

## **Development of Non-Destructive Image Analysis Protocol to Assess the Quality of *In-Vitro* Propagated Orchid Plantlets**

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*In-vitro* propagated plants are vulnerable to external changes leading poor growth performances depends on the vigour at hardening stage. The accurate procedure to evaluate *in-vitro* propagated plants, prior to hardening is vital through incorporating modern technologies such as image processing, to mitigate the drawbacks in visual evaluation which is used at present. Therefore, this study focuses on developing an image analysis protocol to assess the quality of *in-vitro* propagated Orchid plants. Digital photographs of the culture vessels of Orchid plants which were at the stage of acclimatization were taken inside an illuminated chamber. A visual scoring scale was developed in a scale of 0 to 5 to rank individual plantlets in each vessel. Plant images were isolated from the background and mean red (R), green (G) and blue (B) values were calculated from the segmented images. Through the RGB values, Yellowness, Hue (H), Saturation (S) and Intensity (I) values and thereby Dark Green Colour Index (DGCI) were calculated. Further vegetation indices such as Photosynthetic Vigour Ratio (PVR), Plant Pigment Ratio (PPR), Redness Index (RI), and Green Leaf Index (GLI) were also calculated. The visual evaluation score, DGCI, Yellowness and vegetation indices values were tested to determine the correlation between visual evaluation score and other values obtained using digital photographs. Among all indices, strong correlation ( $p < 0.05$ ) was observed in PVR and RI and then the composite of the values were accordingly regressed with visual evaluation score to select healthy and chlorotic tissues. The high possibility of the use of PVR and RI values composite (PR) to determine the quality of *in-vitro* propagated plantlets, was determined by high R squared value. The function for the quality of the Orchid plants was developed using constant coefficient and coefficient of PR, obtained through regression that could easily apply to select the high quality plantlets.

**Keywords:** Image processing, Orchids, *In-vitro* propagated plantlets, Vegetation indices, Visual score