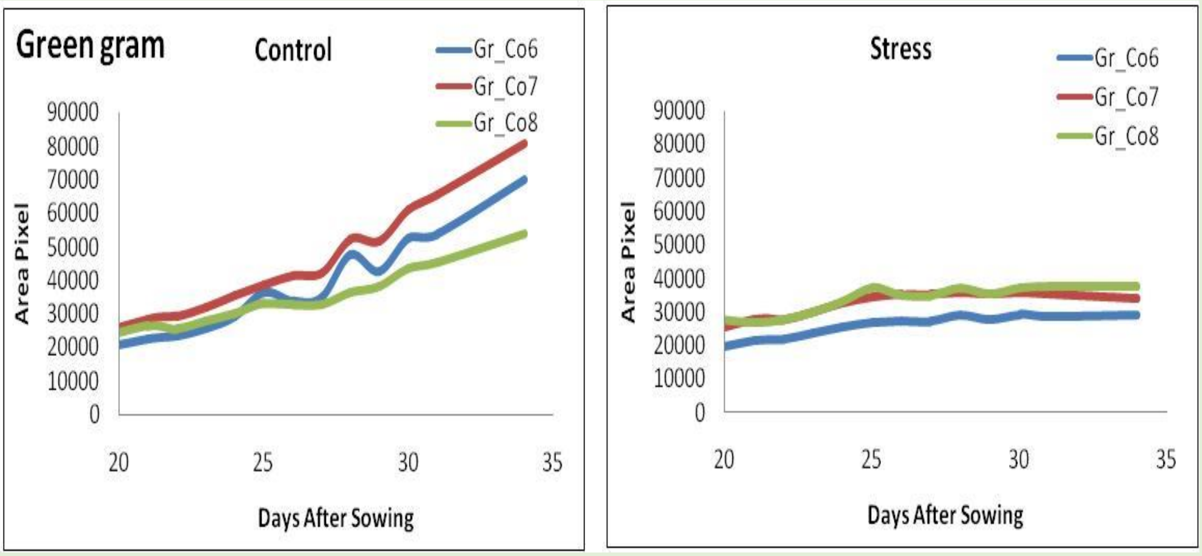


Fig. 37: Digital area (Pixels)differentiating plant responses to water stress and control conditions in mungbean

vii. Assessment of the efficacy of affordable image-based tools to differentiate drought responses of pulse crops at the seedling stage

An experiment was conducted to develop phenotyping protocols to assess drought responses of different pulse crops at the seedling stage using easily affordable sensors such as mobile phones. Image acquisition protocols were optimized to ensure the high-quality repeatable images of the plants grown in pots from top and side views simultaneously with mobile phones fixed at the top and side of the object (Plant) at a convenient distance to cover the whole plant throughout growth and development. For image analysis, open-source software ImageJ was configured for deriving image parameters including digital area. The images were captured by using a phenomics facility and simultaneously by using the mobile phone-based camera outside the imaging chamber. The Phenomics images were analyzed using Lemnagrid software and the image-derived parameters were extracted for further analysis of the response of pulse crop to the moisture deficit during the vegetative stage. Further, digital biomass calculated by both phenomics data and ImageJ data were validated for concluding that mobile camera-based phenomics protocol can capture the response of pulse crop at par with phenomics. This experiment validates the possibility of using mobile phones as an affordable phenomics tool for assessing the digital biomass of legume crops.

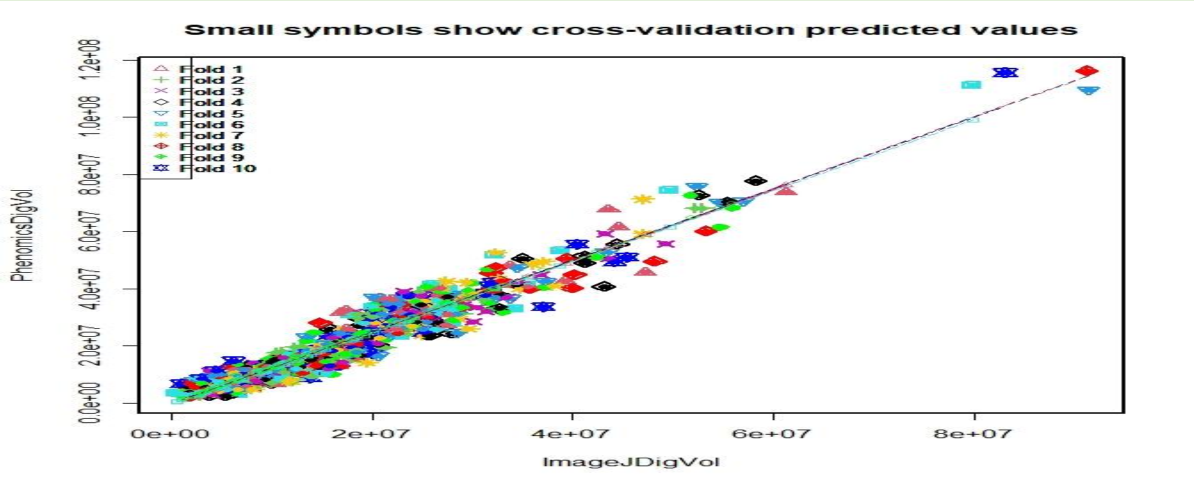
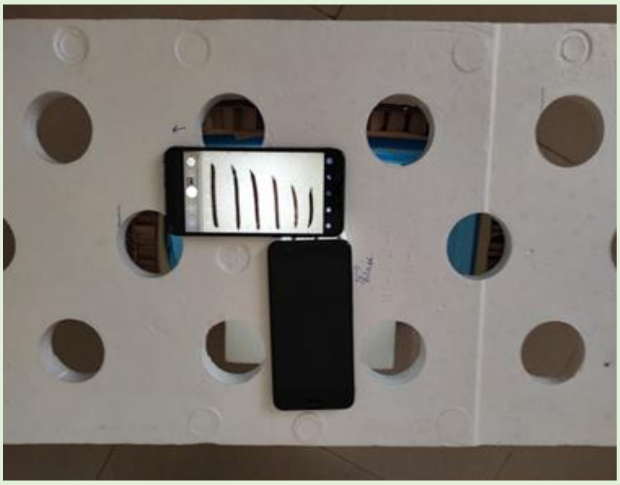


Fig. 38: Validation of Digital biomass analyzed from LemnaTech phenomics and Mobile phone-based Affordable Phenotyping tools

viii. Optimization of protocol to predict seed yield of mungbean by employing pod images

An attempt was made to develop and test high throughput phenotyping of pods and seeds of mungbean by employing an imaging system that can be affordable and reliable. The imaging system was designed with cost-effective materials like Polystyrene (Thermocol), a background luminescent light source, and a smartphone with a high-resolution camera. This system could enable the acquisition of images of more than 300 pods and about 1500 seeds without any background noise. Images were then analyzed by using ImageJ open-source software. There was a significant association between the manually measured pod length and the digital pod length as revealed by R^2 value of 0.98. Correlation analysis revealed that digital features of pods extracted from images could explain the number of seeds per pod and also the manually measured seed weight per pod. Digital seed parameters were strongly correlated with the number of seeds per pod (R^2> 0.93); and seed weight per pod (R^2> 0.9); along with this, digital pod parameters i.e. area, perimeter, and length were closely related with digital seed parameters (R^2> 0.8), seeds per pod (R^2> 0.7) and seed weight per pod (R^2> 0.82) Results from this experiment could establish strong relation between pod geometry and seed yield. This experiment proves the potential utility of a highly economic and non-invasive protocol developed for accurate, reliable, and rapid phenotyping of at least 2000 pods of Greengram within 4-5 hours.



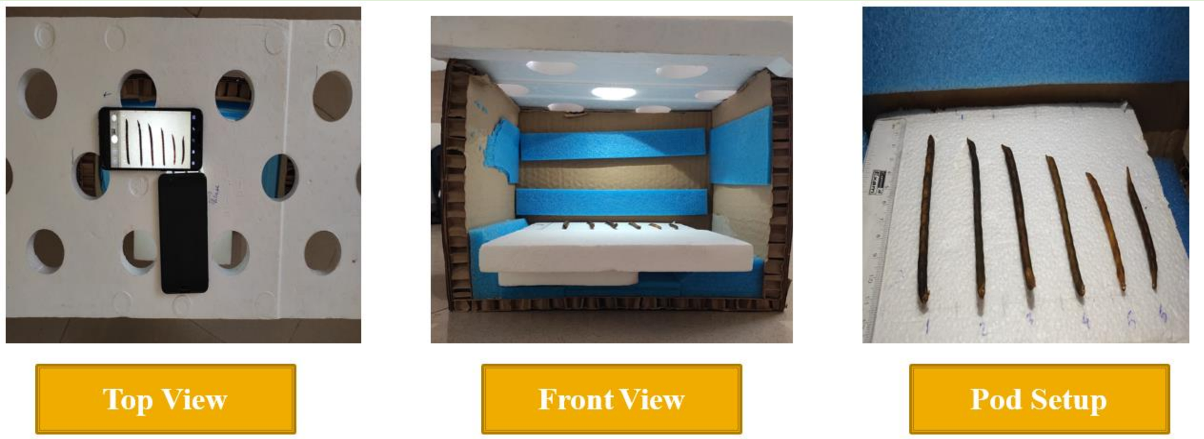


Fig. 39: Setup for the acquisition of pod images

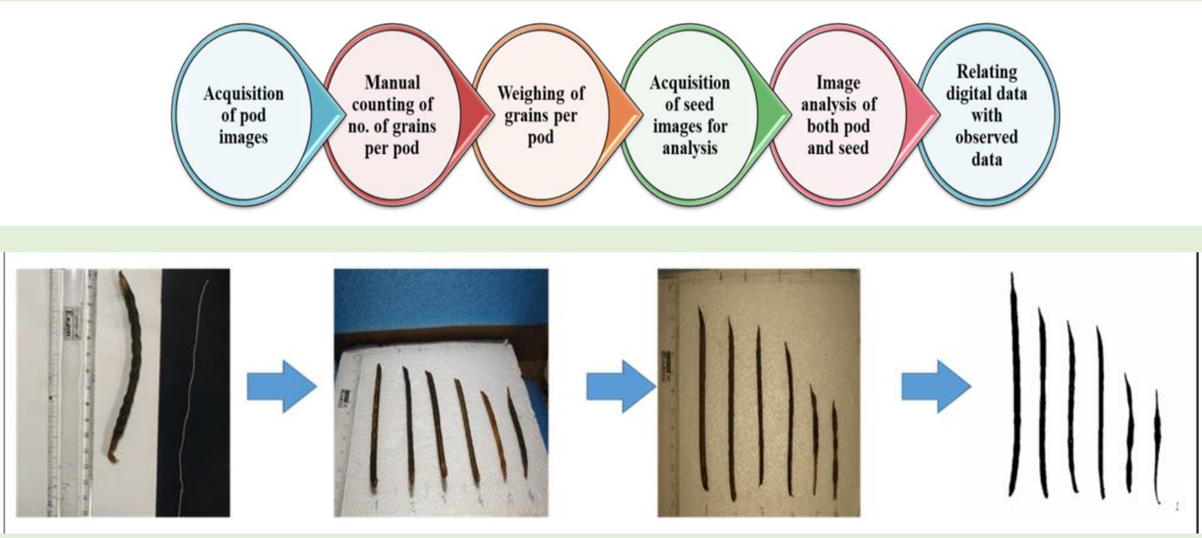


Fig. 40: Optimising protocol for pod length by image analysis

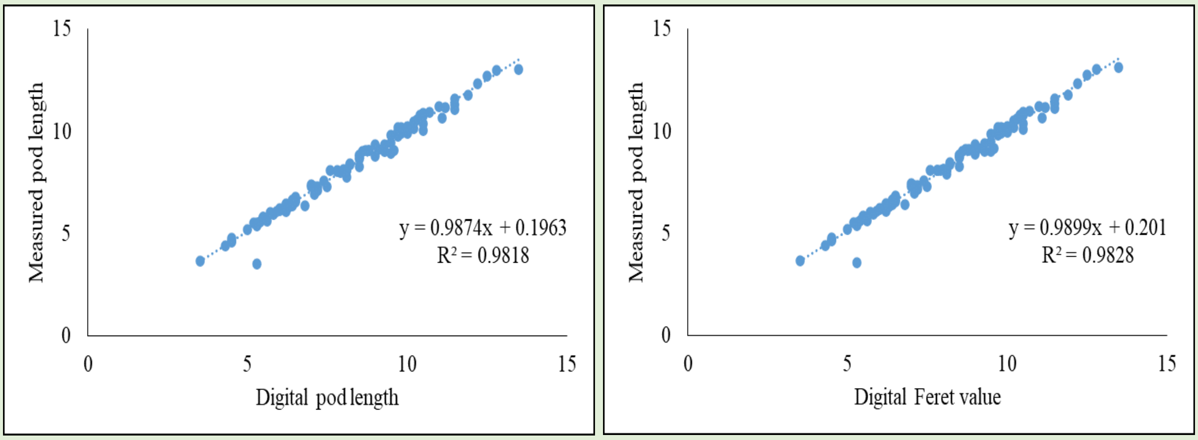


Fig. 41: Correlation between manually measured pod length and digital pod length

ix. Optimization of in vitro phenotyping of root traits to differentiate PEG-induced stress responses of chickpea seedlings

An experiment was conducted with 22 chickpea genotypes with local check Digvjay in a test tube In *vitro* culture using MS media. Osmotic stress was induced by 5% PEG 6000 (Polyethylene glycol 6000) treatments. Morpho-physiological parameters and root observations were taken by imaging with the high-resolution mobile camera up to 18 Days after treatments. Chickpea genotype JG 16, the stress-tolerant genotype, had a better root system than the local check Digvijay and this difference could be detected by the protocol optimized for *in vitro* phenotyping.

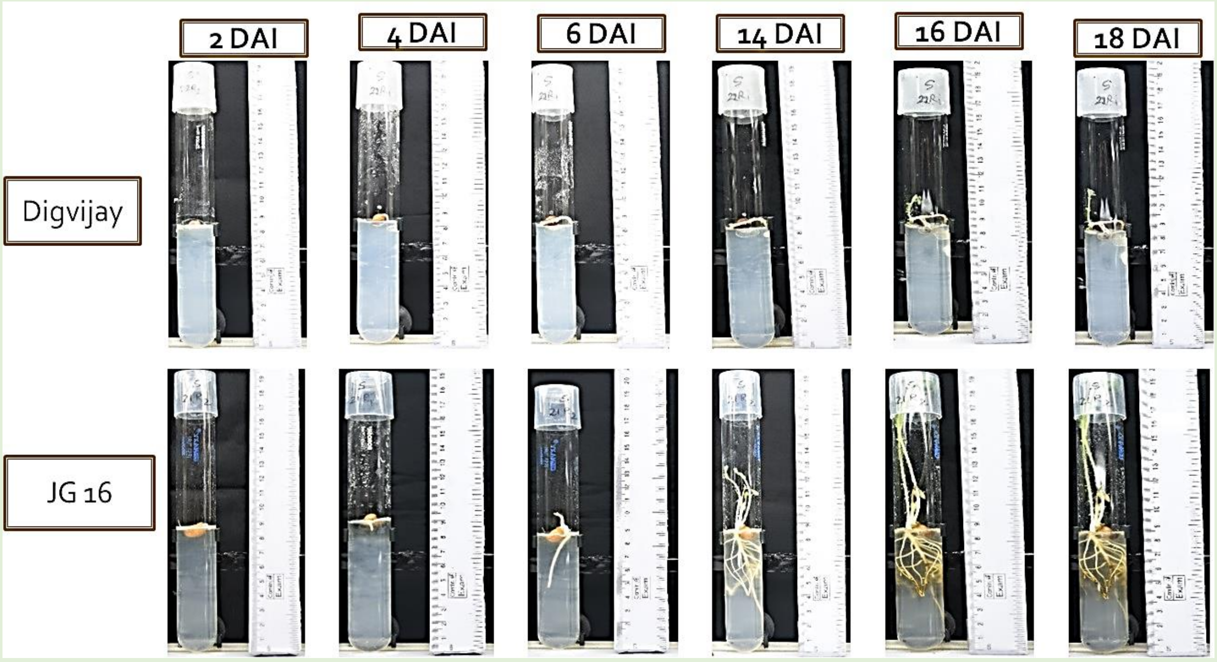


Fig. 42: Genotypic Comparison between different genotypes.

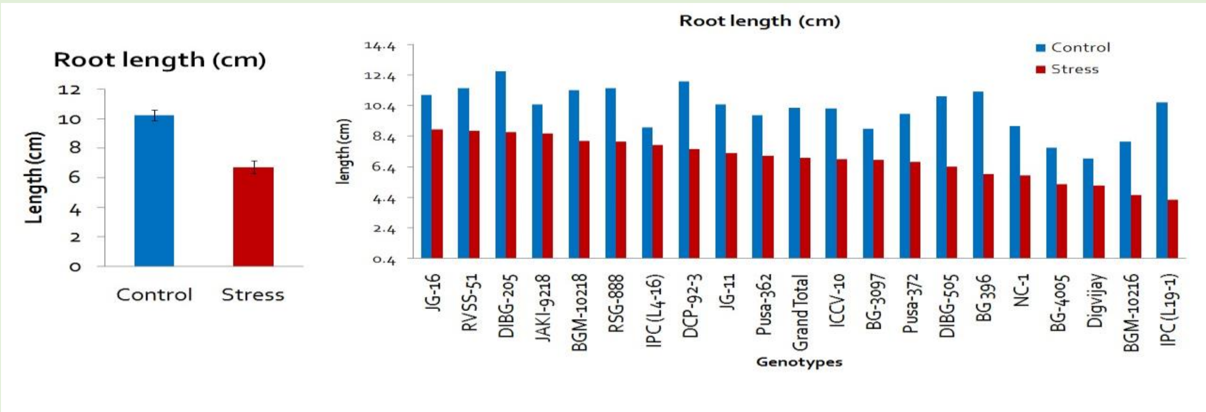


Fig. 43: Root length of different chickpea genotypes.

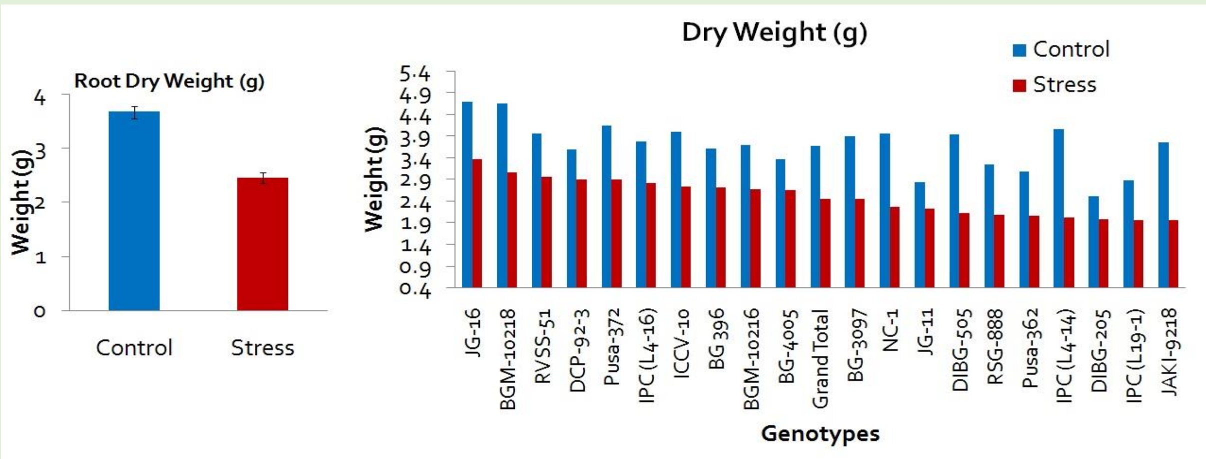


Fig. 44: Root dry weight of different chickpea genotypes.

PROMISING SURROGATE TRAITS

I. Cooler canopy leverages sorghum adaptation to drought and heat stress

In this study, individual and combined effects of drought and heat stress were investigated on key physiological parameters (canopy temperature, membrane stability index, chlorophyll content, relative water content, and chlorophyll fluorescence) in two popular sorghum cultivars (Phule Revati and Phule Vasudha) during the seedling stage. Estimating canopy temperature through pixel-wise analysis of thermal images of plants differentiated the stress responses of sorghum cultivars more effectively than the conventional way of recording canopy temperature. Cultivar difference in maintaining the canopy temperature was also responsible for much of the variation found in critical plant physiological parameters such as cell membrane stability, chlorophyll content, and chlorophyll fluorescence in plants exposed to stress. Hence, the combined stress of drought and heat was more adverse than their impacts. The continued loss of water coupled with high-temperature exposure exacerbated the adverse effect of stresses with a remarkable increase in canopy temperature. However, Phule Vasudha, being a drought-tolerant variety, was relatively less affected by the imposed stress conditions than Phule Revati. Besides, the methodology of measuring and reporting plant canopy temperature, which emerged from this study, can effectively differentiate the sorghum genotypes under the combined stress of drought and heat. It can help select promising genotypes among the breeding lines and integrating the concept in the protocol for precision water management in crops like sorghum.

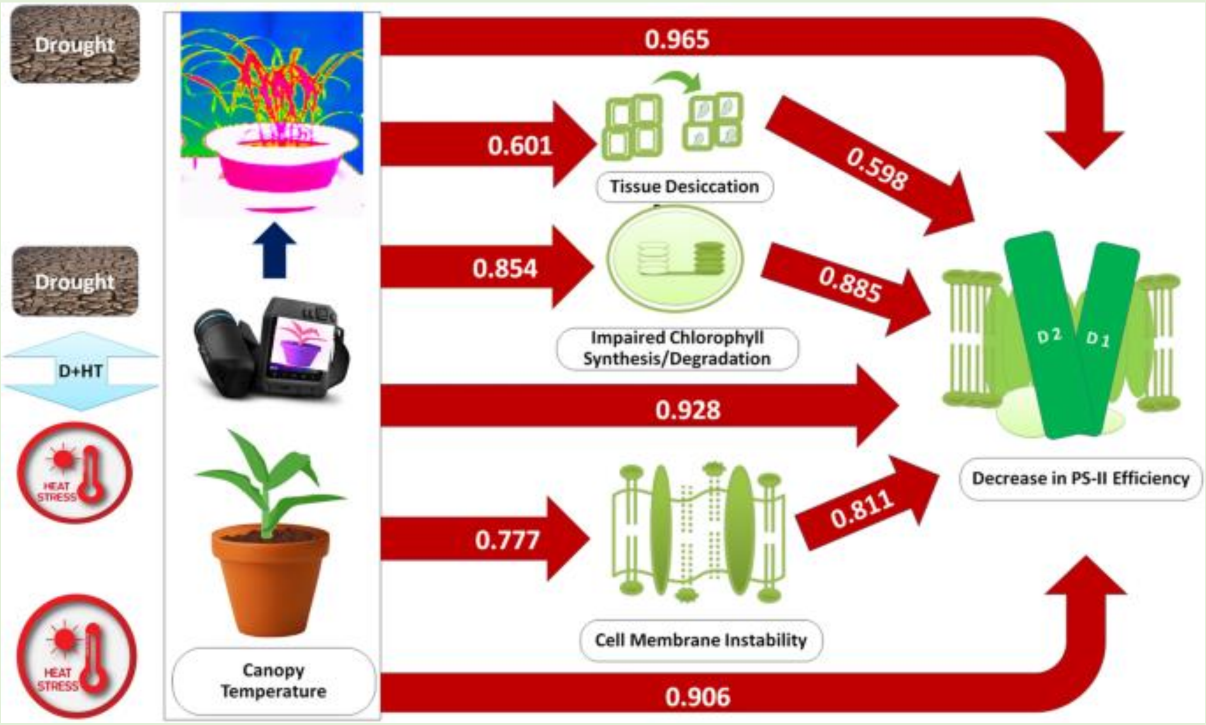


Fig. 46: Schematic diagram of the impact of individual and combined effects of drought and heat stress on canopy temperature and other critical physiological parameters in sorghum. Values represent the Pearson correlation coefficient between the respective pair of traits.

II. Physiological traits reveal the potential for identification of drought-tolerant mungbean [Vigna radiata (L.) Wilczek] genotypes under moderate soil-moisture deficit

Canopy temperature is an important physiological trait used for screening drought tolerance in several crop plants. Mungbean being often exposed to post-flowering drought, we evaluated a set of 48 genotypes for variability in post-flowering canopy temperature and its association with root traits and other physiological parameters contributing to drought tolerance under soil-moisture deficit stress conditions. Overall, canopy temperature depression revealed a significant association with seed yield. Root traits like a number of lateral branches and dry root weight exhibited a significant negative correlation with canopy temperature. Leaf SPAD readings were positively associated with grain yield and most of the high SPAD genotypes maintained hot canopies under drought. Some genotypes with contrasting variations in SPAD levels (DMG-1050 and SML- 1628) maintained their photosystem PSII health at par. Moreover, cool canopy was no guarantee for better PSII health or vice versa. This study identified some cool canopy genotypes (VC-6173-C, IC-325770 and ML-2082) and a genotype (DMG-1050) with novel trait combinations like high SPAD and better PSII health despite high canopy temperature which can be used as donors in mungbean breeding programs. Present study explores genetic variation in these adaptation traits contributing to plant performance under soil-moisture deficit stress conditions and potential of physiological breeding approaches for genetic enhancement of this legume crop.

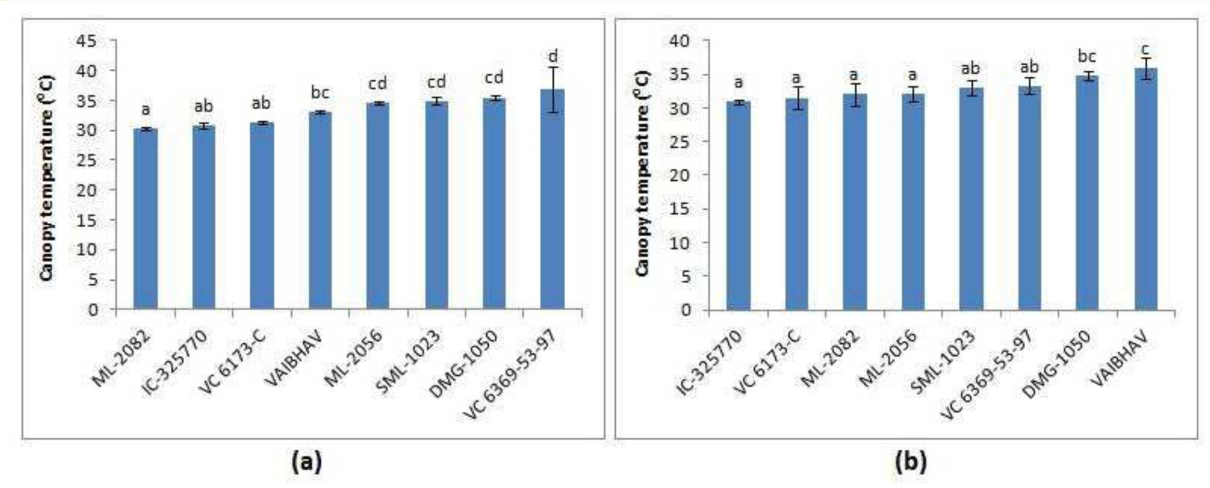


Fig. 47: CT comparison of selected mungbean genotypes. (a) Comparison of CT of cool canopy genotypes with high SPAD genotypes during crop season of 2014 and (b) 2015. Error bars indicate standard deviation with n= 15. Means with same letters are not significantly different at $p < 0.05$

III. Identification of promising image-based traits

Attempts were made to analyse about 36000 data set for different image parameters recorded to assess response of mungbean genotypes to soil moisture stress. This experiment was conducted during previous year. The objective was to identify promising parameters by employing machine learning algorithms. A total of 19 image parameters were assessed for their effectiveness in explaining the variation in area of the images at different growth stages.

Salient finding: convex hull area, absolute z rotation second moment, minimum rectangle area was found to be important in mungbean.

IV. Ascorbic acid rich chickpea cultivars as a source of drought tolerance for genetic improvement of chickpea

An experiment was conducted to optimize phenotyping protocol to assess genetic variation in endogenous ascorbic acid with 106 genotypes of chickpea. Dynamics of ascorbic acid accumulation in the fresh leaves of popular cultivars of chickpea were measured to determine the appropriate time and level of soil moisture to collect sample for ascorbic acid during soil moisture depletion. Experiments revealed that ascorbic acid accumulation reached its peak when the soil moisture reached 30 % field capacity. Then this protocol was employed to screen

106 diverse chickpea genotypes for genetic variation in ascorbic acid accumulation in response to depleting soil moisture. High resolution visible (400-700 nm) and NIR (700-1700 nm) sensors were used to capture shoot image parameters. Two distinct sets of genotypes differing in levels of ascorbic acid were selected based on the experiments conducted for evaluating their yield performance in field. The field trial revealed that high ascorbic acid accumulating genotypes *viz*., BDNG-2018-15, PG-1201-20, C-19315, C-19186, BDNG-2017, PG-1012-15, C-19190, C-19291 exhibited more drought tolerance with minimum reduction in yield attributes when compared with popular cultivars. These high endogenous ascorbic acid accumulating chickpea genotypes can serve as potential genetic resource for breeding program that aim at improved chickpea cultivars for drought prone areas.

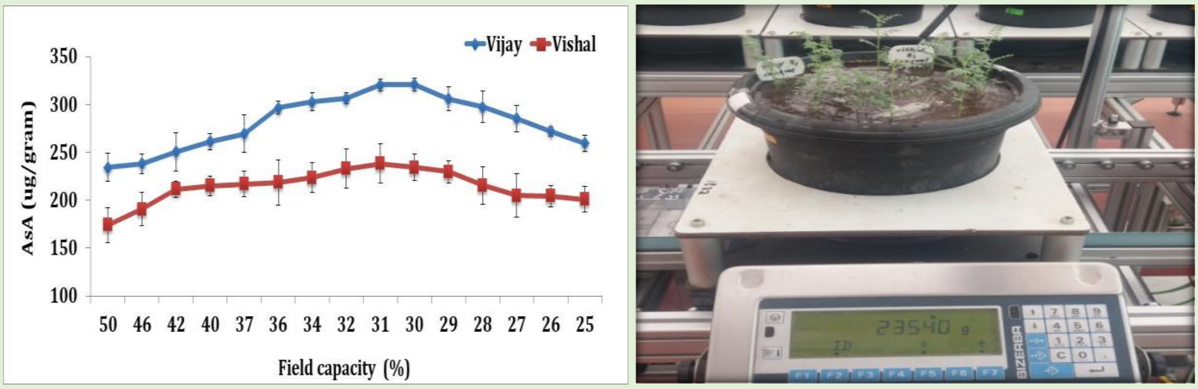


Fig. 49: Determined appropriate soil moisture level for determination of Ascorbic acid in different chickpea genotypes.

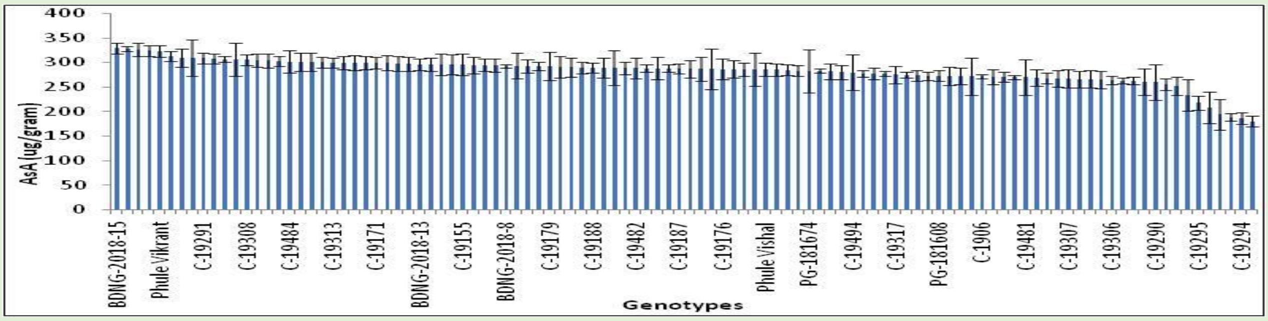


Fig. 50: Genetic variation in 106 chickpea genotypes (including genotypes tested in coordinated trials) at 30% FC

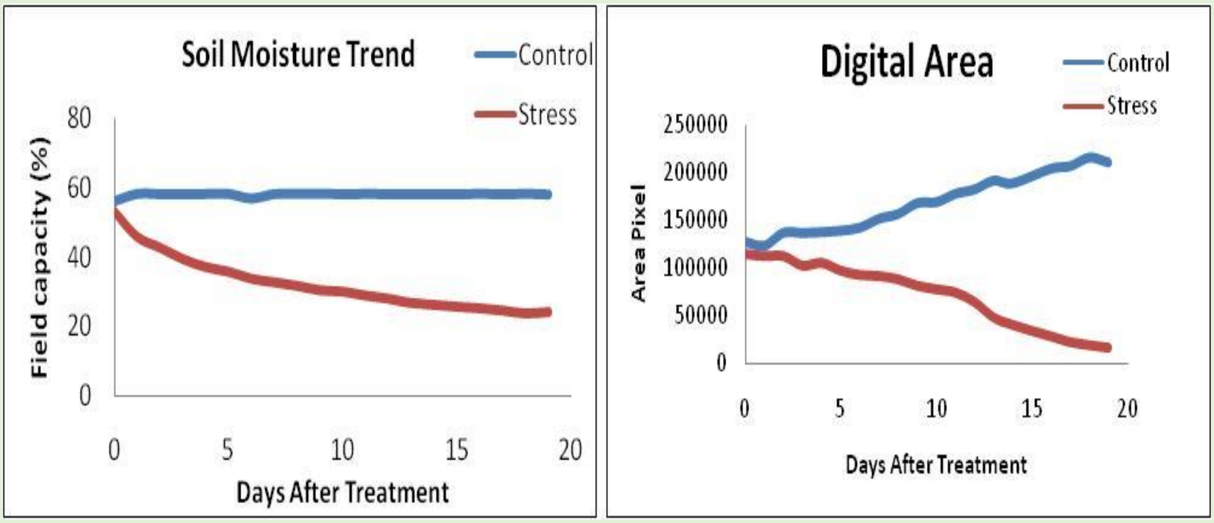


Fig. 51: Difference in Soil moisture trends and digital biomass during the experiment in control and drought stress treatment

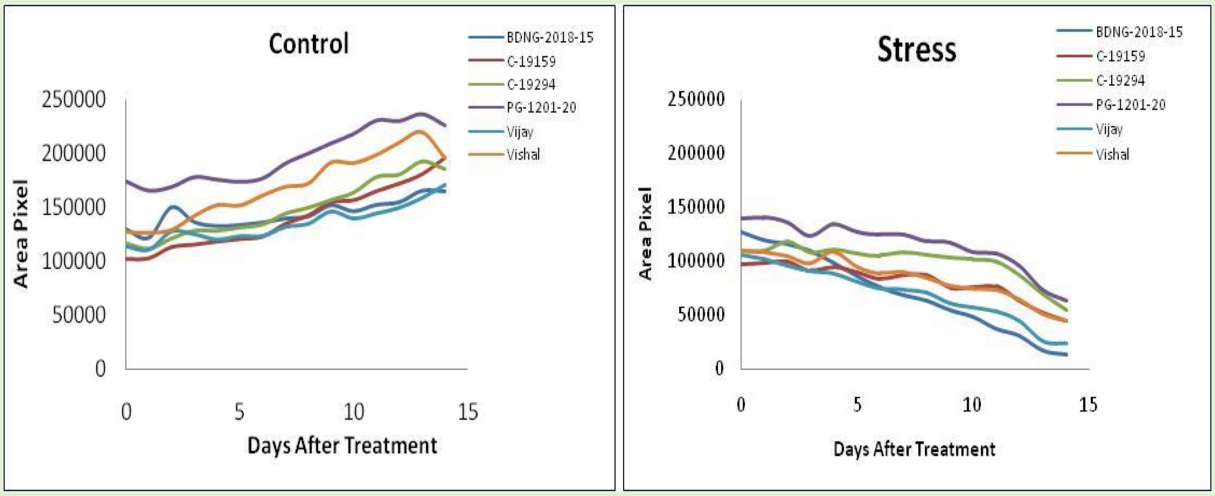


Fig. 52: Genetic variation in drought stress responses of chickpea genotypes as revealed by digital area.

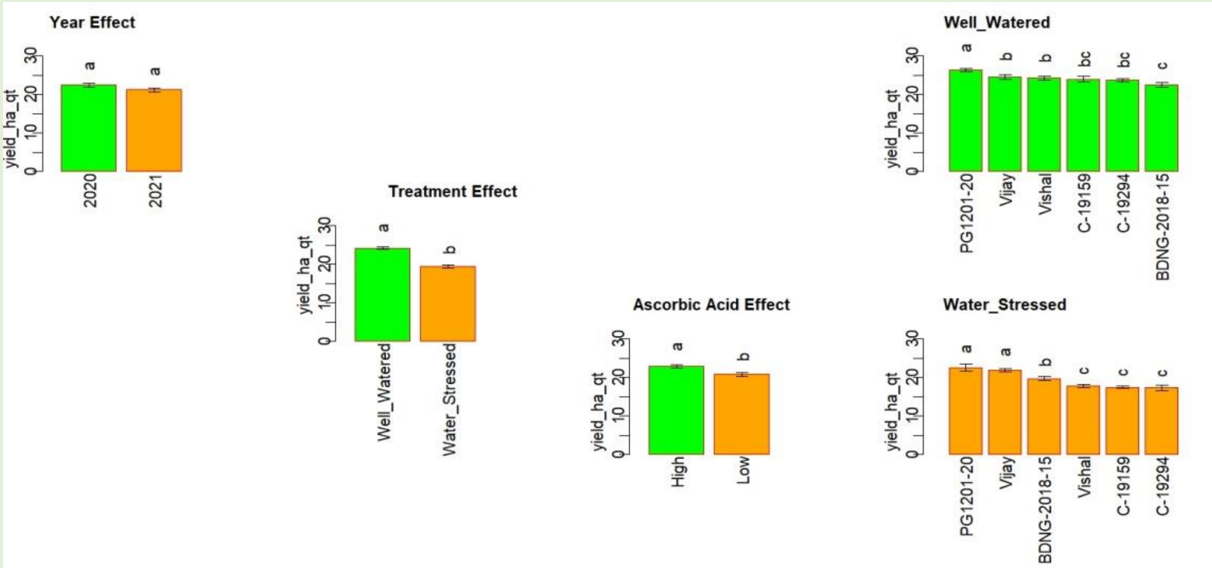


Fig. 53: Seed yield (q/ha) in AA rich and AA poor chickpea Genotypes. Under well-watered and water stressed conditions.

V. Evaluation of pod pedicel as a component trait to facilitate photosynthate supply to developing grains during soil moisture depletion in chickpea

An experiment was conducted with 35 chickpea genotypes to study the genetic variation in contribution of non-leaf green parts to grain yield under soil moisture stress and to assess the association of pod-pedicel features with grain size. In general, leaves are the principal sites of photo-synthate production. However, non-leaf green organs (NLGO) can be photo-synthetically active to some extent, and their contribution to developing grains cannot be ignored, particularly during stress imposed due to less soil moisture. Investigating and increasing the photosynthetic ability of NLGOs, especially under stress conditions, is a novel way to increase the photosynthetic ability of the whole plant and finally increase the grain yield. In this experiment, we investigated the role of pedicel that connects pods and the branch of plant Phenomics approach was adopted to extract features of pedicel and then to correlate with seeds of different genotypes. The experiment revealed genetic variation in pedicel parameters under both water stressed and well-watered conditions. Perimeter and seed weight were found to be correlated giving an indication that the pedicel can play a crucial role in determining yield of chickpea.

VI. Deciphering endurance capacity of mango tree (Mangifera indica L.) to desiccation stress based on efficiency of PSII

Capacity of mango tree to withstand drought (absence of soil moisture) can be attributed to stress resilient physiological processes inside the cell and also at whole plant level. To test this hypothesis, photosynthetic traits were recorded over the period of time. Further, desiccation tolerance of photosystem II (PSII) in excised mango leaves were measured by employing chlorophyll fluorescence imaging system. Beside this, the capacity of mango tree to keep its canopy cool was monitored in every 10 min interval throughout the day during dry and rainy season in the field by employing thermal imaging system. Finally, phenomics platform was used to monitor depletion of tissue moisture level as well as changes in structural attributes during desiccation in excised shoots of the tree. It was inferred that mango tree can maintain its carboxylation efficiency over the period of time. IR studies confirmed that mango tree maintained its canopy coolness during dry season. In addition, the chlorophyll fluorescence experiments revealed that mango leaves retained 50% of initial PSII efficiency for as many as 4 days after desiccation and chlorophyll fluorogram also depicted the observations. Phenomics studies concluded that mango twig retained tissue water content even up to the 164 h of desiccation with gradual decrease in canopy area. Hence, it is interpreted that this physiological resilience are amongst the various reasons for evergreen feature of mango tree which has tendency to survive severe soil moisture deficit particularly during the summer in tropical and subtropical regions, which has been revealed for first time using phenomics platform.

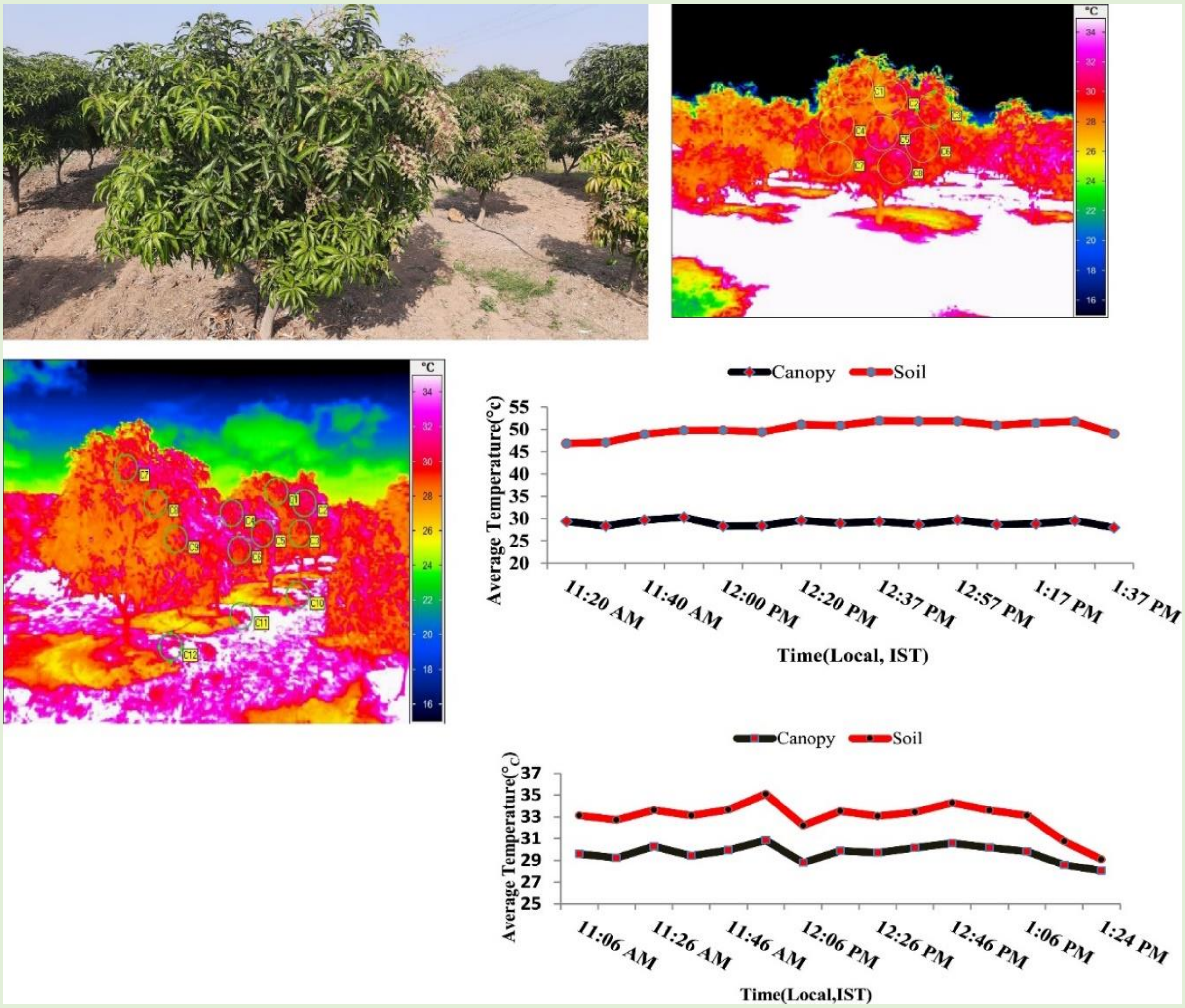


Fig. 54: Visual image (A), thermal image during dry (B) and rainy (C) season, temperature dynamics of canopy foliage during dry (D) and rainy (E) season of *M.indica*.

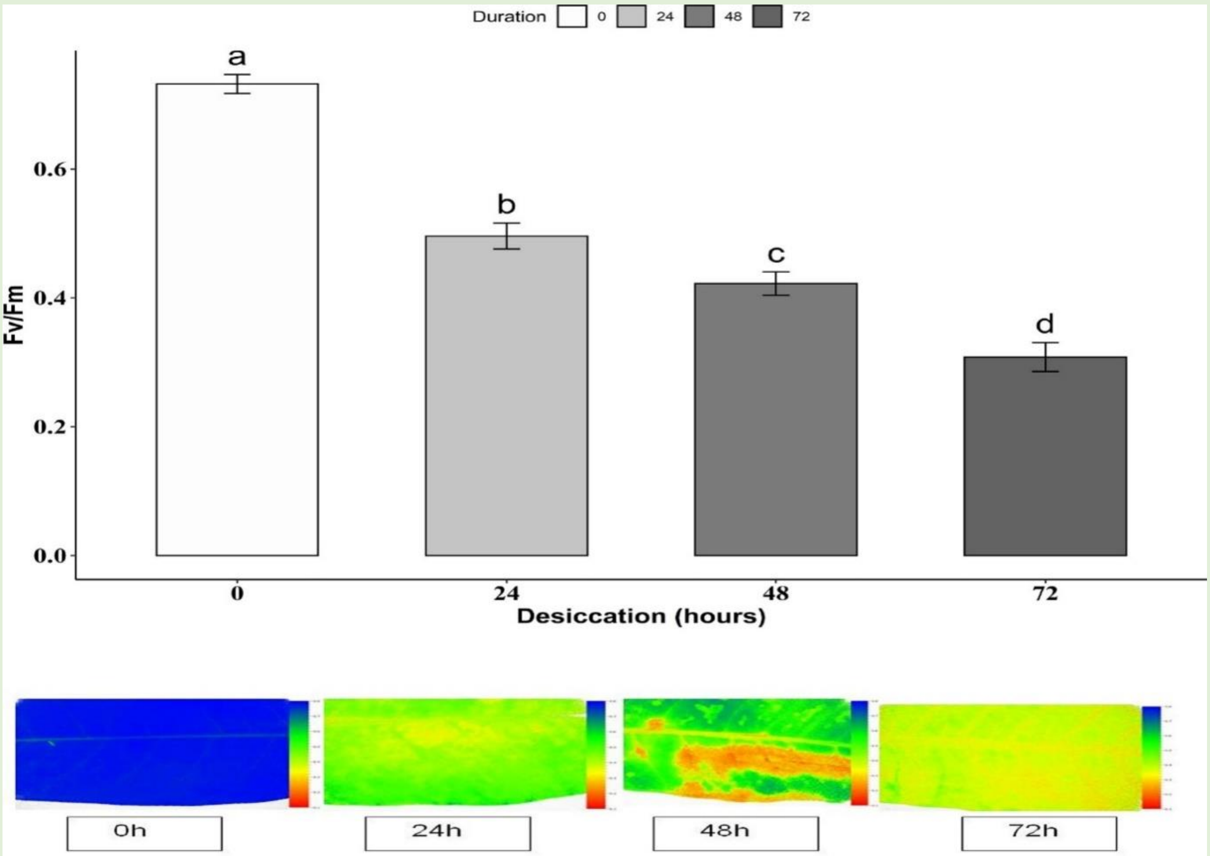


Fig. 55: Change in Fv/Fm (A) and florescence images (B) over desiccation periods in *M.indica*. Different letters denote significant difference between treatments. Vertical bars represent standard errors.

VII. Desiccation tolerance of Photosystem II in dryland fruit crops

Dryland fruit crops are highly prone to stresses caused by depleting soil moisture coupled with high ambient temperatures, particularly during summers. This is more conspicuous and seldom deleterious during droughts, which recur periodically. However, some of the crops sustain and recover their growth after the drought. Since desiccation of leaf is one of the consequences of depleting soil moisture and high temperature, we predicted that those crops that can maintain their photosynthetic efficiency during such stresses could outperform others. We hypothesised that the variation exists in the sensitivity of photo system II (PS-II) component of the photosynthesis system among the dry land fruit crops. A series of experiments were conducted for assessing the desiccation responses of leaves of six fruit crops by employing chlorophyll fluorescence imaging, which reveals PS-II efficiency. As expected, there was a drastic reduction in the maximum quantum efficiency of PS-II (QYmax) of leaves of all the fruit crops with the decrease in tissue water content. However, there were significant differences among the crops in their responses to the desiccation of leaves. The PS-II tolerance to tissue dehydration observed in karonda (*Carissa carandas* L) and sweet orange (*Citrus sinensis*), was higher than that of mango (*Mangifera indica* L) and grape (*Vitis vinifera* L). This study reveals the method to assess the sensitivity of fruit crops to desiccation, which can be useful in water management, and in assessing the efficacy of novel chemicals for alleviating abiotic stresses

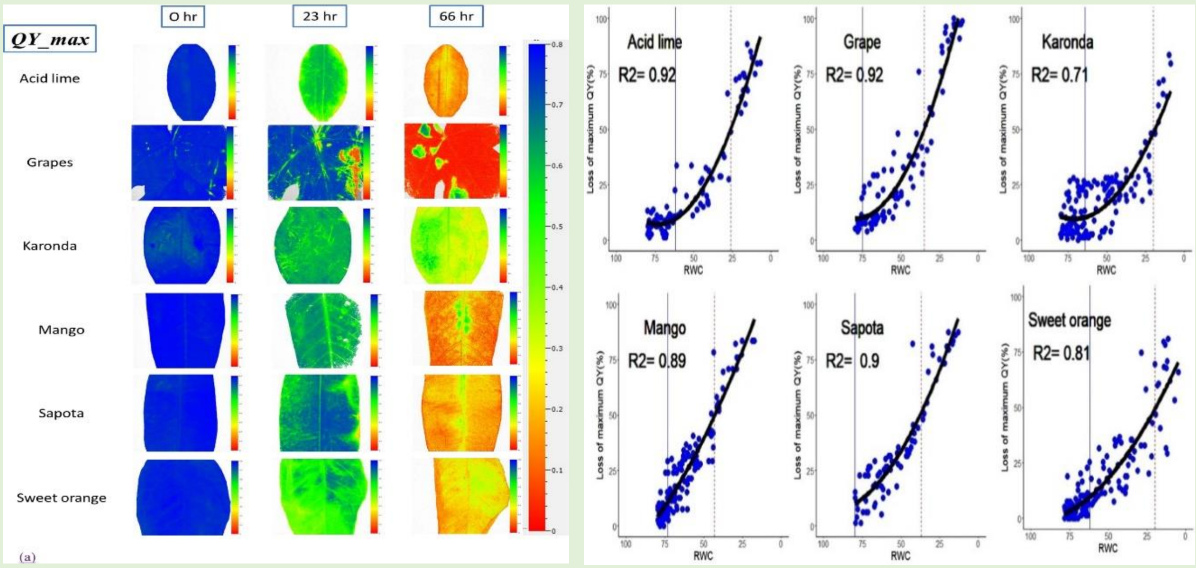


Fig. 56: Chlorophyll fluorescence imaging of representative leaf samples of diverse dryland fruit crops depicting the desiccation sensitivity of PS-II as revealed by QYmax at 0, 23 and 66 hours after desiccation treatment and loss of QYmax of photosystem II (Fv/Fm) over decreasing relative water content (RWC) of six dryland fruit species

VIII. Canopy temperature depression (CTD) and canopy greenness associated with variation in seed yield of soybean genotypes under soil moisture stress

The method to measure canopy temperature depression (CTD) by employing a thermal imaging system for crops like soybean, which is sensitive to low soil moisture has not been standardised/optimised. Hence, the present study was conducted to optimise the thermal imaging method and evaluated the CTD along with canopy greenness-based physiological traits in screening/selecting soybean genotypes suitable for semi-arid environment. The CTD and canopy greenness were measured six to eight times during different growth phases/stages using infrared (IR) and visible cameras mounted on a semi-automatic trolley that allowed rapid acquisition of high quality thermal and visible images, respectively. The CTD measured at the reproductive stage explained a major proportion of the variation in grain yield under both well-watered and water-stressed conditions. This could be attributed to close association between plant's capacity to keep its canopy cooler (low canopy temperature) and canopy greenness (higher chlorophyll content) as indicated by efficient photosynthesis which leads to grain yield. These results indicated that in addition to assess stay green features, CTD along with canopy greenness can also be used as a key trait of leaves in the selection of soybean genotypes for higher adaptability to low soil moisture stress conditions, a common feature exists under semi-arid regions.

IX. Relative tolerance of photosystem II in spike, leaf, and stem of bread and durum wheat under desiccation

In dryland regions, soil moisture stress often leads to desiccation and causes injury to photosynthetic machinery. Recently, chlorophyll fluorescence (ChlF)-based assessment of photosynthetic efficiency under drought stress is gaining attention due to advances in instrument development and methodology optimization. Our study was designed to explore the use of spike photosynthetic efficiency as a trait to differentiate drought responses in wheat. Bread and durum wheat were assessed for spike, stem, and leaf tissue photosynthetic efficiency in response to progressive desiccation using ChlF imaging. Results showed that durum wheat had higher quantum efficiency and lower photoinhibition of PSII relative to bread wheat across spike, stem, and leaf. Rate of decline in maximum photochemical efficiency of PSII with increased desiccation was seen higher in bread wheat spikes as compared to durum wheat. Our investigation clearly demonstrated that ChlF imaging could be effectively deployed as phenotyping tool to differentiate wheat genotypes for their photosynthetic performance under desiccation.

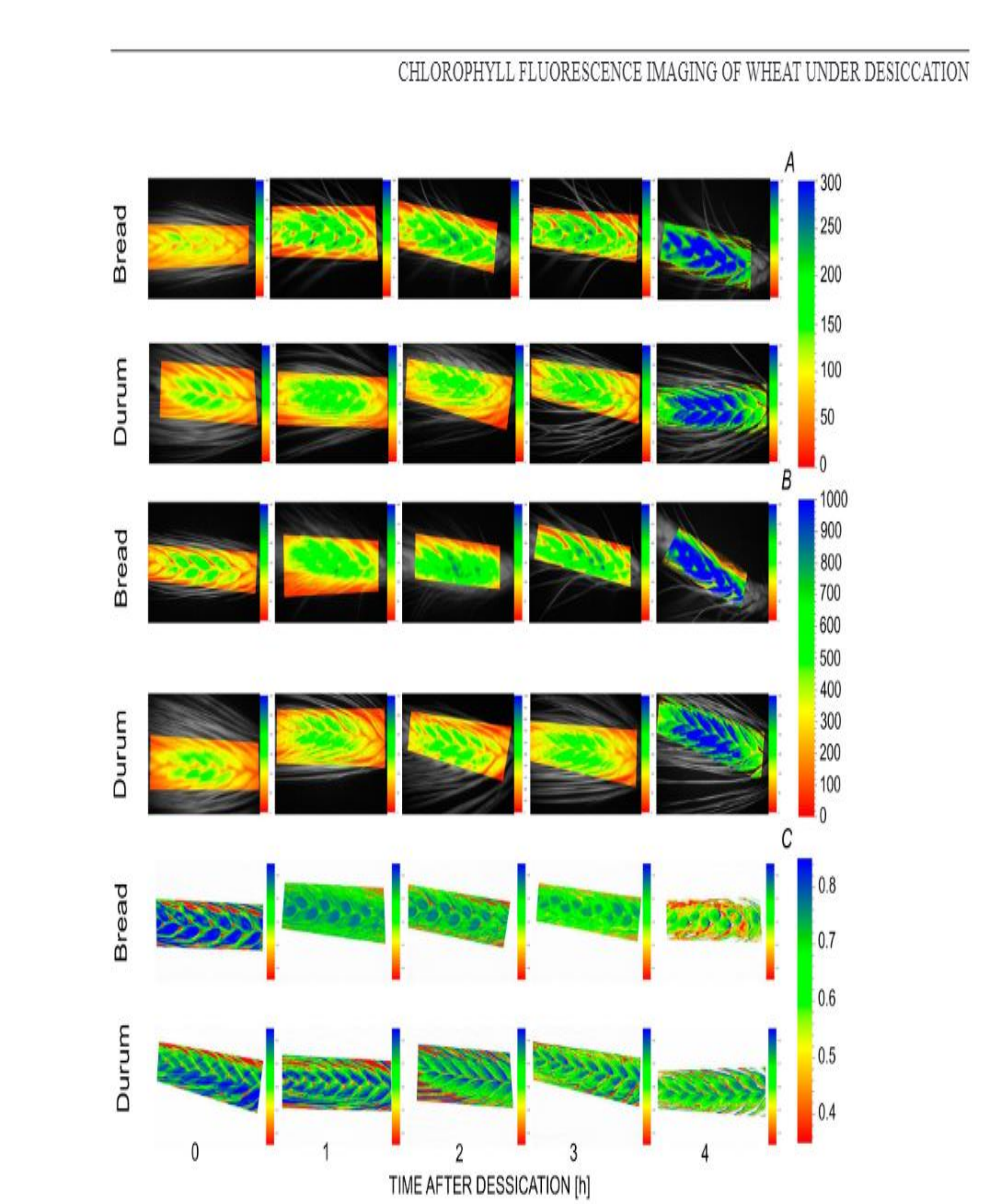


Fig. 57: Chlorophyll fluorescence images of initial fluorescence (F0) (A), maximum fluorescence (Fm) (B), and maximum photochemical efficiency of PSII (Fv/Fm) (C) in spikes of bread (HI1531) and durum wheat (HI8498) over the 4 h of desiccation. Pixel value images of Fv/Fm were displayed as a false colour code ranging from red, through green and yellow to blue. The scale ranged from 0 (red) to 300 (blue) for F0; from red (0) to blue (1,000) for Fm, and from 0.35 (red) to 0.85 (blue) for Fv/Fm.

SCREENING OF GERMPLASM ACCESSIONS FOR VARIOUS ABIOTIC STRESSES

I. Genetic variation in physiological responses of mungbean to drought

Mungbean is a relatively drought-tolerant leguminous crop with a short life cycle. Using leaf water loss (LWL) as a screen for drought tolerance, two mungbean genotypes exhibiting more than two–fold variation in leaf water loss were explored for the genetic variation in their physiological and molecular responses to drought. Efficient stomatal regulation together with better photosynthetic capacity constituted an important trait combination for drought adaptation in water saving low LWL genotype. The stomatal closure under drought was accompanied with a concomitant down-regulation of farnesyl transferase gene. However, cooler canopy temperature, a well branched root system coupled with a relatively higher proline accumulation in water spending high LWL genotype constituted another set of adaptive traits operating when exposed to deficit soil moisture conditions.

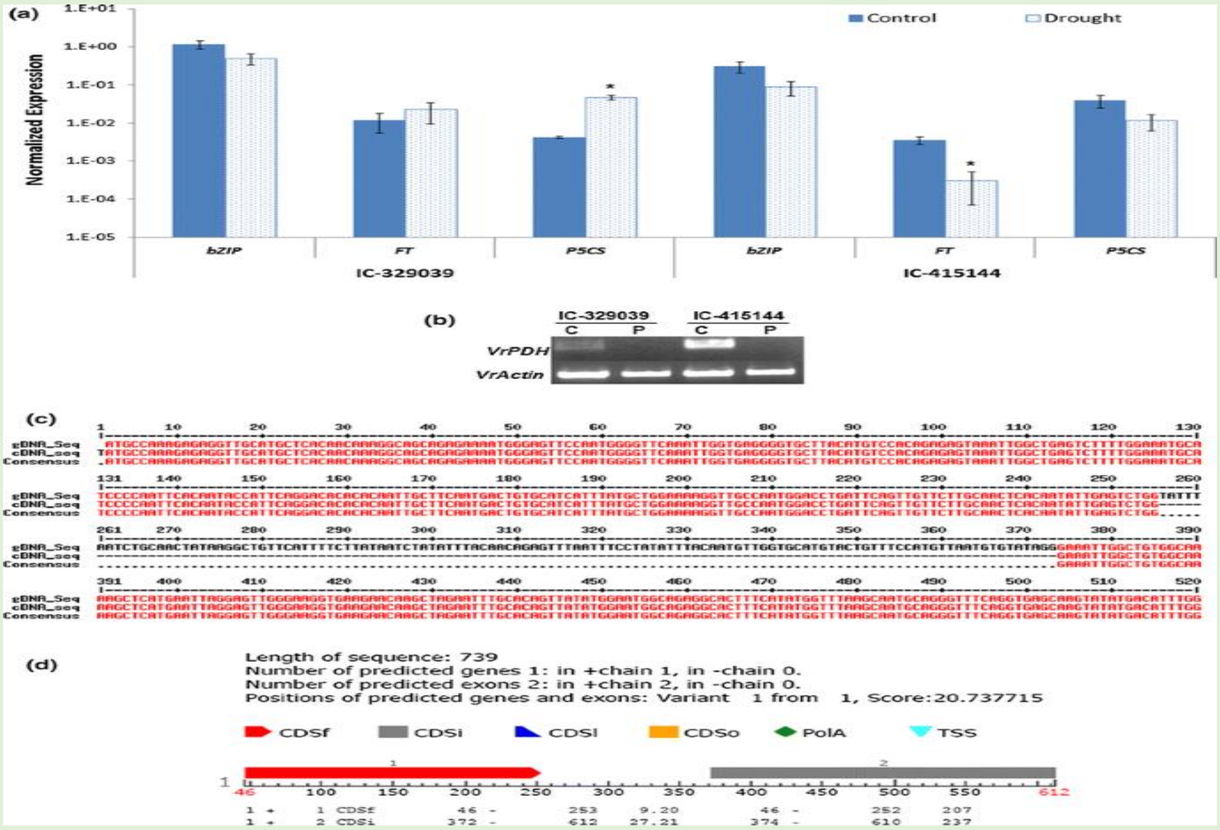


Fig. 58: Changes in gene expressions in mungbean's exposed to drought treatment. a Transcript accumulation of VrFT, VrbZIP and VrP5CS genes in high and low LWL type mungbean genotypes in response to drought stress.. b Semiquantitative RT-PCR revealing down-regulation of VrPDH expression upon drought stress in high and low LWL genotypes. c Sequence alignment of VrPDH amplicons amplified from cDNA (cDNA_seq) and genomic DNA (gDNA_seq) of mungbeans. d Figure showing identification of intron in the genomic DNA sequence of VrPDH.

II. Responses of chickpea genotypes to soil moisture deficit imposed under field condition

This experiment was conducted with two level of soil moisture stress created by varying number of irrigations. The well-watered plants had two irrigations while stressed plots had only one irrigation. Each of the treatment had three replications and each replication had 78 genotypes including a local check named Digvijay. Sowing was carried out in 15th Nov 2018.

The irrigated treatment received the last irrigation on 30 th Dec.2018 while the stressed plots did not receive any irrigation till the harvest. There were no rains during the experiments. Experiment was conducted with Randomized Block design and with 1.8 m ×1.5 m plots. Crop was provided with 20-60-00 Kg/ha of N-P-K as basal doze. Most of the time the soil upto 30 cm was dry with only about 8% moisture in stressed plots and about 10-18% moisture in irrigated plots.

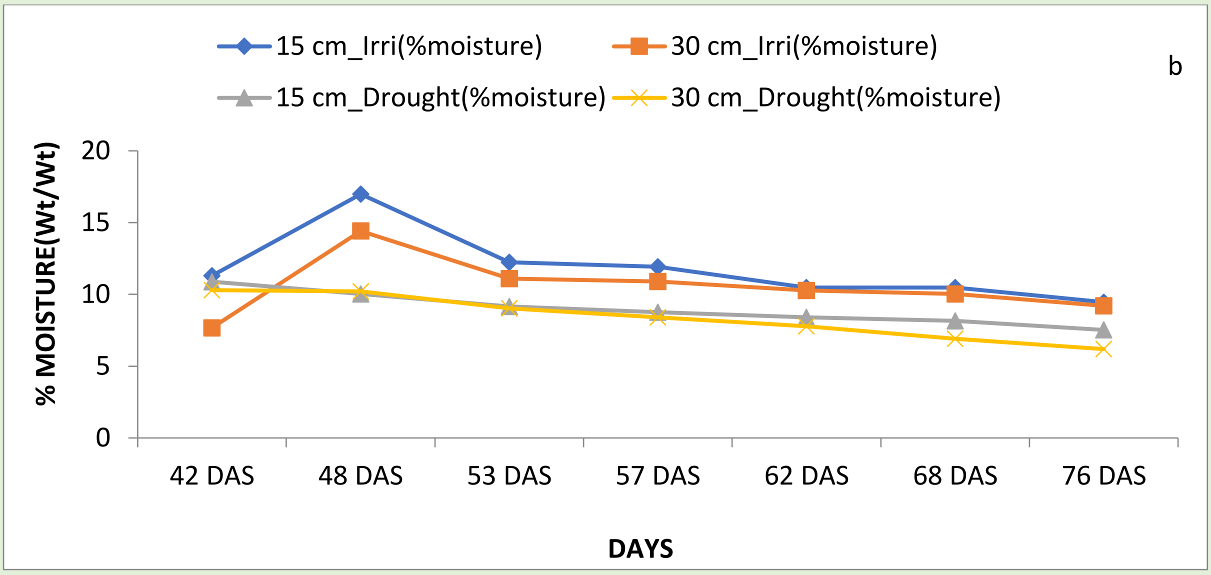
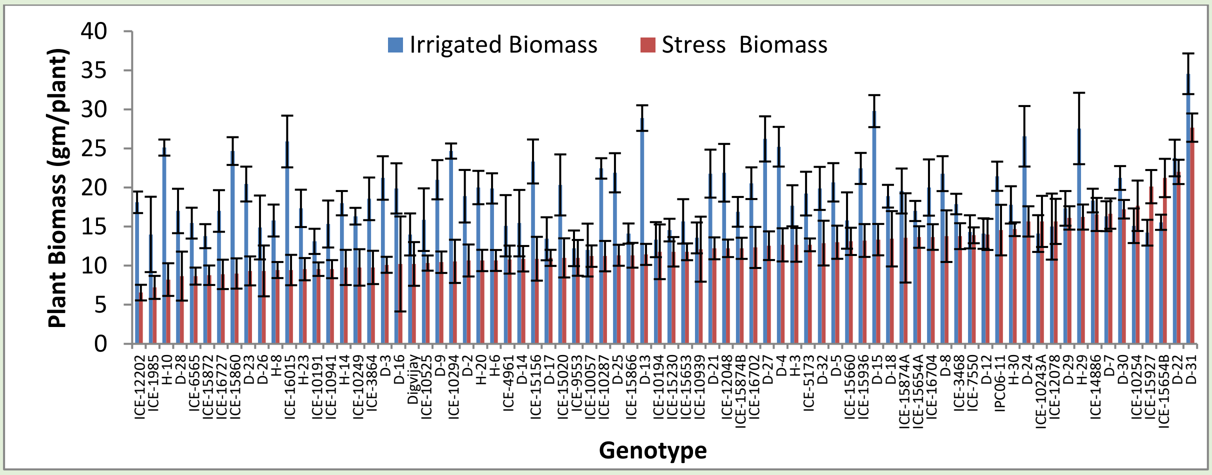


Fig. 59: (a) View of field experiments conducted with 78 genotypes and (b) the soil moisture levels at 15 and 30 cm during the experiments.



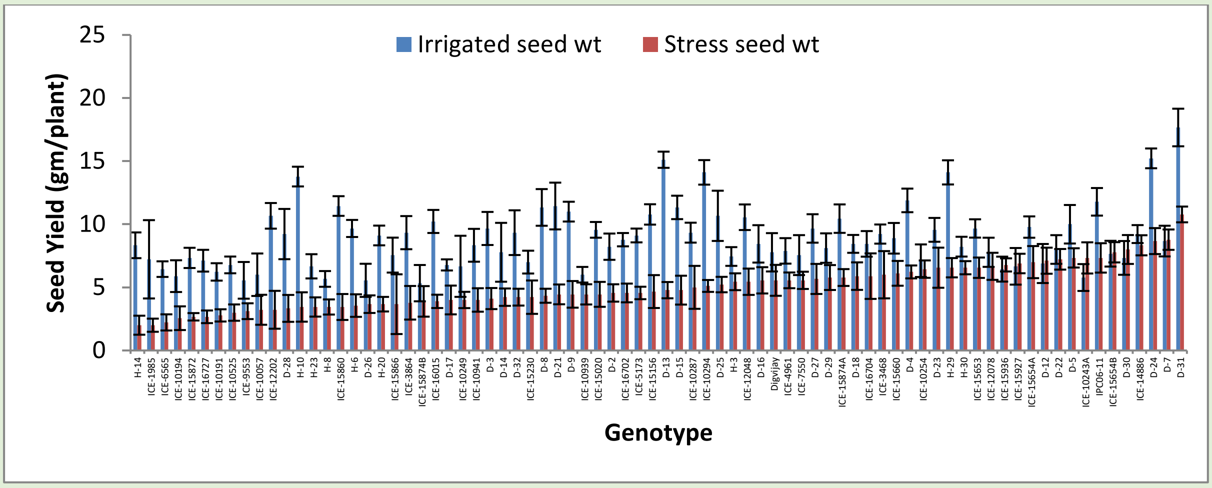


Fig. 60: (a) Variation in canopy temperature, (b) biomass (c) seed yield per plant in chickpea genotypes tested under field conditions.

Conclusions: Early flowered genotypes D13, D15, D30& D31 maintained cooler canopy & high Vegetative index than Digvijay, the local check. Genotypes ICE15654A, ICE15654B, IPC06-11 & ICE15660 revealed a high Vegetative index as well as maintained their canopy cooler than Digvijay. Genotypes Such as D24, D5, D15, IPC06-11, and ICE15654B had high seed yield and could maintain their canopy cooler in stress conditions induced by soil moisture deficit. Higher biomass accumulation was observed among D24, D15, D5, D22 and ICE 10294 under well water conditions while D22, D29, and ICE 15654B under water stress conditions.

III. Responses of pigeon pea genotypes to soil moisture deficit imposed under field condition

Experiments conducted with genotypes obtained from VNMKV, Parbhani Pulse Research Station, Badnapur were considered for detailed analysis. How the plants responded to soil moisture stress and how it was reflected by different image parameters were investigated. Water consumed by each of the genotypes were analysed. Attempts were made to assess potential of different image parameters to differentiate the 24 genotypes of pigeon pea.

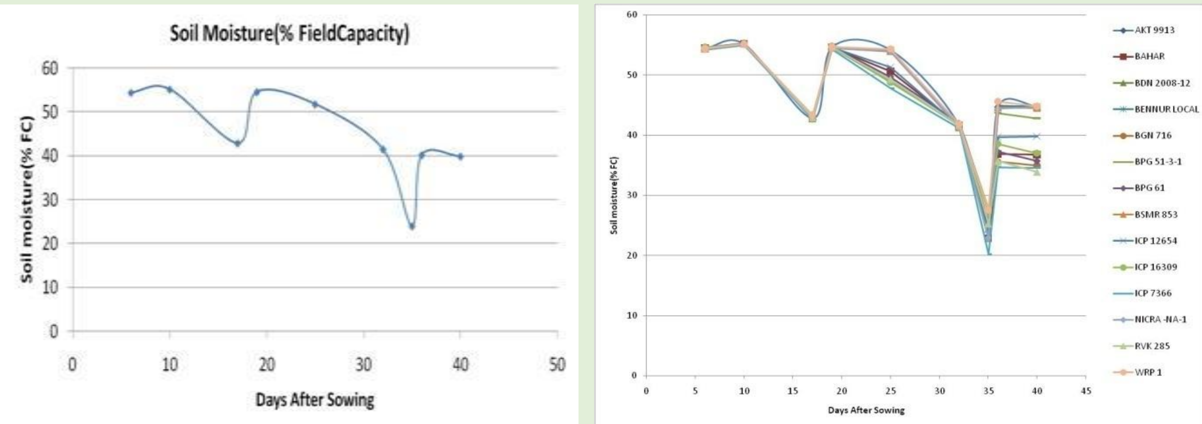


Fig. 61: (a) Trend in soil moisture depletion and (b) Genetic variation in soil moisture depletion by pigeonpea genotypes

These experiments were conducted to know the trend in depletion of soil moisture stress and genetic variation among the genotypes in utilization of soil moisture.

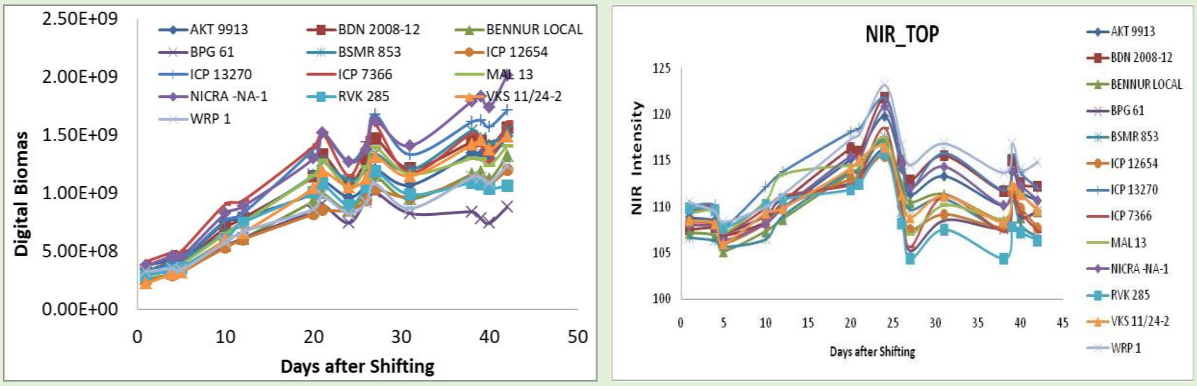


Fig. 62: Digital Biomass and (b) NIR Intensity in Pigeonpea genotypes

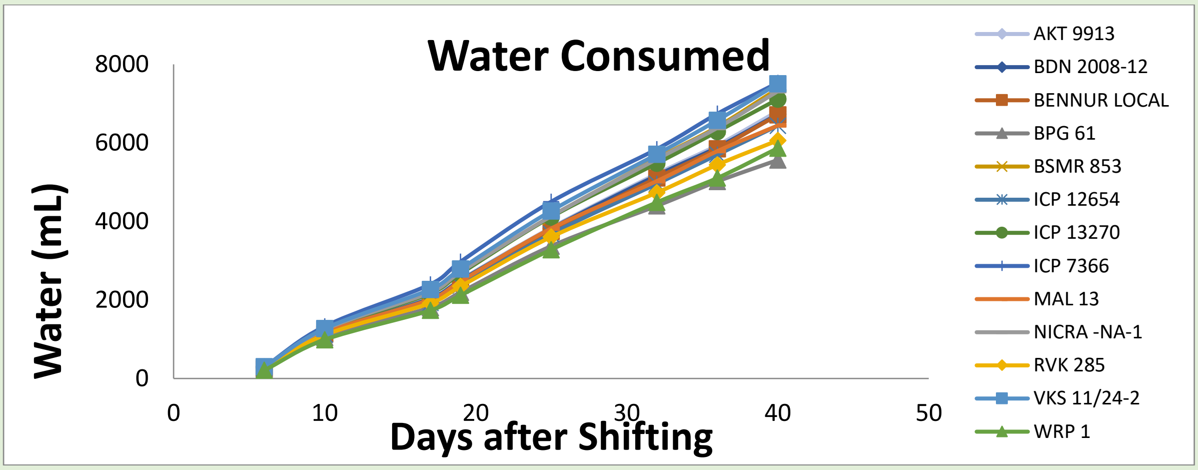


Fig. 63: Water consumption of different genotypes of pigeonpea

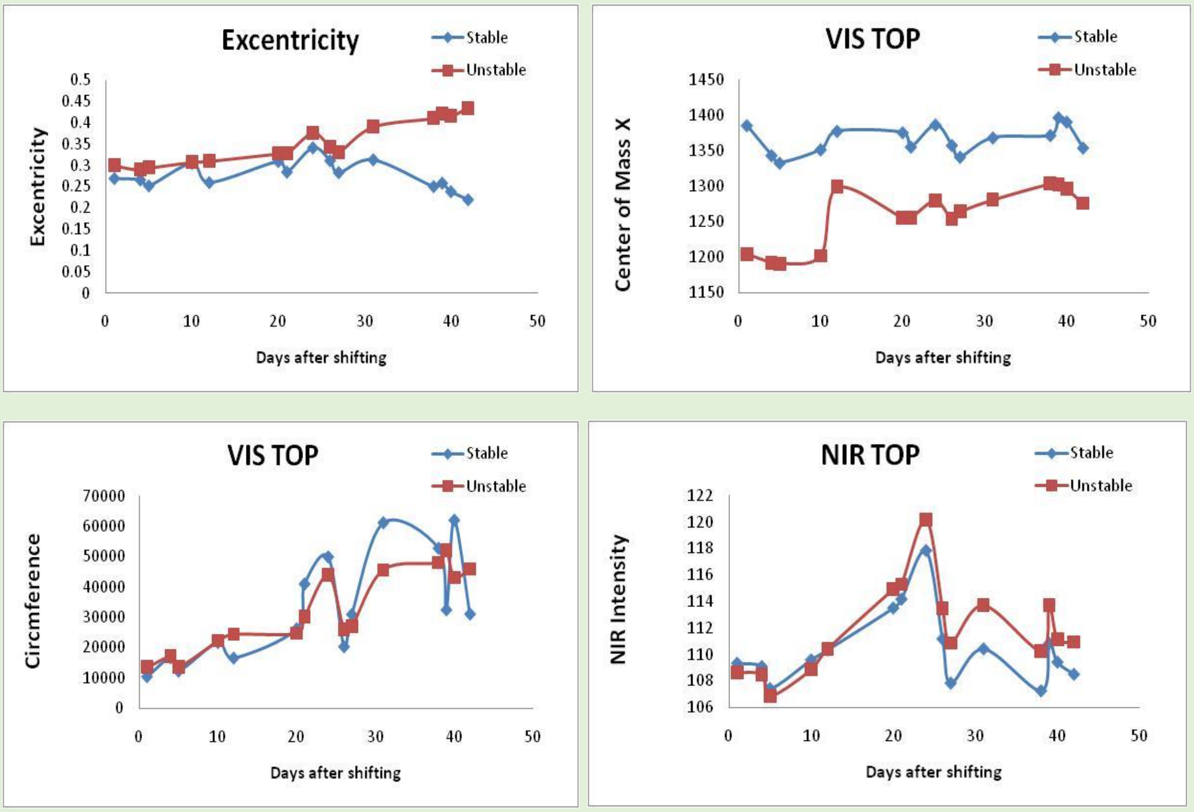


Fig. 64: Significant difference in image parameters (a) Excentricity (b) Center of mass X (c) Circumference and (d) NIR intensity between stable group (RVK-285, AKT9913) and unstable group (Bennur Local, BDN 2008-12, ICP 7366, WRP-1) of genotypes tested across the environments.

Conclusions: Genetic vitiation in pigeonpea genotypes exist for the depletion of soil moisture. Digital biomass, excentricity, and compactness could differentiate the responses of pigeonpea genotypes to depleting soil moisture conditions. RVK 285 produces higher dry biomass after consuming low water. RVK 285 Showed higher water content but lower digital biomass as an adaptive strategy under depleting soil moisture conditions and hence had better recovery. The significant difference found in some image parameters between the stable group (RVK-285, AKT9913) and unstable group (Bennur Local, BDN 2008-12, ICP 7366, WRP-1) of genotypes tested across the environments. Center mass of X showed the highest difference between stable group (RVK-285, AKT9913) and the unstable group (Bennur Local, BDN 2008-12, ICP 7366, WRP-1) of genotypes followed by Excentricity, circumference, NIR Intensity, Roundness, and sub object count.

IV. Screening of Pigeonpea genetic resources for waterlogging tolerance

The experiment was conducted with 166 diverse pigeonpea genotypes including 4 checks under laboratory conditions. In the lab conditions, 10 seeds of each genotype were kept in a Petri plate filled with water (complete submergence) for about 2, 4, 6, 8 days with 3 replications each. After the respective days of stress treatment, seeds were taken out and excess water was drained out and seeds were kept for germination under normal conditions. The germination percentage and root and shoot length were measured and waterlogging tolerance coefficient were estimated. It was found that ICP-5863, ICP-6370, ICP-16309, ICP-6128 and ICP-7869 showed higher waterlogging tolerance coefficients than check ICP-5028, MAL-15 under different durations of waterlogging stress. Similarly, genotypes *viz*, GRG-811, ICP-6815, ICP-6845, ICP-7375, ICP-7507, ICP-7314, ICP-16309, ICP-5863, ICP-6128, ICP-6370, ICP-7223, ICP-10228, ICP-7803, ICP-8255 and ICP-7366 recorded 100 percent survival rate under all (control, 2, 4 ,6, 8 days) of submergence stress treatments. Seedling length was also varied among genotypes for different durations of submergence treatment. Among the genotypes, GRG-811, ICP-6815, ICP-6845, ICP-7507, ICP-7314, ICP-16309, ICP-5863, ICP-6128, ICP-6370, ICP-7223 and ICP-10228 recorded higher seedling length after stress treatment for 8 days.

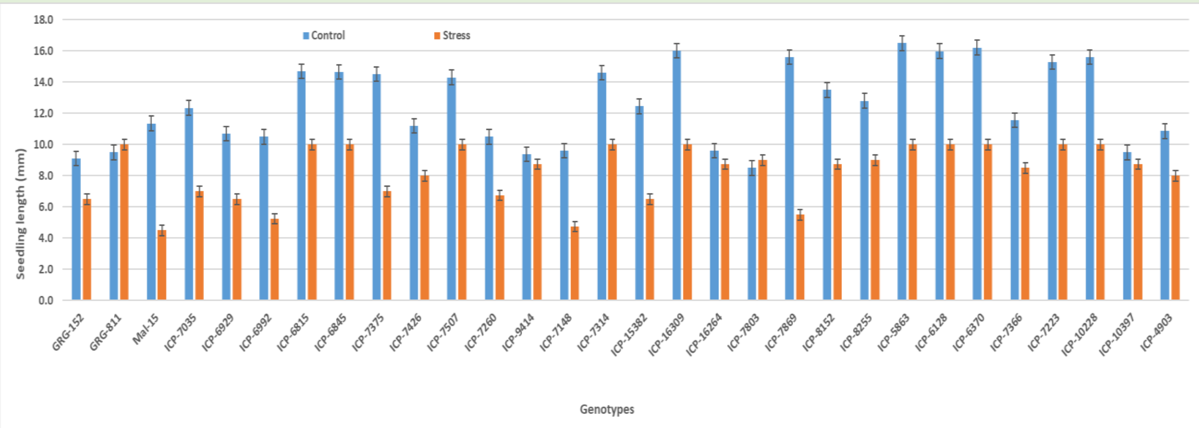


Fig. 65: Genetic variation for seedling length after 8 days of submergence treatment among pigeonpea genotypes

Further, a pot experiment was conducted for two years (2020 and 2021) to know the performance of selected genotypes for transient waterlogging stress treatment at early seedling stage of the crop. Each pot was sown with 5 seeds/pot at 17-20 mm depth following a completely randomized design. Before the imposition of stress treatment, the number of plants in each pot was counted. Transient waterlogging was achieved by immersing five pots (20 days old seedlings) in a cement tank filled with water for ten days, with the pot surface remaining at least 20mm under water for the duration of the experiment (10days), while the sixth pot was kept at normal moisture as a control. Throughout the stress treatment, the water level in the pot was maintained at the same level for ten days. After 10 days of stress treatment excess water in the pots were drained and allowed to recover. The number of plants that survived in each pot 10 days after the waterlogging stress treatment was counted, and the rate of survival was calculated based on the number of plants in each pot before treatment. The genotypes showed broad range of variation for stress treatment among the 54 genotypes including checks used in the present study only 33 genotypes were survived 10 days transient waterlogging stress. Observations on days to 50% flowering, number of primary and secondary branches per plant, plant height and pods per plant, seeds per pod 100 seed weight and grain yield per plant was recorded from both control plants and stress-imposed plants. There was significant variation among the genotypes for the all the traits studied among the stress and control treatments.

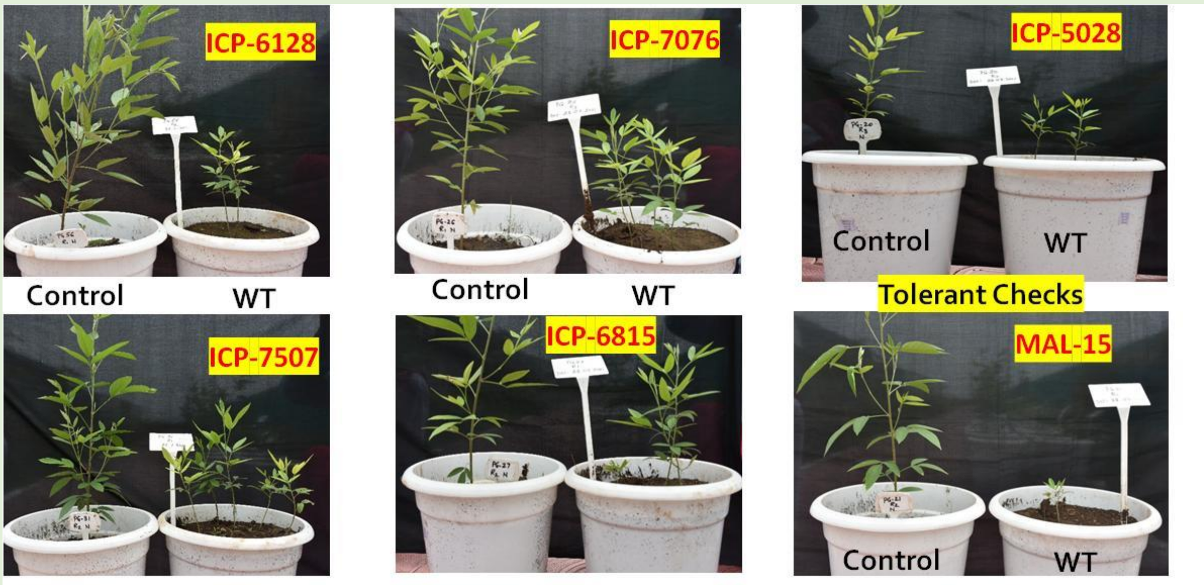


Fig. 66: Waterlogging tolerant pigeonpea germplasm accessions

V. Non-destructive assessment of seed phenotypes in Mungbean Mini-Core collection (MMC) by image analysis

Phenotyping plays a key role starting form identification of sources for the trait, to selection and advancement of lines, and evaluation of cultivars prior to release. Grain weight and number per pod are the routine post-harvest parameters used by crop scientists for assessing performance of genotypes while other features of grains are often ignored due to constraints in phenotyping. Recent image analysis techniques offer opportunities to bridge this gap. Such techniques need to be optimized for high throughput assessment of individual seed which is practically difficult to do manually for large number of samples. Hence, experiments were conducted to optimize image based protocols for phenotyping seed morphological features in addition to grain number and grain weight. Seeds of 296 accessions of mungbean obtained from the World Vegetable Center, Taiwan were used for image analysis. For image acquisition, light illuminated setup was used to capture the images of 12192 seeds. Images were analysed by using ImageJ, an open-source software. Different seed parameters *viz*. Roundness, Feret Y, Feret angle, Feret X, Circularity, Solidity, Raw integrated density, Aspect ratio, Integrated density, Perimeter, Width, Height, and Feret diameter. These data could differentiate the origin of the seeds as the originating countries could be classified into different clusters. Among the seed parameters, roundness, solidity, feret angle and circularity showed high genotypic variation. The present experiment could demonstrate that digital images can facilitate breeders attempt to phenotype large number of seeds in rapid and robust manner. Additionally, results from this experiment could reveal that seeds originating from the cluster of countries like USA, Australia, and Philippines were different from those originated from the Afghanistan, Nigeria, Taiwan and Thailand. Seeds originated from Brazil, France, Kenya, Iran, Iraq, India, Mexico, Netherland, S. Korea, Turkey were grouped in separate cluster. Thus, the image-based protocol developed by us offer potential to provide better insights into the genotypic variation as well as origin of seeds, which needs to be further validated by assessing core collections.

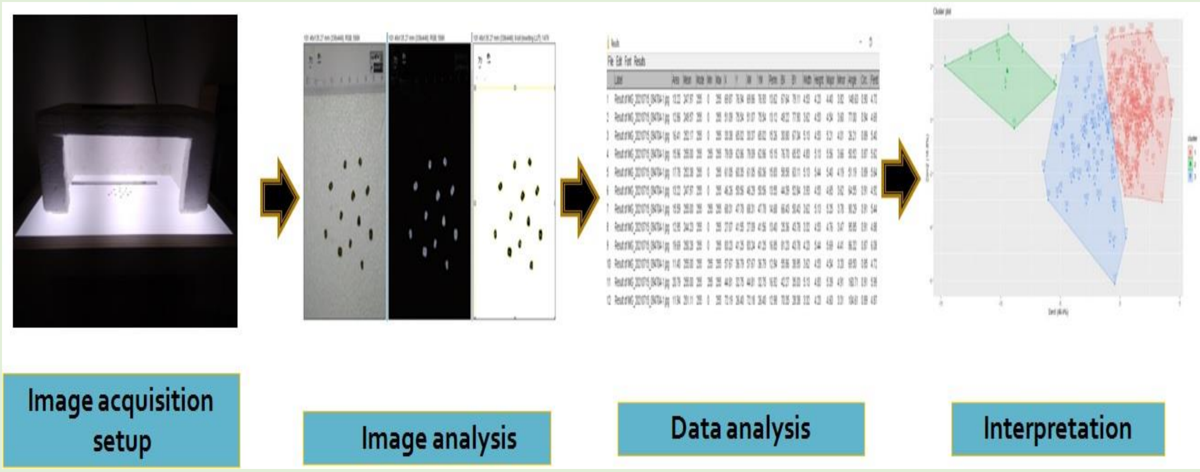


Fig. 67: Protocol optimized for image parameters

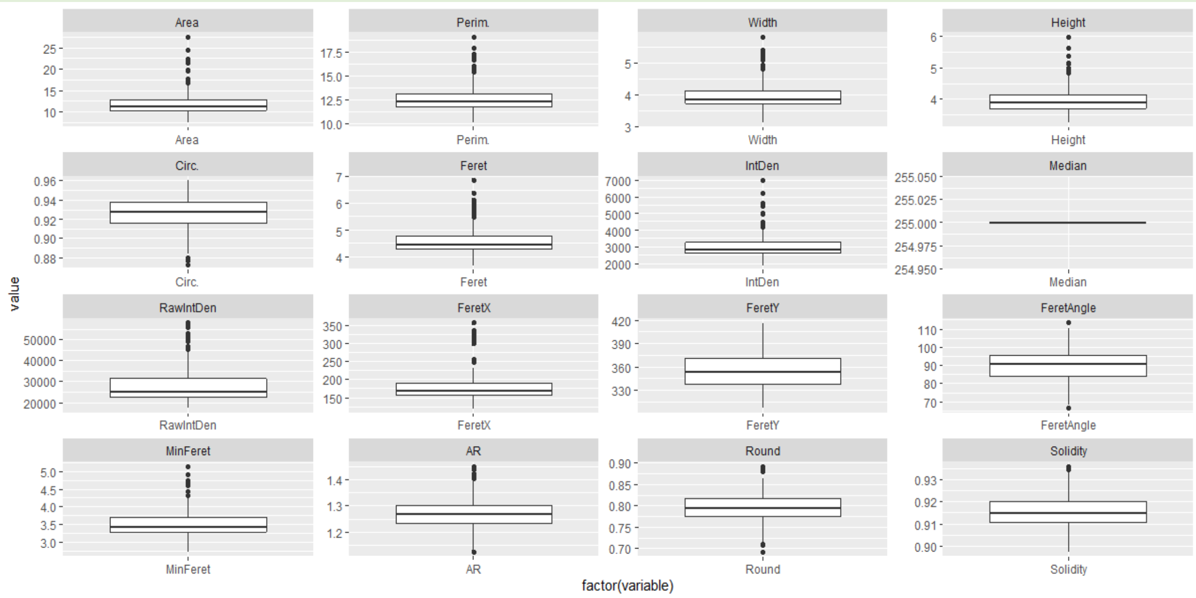


Fig. 68: Genetic variation in different seed parameters

VI. Genetic variation for Invitro RSA in diverse genotypes of Lentil:

The experiment was conducted with 24 diverse genotypes of lentil. The seeds of lentil genotypes were surface sterilized with 0.5% sodium hypochlorite and all the glassware were washed in distilled water and autoclaved. 10 Litre of 1/2 MS media (Murashike and Skoog) media was prepared using Sucrose, agar and MS media. Melted media was poured into glass test tubes of 60% volume. Each test tube was inoculated with one healthy seed. A total of 4 replication for each genotype was maintained. Observations (Root length, no. of secondary roots, shoot length) were recorded at every 15 days interval. At 15 days after inoculation (15 DAI) genotypes, BL3, BL9 and BL23 showed faster root growth and a greater number of secondary roots. Again after 30 DAI the root traits were measured. It was found that genotypes BL6 and BL23 recorded higher root length and a greater number of secondary roots. Hence it is observed that among the 24 lentil genotypes there is great genetic diversity exist for root traits under *invitro* growth conditions

VII. Identification of trait specific germplasms for high temperature stress tolerance in cowpea

A total of 250 germplasm and five checks were screened for high temperature stress tolerance under field conditions during summer month of 2022. Among them 50 germplasm found better than checks for the traits such as canopy temperature, leaf and pod florescence, grain yield and other physiological traits.

Type of Germplasm	Trait	Genotypes
Vegetable type	Very early (Escape Mechanism) under both stress and control	EC-724484, EC-724740, EC724484, EC-723684, IC-488084, IC488077, EC-243999, IC-259159, IC410043, IC554414
Grain type	High yield under stress and control	Ec240930,EC-240926, EC-240884, EC-107151, EC-240874, EC-121826, EC24081, EC240741, EC240829, IC-488085, EC-240902, EC241078, EC 244133, IC 488268, IC 488222, EC 149469, EC 241035, EC 240995, EC 240989-A, EC 240878, IC 400155, IC 402101, IC 426824, IC 472252, EC 240900, EC 243995, IC 488124, EC 243927, EC 240625, IC 402176, IC 596961, IC 426824, IC 488067, IC 548288, IC 472254, IC 488124, EC 240897-1, EC 240924, EC 240625, IC 605507, IC 421917, IC 596961
Fodder type	High Biomass under stress and control	EC 240891, EC 107182, EC 240917, EC 240875, EC 240890, EC 240801, IC 488112, IC 488119, IC 488131, IC 488085, EC 242128, EC 149458, EC 243938, EC 723742, EC 244121, EC 723851, EC 241058, EC 723735-B, IC 488270, IC 488239, IC 488109, EC 244175, EC 724252, EC 723796. EC 723836, EC 244148, EC 723674, EC 23850, EC 240648, IC 397983, EC 240630, EC 240891, EC 100094, EC 240966-A, IC 402125, IC 402103, IC 488195, IC 488146, IC 598466, IC 569092, IC 594504, IC 560928, IC 369857, IC 488246, IC 471387, IC 488171, IC 561238, IC 590841, IC 471435
Dual Purpose	High grain yield and Biomass under stress and control	IC 488095

Type of Germplasm	Trait	Genotypes
Grain Type	Significantly higher leaf PS-II efficiency under stress and control	EC 724764-B, IC 560916, IC 548288, EC 240966-A, EC 724905, EC 724484, EC 240989-A, EC 241058, IC 402097, EC 240868, ,IC 548860, IC 507157,EC 724805, IC 488185, IC 418505,C 402161, ,EC 723908, IC 402111, IC 554414, ,EC 24081, IC 58905,IC 488085, IC 488135,EC 97167, EC 723743, EC 240824, IC 554347, IC 420660, IC 471955, EC 149469, IC 397397,EC 243999, EC 724742, IC 401315, IC 548859, EC 240676, EC 240902, IC 488065, EC 240635, EC 724547, IC 402125, IC 488067, IC 426809, IC 472254, EC101775, IC 488264, IC 560917, EC 724872,
Grain Type	Significantly higher Pod florescence under stress and control	EC 148709, EC 240900-A, EC 240861, EC 240890, EC 244133, EC 244175, EC 244075, IC 397397, ,IC 402105, IC 401315, IC 402090, IC 402159, IC 402099, IC 418505, IC 488146, IC 488065, IC 488063, IC 554414, IC 512204

STUDENTS TRAINED

Sl.No.	Name of Student	Degree	University	Title	Year
1.	Gare Suvarna Somanath	Ph.D.	MPKV, Rahuri, Maharashtra	Influence of temperature on stem reserve mobilization for grain development in wheat	2019
2.	Babar Rohit Rajiv	M.Sc.	VNMKV, Parbhani, Maharashtra	Assessment of Drought Responses of Chilli (*Capsicum annum* L.) Genotypes By Using Modern Plant Phenomics Tools	2019
3.	Vitnor Sushil Sarjerao	Ph.D.	MPKV, Rahuri, Maharashtra	Assesment of Genetic Variability of Wheat For Early Establishment Under Moisture Stress Condition By Using PEG	2019
4.	Chahande Rahul Vinod	Ph.D.	MPKV, Rahuri, Maharashtra	Marker Assisted Selection for drought tolerance in Chickpea (*Cicer aritinum* L.)	2020
5.	Tamilselvan A	M.Sc.	IARI-NIASM, New Delhi	Assessment of efficacy of image-based tools to differentiate drought responses of pulse crops at seedling stage	2022
6.	Vidisha Thakur	Ph.D.	BanasthaliVidyapeeth, Jaipur, Rajasthan	The role of *bZIP* transcription factors in morpho-physiological	2022

Sl.No.	Name of Student	Degree	University	Title	Year
				responses to drought and heat stress during grain filling in wheat (*Triticum aestivum*L.)	
7.	Sagar P	M.Sc.	IARI-NIASM, New Delhi	Evaluation of pod pedicel as a component trait to facilitate photosynthate supply to developing soil moisture depletion	2022
8.	Madhavi Prakash Sonone	Ph.D.	BSKKV Dapoli	Development of phenomics protocol and identification of genetic resources for sodium exclusion in rice (*Oryza sativa* L.)	2023
9.	Shubhangi KshirsagarMaraskole	Ph.D.	BSKKV Dapoli	Genomics intervention to identify candidate genes for salinity tolerance in Rice (*Oryza sativa* L.)	2023
10.	Dnyaneshwar Ambadas Raut	Ph.D.	MPKV, Rahuri, Maharashtra	Genetic Variation in endogenous Ascorbic Acid accumulation and it's influence on physiology and seed yield of Chickpea genotypes under water stress	2023
11.	Vinay Mahadev Hegde	Ph.D.	PDKV, Akola, Maharashtra	Sugarcane ecophysiology investigations to explore water	Cont.

Sl.No.	Name of Student	Degree	University	Title	Year
				saving avenues for reducing ecological cost of sugar production through AI facilitated micro irrigation technology	
12.	Priyanka Negi	Ph.D.	MPKV, Rahuri, Maharashtra	Investigations on traits contributing to early crop establishment under direct seeded rice conditions	Cont.
13.	Girish Chopade	M.Sc.	MPKV, Rahuri, Maharashtra	High-throughput Phenotyping for Identification of Cowpea Accessions for Drought Tolerance.	Cont.
14.	Prasad	M.Sc.	UAS, Raichur, Karnataka	Genetic Analysis for Drought tolerance in Breeding lines of Pigeonpea (*Cajanus cajan* L)	Cont.

RESEARCH PUBLICATION

Research Papers

1. Raina SK, Govindasamy V, Kumar M, Singh AK, Rane J, Minhas PS (2016). Genetic variation in physiological responses of mungbean (*Vigna radiata* (L.) Wilczek) to drought. Acta Physiologiae Plantarum. 38, 1-12.

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Germplasm Registered

Certificate

Plant Germplasm Registration

It is certified that germplasm EC-0398949 of Mung Bean (INGR21224) developed by

SK Raina, Jagadish Rane, Nikhil Raskar, AK Singh, V Govindasamy, Mahesh Kumar, SC Ekatpure, PS Minhas and SM Sultan
ICAR-NBPGR Regional Station, Srinagar, Jammu & Kashmir

has been registered by Plant Germplasm Registration Committee (PGRC) of Indian Council of Agricultural Research on December 24, 2021.

Member-Secretary
PGRC

Chairman, PGRC
DDG (CS) ICAR

TRAINING ORGANISED

ICAR Sponsored Short course on "Phenomics: Perspectives for application in improvement of abiotic stress tolerance in crop plants" 20th -29th July 2017.

Advanced Training on "Application of plant Phenomics tools for assessing responses of crop plants to drought and high temperature" Ministry of External Affairs, and DARE, New Delhi under Indo Africa Forum Summit III 15th - 28th, February 2018

ICAR Sponsored short course on " Advances in application of Phenomics tools for assessment of abiotic stress responses of crop plants' from 28th Feb to 9th March 2022.

VISITORS AND MEETINGS

NICRA (NRM) Review Meeting held during 8-9 February 2018 at ICAR-NIASM

A two-day Review meeting of National Innovations in Climate Resilient Agriculture (NRM) was held at ICAR-NIASM, Baramati during February 8-9, 2018. The meeting was aimed at reviewing the consolidated work progress of all the NICRA partner institutes and to discuss future work plan. The meeting was attended by Principal Investigators of 15 different institutes of Natural Resource Management (NRM) division located across the country. The meeting started with a welcome address by Dr. N.P. Singh, the Director of NIASM, who gave a brief introduction about the institute. Dr Alugusundaram, DDG (Deputy Director General (Agril. Engg. & NRM) ICAR, New Delhi in his introductory remark. The progress made by NICRA projects as 15 ICAR research institutes were presented by principal investigators of respective institutes. The DDG specifically stressed for extending the knowledge and technologies generated under NICRA programme for the benefit of farmers. He also suggested avoiding duplication and ensuring complementarity of works and suggested encouraging young generation of scientists. Dr B. Venkateshwaralu, Hon'ble VC of VNMKV, Parbhani was the external expert for this review meeting of NICRA(NRM). He was critical about the contribution of each of the institute in achieving the objectives of the NICRA that focuses largely on climate change related issues and concerns. He expected that the who substantially contributed to the knowledge generation and establishing facilities under the NICRA to be encouraged. He felt that a huge set of data have been generated in the project and that has to be documented for the benefit of policy makers and adaption to climate change while mitigation options need to be implemented. Dr. S. Bhaskar, ADG (AAF&CC) joined the meeting on 8th February, 2018 and he opined that significant achievements should be published in the best possible format to provide decision making support for policy makers and also climate change adoption and migration for farmers. He suggested to add new dimension to the research on climate change in participating institutes without duplicating their mandated research activities.

NICRA (NRM) Review Meeting held during 8-9 February, 2018 at ICAR-NIASM

Visit of NICRA (NRM) Review Meeting Team on during 8-9 February, 2018

Visit of PI NICRA and Head, Division of Crop Science, CRIDA to ICAR-NIASM on October 11, 2022

Visit of Hon'ble Member of Parliament Shri. Sharadchandra Pawar along with Dr. Ranveer Chandra, CTO, Agri-Food and Executive Director, Microsoft Networking Research and Dr. Ajit Jaokar, Course Director (AI), Oxford University on January 03, 2023

Visit of Mr. Stewart Collis of Gates Foundation to National Plant Phenomics Facility on 6th March 2023.

हर कदम, हर डगर

किसानों का हमसफर

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