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Next generation phenotyping for developing climate resilient rice varieties

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ABSTRACT

Present paper deals with different components of next generation phenomics for characterizing rice genotypes for water deficit stress. Major sensors used in the study were non-imaging hyperspectal remote sensing, thermal imaging at ground platform and RGB and multispectral imaging sensors from drone platform. Different spectral indices were evaluated along with new proposed index and different multivariate models were studied for non-invasive estimation of relative water content (RWC) and sugar content in rice plant using spectral reflectance data collected in spectral range 350 to 2500 nm. Spectral data were further used for spectral discrimination of rice genotypes. Crop water stress index derived from thermal images acquired for rice genotypes could well characterize the drought resistant and sensitive genotypes. Initial study on field phenotyping through drone remote sensing using multispectral and RGB sensor was also explored to capture differential response of genotypes, trait and heat map mapping. All developed protocols as reliable alternative to conventional methods are fast, economic and non-invasive and in use in plant phenomics centre for high throughput plant phenotyhping for water deficit stress studies.

Key words: *Water deficit stress, RWC, phenomics, sugar, hyperspectral, thermal, drone remote sensing, multivariate models*

INTRODUCTION

Rice is one of the staple food crops for approximately half of the global population and rice production must increase by 70% by 2050 to satisfy the requirements of the growing world population (Godfray et al., 2010). India accounts for 20% of the world rice production (Ministry of Agriculture & Farmers Welfare, 2017). Hence it is necessary to maintain rice productivity for the food security of our country and the world. Further, it is estimated that rice production must be enhanced by 40% to feed 5 billion people by 2030 (Khush, 2005). Fresh water scarcity is a looming threat for agriculture production and specifically rice cultivation in near future. Rice accounts for about 50% of irrigation water, and thus rice cultivation is expected to be unsustainable in future as the per- capita water availability is expected to decline by 15 to 54% in most river basins of by the year 2025 (Guerra et al., 1998). Besides, rainfed lowland and upland rice is cultivated in about 45% of the rice-grown area in the country, which are subjected to intermittent soil moisture deficit that causes severe yield loss. Breeding crops for high water use efficiency (WUE) is essential to produce more crops in the dwindling fresh water scenario to secure food security, pursuing the slogan "more crop per drop" (Monaghan et al., 2013). Drought which is very frequent in tropical country like India is the most devastating abiotic stress affecting crop productivity (Toker et al., 2007). Therefore, genetic improvement in water use efficiency (WUE) and drought tolerance of rice is a priority research target for food security as well as minimizing agricultural water use.

Recent advances in crop physiology, systematic plant phenotyping and genomics helps to gain new insights in drought tolerance, thus providing crop breeders with greater knowledge of the gene networks and providing new tools for plant improvement to increase crop yield (Tuberosa and Salvi, 2006). The advances in the genomics during the past one decade offer great potential for genetic enhancement in yield and adaptability of crops. With the availability of next generation sequencing and automated genotyping technologies, generating accurate genotypic data for a large set of germplasm and breeding population has become easier. However, a limitation in using the genomic information for crop improvement is the costly and time-consuming processes of deciphering the phenotype that arises from the interaction of genome with environment. Conventional phenotyping methods are laborious, time consuming and not very precise. Thus phenotyping is the major bottleneck that limits utilization of the power of genomics for identification and use of novel genotypes, high-resolution linkage mapping, genome-wide association mapping and training genomic selection models for crop improvement. The multi-disciplinary science of phenomics, the sensor aided non-destructive high throughput automated acquisition and analysis of high-dimensional phenotypic data on an organism-wide scale, emerged recently to bridge the phenotype-genotype gap and enhance the pace of analytical breeding (Kumar et al., 2014). Next generation phenomics, the study of plant growth, performance, and composition, utilizes new technologies to better characterize plant responses to the environment and also better describes the growth environment itself (Furbank and Tester, 2011). Phenomics can also provide a platform wherein non-invasive biological data can be collected on a large number of plants simultaneously, providing observations of plant behaviour that have been unavailable via traditional phenotyping techniques and destructive harvests, *e.g.*, chlorophyll fluorescence for photosynthetic performance or hyperspectral imaging for measuring leaf constituents. Further, introduction of time axis by the use of non-destructive phenomics methods in QTL analysis revealed genetic dynamics of complex traits such as biomass, yield and stress responses.

Next generation phenomics for mapping crop traits has majorly three components such as (i) Phenotyping platform - growth chamber, climate controlled greenhouse and experimental fields, (ii) Imaging sensors and image acquisition and (iii) data analytics and algorithms for phenotyping. High-throughput phenomics advances needs to occur across scales of phenotyping platforms for sustained and increased crop yields. Field trials and growth chamber has its own advantages, disadvantages and application. Growth-chamber and greenhouse-based phenotyping platforms offers the advantage of increased experimental cycling and greater environmental control, but are often restricted by pot growth and the spectrum of environmental conditions (Fahlgren et al., 2015). Field platforms on the other has the advantage of growing crop-sized plants under natural settings, but are constrained to seasonal growing conditions. Even after this, field trials remain essential for the studies relating to growth and yield assessment for which imaging solutions are being developed (Corp et al., 2003; Rodriguez et al., 2005). Now days, aerial-based phenotyping platforms are increasingly becoming popular as it enables the rapid characterization of many plots within minutes unlike, ground based phenotyping. Unmanned aerial platforms like-drones have greater flight control and autonomy and are becoming increasingly affordable (Araus and Cairns, 2014). It also offers us with the capability to record data of crop traits throughout the crop life cycle. The potential to assess traits, such as adaptations to water deficits or acute heat stress, several times during a single diurnal cycle is especially valuable for quantifying stress recovery (White et al., 2012). Digital camera technology has become relatively inexpensive and ubiquitous, leading to a recent surge in high-throughput phenotyping systems that utilize plant imaging to capture data (Fahlgren et al., 2015). This allows measurement of the physiological, growth, development, and other phenotypic properties of plants through automated processes. Imaging techniques are used to quantify complex traits under related growth, yield and applications to stress for plant phenotyping in controlled environmental systems (in growth chambers or in the greenhouse) or in the field for example Berger et al. (2010) used high-throughput shoot imaging to study drought responses, White et al. (2012) used field based phenomics for plant genetics research etc. Hyperspectral imaging is a promising technology for the detection of abiotic and biotic stresses. Multispectral and hyperspectral cameras are of high importance for phenomics as it collects information in a wide range of wavelength and thus helping in better characterization of traits. ICAR- Indian Agricultural Research Institute (IARI) has state of the art Nanaji Deshmukh Plant

Phenomics Centre for carrying out plant phenotypic experiments on major crops. The phenomics centre has different imaging platforms to capture wide range of images from visual color imaging, thermal infra-red imaging, near infra-red (NIR) imaging, chlorophyll fluorescence imaging, visible-near infra-red (VNIR), hyperspectral imaging and short-wave infra-red (SWIR) hyperspectral imaging at different angles. In the meanwhile, many methodologies have been developed for analysis of the images from all these sensors. For example, image analysis pipeline, HTPheno has been developed which is implemented as a plugin for ImageJ for high throughput plant phenotyping (Hartmann et al., 2011). Methods for the segmentation and the automated analysis of time-lapse plant images from phenotyping experiments in a general laboratory setting has also been developed (Minervini et al., 2014). An open source, flexible image analysis framework, called image harvest (IH) has been developed for processing images originated from high throughput plant phenotyping platforms and its application has been presented using rice crop (Knecht et al., 2016). On this backdrop, our effort extends to develop a high throughput non-destructive sensor (RGB, multispectral, hyperspectral and thermal) based phenotyping of rice and to access the plant water status under variable degree of water-deficit stress. Some of the works discussed in this paper are (i) Predictive model for relative water content (RWC) and sugar content through spectrometry, (ii) differential response of rice genotypes to water stress and discrimination of rice genotypes, (iii) Thermal image based study for water stress and (iv) drone remote sensing for field phenotyping under differential treatment of water and nitrogen content.

MATERIALS AND METHODS

The study was carried out at ICAR-Indian Agricultural Research Institute (IARI), New Delhi research farms (28°38'28.59"N, 77° 9'28.09"E). The soil is mostly well-drained sandy loam. The minimum temperature is recorded between 0°C to 7°C during the winter season and the maximum temperature ranged between 41°C to 46°C. The average annual rainfall is about 750mm. The relative humidity (RH) is found to be the highest during the monsoon season. In the summer months, the RH is observed between 40 to 45%. Ten rice genotypes, five drought sensitive *i.e.*, MTU 1010, Patchaiperumal, Pusa Basmati-1, Pusa Sugandha-5, IR 64 and five drought tolerant *i.e.*, Sahbhagidhan, CR-143, Nerica L44, Moroberekan, APO were grown with three replications. All the genotypes were grown with two moisture conditions *i.e.*, maximum moisture stress and well irrigated.

Excised leaf water loss experiment

The experiment was conducted to understand differential genotypic response to stress and discrimination of rice genotypes. Thirty leaf samples from another set of 14 rice genotypes (3 replicates each) were collected in well-watered conditions from the experimental fields for spectral measurements followed by all biochemical analysis including relative water content (RWC).

The thermal imaging synchronised with the digital image, were taken for all the genotypes grown in field. The camera used was JenopticVariocam, sensitive to the spectral range 7.5 µm to 14 µm with spatial resolution 1024 x 768 pixels (0.270m x 0.2032m) and thermal resolution of 50 mK and ±2.0% accuracy. The thermal images from the middle of all the plots were captured so that proper crop area from the plot can be captured by the camera. The thermal images from lateral view were also captured but subsequent analysis showed that in a lateral view of a plot, leaves of an adjacent plot may get captured from the gap among plants. Therefore, all the analysis was done using top canopy view of the genotypes. Both thermal and digital images were clicked from nadir position at about 1.00 to 2.00 pm, when the crop plants were having maximum day temperature, on a perfect sunny day and less wind flow.

Crop water stress index (CWSI) images of rice genotypes

The temperature based crop water stress index was computed as proposed by Idso et al. (1981) and is given as

$$CWSI = \frac{(T_c - T_{air}) - (T_l - T_{air})}{(T_u - T_{air}) - (T_l - T_{air})} \; or \; \frac{dT - dT_l}{dT_u - dT_l} \; .....\text{Eqn.1}$$

Where, T_C= Temperature of canopy

T_{air}= Air Temperature,

T_l= Lower limit of canopy temperature from well-watered crop

T_l = Upper limit of canopy temperature from water stressed crop

dT = difference between canopy temperature and air temperature

dT_l = lower limit of canopy temperature minus air temperature (well watered plants)

dT_u = upper limit of canopy temperature minus air temperature (completely closed stomata or Non-Transpiring plants)

Four leaves sample per genotype for above mentioned 10 genotypes were collected from the field experiment site. Leaves were quickly placed in plastic bags in an airtight container and immediately transferred to the laboratory for spectroscopic measurements at predetermined time intervals after making all samples to a fully turgid condition till maximum water loss condition. In the laboratory, the spectroscopic data of above mentioned 10 genotypes were collected using an ASD Field Spec-3 spectroradiometer for the spectral range 350 to 2500 nm.

The water content in the leaves was analyzed using relative water content (RWC) computation as given below

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} X100 \text{.............Eqn.2}$$

Where, FW = Fresh weight of leaves,

DW = Dry weight (after oven drying),

TW = Turgid Weight

Spectral indices

Spectral indices for plant water studies utilize simple ratios between the reflectance of a wavelength located within an range of theelectromagnetic spectrum strongly absorbed by water,described as water absorption bands, and another wavelength located outside the water absorption band typically used as a control (Sims and Gamon, 2003; Eitel et al. 2006). Several water sensitive vegetation indices were evaluated for estimation of RWC. Also new indices were proposed based on lambda versus lambda contour plotting approach and identifying wavelength combination for maximum R^2 for RWC (Sahoo et al., 2015). New proposed indices has further been used for characterizing differential response of rice genotypes to water deficit stress.

Multivariate analysis

Some of recently developed multivariate techniques such as support vector regression (SVR), artificial neural networks (ANN), random forest (RF) and the partial least square regression (PLSR), PLSR followed by multiple linear regression (MLR) and PLSR followed by ANN were evaluated to determine the best suitable multivariate model for spectral based prediction of RWC. The overall performance and robustness of the models were appraised by the coefficient of determination (R^2), root mean square error of cross-validation (RMSECV), root mean square error of prediction (RMSEP) and ratio of prediction deviation (RPD) and upper and lower confidence intervals of regression at 95% confidence level.

Spectral discrimination analysis

An hierarchical spectral analysis method based on three integrated levels for spectral discrimination analysis and these were one-way ANOVA to test if the differences in the mean reflectance of 14 rice genotypes were statistically significant. Following the one way ANOVA with post-hoc Tukey HSD test, important wavebands for spectral discrimination for the 10 rice (91 pairs) genotypes were identified by counting, for each waveband, the genotype pairs where the mean reflectance difference was statistically significant. Classification and regression trees (CART) as a part of this second level of the hierarchical method to further lessen the number of significant wavelengths acquired from ANOVA analysis, with the purpose of reducing data dimensionality using a nonparametric statistical technique developed by Breiman et al. (1984) followed by the separability index. *i.e.* Jeffries-Matusita (J-M) distance analysis (Schmidt and Skidmore, 2003; Ismail et al., 2007; Vaiphasa et al., 2007). The JM distance between a pair of probability functions is the average distance between two class density functions (Richards, 1993; Schmidt and Skidmore, 2003). The square of JM varies between 0 and 2, with the higher values indicating the total separability of the class pairs in the bands being used (Richards, 1993; ERDAS Field Guide, 2005). In this study we have decided to use separability values ≥1.94 (≥97% of 2) as a JM distance threshold for spectral separability between class pairs, which is even stricter than the value of 1.90 that is normally utilized in remote sensing. The formula for calculating JM distance is as follows (ERDAS Field Guide, 2005):

$$J\text{-}M_{ij} = \sqrt{2\left(1 - e^{-\alpha}\right)}$$

$$\alpha = \frac{1}{8}\left(\mu_i - \mu_j\right)^T \left(\frac{C_i + C_j}{2}\right)^{-1} \left(\mu_i - \mu_j\right) + 2\ln\left(\frac{\left(\frac{1}{2}\right)\left|C_i + C_j\right|}{\sqrt{\left|C_i\right| \times \left|C_j\right|}}\right)$$

where i and j are the two species being compared; C_i=the covariance matrix of the spectral response of i species; μ_i=the mean vector of signature of i; T=transposition function; ln=natural logarithm function; $|C_i|$=the determinant of C_i.

Drone image acquisition and processing

Drone remote sensing was done in IARI experimental field having 183 rice genotypes with 2 parental lines grown during *kharif* season 2018 for acquisition of RGB image and multispectral images using Micasense with red edge. Image acquired was pre-processed and different spectral indices were computed and evaluated for biomass mapping and generated heat map for all genotypes.

RESULTS AND DISCUSSION

Evaluation of rice genotypic response to water deficit stress through thermal imaging

CWSI images of the ten rice genotypes were derived from the thermal images. The mode values were also computed for all the images so that the highest occurring CWSI value can be quantified for a particular image.The CWSI images of well watered or non-stressed plants (Fig. 1a) prove the fact that lower CWSI values exhibit no or very low water deficit stress in plants. The CWSI values were observed in 0.00 to 0.5 ranges. The CWSI images of water deficit stressed genotypes (Fig. 1b) demonstrated high CWSI values and were found between 0.17 to 1.00 ranges. The highest CWSI value depicting genotype was MTU1010.

Frequency distribution (Fig. 2) of CWSI values for each genotype in both the stressed and non-stressed conditions, were plotted. The plants with CWSI value near zero reflect no water deficit stress whereas CWSI value as 1 shows highest water deficit stress in the plant. The frequency distribution plots clearly demonstrated the response of all genotypes during water deficit stress condition. The genotypes which were kept under water stress exhibited a shift in frequency towards the higher side (Fig. 2b) of the CWSI values whereas genotypes of normal irrigation condition were observed to stick around lower values of CWSI (Fig. 2a). In this study, the crop canopies with water deficit stress condition exhibit CWSI values between 0.4 to 1.0 ranges reflecting considerable drought condition whereas CWSI values between 0.0 and 0.4 ranges reflect that the plants are showing negligible or very low water deficit stress.

The rice genotypes Pusa Sugandha and APO expressed high tolerance to water deficit stress whereas CR-143, MTU 1010 and Pusa Basmati were discovered to be highly susceptible to the drought condition (Fig. 3). The higher susceptibility to drought shown by MTU 1010, Pusa Basmati, IR-64 and Patchaiperumal was found to be coinciding with their genetic trait of being drought sensitive rice genotypes. The CR-143 is genetically a drought tolerant genotype but in this study, it demonstrated the highest drought affected characteristics that could be due to a multitude of unfavourable factors such as highest reduction in RWC, lower membrane, and lower osmotic potential etc. In this study, genetically drought sensitive genotypes i.e. MTU 1010, Patchaiperumal, Pusa Basmati-1, IR 64 exhibited the same drought susceptibility also by CWSI method.

Effect of water deficit stress on spectral signature

Normally the plants of a particular crop show a similar pattern of reflectance spectra. But water deficit stress conditions bring noticeable changes in reflectance spectra. The study shows the reflectance patterns of plants with different water deficit stress conditions i.e. decline in relative water content. The water content varies from 96.5% to 0.7%. The reflectance of the fresh plant was less whereas the reflectance of the dry plant was high (Fig. 4). The reflectance in SWIR region increases as the RWC decreases from the highest to lowest. The reason behind the increase in reflectance is weakening of the water absorption

(a) (b)

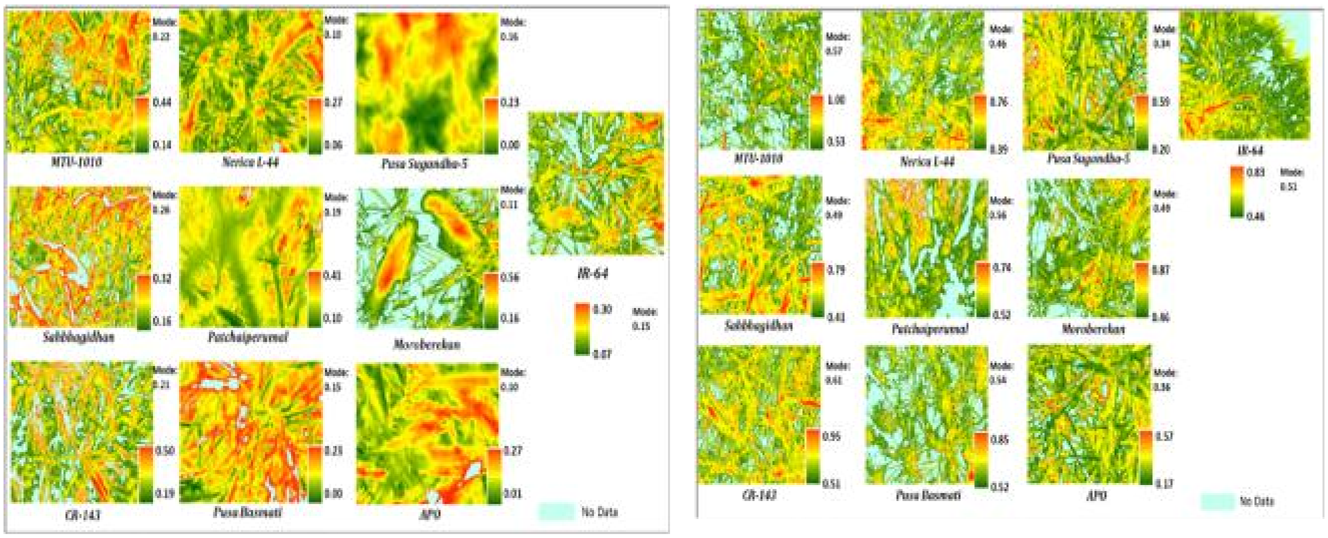

Fig. 1. CWSI image for Ten Genotypes of Rice CropFields in (a) Well watered condition and (b) Water Deficit Stress condition depicts sensitivity of the crop to drought condition. Higher values of CWSI reflect high water deficit stress condition.

(a) (b)

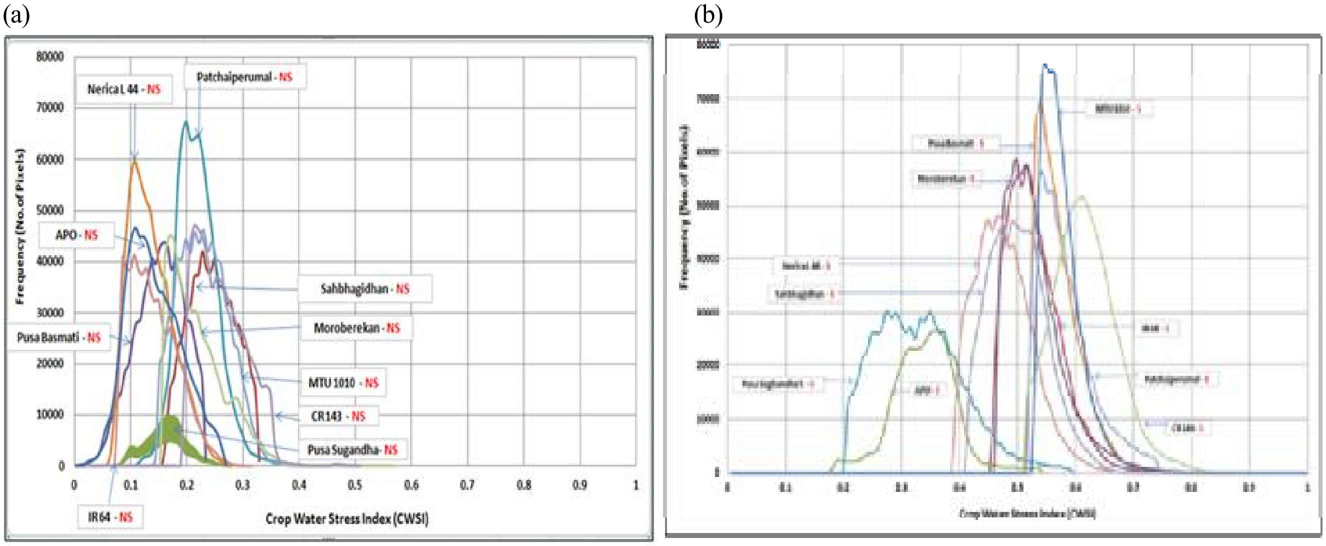

Fig. 2. Quantification of response of rice genotypes to (a) the non-stressed (NS) and (b) stressed condition using CWSI images.

features at1400 nm and 1900 nm. A similar pattern of increasing reflectance with a decrease in water content was observed at 350 to 700 nm wavelength region. The spectrum in the blue and red regions (chlorophyll a and b absorption ranges) was showing a trend of higher reflectance with decreasing water content due to loss of chlorophyll. A shift of 1400-1925 nm wavelength range towards shorter wavelengths was observed with the drying of leaves and increase in spectral reflectance is also visible. With the decrease in relative water content, the absorption features in 1400 to 1500 nm and 1850 to 1900 nm were seen as becoming shallow. The reason behind the decrease in absorption is weakening of water absorption features due to the decrease in water content. The scattering in spongy mesophyll at 810 to 1350 nm was also reflected a similar trend of increasing reflectance with the decrease in water content. In addition, absorption at the middle

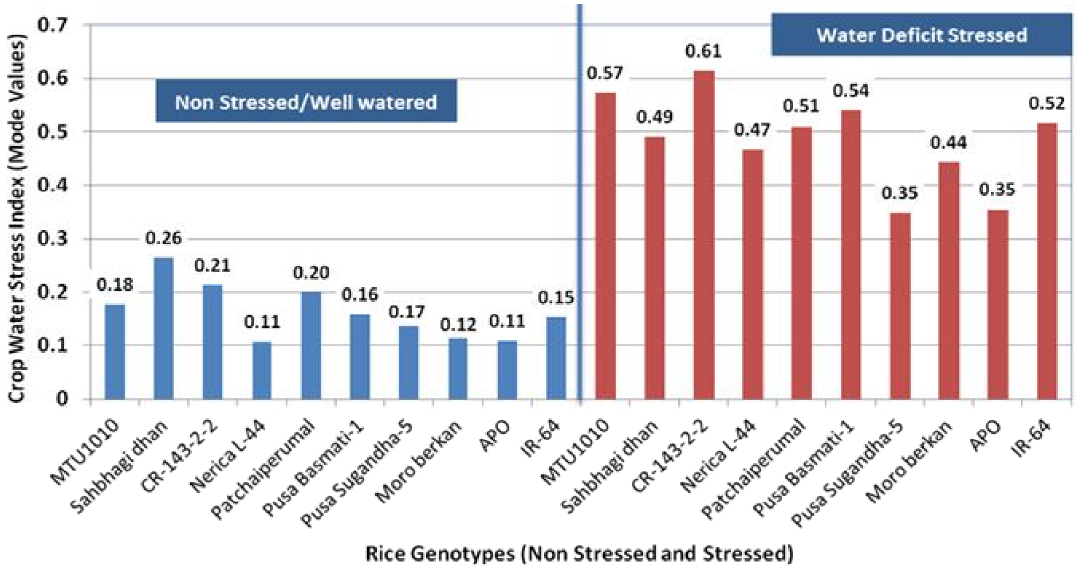

Fig. 3. Identification and quantification of the highest drought sensitiveness in the ten rice genotypes thorough mode values from CWSI images. Figure shows that during water deficit stress condition, APO and Pusa Sugandha-5 exhibited the highest resistance to the drought whereas CR-143, MTU-1010 and Pusa Basmati-1 ascertained highest sensitiveness to the drought.

infrared (1100-2500 nm) is also a zone of strong absorption, primarily by water in a fresh leaf and secondarily by dry matter (*e.g.*, protein, lignin and cellulose) when the leaf wilts (Jacquemoud and Ustin, 2001), become more visible with decrease in RWC.

Relationship between spectral index and RWC

The relationship between conventional water band indices with RWC was evaluated and revealed that the

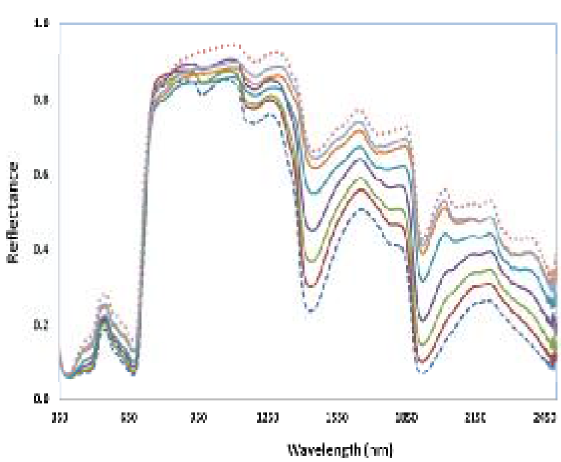

Fig. 4. Representative mean spectral reflectance observations of the genotypes with decreasing RWC (%) in leaves of rice, showing percentage of RWC and corresponding spectra at different time intervals.

maximum difference water index (MDWI) exhibits the strongest correlation with R^2 as 0.92 for both calibration and validation sets (Fig. 5). The MDWI is computed using the maximum reflectance value from max1500-1750 nm and minimum reflectance value from min1500-1750 nm located at the atmospheric window between 1500 and 1750 nm. The MDWI performed well because it allows the best combination of numerator and denominator from 1500 and 1750 nm wavelength range. This dynamism of choosing better absorption features, under varying plant water-deficit stress conditions provides better results (Eitel et al., 2006; Peñuelas et al., 1997).

The lambda versus lambda contour plotting approach has the advantage of providing an efficient selection of the optimal combination of wavebands for development of the effective spectral indices. The contour maps of R^2 values from linear regression between RWC and all possible combinations of RSI (Ratio spectral index-ratio approach) and NDSI (Normalized difference spectral index -normalized difference approach) reveal hotspot positions that have high correlation values. In the present study, one highest R^2 value each for RSI and NDSI was extracted from the hotspots which were found at 1233 and 1305 nm combination. Therefore, on the basis of highest R^2, the

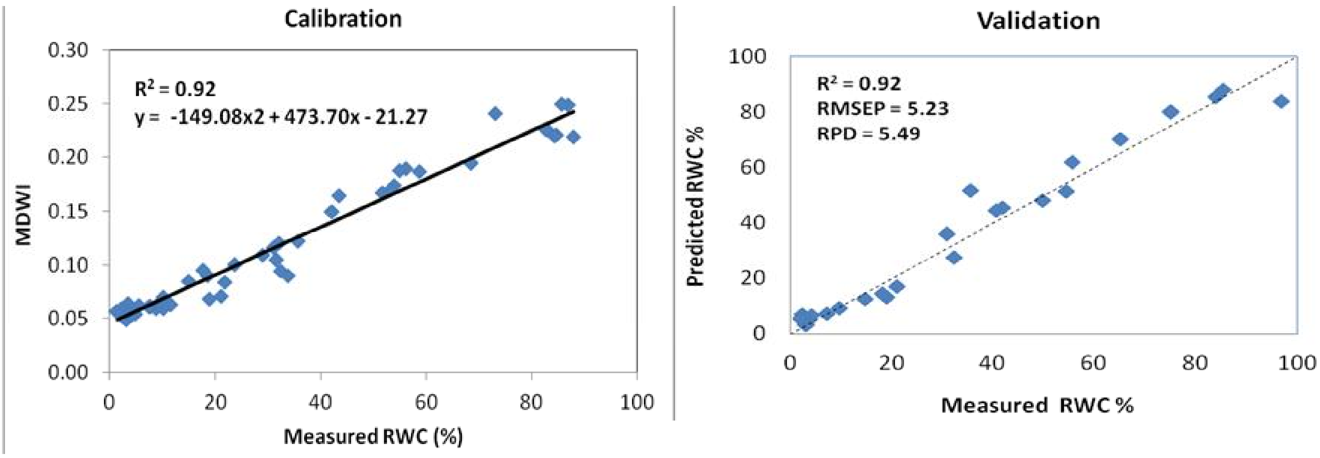

Fig. 5. The Calibration model developed through the relationship between MDWI and Measured RWC (%) and its validation. (Calibration - N=55 & validation - N=25). The solid black line is regression line and dotted line is 1:1 line.

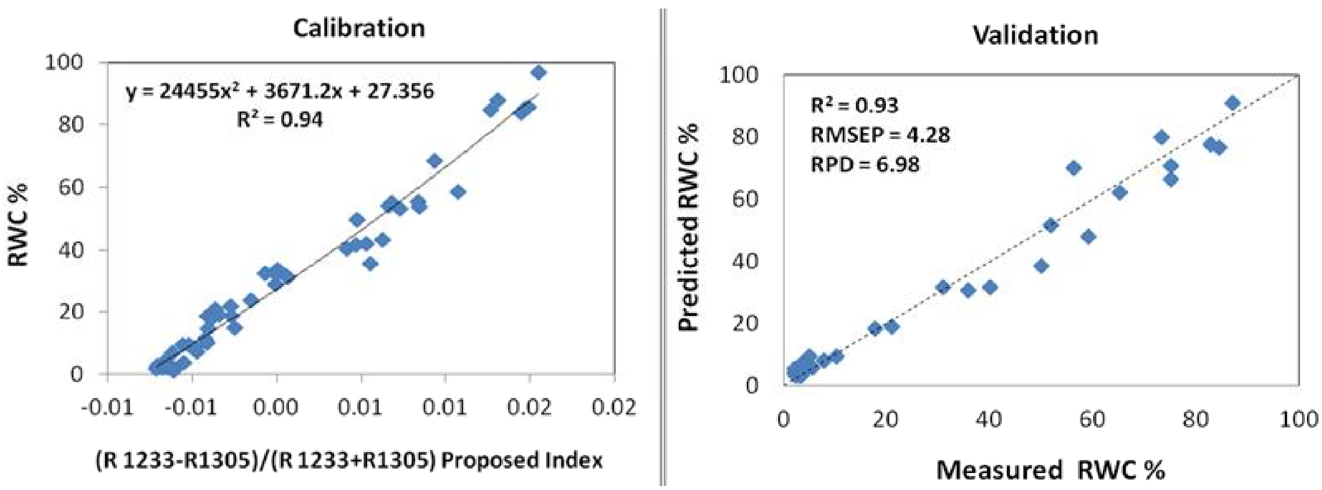

Fig. 6. The proposed Normalized Difference Ratio Index (R1233-R1305)/(R1233+R1305) for prediction of RWC. (Calibration - N=55 & validation - N=25). The solid black line is regression line and dotted line is 1:1 line.

best combinations selected were ratio index (R1233, R1305) and normalized difference ratio index (R1233, R1305) for RWC. The 2nd order polynomial equation was found to be the best in predicting RWC with both ratio index and normalized difference ratio index (R^2_{Cal}= 0.94, RMSEP = 4.27; R^2_{Cal} = 0.94, RMSEP = 4.28, respectively) (Fig. 6). The RSI and NDSI R^2 values for both calibration and validation have P-value as <0.00001 and the result is significant at $p < 0.05$.

Multivariate techniques for RWC estimation

The PLSR followed by MLR was proved as the best technique for RWC prediction model development, out of all multivariate techniques evaluated through this

This study evaluated multivariate techniques and indices based approach including contour plotting. The comparison of results clearly reflects that use of multivariate techniques enhances the prediction capability of models significantly. The multivariate techniques have many positive approaches compared to conventional indices based approach like self-identification and removal of outliers, use of principalcomponents, ability to deal with multi-collinearity, use of decision tree approach etc. Multivariate techniques utilize all the water absorption related bands which increase model's accuracy considerably by unveiling improved sensitivity to

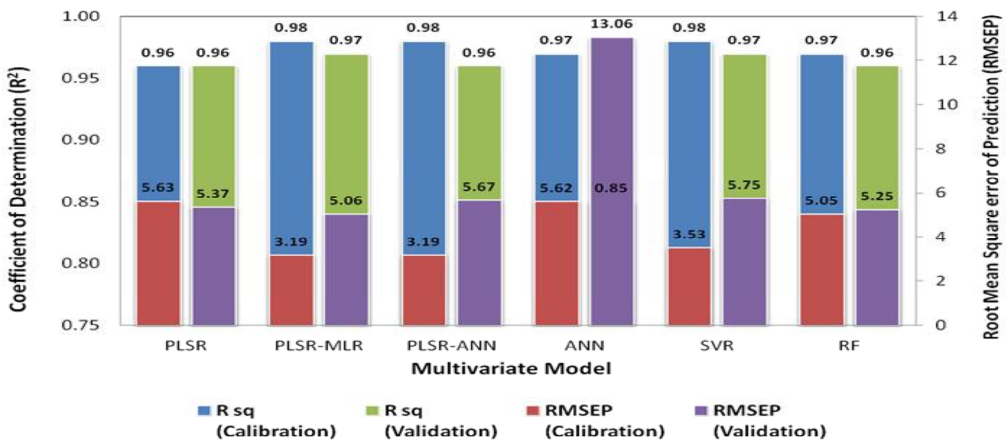

Fig. 7. Performance assessment of multivariate models as well as neural network models using R2and RMSEP of calibration and validation.

changes in the RWC whereas index-based approaches use only two or three prominent water absorption bands. This study has successfully applied the MLR and ANN models on PLSR selected optimum wavebands which increased the accuracy of model significantly. Use of PLSR selected optimum wavebands as input removed the multi-collinearity problem in MLR, and provided outliers free x variables to ANN; consequently, improving the efficiency of the PLSR model. study. The model equation developed through PLSR-MLR techniques is also useful in monitoring water content in rice crop. The second best model developed was the combination of PLSR and ANN. The support vector regression was also proved to be a useful technique with satisfactory results. The SVR determines maximum-margin hyperplane; therefore, it reduces the prediction error. The ANN is vulnerable to outliers, therefore when applied on the whole dataset; its prediction was very poor. The random forest is an ensemble tree classifier and has the goodness of decision tree system. The RF proved as an intermediate classifier compared to others. It was proved slightly better over PLSR in this study. In the PLSR equation, every coefficient has a RMSEerror associated with it which makes it more susceptible todeviation (Krishna et al., 2014), therefore PLSR model developed through all of the x variables, produced intermediate results compared to PLSR-MLR combination. The order ofperformance of the multivariate models with respect to R2 and RMSEP isas follows: PLSR-MLR>PLSR-ANN> SVR >RF > PLSR > ANN (Fig. 7). This order of performance is also supported by the value of RPD for all models.

Multivariate technique for plant sugar estimation

Similar tospectroscopy based RWC estimation, the potential of VNIR and SWIR spectra was also explored for rapid quantification of sucrose, reducing sugars and total sugars in rice as affected by water-deficit stress without any chemical reagents. New proposed RSI and NDSI were able to predict sugar content with good accuracy. Among the multivariate models, the best results were obtained using the ANN, SVMR and MARS for reducing sugars, sucrose and total sugars, respectively with respect to R^2 values. Combing VNIR and SWIR spectroscopy with chemometrics can provide a rapid method for selection for rice genotypes for water-deficit stress phenotyping (Das et al., 2018).

Differential response of rice genotypes to water stress

The water loss behaviour of the different rice genotypes with time starting from 1st spectral measurement within 2 hours of harvesting, T_0 and last spectral measurement after 27 hours at T_{11}, is shown in fig.8. Among the rice genotypes Pusa44 and IR64 showed least change in the difference of RWC and Vandana showed the highest change. But when the difference of index was calculated for consecutive measurements, Vandana showed the least change followed by IR64 and Pusa44; Nagina22 and Pusa Basmati6 showed the highest change. This happens due to least change in the plant water content or least difference in leaf reflectance at

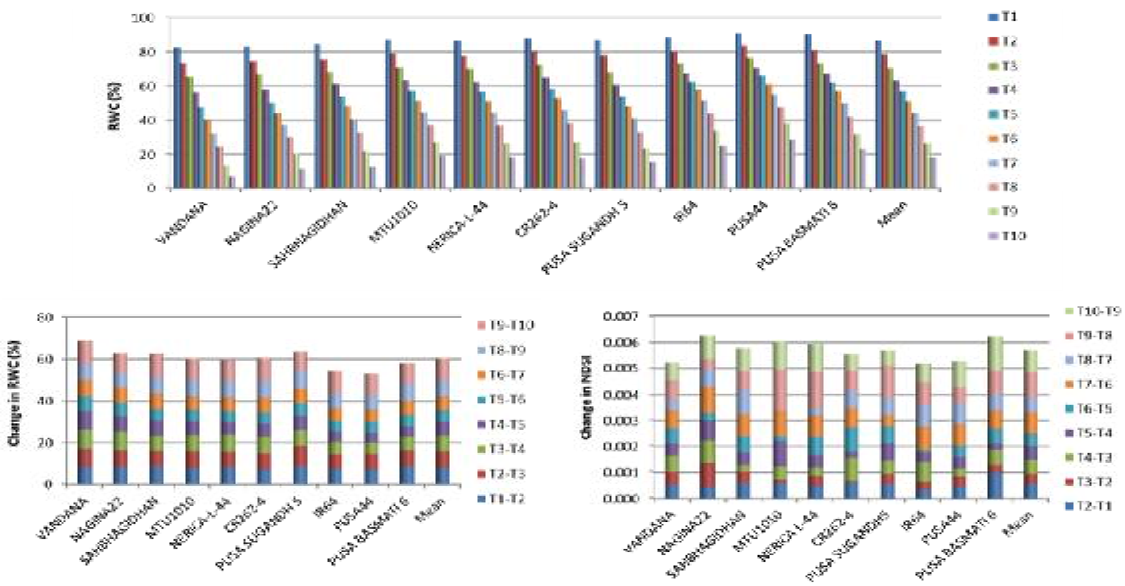

Fig. 8. Differential response of rice genotypes to different stress level due to water loss at different time interval (T1 to T9) as measured through RWC and proposed spectral index, NDSI.

fully turgid and highest water loss conditions. So, these genotypes were found to have more resistance towards water stress. Dasgupta et al. (2015) also reported of all multivariate techniques evaluated through this study. The model equation developed through PLSR-MLR techniques is also useful in monitoring water content in rice crop. The second best model developed was the combination of PLSR and ANN. The support vector regression was also proved to be a useful technique with satisfactory results. The SVR determines maximum-margin hyperplane; therefore, it reduces the prediction error. The ANN is vulnerable to outliers, therefore when applied on the whole dataset; its prediction was very poor. The random forest is an ensemble tree classifier and has the goodness of decision tree system. The RF proved as an intermediate

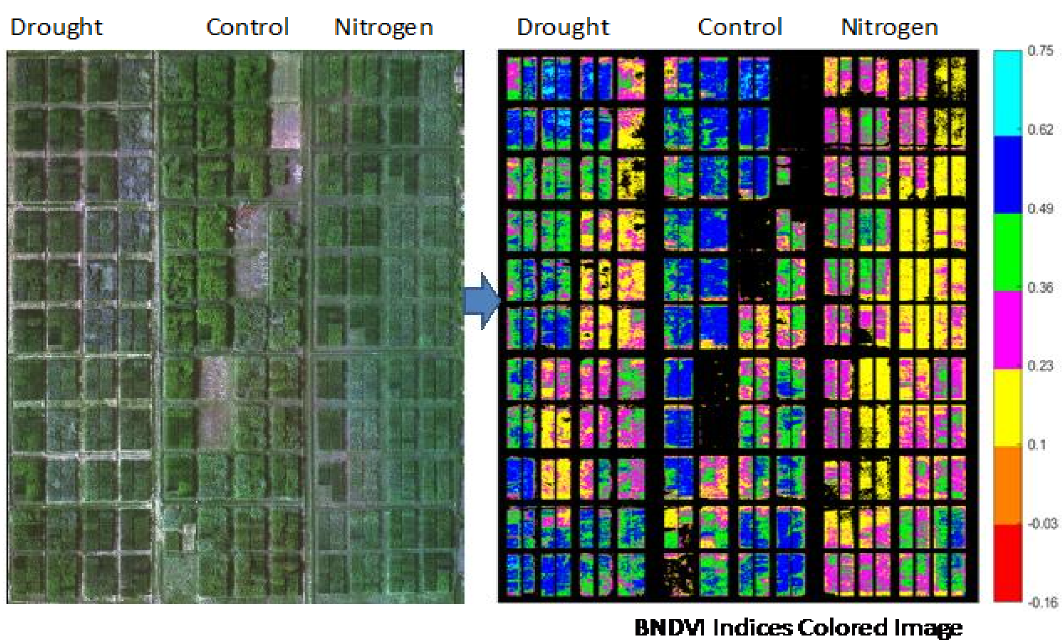

Fig. 9. Multispectral (MicaSense) image of Rice 2018 Field Experimentation taken from drone on 6 th October 2018.

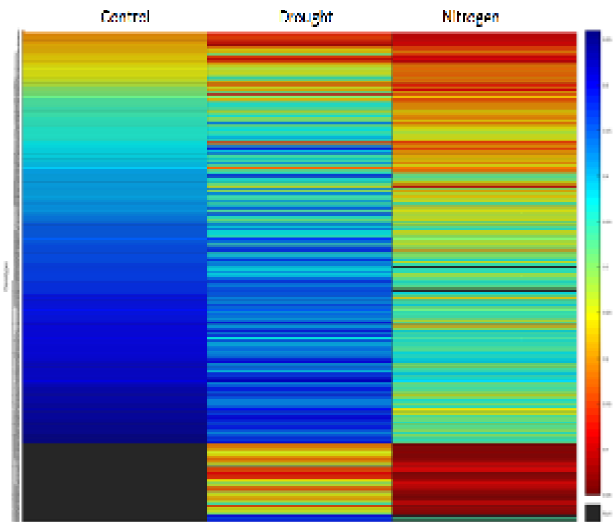

Fig.10. Heat map of BNDVI processed from drone multispectral image of rice 2018 field experiment.

classifier compared to others. It was proved slightly better over PLSR in this study. In the PLSR equation, every coefficient has a RMSE error associated with it which makes it more susceptible to deviation (Krishna et al., 2014), therefore PLSR model developed through all of the x variables, produced intermediate results compared to PLSR-MLR combination. The order of performance of the multivariate models with respect to R^2 and RMSEP is as follows: PLSR-MLR>PLSR-ANN> SVR >RF > PLSR > ANN (Fig. 7). This order of performance is also supported by the value of RPD for all models.

This study evaluated multivariate techniques and indices based approach including contour plotting. The comparison of results clearly reflects that use of multivariate techniques enhances the prediction capability of models significantly. The multivariate techniques have many positive approaches compared to conventional indices based approach like self-identification and removal of outliers, use of principal Vandana as water-deficit stress tolerant variety under field conditions. So the results of the spectral indices based approach were found to be more effective to differentiate the susceptible and tolerant genotypes as compared to conventional destructive methods.

Spectral discrimination of rice genotypes

CART analysis was used to reduce the numbers of significant bands (n = 1759) selected by ANOVA analysis to fewer bands that could optimally separate the rice genotypes. These spectral bands are: 350, 532, 553, 665, 717, 730, 823, 887, 910, 960, 1440, 1960, 1973, 1979, 2009, 2296 and 2313 nm for rice. From this analysis we can infer that these seventeen wavelengths for rice could potentially discriminate genotypes from each other. The separability index (J-M distance) was computed between each genotype pair as well as stress levels utilizing the CART selected wavelengths. The results revealed (Table 1) that separability between the genotype pairs was greater than the required J-M distance value of 1.94.

Field phenotyping using multispectral imaging from drone platform

RGB images and multispectral image from micasense with red edge camera captured from 60 m height on 6th October, 2018 was pre-processed and mapped as true color composite and Blue band based normalized difference vegetation index (BNDVI) (Fig. 9). Some of the fields were found fallow as some early genotypes were harvested in control treatment with recommended dose of irrigation and nitrogen. BNDVI was found the best for biomass mapping having highest R^2 of 0.59 (Fig. 10). It is the normalized difference ratio of energy received in NIR and Blue bands of the sensor and expressed as BNDVI = (NIR-Blue)/(NIR+Blue). Based on BNDVI value, heat map of 182 genotypes was prepared (Fig. 11) capturing their response to differential treatment of drought and nitrogen.

CONCLUSION

In this study, thermal imageries were used to evaluate the behaviour of genotypes during drought condition. The susceptibility and resistance of genotypes to water deficit induced stress were evaluated through analysis. The mode values of normalized temperature and frequency distribution curves of ten rice genotypes were analysed and their behaviour to water deficit stress was identified. CR-143, MTU-1010 and Pusa Basmati-1 genotypes were found to be the highest sensitive whereas APO and Pusa Sugandha-5 genotypes were ascertained as the most tolerant genotypes to the drought condition. This study successfully evaluates the indices based, multivariate techniques based and neural networks based approaches to predict relative water content (RWC) under water deficit stress condition of

Table 1. Jeffries-Matusita (JM) distance analysis for 14 rice genotypes indicating all pair potentially separable being values are more than 1.94.

	JM distance													
	CR262-4	SAHBHA GI DHAN	VANDANA	IR64	CROSSA	SWARNA	MTU	CR143 1010	RASI -2-2	N22	PS5	NL44	PUSA44	PB6
CR262-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAHBHAGI DHAN	1.99	-	-	-	-	-	-	-	-	-	-	-	-	-
VANDANA	2	1.99	-	-	-	-	-	-	-	-	-	-	-	-
IR64	1.99	1.99	1.99	-	-	-	-	-	-	-	-	-	-	-
CROSSA	1.99	1.99	1.99	1.99	-	-	-	-	-	-	-	-	-	-
SWARNA	2	1.99	1.99	1.99	1.99	-	-	-	-	-	-	-	-	-
MTU1010	1.99	1.99	1.99	1.99	1.99	1.99	-	-	-	-	-	-	-	-
CR143-2-2	1.99	1.99	2.00	1.99	1.99	2	1.99	-	-	-	-	-	-	-
RASI	2	1.99	1.99	1.99	1.99	1.99	1.99	1.99	-	-	-	-	-	-
N22	2	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	-	-	-	-	-
PS5	2	1.99	1.99	1.99	1.99	1.99	1.99	2	1.99	1.99	-	-	-	-
NL44	2	1.99	1.99	1.99	1.99	1.99	1.99	2	1.99	1.99	1.99	-	-	-
PUSA44	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99	-	-
PB6	2	1.99	1.99	1.99	2.00	1.99	1.99	2	1.99	1.99	1.99	1.99	1.99	-

rice genotypes with significant accuracy. Existing water band indices were evaluated and new water band indices sensitive to water stress were proposed. The MDWI was found to be the best index among all conventional existing indices. The newly proposed indices outperformed all existing indices.

The multivariate model developed through PLSR and MLR techniques (PLSR-MLR model) proved to be the best ((yielded high R2 and low RMSEP) followed by the model developed through PLSR and ANN techniques (PLSR-ANN model) for estimation of RWC in rice crop. Thus from this study it may be concluded that timely detection of water deficit stress is quite important for precision agriculture. Same approach was used by authors for assessing sugar content in rice plant through spectroscopy for selecting rice genotypes for water deficit stress phenotyping. Spectral based new proposed index (NDSI) for RWC estimation was also evaluated for differential response of genotypes to water deficit stress and was useful to identify genotypes found to be relatively sensitive and resistant to drought. Spectral signatures were could be used to discriminate 14 rice genotypes and also their stress levels using three tier hierarchical approach. Drone based multispectral imaging could be used to capture differential response of 182 rice genotypes having treatment of drought and nitrogen.

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