Under challenging environmental situations, significantly improved crop varieties are needed to cope up with the rapidly growing human population scenarios (Furbank et al. 2009, Sticklen et al. 2007). The greatest challenge facing agricultural scientists and policy makers is to meet the future demand of agricultural production under challenging environmental situations (Bruinsma 2003). For this purpose, systematic quantification of phenotypic traits or components of a particular genotype in a given environment is necessary. Plant phenomics is the study of plant growth and performance based on morphology, physiology and phenotypic traits or characteristics of the plant. But the conventional measurement of these traits are recorded either manually or visually which is not only time-consuming and labour-intensive but may also error prone to acquire large amount of dataset. Therefore, focus has been shifted on precise, accurate and rapid phenotyping from the last few years. In this context, high-throughput image analysis (Furbank et al. 2009, Jansen et al. 2009) is being used to extract several phenotypic parameters related to plant growth, development, physiology, yield and the basic measurement of individual quantitative parameters that form the basis for the more complex traits.

Plant biomass plays a vital role in the study of functional plant biology and growth analysis. Growth rate of the plant as well as net primary production are determined on the basis of plant biomass. Shoot fresh weight is used to estimate yield as well as biomass (Poorter and Nagel 2000, Niklas and Enquist 2002). Conventional biomass is measured by gravimetric weighing of harvested sample. This method is very time consuming, labor intensive and destructive. In this context, digital image analysis has been developed as an alternate approach. VIS image is widely used to estimate biomass as a linear function of projected shoot area (Paruelo et al. 2000, Mizoue and Masutani 2003, Golzarian et al. 2011, 2012).
Schirrmann et al. 2016). Biomass has significant amount of water and hence it is key determinant of fresh biomass (Seelig et al. 2008). Water in biomass absorbs radiation in the NIR region, so, NIR reflectance image can be used to estimate moisture content (Stenberg et al. 2010, Fernández et al. 2015). Hence, prediction models developed by only using projected shoot area from VIS may not estimate fresh biomass accurately.

According to Tsaftaris et al. (2016), future trend in image-based plant phenotyping will be a combined effort of image processing and machine learning technique for feature extractions and data analysis purpose. The main drawback of conventional regression approach is that it assumes the dependent variable as a linear function of independent variables but real data set is rarely pure linear. On the other hand, Artificial Neural Networks (ANNs) model is considered as a class of generalized nonlinear model that can capture various nonlinear structures present in the data set. The main advantage of ANN model is that it does not require prior assumption of the data generating process, instead it largely depends on characteristics of the data, and popularly known as data-driven approach (Behera et al. 2013, Ray et al. 2016, Ebrahimi et al. 2017). Hence, in this study ANN approach was employed to estimate the biomass by using combination of VIS-NIR image data to estimate leaf fresh weight (LFW).

**MATERIALS AND METHODS**

*Image acquisition:* Rice leaves from mini-core rice genotypes were harvested and subjected to dehydration at room temperature to generate samples of rice leaves with different fresh mass. The leaves were arranged in a hanger as shown in Fig 1. Images of 104 set of leaf samples were collected using VIS and NIR sensors (LemnaTec GmbH, Aachen, Germany) at Nanaji Deshmukh Plant Phenomics Centre, ICAR-IARI, New Delhi, India under the experiment carried out in year 2017–18. Total 26 images were taken and each image had 4 sets of leaves (i.e. 104 sets) with 3 leaves per set. RGB (Red Green Blue) camera of spectral response 400 to 700 nm with sensor (6576 × 4384 pixels) was used to collect VIS images, whereas GoldEye P-032 SWIR camera of spectral response 900 to 1700 nm with InGas sensor (640*480 pixel) was used for taking NIR images. A uniform background of white colour was maintained at the time of imaging to easily separate the background and leaf regions. LFW was measured for each set with the help of weighing machine which was used as ground truth value to validate the approach.

*Proposed methodology of leaf fresh weight estimation* 

GLP from VIS image and NIR_MGI from NIR image were obtained by using the following methods:

**Computation of GLP from VIS image:** It was done as per the following steps:

Step 1: Apply operation ‘O’ for background extraction and Otsu’s thresholding(Otsu, 1979) was used for background removal.

\[
O = \frac{(G-R)}{(G+R)}
\]

Where G, Green content per pixel; R, Red content per pixel

Step 2: Multiply outcome image of step 1 with the original image for extracting the original gray values.

Step 3: Extract that portion of the resulting image where G>B and G>R, it will be Green Leaf Area (GLA).

Step 4: Green Leaf Proportion (GLP) was calculated by using the following formula:

\[
GLP = \frac{GLA}{TA}
\]

Where GLA, Green Leaf Area; TA, Total Leaf Area

**Computation of NIR_MGI from NIR image:** For background subtraction and mean estimation of gray value of foreground from NIR image, NIR imaging algorithm of PlantCV v2 (Gehan et al. 2017) was implemented in MATLAB software (version 2015b, MathWork, Natick, MA) along with several image processing techniques (Lillesand et al. 2015). High gray values represent high reflectance and indicate low water content, while low gray-scale values represent high absorption and high water content in the leaf. This algorithm was slightly modified as per our requirement on the basis of background. The modified algorithm is given here.

Step 1: Subtraction of original image (leaf image) from background image.

Step 2: Thresholding of the subtract image using Otsu’s thresholding algorithm (Otsu, 1979): In this step, pixels that have signal value less than the threshold value will be set to 0 (black) and else set to 1 (white).

Step 3: Image Sharpening: This step was done for capturing maximum amount of object of interest (leaf) especially when the background pixel intensity is problematic (full of noise). For this purpose Second derivative Laplacian filter was applied to the original image.

Step 4: Subtraction of the filtered image from the original image for increasing the contrast between object and background.

Step 5: Sobel filtering of the original image was done for highlighting more ambiguous boundaries within the image.

Step 6: Median blur filtering was applied on the Sobel filtered image to decrease the amount of noise present in that image.


Step 8: Thresholding of the output image using Otsu’s thresholding algorithm.

Step 9: Erode the output image of step 8 using 3*3 filter.

Step 10: Add outcome image of step 2 and step 5.

Step 11: Multiply outcome image of step 10 and the original image for extracting the original gray values.

**Flow diagram to predict leaf fresh weight:** Two image derived parameters (GLP & NIR_MGI) were used as input for building artificial neural network model (ANN) to estimate LFW (Fig 1) in rice plant.

**Artificial Neural Network:** Artificial Neural Network (ANNs) model is able to capture various non-linear structures present in the data set. It depends on characteristics of the
data. Feed forward multilayer network is the most popular network for regression problem. This model is characterized by a network of three layers of simple processing units; the first layer is one input layer, the middle layer is the one or more hidden layer and the last layer is one output layer.

The relationship between the output \( (y) \) and the inputs \( (x_1, x_2, ..., x_p) \) can mathematically be represented as follows:

\[
y = f\left(\sum_{i=1}^p \omega_j g\left(\sum_{m=0}^q \omega_{ij} x_m\right)\right)
\]

where \( \omega_j \) (\( j = 0, 1, 2, ... , q \) ), and \( \omega_{ij} \) (\( i = 0, 1, 2, ... , p, j = 0, 1, 2, ... , q \) ), are the model parameters often called the connection weights, \( p \) is the number of input nodes and \( q \) is the number of hidden nodes, \( g \) and \( f \) denote the activation function at hidden and output layer respectively. Activation function defines the relationship between inputs and outputs of a network in terms of degree of the non-linearity. For regression problem generally sigmoid activation function is employed in hidden layer and identity activation function is employed in the output layer.

The selection of appropriate number of hidden layer and hidden nodes in each layer is important in ANN modeling. Though there are no established theories available for the selection of hidden layers and hidden nodes, hence, experiments are often conducted for the determination of number of hidden layer and hidden node in each layer. In this study, Gradient decent back propagation has been employed for estimation of weight in neural network.

**Model development:** For developing the model, the dataset was divided into two parts randomly: 85% for training and 15% for testing. Feed-Forward Multilayer Perceptron Neural Network (MLPNN) was fitted using the NIR_MGI from NIR image and GLP from VIS image as the input and the corresponding actual LFW as the output for the training data set. As two independent variables (NIR_MGI and GLP) and one dependent variable (LFW) were used in this study, the ANN architecture consists of one input layer with two input nodes and one output layer with one node. The choice of suitable number of hidden layer and hidden node within each layer is critical in ANN modelling. Despite the fact that there are no settled hypotheses accessible for the choice of hidden layer and hidden node, consequently tests are frequently used for the determination of the ideal estimations of hidden layer and node. Hence, distinctive combinations of hidden layer and hidden nodes in each layer has been attempted, out of which best fitted ANN model was selected. “Neuralnet” package of R software (Teodoro 2015) has been employed for the model development.

**Measurement of the performance:** Performance of the model has been judged by computing Mean Absolute Percent Error (MAPE) and Root Mean Square Error (RMSE). The model with less MAPE and RMSE is preferred for prediction purposes. The MAPE and RMSE are computed as follows:

\[
MAPE = \frac{1}{n} \sum_{i=1}^n \left| \frac{y_i - \hat{y}_i}{y_i} \right| \times 100
\]

\[
RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2}
\]

Where, \( n \) is the total number of observations to be estimated, \( y_i \) is the actual value of observation \( i \) and \( \hat{y}_i \) is the corresponding estimated value.

**RESULTS AND DISCUSSION**

For analysis purpose, the experimental dataset was randomly divided into two parts: 89 (85%) for training and 15 (15%) for testing. ANN model has been developed by using two independent parameters (GLP from VIS image and NIR_MGI from NIR image) and one dependent parameter (ground truth LFW). Distinctive combinations of hidden layer and hidden nodes in each layer of ANN have been attempted and out of which one hidden layers with 5 hidden nodes were performing superior than other combinations. The architecture of the best fitted ANN model is given in Fig 2.

Performance of the proposed approach has been compared with the conventional approach which is based on linear function of projected shoot area (Paruelo et al.)
The proposed approach has also been compared with the regression approach by taking the same input (i.e. GLP & NIR_MGI) as independent parameter and ground truth LFW as dependent parameter. RMSE and MAPE was measured to compare the performance of the approaches both in training and testing dataset and the results are given in Table 1. The above table reflect that the proposed approach is outperformed than the other approaches in both training and testing dataset. The graphical plot of actual fresh weight of leaf versus fresh weigh predicted with different approaches (ANN, regression and conventional) are plotted in Fig 3. This also showed that the hypothesized approach with ANN modeling is superior as compared to other approaches.

### Conclusion

In this study, a new approach has been proposed to estimate LFW by using ANN model with combination of VIS-NIR images. For background subtraction and estimation of mean gray value of the foreground, algorithm of NIR imaging pipeline of Plant CV has been successfully implemented in MATLAB software. The main feature of the proposed approach is that the concept of moisture content has been included which is the most important basis of LFW. This study uncovered that GLP from VIS image and

### Table 1 Comparison of proposed ANN based, regression and conventional approach in training and test dataset

<table>
<thead>
<tr>
<th>Indices of Prediction accuracy</th>
<th>Training</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional approach based on linear function of projected shoot area</td>
<td>Regression approach based on GLP and NIR_MGI</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>MAPE</td>
<td>27.66</td>
<td>23.44</td>
</tr>
</tbody>
</table>

RMSE, Root Mean Square Error; MAPE, Mean Absolute Percentage Error
NIR_MGI from NIR image can be effectively used for estimating LFW. The ANN approach proposed in this study outperforms over projected shoot area based conventional approach and approach based on GLP and NIR_MGI. This approach may have wider applicability in estimation of fresh biomass in other cereal crops like wheat, barley, maize etc. as its all all having almost similar characteristics.

ACKNOWLEDGEMENTS

The first author acknowledges the fellowship received from IASRI, New Delhi, India to undertake this research work as a part of PhD work. This work was supported by National Agriculture Science Fund (NASF), ICAR, Grant No. NASF/Phen-6005/2016–17.

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