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Optimizing hydroponic salinity screening: a novel approach using image-based phenotyping for double haploid rice selection

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The development of effective selection criteria and models under hydroponic salinity screening can be used image-based phenotyping (IBP) and statistical analysis to detect double-haploid rice with high adaptability to saline environments. Therefore, this study aimed to identify the IBP selection criteria and develop a model for potential tolerance in double-haploid rice under hydroponic salinity screening. The experimental design was a split-plot randomized complete block design. The main plot was NaCl concentration (0 mM and 120 mM), and the subplots contained ten genotypes with three replications. Selection criteria and model development were identified systematically and comprehensively through the best linear unbiased estimation, stress tolerance index, principal component analysis, factor analysis, and selection index. In addition, validation was also carried out based on conventional morphological characteristics, physiology, Na⁺ and K⁺ contents, and yield in saline land. The results showed that there are two tolerance index models: a morphometric (geometric) index represented by the total area and green area, and a colorimetric index defined by the green area percentage, CIVE, and GLI. The interaction of these indices effectively mapped the double-haploid rice genotypes based on their tolerance levels and adaptability to salinity stress. The colorimetric index was a reliable indicator of the potential adaptability of double-haploid rice lines in saline fields. This study provides a novel approach for developing effective selection criteria and models for rice tolerance, especially double-haploid line, under hydroponic salinity screening, which can accelerate the identification of genotypes with high adaptability to saline environments.

KEYWORDS

BLUE, colorimetric index, image processing, morphometric index, *Oryza sativa*

1 Introduction

Rice development is a priority in maintaining global food security. This commodity is the main food source for most of the world's population, particularly in Asia (Fukagawa and Ziska, 2019; Mohidem et al., 2022). However, this commodity faces various production challenges, including the impact of global warming (Arifah et al., 2022; Jamal et al., 2023). An increase in Earth's temperature will induce an increase in sea level, which will indirectly increase salinity levels on land (Griggs and Reguero, 2021; Jabir et al., 2021; Mazhar et al., 2022; Olson et al., 2022). This is crucial for island regions, such as Indonesia (Rumanti et al., 2018; Anshori et al., 2021; Dewi et al., 2022). This impact is becoming more serious with expert changes in the function of agricultural land in buildings, so that agricultural areas lead to suboptimal areas, such as coastal areas (Azadi et al., 2021; Nong et al., 2021; Sekaranom et al., 2021). Rumanti et al. (2018), stated that 29% of the total rice land in Java (Indonesia's rice production center) is located in coastal areas. This phenomenon reduces national rice production, which is correlated with a threat to food security in Indonesia (Rumanti et al., 2018; Anshori et al., 2021; Arifah et al., 2022). This will also have an increasingly negative impact on global warming, which will continue to occur dynamically (Rumanti et al., 2018). Therefore, the development of rice varieties that are tolerant and adaptive to salinity stress is crucial for mitigating the effects of global warming on rice cultivation in coastal areas.

The assembly of salinity stress-tolerant varieties is largely determined by three components: the screening method, selection environment, and selection characteristics (Reddy et al., 2017; Anshori et al., 2018; Singh et al., 2021). Screening approaches have been strongly linked to the critical phase of plants (Reddy et al., 2017; Wu et al., 2019; Chen et al., 2021; Farid et al., 2021; Singh et al., 2021; Afzal et al., 2023). According to a study by Bado et al. (2016), Gerona et al. (2019), and Singh et al. (2021), there are two critical phases in rice exposed to salinity: the early seedling and reproductive phases. However, the most widely used screening method is rapid screening in the seedling phase using the concept of hydroponics (Liu et al., 2019; Chen et al., 2020; Anshori et al., 2021; Arifuddin et al., 2021; Farid et al., 2021; Singh et al., 2021; Lutambi et al., 2024). This screening method was recommended by the IRRI for rapid salinity selection (Bado et al., 2016; Farid et al., 2021; Singh et al., 2021). The selection environment for this screening has also been reviewed and recommended as 12 dS/m (Anshori et al., 2021; Singh et al., 2021). However, this concept still needs development concerning the selection criteria. In general, tolerance levels are determined only by assessing the tolerance scores (Bado et al., 2016; Anshori et al., 2021). This is considered less objective when describing the tolerance level of a genotype. The tolerance score is considered a categorical approach in assessing tolerance potential (Purbajanti et al., 2019), whereas salinity stress is controlled by minor genes that are quantitative (Rahman et al., 2019; Kumar et al., 2022; Sugasi et al., 2024). In addition, salinity stress can induce other secondary stresses such as osmotic stress, ion homeostasis, and oxidative stress (Reddy et al., 2017; Wu et al., 2019). This phenomenon makes salinity tolerance patterns more complex (Erythrina et al., 2021). Moreover, tolerance during the seedling phase is not necessarily related to tolerance potential during the reproductive phase (Bado et al., 2016; Chen et al., 2021; Singh et al., 2021). Therefore, comprehensive selection criteria and models are required for screening hydroponic salinity.

The development of the selection criteria and models could not be separated from the included genotypes. Each genotype has a different genetic background and correlates with its selection response (Goldstein and Ehrenreich, 2021; Čalić et al., 2022). The more homozygous the genetic construct of a genotype, the more additive is the selection response (Karavolias et al., 2020; Villiers et al., 2024). Thus, response patterns can be easily understood and inherited. This differs when selection is performed on genotypes with heterozygous genetic constructs, and the potential dominant variant determines the selection response. This reduces the consistency of the selection response for each generation (Liu et al., 2020; So et al., 2022; Li et al., 2023). One genotype with a highly homozygous genetic construct was the double haploid line. These lines result from chromosome doubling in haploid cells due to anther or ovule culture (Mishra and Rao, 2016; Ren et al., 2017; Niazi and Shariatpanahi, 2020; Lantos et al., 2022). The development of double haploid lines can accelerate the variety development process; therefore, this concept is an important part of accelerating the rice variety breeding (Dewi et al., 2017; Ren et al., 2017; Gudi et al., 2022; Lantos et al., 2022). In addition, the homozygous nature of these lines makes them suitable for genetic studies and method development such as hydroponic salinity screening (Arifuddin et al., 2021; Ivanova et al., 2025). The development of DH rice lines was reported by Anshori et al. (2021), who identified several DH rice lines with various levels of tolerance and adaptability. These lines can be used as the basis for the development of selection criteria for hydroponic salinity screening.

Image-based phenotyping (IBP) is a new approach for assessing and evaluating genotype responses. This concept utilizes the imaging analysis of an image that is translated into various observation characters, either area-based or morphometric (geometric), or based on color reflection or colorimetry (Mochida et al., 2018; Li et al., 2021; Vârbă et al., 2022; Anshori et al., 2023; Sakinah et al., 2024). The concept of IBP can be used to assess the overall morphological potential of a plant in an image. Therefore, this approach is efficient and effective for determining the potential of a genotype, including its tolerance potential (Anshori et al., 2023). Several studies have reported the effectiveness of IBPs under salinity stress, including in rice (Anshori et al., 2021; Pabuayon et al., 2022; Sakinah et al., 2022). Anshori et al. (2021) and Sakinah et al. (2022) also reported the development of IBP selection criteria for hydroponic salinity stress screening. However, the development was still simply analyzed and did not use double-haploid rice genotypes. In addition, the analysis was not completed through systematic validation. Therefore, the IBP approach needs to be comprehensively deepened, based on both the concepts of model development and validation.

Statistical analysis is an important part of data interpretation. The more complex the data and objectives, the more complex is the statistical analysis. This can be an indicator of the comprehensiveness of the evaluation (Nayak et al., 2018; Reed et al., 2021; Soltabayeva et al., 2021; Phanniphong and Na-Nan, 2025). However, the results of the analysis still require validation to increase confidence in interpreting drawings. Validation is key to the effectiveness of a systematic set of statistical approaches (Nelson et al., 2019; Manavalan et al., 2021; Myllyaho et al., 2021; Baharuddin et al., 2025). Such validation should be performed incrementally, both on approaches with higher accuracy and on different populations (LaBudde and Harnly, 2012; Nelson et al., 2019; Anshori et al., 2021, 2022; Myllyaho et al., 2021; Satpathi et al., 2023; Baharuddin et al., 2025). Validated

statistical analysis in the development of a crop model will increase the comprehensiveness of the study (Nelson et al., 2019; Anshori et al., 2021, 2022; Baharuddin et al., 2025). Therefore, systematic statistical development and validation are required to develop IBP-based selection criteria and models for double-haploid rice tolerance under hydroponic salinity screening. The purpose of this study was to identify IBP selection criteria and develop a model for potential tolerance in double-haploid rice under hydroponic salinity screening.

2 Materials and methods

2.1 Experimental design

This experiment was conducted at BB Biogen Greenhouse, Bogor, for 3 months from February to April 2019. The test was conducted with a hydroponic culture using Yoshida solution (Platten, 2018). The experimental design used was split-plot randomized complete block design, the main plot was NaCl concentration (0 mM (0.77 dS m⁻¹) and 120 mM (13.42 dS m⁻¹), and the subplots were ten genotypes 7 double haploid lines (HS4-15-1-63, HS4-15-1-70, HS4-15-2-4, HS4-15-2-6, HS4-45-1-66, and HS17-33-1-8) and 3 check varieties [Ciherang, IR 29 (sensitive), and Pokkali (tolerant)]. This study was repeated 3 times, so there were 60 experimental units. Each experimental unit consisted of 20 seedlings.

2.2 Research procedure

Seeding was carried out in stages according to the method of IRRRI (2021), namely, seeding in Petri dishes for 2 days and then transferred to the nursery until 7 days after sowing (HSS). The 7-day-old seedlings were removed from the nursery media and transplanted into the nutrient culture media using a Styrofoam tray that had been perforated with a diameter of 18 mm, with a distance of 5 cm × 4 cm between holes. The seedlings were planted by rolling the seedling stems with a thin foam sheet and placing them into holes that had been made in the Styrofoam. The styrofoam containing the seedlings was then placed (until it floated) in Yoshida medium (8 L per tub) (IRRRI, 2021). Each tub consisted of two genotypes and two sensitive and tolerant controls to compare the tub microenvironment. NaCl was applied when the seedlings had an average of three leaves. The first stage of NaCl application was 60 mM NaCl; after 2 days, it was increased to 120 mM NaCl. The pH of the solution was maintained in the range of 5.0–5.1 by adding 1 N NaOH or HCl every other day. The nutrient culture medium was replaced once a week until observations were made.

Images were taken with a Canon EOS 1200D RGB camera in a portable photo studio measuring 75 cm × 75 cm × 75 cm. A white background with two 8-watt white LED lights in the studio with camera settings (5.6 f-stops, 1/160 s exposure time, ISO 800, and no flash) was used. The side view (90°) from which the images were taken, is in the visible spectrum, commonly known as the RGB range (400–700 nm). Images were taken twice at 28 DAS (days after planting) or 14 days after treatment. Geometric characteristics were analyzed using ImageJ 1.54c. Excess area was cropped from all four sides to focus on the plant canopy and reference ruler, and a straight line of known length was drawn across the ruler to calibrate pixel

measurements to real-world units. A color threshold based on saturation was then applied to separate the plants from the background, resulting in a binary mask that retained only plant pixels. The entire canopy area was extracted through this segmentation, and the green area was identified using the Hue, Saturation, and Brightness (HSB) model with a hue value of 50. All measured areas were converted from pixels to cm² using the calibrated scale. Additionally, R (Red), G (Green), and B (Blue) channel data are obtained from the segmented image, where R, G, and B represent the raw digital values of each color channel, while r (Normalized Red), g (Normalized Green), and b (Normalized Blue) are the normalized values used to calculate the colorimetric index for IBP characteristics.

The association between RGB image data and salinity tolerance in double-haploid rice lines were defined through image-based phenotyping (IBP) combined with statistical modeling. Rice seedlings were grown under optimal and saline hydroponic conditions, then photographed using a Canon EOS 1200D camera in a controlled photo studio to maintain consistency in lighting and background. From each image, morphometric traits and colorimetric indices were extracted using ImageJ through segmentation and color thresholding. These traits were analyzed using Best Linear Unbiased Estimator (BLUE) and Stress Tolerance Index (STI) to evaluate performance across different environments. Properties significantly associated with salinity tolerance were identified through Spearman correlation with IRRRI salinity scores, followed by Principal Component Analysis (PCA) and factor analysis to select the most informative IBP criteria. The selected traits were verified against physiological measurements (chlorophyll content, relative water content, malondialdehyde, and proline), and integrated into a morphometric and colorimetric selection index to predict and classify salinity tolerance directly from RGB images. To enhance clarity, the entire process is explained in Figure 1.

2.3 Observation parameters and data analysis

The observed data included the morphology, image-based phenotyping, and physiology. The conventional morphological characteristics observed were those reported by Anshori et al. (2021), namely crown height and crown wet weight. The observation of image-based phenotyping characters adapts the concepts of Laraswati et al. (2021) and Sakinah et al. (2022, 2024), both for morphometric (geometric) and colorimetric potentials. The observed IBP characters include total area, green area, green area percentage, red index, green index, blue index, green-red distance (GRD), red-green ratio (RGR), red-blue ratio (RBR), vegetation index (VEG), color index of vegetation extraction (CIVE), visible atmospheric resistance index (VARI), red-green-blue vegetation index (RGBVI), modified green-red vegetation index (MGRVI), green leaf index (GLI), and normalized green-red difference index (NGRDI). Information regarding colorimetric index are provided in Table 1. The observed physiological characteristics included chlorophyll content (total chlorophyll, chlorophyll a, and chlorophyll b), carotenoids, relative water content (RWC), malondialdehyde (MDA), and proline. All analyses were conducted at the Physiology Laboratory, Biology, IPB University, with specific protocols. Meanwhile, Na⁺ and K⁺ ion content in the canopy was observed on selected genotype samples based on

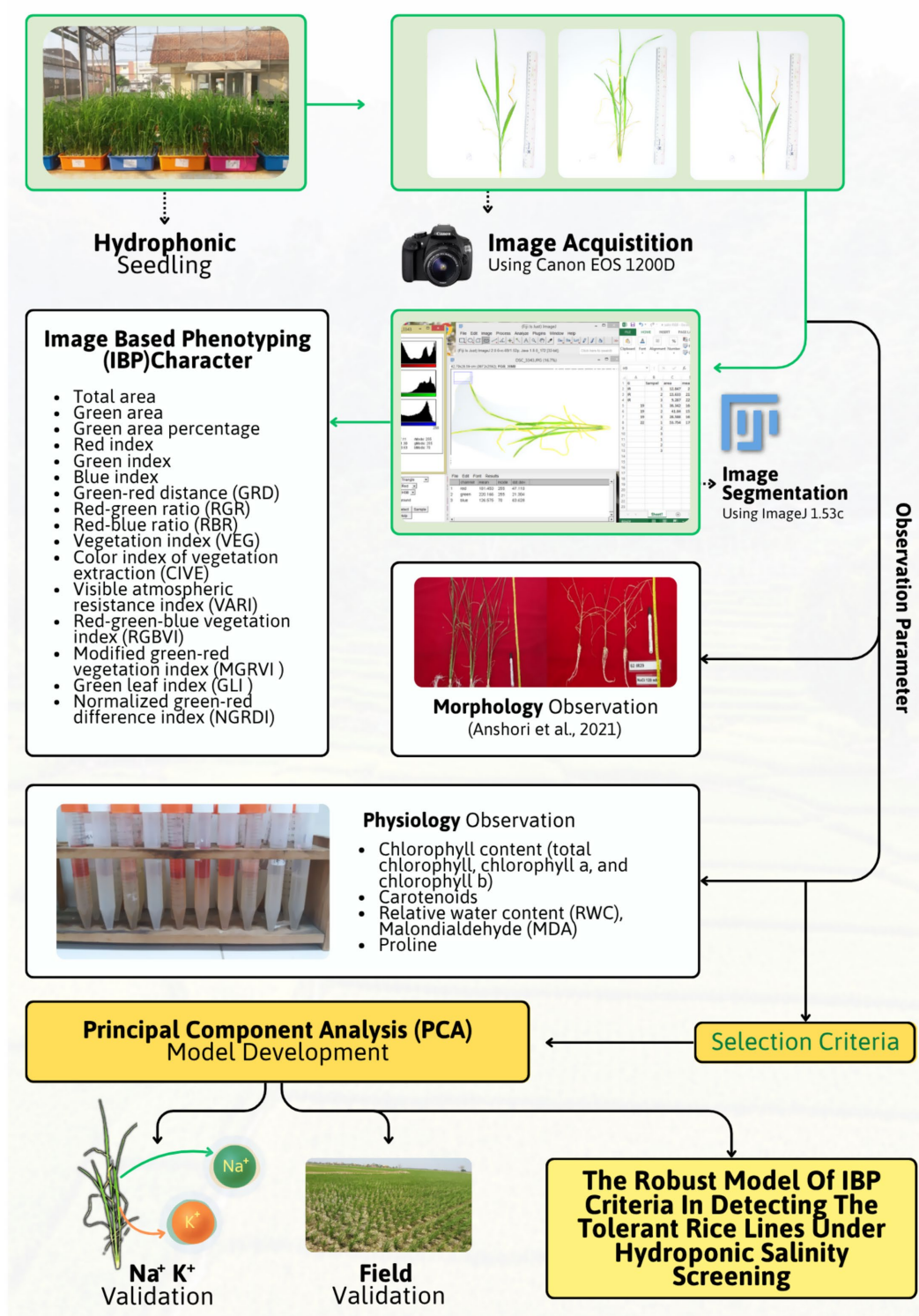


FIGURE 1
Research scheme flow.

the cluster. Analysis of Na^+ and K^+ ion content was performed by atomic absorption spectrophotometer (AAS) analysis at the Integrated Laboratory, Department of Agronomy and Horticulture, IPB University. Samples of each genotype were taken with as much as 5 g of dry matter for Na^+ and K^+ ion analysis.

Chlorophyll determination was carried out using fresh leaves from the oldest leaves (0.1 g at the center of the leaf). The leaves were then crushed, 2 mL of 80% acetone was added, and the extract was placed in a falcon until 10 mL. Then the mixture was centrifuged with a rotational speed of 3,000 rpm for 10 min at 4 °C. The

TABLE 1 Description and formulas of selected colorimetric index.

Characters	Abbreviation	Formula	Reference
Red	R	–	Sakinah et al. (2024)
Green	G	–	Sakinah et al. (2024)
Blue	B	–	Sakinah et al. (2024)
Red Index	r	$R/(R + G + B)$	Sakinah et al. (2024)
Green Index	g	$G/(R + G + B)$	Sakinah et al. (2024)
Blue Index	b	$B/(R + G + B)$	Sakinah et al. (2024)
Green-Red Distance	GRD	$ g - r $	Kior et al. (2024) and Wiwart et al. (2009)
Red-Green Ratio	RGR	r/g	Kior et al. (2024)
Red-Blue Ratio	RBR	r/b	Cen et al. (2019) and Kior et al. (2024)
Vegetation Index	VEG	$g/r^a * b^{(1-a)}$	Sakinah et al. (2024)
Color Index Vegetation Extraction	CIVE	$0.441r - 0.811g + 0.385b + 18.787$	Kawamura et al. (2021)
Visible Atmospheric Resistance Index	VARI	$(g - r)/(g + r - b)$	Coswosk et al. (2024)
Red-Green-Blue Vegetation Index	RGBVI	$(G^2 - R * B)/(G^2 + B * R)$	Sakinah et al. (2024)
Modified Green-Red Vegetation Index	MGRVI	$(G^2 - R^2)/(G^2 + R^2)$	Sakinah et al. (2024)
Green Leaf Index	GLI	$(2 * G - R - B)/(R + 2 * G + B)$	Sakinah et al. (2024)
Normalized Green-Red Difference Index	NGRDI	$(G - R)/(G + R)$	Sakinah et al. (2024)

G, Green channel; R, Red channel; B, Blue channel; *, Multiplication, a = 0.667.

chlorophyll content in the supernatant was measured with a spectrophotometer at wavelengths of 470 nm, 464 nm, and 663 nm. Chlorophyll a, b, and carotenoid contents were calculated using the formula described by Lichtenthaler (1987).

RWC measurements were performed on salinity-stressed leaves. Fresh leaves were cut to a length of 2 cm, and the leaves were immediately weighed as wet weight. The leaves were placed in distilled water for 24 h in the dark. The next day, the leaves were dried with a hard filter, and the turgid leaves were weighed. The leaves were then dried again at 50 °C for a day and weighed again as dry weight. RWC was determined using the following formula:

$$RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}}$$

MDA testing can be performed on leaves. The weight of the samples used was 0.5 g. The oldest leaf sample was used. MDA extraction began with grinding and the addition of 5 mL of 5% *trichloroacetic acid* (TCA). The extract was centrifuged at 3000 rpm for 30 min at 4 °C. Then, 2 mL of the supernatant was added to a new phial and mixed with 2 mL of 0.5% *thiobarbituric acid* (TBA): 5% TCA. The mixture was heated at 80 °C in a *water bath* for 45 min. After 45 min, the reaction mixture was cooled on ice for 5 min and resuspended at the same speed, time, and temperature. The absorbance of the mixture was then measured with a spectrophotometer at wavelengths of 450 nm, 532, and 600 nm. MDA was measured using the following formula (Wang et al., 2013):

$$[MDA] = 6.45 \times (A^{532} - A^{600}) - (0.56 A^{450})$$

Fresh leaves in young leaves were taken as much as 0.25 g then crushed and added 3% Sulfosalicylic Acid as much as 5 mL. The sample was then centrifuged at 3600 nm for 25 min at 4 °C. Then, 2 mL of the supernatant was transferred to an empty phial and mixed with 2 mL of ninhydrin, which had been dissolved in glacial acetic acid, 6 M orthophosphoric acid, and 2 mL of glacial acetic acid. The reaction mixture was then incubated for 1 h at 80 °C in a water bath and cooled with water at room temperature. Subsequently, 4 mL of toluene was mixed with each phalcone and vortexed. The reaction mixture was allowed to stand for 5 min at room temperature until a boundary formed between toluene and the other mixtures. Proline dissolved in toluene was measured using a spectrophotometer at 520 nm, and the amount of proline in the sample was estimated using a standard curve (Ali et al., 2014).

The data were analyzed using systematic and comprehensive concepts. In general, the statistical concepts used focus on the identification of selection criteria and the development of selection models. The identification of selection criteria began with a variance analysis of IBP characteristics using STAR software, version 2.0. Subsequently, significant data, especially in the interaction variance, were followed by analysis of the best linear unbiased estimator (BLUE) and stress tolerance index (STI). This combination is a part of the development of standard values for salinity tolerance analysis. In general, BLUE is used as the basic approach for determining selection criteria. This approach provides fixed effect estimates that are linear and unbiased, and have the minimum variance among all unbiased linear estimators, thus minimizing the impact of errors in the estimates (Kleinknecht et al., 2013; Dieng et al., 2024; Ridwan et al., 2025). The formulation of the BLUE concept is as follows:

$$\hat{\beta} = (X^T \cdot X)^{-1} \cdot X^T \cdot y$$

Where y is the vector of dependent variables ($n \times 1$), X is the matrix of independent variables ($n \times k$), and is the parameter estimation using ordinary least squares (OLS). Meanwhile, the STI concept focuses on correlating the differences in the response of genotype potential on optimal land and salinity to the average potential of genotypes. This concept considers the general response of a genotype, so that the assessment is dynamic and objective in determining the adaptability of a genotype to abiotic stress (Anshori et al., 2018, 2022; Hooshmandi, 2019). The formulation of STI according to Fernandez (1992) is as follows:

$$\text{Stress Tolerance index (STI)} = \frac{Y_s \times Y_n}{\overline{Y_n}^2}$$

Y_n = character value in normal condition; Y_s = character value in saline condition; $\overline{Y_n}$ = average value in normal condition.

The combination results of BLUE and STI of IBP characteristics were followed by Spearman correlation analysis using STAR 2.0.1 software. This analysis focused on the IRR tolerance score. The use of Spearman's correlation is considered effective for the combination of ordinal data (tolerance score) with quantitative IBP characters (Anshori et al., 2021). The characters that correlated significantly were further analyzed by Principal Component Analysis (PCA) biplot analysis through Rstudio with the factorextra package (Sakinah et al., 2022, 2024). This analysis aims to simultaneously map the diversity between characters into two main dimensions by minimizing the effect of overlap between sources of diversity. This allows the pattern of relationships between characters to be interpreted more clearly and informatively (Jolliffe and Cadima, 2016; Anshori et al., 2019). The interpretation of this analysis focused on the clustering of IBP characters and their relationship with important morphological characteristics [shoot height (SH) and shoot fresh weight (SFW)] (Anshori et al., 2021). IBP characteristics related to SH and SFW are indicated as morphometric (geometric) IBP criteria, while other RGB-based characteristics are considered colorimetric IBP characteristics. Especially for characters related to colorimetry, all characters were analyzed more in depth with factor analysis the minitab v 17 software. This analysis was intended to reduce factors that are considered to have a low covariance on a dimension (Rocha et al., 2018; Anshori et al., 2022). Colorimetric IBP characteristics that are consistently high on both dimensions of the factor become a reference for estimating the selection criteria for selected colorimetric IBPs. The selected morphometric and colorimetric IBP criteria were validated using physiological parameters. The physiological parameters were first changed through a combination of BLUE and relative increase. The relative increase in this study was calculated using the formula (Anshori et al., 2020):

$$\text{relative increase (ratio)} = \frac{Y_s - Y_n}{Y_n}$$

The overall physiological relative gains were reanalyzed using a PCA biplot against the candidate colorimetric and geometric IBP selection criteria. Subsequently, the validated candidates were developed into IBP selection models based on morphometric (geometric) and colorimetric methods. The selection model was developed through the concept of a selection index. The selection

index is a method that utilizes several selection criteria that are used as a multiple regression formulation (Alsabah et al., 2019; Anshori et al., 2019, 2021). This index can efficiently increase the genetic value of the population by considering the priority value of each selection criterion through the concept of weighting criteria (Rahimi and Debnath, 2023; Wellmann, 2023). This has also been reported in the development of selection for salinity stress tolerance (Anshori et al., 2019, 2021, 2022; Arifuddin et al., 2021; Farid et al., 2021; Sakinah et al., 2022). The selection index was formulated as follows (Anshori et al., 2019):

$$I = b_1x_1 + b_2x_2 + \dots + b_nx_n$$

Where I is the index value; $b_1, b_2, \dots, b_n$ = selection weight (coefficient) for each trait; and $x_1, x_2, \dots, x_n$ = observed trait values. Weighting development of the selection index values was performed using the concept of eigenvectors on the PCA values. The selected IBP selection criteria were re-analyzed using PCA without biplots. This concept was developed by Anshori et al. (2019, 2021), Laraswati et al. (2021), Sarwendah et al. (2022), Hadiananto et al. (2023), and Nasaruddin et al. (2024). The determination of eigenvectors is based on PCs, which can describe the differences in the potential of colorimetric and geometric selections in shaping the weighting of selection indices from the two IBP approaches (Anshori et al., 2019, 2021). Subsequently, the two indices were interacted to determine the mapping pattern of the selection potential. The index interaction was visualized by scatter plot analysis using Excel 365 software and Rstudio with the package ggplot2. The Na⁺ and K⁺ ion contents of the samples from each interaction group were analyzed. The Na⁺ and K⁺ contents can be used as indicators to determine the mechanism of tolerance of rice to salinity stress (Reddy et al., 2017; Gerona et al., 2019; Chen et al., 2021; Paul et al., 2024), including in this study. In addition, the geometric and colorimetric indices were validated against their production potential under salinity stress, except for IR 29 and Pokkali. This study aimed to identify the relationship between tolerance potential and adaptability in a salinity field. This concept is an indication for the development of criteria and selection models for salinity tolerance, especially through hydroponic salinity screening of DH rice lines.

3 Result

3.1 Identification of image-based phenotyping (IBP) selection criteria for DH Rice tolerance to salinity stress based on conventional phenotypic validation

The analysis of variance for image-based phenotyping (IBP) characteristics is shown in Table 2. Genotype variation had a highly significant effect on all IBP characteristics of double-haploid rice in the hydroponic salinity test. This is also followed by environmental variation and $G \times E$ interaction, which also show significant effects on almost all characters, except for the blue index and red-blue ratio. The blue index character does not show a significant effect from environmental variation, and the red-blue ratio does not show a significant effect from environmental variation and $G \times E$ interaction.

TABLE 2 Analysis of variance of image-based phenotyping characters of double haploid rice lines under hydroponic salinity screening.

Characters	Env	Geno	GxE	Error
Total area	18622.23**	621.91**	373.39**	73.50
Green area	26582.78**	590.25**	345.86**	64.15
Green area percentage	1.9189**	0.0802**	0.0731**	0.0041
Red Index	0.0147**	0.0006**	0.0004**	0.0000
Green Index	0.0510**	0.0008**	0.0012**	0.0002
Blue index	0.0112*	0.0007**	0.0004 ns	0.0002
GRD	16617.70**	380.45**	445.50**	37.2322
RGR	0.4878**	0.0116**	0.0142**	0.0011
RBR	0.0807 ns	0.1001**	0.0118 ns	0.0164
VEG	1.8550**	1.8550**	0.0428**	0.0065
CIVE	8621.29**	159.49**	205.33**	28.24
VARI	0.1270**	0.0021**	0.0029**	0.0003
RGBVI	0.5396**	0.0117**	0.0163**	0.0025
MGRVI	0.6573**	0.0136**	0.0170**	0.0015
GLI	0.1870**	0.0035**	0.0052**	0.0007
NGRDI	0.1771**	0.0037**	0.0046**	0.0004

** significant effect at 1% error level, * significant effect at 5% error level, Env (E), Environment; Geno (G), Genotype; GRD, green-red distance; RGR, red-green ratio; RBR, red-blue ratio; VEG, vegetation index; CIVE, color index of vegetation extraction; VARI, visible atmospheric resistance index; RGBVI, red-green-blue vegetation index; MGRVI, modified green-red vegetation index; GLI, green leaf index; NGRDI, normalized green-red difference index.

The IBP characteristics with potential as selection criteria were further analyzed using BLUE analysis and stress tolerance indices. BLUE analysis for all IBP characteristics of diploid rice in the hydroponic salt tolerance test is presented in [Supplementary Table 1](#). The results of the BLUE analysis were used as the basis for developing stress tolerance indices ([Table 3](#)). The table shows that the double haploid line HS4-15-1-63 (F19) showed good potential for the green index (GI) (0.93), green-red distance (GRD) (0.65), vegetation index (VEG) (0.87), color index of vegetation extraction (CIVE) (0.70), visible atmospheric resistance index (VARI) (0.60), red-green-blue vegetation index (RGBVI) (0.83), modified green-red vegetation index (MGRVI) (0.64), green leaf index (GLI) (0.79), and normalized green-red difference index (NGRDI) (0.63). The Ciherang and IR 29 varieties have a high potential in the red-green ratio (RGR) (1.49) and red index (1.17), respectively. Meanwhile, the Pokkali variety has high potential in terms of area (1.00), green area (0.90), percentage of green area (0.85), plant height (1.61), wet canopy weight (1.39), and IRRI tolerance score (1.00).

The results of the Spearman analysis are shown in [Figure 2](#). This correlation focused on the IRRI tolerance score. Based on the figure, the tolerance score has a significant negative correlation with shoot height (−0.85), green area (−0.96), total area (−0.91), shoot fresh weight (−0.87), green area percentage (−0.85), VARI (−0.87), GRD (−0.86), MGRVI (−0.83), NGRDI (−0.83), CIVE (−0.88), and GLI (−0.82). Additionally, green area had a significant positive correlation with other characteristics related to the assessment, namely shoot height (0.92), total area (0.95), shoot fresh weight (0.95), green area

percentage (0.89), VARI (0.88), GRD (0.88), MGRVI (0.85), NGRDI (0.85), CIVE (0.88), and GLI (0.84).

The results of the biplot analysis based on principal component analysis (PCA) showed two groups of characteristics based on vector direction ([Figure 3](#)). The first group consisted of shoot height, shoot fresh weight, total area, and green area. The Pokkali variety was the only variety aligned with the first vector group with a high individual contribution value. Conversely, the second vector group consisted of CIVE, GRD, VARI, MGRVI, GLI, green area percentage, and NGRDI. This vector group also depicts several double haploid lines, namely HS4-15-2-4, HS4-15-2-6, HS4-15-1-70, and HS4-15-1-63. These lines have significant individual contributions to the biplot analysis. The other genotypes were grouped in different quadrants. Varieties IR 29 and Ciherang are in quadrant 2, with high individual contribution values. Conversely, HS17-33-1-8, HS4-45-1-66, and HS4-15-1-26 were grouped in quadrant 3 with low individual contribution values.

The results of factor analysis of the PCA biplot of vector group 2 ([Figure 3](#)) are presented in [Table 4](#). Based on the table, two factors describe the diversity of vector group 2 or colorimetric group, with a total diversity of 99.30%. Based on the intersection of the two factors, the characteristics of green percentage, CIVE, and GLI had high and consistent factor loadings or variances for both factors. The green percentage had factor loadings of 0.728 and 0.614 for factors 1 and 2, respectively. CIVE had factor loadings of −0.541 and −0.836 for factors 1 and 2, respectively. Meanwhile, GLI had factor loadings of −0.676 and −0.988 for factors 1 and 2, respectively.

3.2 Identification of image-based phenotyping selection criteria for DH Rice tolerance to hydroponic salinity screening based on physiological validation

The relative increase in the physiological parameters of the double haploid rice lines under hydroponic salinity screening is shown in [Table 5](#). The table shows that IR 29 had a high relative increase in malondialdehyde (MDA) (2.81) and proline (46.23). Additionally, Pokkali exhibited a relatively high increase in total chlorophyll (0.49), chlorophyll a (0.44), chlorophyll b (0.68), and carotenoids (0.22). The genotype with the highest relative increase in relative water content is HS4-15-1-63 (0.00).

The results of the PCA biplot analysis of the selected IBP and physiological characteristics are shown in [Figure 4](#). According to this figure, there are two vector groups. The first group consisted of the total area, green area, GLI, chlorophyll a, chlorophyll b, total chlorophyll, CIVE, green area percentage, carotenoids, and relative water content. The genotypes around this vector group are Pokkali, HS4-15-2-4, HS4-15-2-6, HS4-15-1-63, and HS4-15-1-70. Conversely, the second vector group contained MDA and proline. The genotypes grouped in this vector were IR 29 and Ciherang.

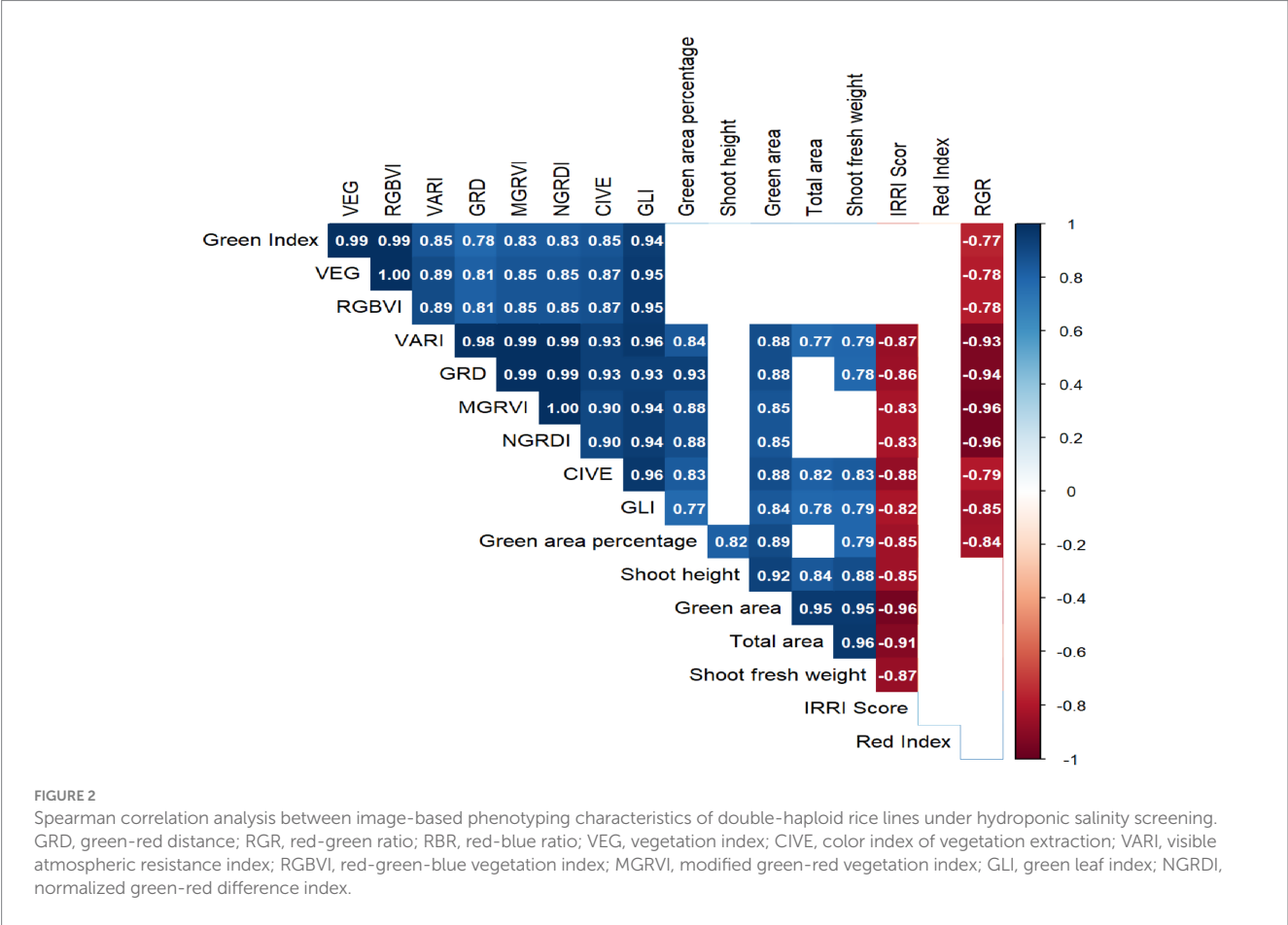
3.3 Development of a Rice tolerance index to salinity stress based on IBP and its validation based on Na and K contents

The results of the PCA involving five selection criteria for IBP double-haploid rice under hydroponic salinity screening are shown in

TABLE 3 BLUE-based stress tolerance index of image-based phenotyping characteristics of double-haploid rice lines under hydroponic salinity screening.

Characters	Genotype									
	F15	F19	F21	F22	F23	F34	F36	Ciherang	IR	PK
BLUE_Total area	0.26	0.52	0.54	0.75	0.65	0.39	0.38	0.30	0.23	1.00
BLUE_Green area	0.20	0.38	0.35	0.59	0.51	0.29	0.18	0.09	0.04	0.90
BLUE_GAP	0.75	0.76	0.70	0.77	0.74	0.73	0.41	0.25	0.15	0.85
BLUE_Red Index	1.04	1.06	1.10	1.05	1.07	1.03	1.17	1.20	1.17	1.07
BLUE_Green Index	0.86	0.93	0.90	0.92	0.93	0.86	0.90	0.80	0.79	0.89
BLUE_GRD	0.47	0.65	0.45	0.62	0.55	0.42	0.20	−0.09	−0.22	0.61
BLUE_RGR	1.20	1.15	1.21	1.15	1.16	1.21	1.28	1.49	1.47	1.21
BLUE_VEG	0.76	0.87	0.84	0.86	0.86	0.76	0.83	0.68	0.67	0.80
BLUE_CIVE	0.40	0.70	0.52	0.66	0.60	0.36	0.49	0.10	0.02	0.68
BLUE_VARI	0.40	0.60	0.45	0.56	0.54	0.36	0.16	−0.07	−0.18	0.49
BLUE_RGBVI	0.60	0.83	0.74	0.80	0.80	0.56	0.74	0.45	0.37	0.72
BLUE_MGRVI	0.47	0.64	0.46	0.61	0.56	0.42	0.18	−0.07	−0.17	0.53
BLUE_GLI	0.56	0.79	0.68	0.76	0.75	0.54	0.65	0.37	0.30	0.69
BLUE_NGRDI	0.48	0.63	0.45	0.62	0.55	0.39	0.17	−0.07	−0.19	0.52
BLUE_Shoot height	0.66	0.76	0.69	0.87	0.79	0.77	0.56	0.60	0.63	1.61
BLUE_SFW	0.21	0.47	0.42	0.68	0.58	0.43	0.30	0.28	0.18	1.39
IRRI Tolerance Score	5	3	3	3	3	5	7	7	9	1

The bold indicates the highest value on each character, BLUE, best linear unbiased estimator; GRD, green-red distance; RGR, red-green ratio; RBR, red-blue ratio; VEG, vegetation index; CIVE, color index of vegetation extraction; VARI, visible atmospheric resistance index; RGBVI, red-green-blue vegetation index; MGRVI, modified green-red vegetation index; GLI, green leaf index; NGRDI, normalized green-red difference index; F 15 = HS4-15-1-26, F19 = HS4-15-1-63, F21 = HS4-15-1-70, F22 = HS4-15-2-4, F23 = HS4-15-2-6, F34 = HS4-45-1-66, F36 = HS17-33-1-8, IR = IR 29, PK = Pokkali.



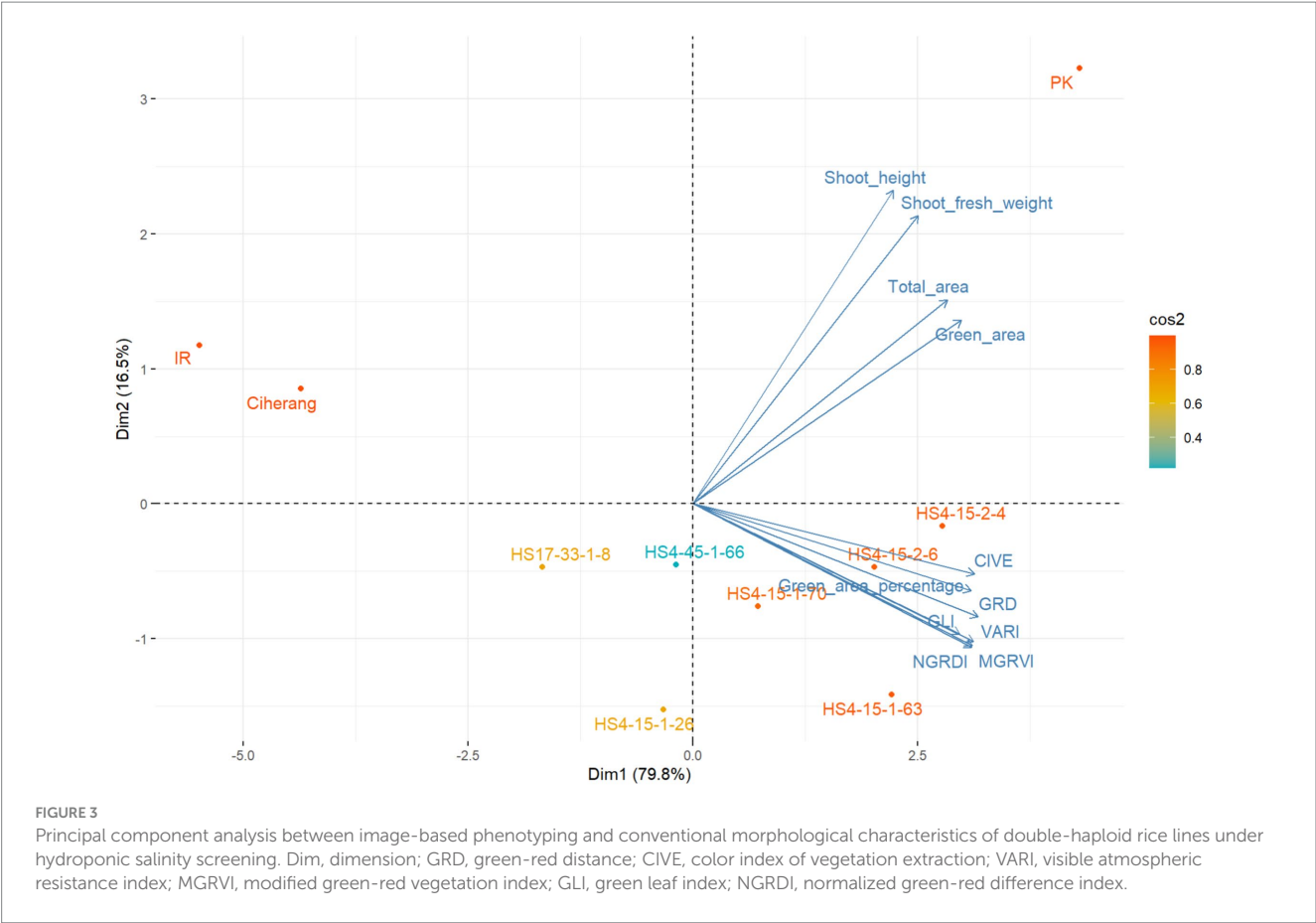


TABLE 4 Factor analysis of selected colorimetric characteristics of double haploid rice lines under hydroponic salinity screening.

Variable	Factor 1	Factor 2	Communality
Green area percentage	0.728	0.614	0.988
GRD	0.296	0.112	0.998
CIVE	−0.541	−0.836	0.987
VARI	0.242	0.05	0.994
MGRVI	0.343	0.166	0.996
GLI	−0.676	−0.988	0.996
NGRDI	0.335	0.157	0.994
Variance	3.9043	3.0483	6.9527
Variance proportion	0.558	0.435	0.993

Bold is the selected character representing colorimetric IBP, GRD, green-red distance; CIVE, color index of vegetation extraction; VARI, visible atmospheric resistance index; MGRVI, modified green-red vegetation index; GLI, green leaf index; NGRDI, normalized green-red difference index.

Table 6. The table shows that two principal components could represent the diversity of the five selection criteria, with a cumulative diversity of 0.95. The determination of these criteria was also based on eigenvalue values in each PC dimension that exceeded 1. PC1 has relatively similar eigenvectors, ranging from 0.4 among the IBP selection criteria. Conversely, PC2 showed two groups of selection

criteria marked by different vector directions. The positive-valued vectors consisted of green area (0.51) and total area (0.58), whereas the negative-valued vectors consisted of green area percentage (−0.31), CIVE (−0.31), and GLI (−0.46). These results can be formulated into two types of indices: morphometric (geometric) and colorimetric indices.

Morphometric (geometric) index = 0.51 green area + 0.58 total area

Colorimetric index = 0.31 green area percentage
+ 0.31 CIVE + 0.46 GLI

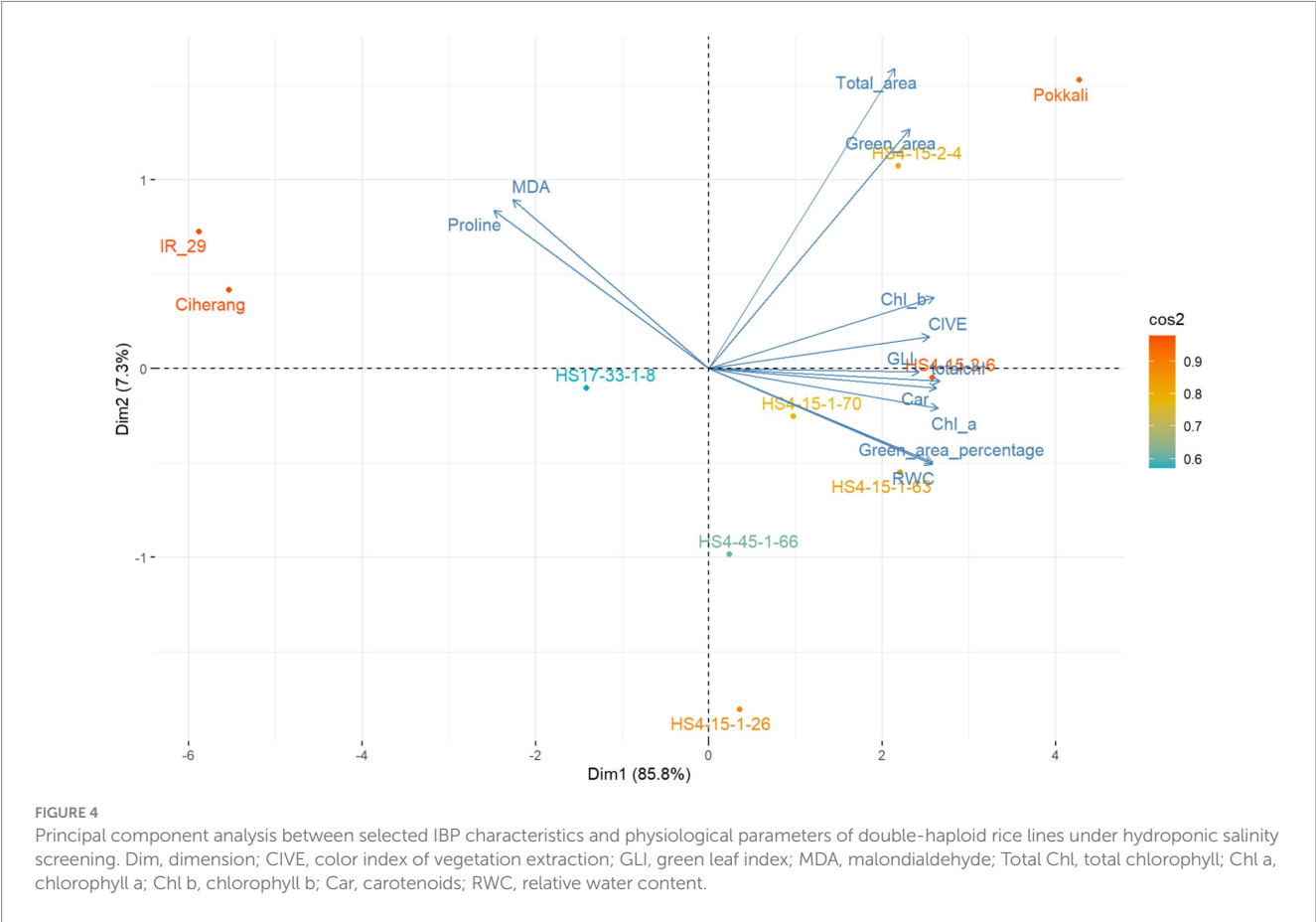
The interaction between colorimetric and morphometric (geometric) indices based on IBP criteria for double-haploid rice under hydroponic salinity stress is shown in **Figure 5**. Based on the figure, there were three groups. The first group consisted of HS4-15-2-6, HS15-2-4, and Pokkali. The second group consists of HS4-15-1-63, HS4-15-1-70, HS4-45-1-66, HS4-15-1-26, and HS17-33-1-8. The last group consisted of Ciherang and IR 29.

The analyses of potassium and sodium contents and their ratios in the IBP interaction index group samples are shown in **Table 7**. Based on the table, HS4-15-2-6 (−0.07) has a low relative increase in potassium content, while Pokkali and HS4-45-1-66 have a high relative increase in potassium, namely 0.31. The double haploid line HS4-15-2-6 (8.29) also showed a low relative increase, while IR 29 showed a high relative increase in sodium (27.84). Furthermore, the lowest relative increase in

TABLE 5 BLUE-based relative increase in physiological characteristics of double haploid rice lines under hydroponic salinity screening.

Genotype	MDA	Proline	Total chl	Chl a	Chl b	Car	RWC
HS4-15-1-26	0.65	0.61	0.13	0.16	0.06	−0.03	−0.02
HS4-15-1-63	0.65	0.88	0.27	0.18	0.50	−0.08	0.00
HS4-15-1-70	0.94	1.98	0.16	0.10	0.32	−0.05	−0.06
HS4-15-2-4	1.98	3.42	0.28	0.21	0.47	−0.02	−0.01
HS4-15-2-6	0.35	0.59	0.34	0.28	0.50	0.05	−0.02
HS4-45-1-66	0.74	3.06	0.14	0.12	0.20	−0.11	−0.05
HS17-33-1-8	2.02	12.78	−0.14	−0.18	0.01	−0.19	−0.07
Ciherang	2.46	30.79	−0.59	−0.61	−0.51	−0.59	−0.16
IR 29	2.81	46.23	−0.44	−0.49	−0.25	−0.54	−0.16
Pokkali	0.21	0.26	0.49	0.44	0.68	0.22	−0.01

The bold indicates the highest value on each physiological character, MDA, malondialdehyde; Total Chl, total chlorophyll; Chl a, chlorophyll a; Chl b, chlorophyll b; Car, carotenoids; RWC, relative water content.



the Na/K ratio is found in HS4-15-2-6 at 8.97, while the highest relative increase in the Na/K ratio is found in IR 29 (27.64).

3.4 Interaction of IBP colorimetric and morphometric (geometric) tolerance index with the yield in saline areas

A summary of the productivity of the genotypes in both salt environments is presented in Table 8. Based on this table, the overall

average productivity of the genotypes in the Truntum and Sukra areas is 6.34 and 5.57 (t ha^{−1}), respectively. The combination of the two regions resulted in an average productivity of 5.95 t ha^{−1}, with HS4-15-1-63 (6.45 t ha^{−1}) as the genotype with the highest productivity compared to the others. Conversely, HS17-33-1-8 (4.12 t ha^{−1}) had the lowest productivity. These results form the basis for the analysis of the 3D interactions of morphometric (geometric) and colorimetric indices.

The interactions among the three approaches are displayed in a 3D plot (Figure 6). Based on the figure, there were four grouping categories. The first group consists of the double haploid lines

TABLE 6 Principal component analysis of selected image-based phenotyping characteristics based on morphometric (geometric) and colorimetric of DH rice under salinity stress.

Variable	PC1	PC2	PC3	PC4	PC5
Green area	0.45	0.51	−0.14	−0.32	−0.65
Total area	0.44	0.58	0.19	0.40	0.53
Green area percentage	0.44	−0.31	−0.82	0.17	0.15
CIVE	0.47	−0.31	0.29	−0.68	0.36
GLI	0.45	−0.46	0.44	0.49	−0.39
Eigenvalue	4.21	0.55	0.22	0.01	0.00
Proportion	0.84	0.11	0.05	0.00	0.00
Cumulative	0.84	0.95	1.00	1.00	1.00

Bold indicates the selected PC for weighting, PC, principal component; CIVE, color index of vegetation extraction; GLI, green leaf index.

HS4-15-2-6 and HS4-15-2-4. The second group consists of HS4-15-1-26, HS4-15-1-63, HS4-15-1-70, and HS4-45-1-66. The third and fourth groups consisted of Ciherang variety and HS17-33-1-8, respectively.

4 Discussions

4.1 Determination of selection criteria in determining DH rice tolerance under hydroponic salinity screening

The identification of selection criteria based on image-based phenotyping characters cannot be separated from the potential of these characters in detecting differences in responses between genotypes to normal and saline environments (Anshori et al., 2019, 2022; Farid et al., 2021; Herawati et al., 2024; Khan et al., 2024). This potential can be seen in the diversity of interactions between the genotype and environment in this study. Based on the source of diversity, all IBP characters showed a significant effect of interaction diversity, except for the blue index and red-blue ratio. In general, screening for stress must include checking genotypes that are tolerant and sensitive to abiotic stresses, including salinity. This aims to control the diversity of a screening to show significant differences in responses between the two checks in the selection environment (Bado et al., 2016; Anshori et al., 2020; Singh et al., 2021). Tolerant check genotypes show a gentle decline in growth characteristics when subjected to salinity stress. Conversely, sensitive genotypes experience a sharp and exponential decline in growth characteristics when experiencing salinity stress (Anshori et al., 2018; Arifuddin et al., 2021; Huqe et al., 2021; Chen et al., 2024). This difference induces a significant effect of genotype and environment interaction diversity on a character. Moreover, the selection also included various other genotypes with varying tolerance levels, as in the present study, (Anshori et al., 2018; Arifuddin et al., 2021; Huqe et al., 2021; Chen et al., 2024). This makes the source of interaction diversity more significant to a growth character, so the determination of the effect of interaction diversity is an early indication in determining the selection criteria in tolerance screening. This concept was also reported by Arifuddin et al. (2021), Farid et al. (2021), Herawati et al. (2024), and Sakinah et al. (2024), in

rice to salinity stress, and for Laraswati et al. (2021), Nasaruddin et al. (2024) on rice against drought stress. Based on this, all IBP characters can potentially be used as selection criteria in screening the tolerance of double haploid rice lines to salinity stress, except blue index and red, blue ratio.

The identification of effective IBP selection criteria in tolerance screening of double haploid rice under hydroponic salinity screening is performed gradually through various approaches and validation. The utilization of several approaches and validations in determining selection criteria was also reported by Anshori et al. (2021, 2022), Huqe et al. (2021), Tabassum et al. (2021), and Chen et al. (2024). In addition, utilization is the basis for developing an effective and precise model for hydroponic-based salinity tolerance screening in this study. The concept of determining the selection criteria in this study is based on the concept of best linear unbiased estimation (BLUE) and stress tolerance index (STI). Utilization of BLUE is considered critical because all genotypes in this study have homozygous and fixed genetic constructs (Dewi et al., 2017; Anshori et al., 2021); therefore, the utilization of BLUE will increase the precision of assessing the tolerance of a genotype. On the other hand, STI also increases the effectiveness of determining selection criteria and developing tolerance-screening models (Lone et al., 2019; Dehnavi et al., 2020; Kandel et al., 2022; Nasaruddin et al., 2024). The STI has a role as a midpoint in assessing the potential adaptability of genotypes to stress (Anshori et al., 2019, 2022; Hooshmandi, 2019; Kumar et al., 2024). The use of this STI in screening rice against stress has also been reported by Lestari et al. (2019), Laraswati et al. (2021), Sakinah et al. (2022, 2024), Hallajian et al. (2024), Kumar et al. (2024), and Nasaruddin et al. (2024), as well as salinity stress (Sakinah et al., 2024). Based on this, the combination of the two is a new and essential approach for increasing the precision of assessing the tolerance potential of double-haploid rice, especially in hydroponic salinity screening.

The determination of IBP selection criteria was first validated by morphological characteristics. The validation concept was also divided into two approaches: validation based on the IRRI SES salinity tolerance score (Anshori et al., 2020; IRRI, 2021; Singh et al., 2021; Fellahi et al., 2024) and conventional morphological selection criteria (Anshori et al., 2021, 2022; Arifuddin et al., 2021; Farid et al., 2021; Sakinah et al., 2022; Alkahtani and Dwiningsih, 2023; Dwiningsih, 2023). The IRRI has developed protocols for the assessment of various tolerances tailored to the developmental stages of the crop, including hydroponic salinity screening in the seedling phase (IRRI, 2021; Singh et al., 2021). This indicates that such an assessment is the minimum requirement for determining the level of plant tolerance. However, the concept of score is more focused on the chlorophyll potential of a genotype under stress (Anshori et al., 2021; Singh et al., 2021; Sakinah et al., 2024). This has limitations in assessing the potential for cell division that occurs when plants experience salinity stress. Some rice genotypes optimize their cell division potential as a form of resilience to minimize the impact of salinity stress (Anshori et al., 2021; Farid et al., 2021). This indicates that the use of conventional morphological characteristics is complementary in assessing the potential tolerance of genotypes to salinity stress. According to Anshori et al. (2021), Farid et al. (2021), Alkahtani and Dwiningsih (2023), and Atta et al. (2023), shoot height and shoot fresh weight are seedling morphological characteristics that are effective as selection criteria in hydroponic salinity screening. Both characteristics can be part of the validation in

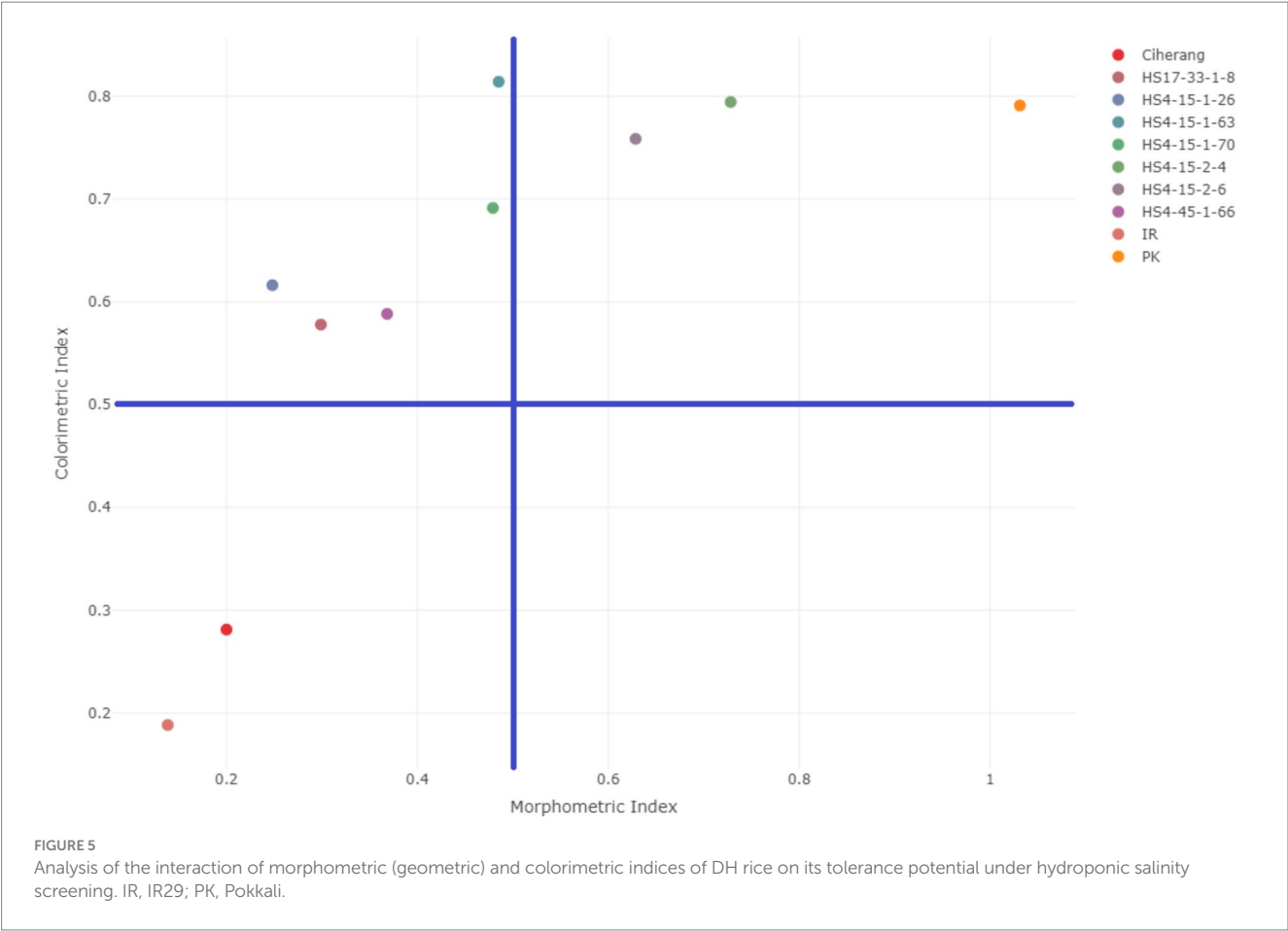


TABLE 7 Analysis of Potassium, Sodium, and Ratio content of sample groups based on the interaction of IBP colorimetric and morphometric (geometric) tolerance indexes.

Genotype	Normal			Saline			Relative increase		
	K ⁺	Na ⁺	Na ⁺ /K ⁺	K ⁺	Na ⁺	Na ⁺ /K ⁺	K ⁺	Na ⁺	Na ⁺ /K ⁺
Pokkali	1.84	0.19	0.10	2.40	3.26	1.36	0.31	16.25	12.18
HS4-15-1-63	2.19	0.25	0.11	2.69	4.22	1.57	0.23	16.14	12.94
HS4-15-2-6	2.56	0.37	0.15	2.38	3.45	1.45	−0.07	8.29	8.97
HS4-45-1-66	1.93	0.22	0.11	2.52	6.13	2.43	0.31	27.38	20.75
HS17-33-1-8	3.19	0.21	0.07	3.00	6.00	2.00	−0.06	27.02	28.84
IR29	2.26	0.23	0.10	2.27	6.55	2.88	0.01	27.84	27.64

TABLE 8 The yield of several double haploid rice lines in Truntum and Sukra, along with the potential tolerance index based on IBP.

Genotype	Truntum (t ha ^{−1})	Sukra (t ha ^{−1})	Mean (t ha ^{−1})	z value	Morphometric (geometric) Index	Colorimetric index
HS4-15-1-26	6.29	5.84	6.07	0.15	0.25	0.62
HS4-15-1-63	6.20	6.70	6.45	0.66	0.49	0.81
HS4-15-1-70	6.87	5.60	6.23	0.37	0.48	0.69
HS4-15-2-4	6.70	6.03	6.37	0.55	0.73	0.79
HS4-15-2-6	6.87	5.59	6.23	0.37	0.63	0.76
HS4-45-1-66	6.25	6.01	6.13	0.23	0.37	0.59
HS17-33-1-8	4.57	3.68	4.12	−2.43	0.30	0.58
Ciherang	6.94	5.13	6.04	0.11	0.20	0.28
Average	6.34	5.57	5.95	0.00	0.43	0.64

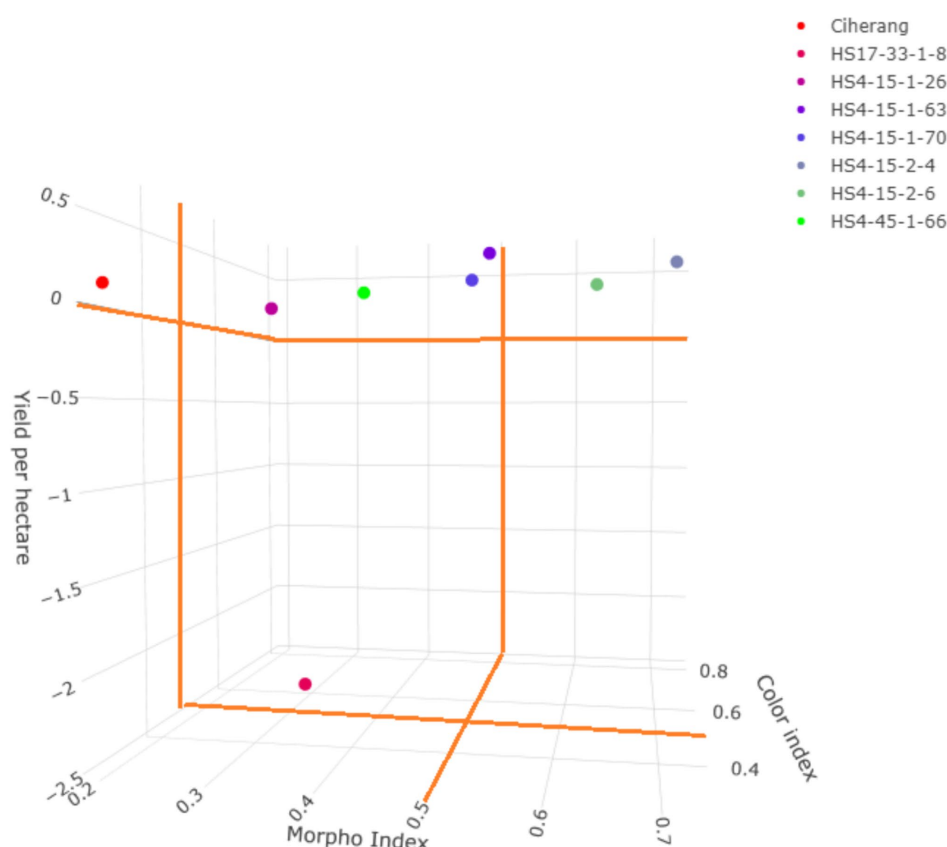


FIGURE 6

Analysis of the interaction of the morphometric (geometric) index, colorimetric index, and yield of DH rice on its potential adaptability to salinity stress. Morpho Index, morphometric (geometric) Index; Color Index, colorimetric index.

determining the IBP selection criteria. Based on this, the IRRI SES salinity tolerance score, shoot height and shoot fresh weight are morphological validation indicators for detecting the potential tolerance of double-haploid rice lines in hydroponic salinity screening.

Based on the morphological validation of the three indicators, the IBP characters total area, green area, green area percentage, CIVE, and GLI can be indicated as potential IBP selection criteria. The five characteristics can be divided into two groups based on PCA analysis, which represent the potential for cell division and chlorophyll color content. The total area and green area represent the morphological characteristics of the shoot height and shoot fresh weight, respectively. This indicates that both characteristics can act as morphometric (geometric) IBP criteria in determining the potential tolerance of DH rice lines in hydroponic salinity screening. In contrast, the green area percentage, CIVE, and GLI became a part of the colorimetric potential of IBP after being analyzed more deeply through factor analysis. This analysis can simplify a large number of variables into fewer factors, based on the clustering of high covariance between variables in one dimension (Rocha et al., 2018; Anshori et al., 2022; Jia et al., 2022); therefore, characters with a large diversity in the PCA dimension can be indicated as potential characters in the group. However, the five characteristics that have been indicated as IBP selection criteria must be further validated based on their physiological potential.

Physiological validation involves various biochemicals that generally undergo changes upon exposure to salinity stress

(Alkahtani and Dwiningsih, 2023). These characteristics included proline, chlorophyll, carotenoids, melandehdhi, and relative water content. Based on the validation, the colorimetric selection criteria (green percentage area, CIVE, and GLI) had the same direction as RWC and chlorophyll content. In contrast, this group of criteria was also in the opposite direction to MDA and Proline contents. In addition, the total area and green area were strongly related to the potential of the Pokkali-tolerant genotypes. In general, plants under salinity stress are exposed to Na⁺ toxicity and free radicals (Atta et al., 2023; Balasubramaniam et al., 2023; Fu and Yang, 2023). This could induce chlorophyll damage. Plants exposed to salinity stress experience chlorophyll depletion (Gerona et al., 2019; Atta et al., 2023; Liu et al., 2024; Ubaidillah et al., 2024). However, plants with salinity tolerance minimize chlorophyll damage to their leaves (Liu et al., 2024; Zuo et al., 2024). This phenomenon may indicate the potential of chlorophyll content as an indicator of tolerance to salinity stress in rice. This was also consistent with the relative water content (RWC). When plants experience salinity stress, it is difficult for cells to obtain water for cell growth (Balasubramaniam et al., 2023; Liu et al., 2024; Zuo et al., 2024). This indicates that sensitive rice plants have lower water content than tolerant plants (Korres et al., 2022; Balasubramaniam et al., 2023). This potential makes RWC an indicator of tolerance to salinity stress. On the other hand, the concepts of malondialdehyde (MDA) and proline can be selection

criteria whose functions are different from those of chlorophyll and RWC. Salinity stress triggers excessive production of reactive oxygen species (ROS) in plant cells (Hasanuzzaman et al., 2021). ROS damage membrane lipid peroxidation, one of the products of which is MDA (Miftahudin et al., 2020). Plants that are sensitive to salinity show a significant increase in MDA levels (Gerona et al., 2019; Miftahudin et al., 2020; Hartatik et al., 2024). This indicates severe cell damage and the inability of the plant antioxidant defense system to overcome these ROS; therefore, this content can be used as an indicator of rice tolerance to salinity stress. Proline is an amino acid that acts as an osmoprotectant and is dominantly expressed when plants, including rice, experience salinity stress (Meena et al., 2019; El Moukhtari et al., 2020; Hosseinifard et al., 2022). In the present study, the highest proline content was found in the sensitive genotypes. This indicates that sensitive genotypes will try to optimally increase their osmotic pressure to meet the needs of water in the crown. This result is different from the statement of Atta et al. (2023), the increase in proline also occurred very highly in tolerant varieties compared to sensitive varieties. However, the assessment focused on the roots, while this study used the crown as an indicator of proline accumulation. This indicates that sensitive genotypes will try to increase the water content in the canopy by increasing the osmotic pressure of the canopy cells (Koc et al., 2024). This role makes proline an indicator of rice tolerance to salinity stress (Nguyen et al., 2021; Koc et al., 2024). Based on this, the five IBP characteristics have great potential as important selection criteria for the tolerance of double-haploid rice lines in hydroponic salinity screening.

4.2 Development of tolerance index model based on IBP selection criteria in double haploid Rice under hydroponic salinity screening

The development of the IBP tolerance index model in double-haploid rice under hydroponic salinity screening was carried out using the concepts of Akbar et al. (2019), Anshori et al. (2019), and Farid et al. (2021). The development of the index is based on the potential eigenvectors of PCA in a PC dimension. The crucial aspect of this concept is to determine the effective PC that can be used as the basis for the weighting value of the selection criteria in the index model. Based on the PCA analysis, PC2 was the optimal PC for distinguishing the potential diversity between the IBP groups. In contrast, PC1 had a unidirectional diversity in the five selection criteria. This indicates that PC cannot be the basis for determining the weighting of the selection index. The development of the IBP index in PC2 is divided into two concepts: the morphometric (geometric) index represented by total area and green area, and the colorimetric index represented by green area percentage, CIVE, and GLI. Although, the colorimetric group showed a negative PC2 value. However, this value is not absolute; therefore, the eigenvectors for each selection criterion can be positive in developing the colorimetric index (Anshori et al., 2019, 2021). Based on this, the two indices developed can be used to assess the potential of double-haploid rice genotypes for image-based phenotyping of hydroponic salinity screening.

The results of the interaction between the colorimetric index and morphometric (geometric) index, along with validation against Na⁺ and K⁺ contents, showed three main quadrants. The first quadrant indicates a group of genotypes with very strong salinity tolerance. This is indicated by the morphometric and colorimetric potential in the same direction so that genotypes in this group can maintain an increase in growth rhythm and chlorophyll content when experiencing salinity stress. This is further reflected in the validation of the Na⁺ and K⁺ contents in the samples of this group. Pokkali and HS4-15-2-6 had low relative increases in Na content and Na⁺/K⁺ ratio. This indicates that quadrant 1 group has tolerance properties by maintaining Na⁺ ions from entering the leaf tissue. This concept is related to the avoidance tolerance mechanism in response to salinity stress (Reddy et al., 2017; Gerona et al., 2019; Paul et al., 2024). The second quadrant genotype group was considered complex. This is a moderate or transitional group between tolerance and sensitivity. Quadrant II was divided into two subgroups, which indicated the direction of tolerance. The first subgroup, consisting of HS4-15-1-63 and HS4-15-1-70, tended to have tolerance potential. This is also reflected in the validation of Na⁺ and K⁺, where the sample group HS4-15-1-63 had a low relative increase, similar to the group in the first quadrant. Both can still be categorized as avoidance mechanisms by minimizing the increase in growth rhythm and maintaining its chlorophyll potential (Reddy et al., 2017; Gerona et al., 2019). On the other hand, the 2nd subgroup has the potential for sensitive genotypes. This can be seen from the close proximity and high increase in Na content in the third quadrant genotype group. This indicates that the genotype uses the tolerance mechanism as a form of tolerance, so that the Na⁺ content is quite high in maintaining the chlorophyll potential in young leaves (Reddy et al., 2017; Paul et al., 2024). Old leaves in these genotypes are sacrificed as part of the organ complementation related to Na⁺ ion toxicity (Reddy et al., 2017; Gerona et al., 2019). The third quadrant is a collection of sensitive genotypes, especially IR 29. These genotypes die because they cannot maintain the balance of division and damage when experiencing salinity stress (Reddy et al., 2017). Based on this, the potential interaction of these two IBP indices is suitable to be an important part in the classification and assessment of the salinity tolerance potential of a Double haploid rice genotype, specifically in hydroponic screening. However, these two index concepts also require field validation as economic and final validation related to the salinity stress adaptability of double-haploid rice.

The interaction of the IBP index (morpho-and colorimetric) on productivity shows that DH rice genotypes that are tolerant have better productivity potential than moderate and sensitive genotypes. The results of this interaction also show that the colorimetric potential has a role in the same direction as the potential productivity of double haploid rice lines in a salinity area. This phenomenon indicates that the chlorophyll potential in the maternity area is related to the potential adaptability of productivity (Wang et al., 2024); therefore, the colorimetric index of IBP plays an important role in identifying the potential productivity of double haploid rice lines in the maternity area. Chlorophyll damage continues until the plant can handle it (Lu et al., 2023; Nisbett et al., 2024). If the increase in chlorophyll damage enters an exponential pattern, the damage will induce a

decrease in productivity and death in plants (Voitsekhovskaja et al., 2020). However, this concept does not apply to the ciherang variety. This variety is considered to be sensitive to hydroponic salinity screening. However, its productivity showed potential value in the salinity area in this study. This is because the Ciherang variety is a megavariety that has a statistically stable production under various environmental conditions (Kinose et al., 2019; Aswidinnoor et al., 2023; Sakinah et al., 2024). In addition, the application of stress in hydroponics has a severe salinity status, reaching 12 dS/m, while in rice-growing areas, the stress status does not reach 12 dS/m. This is why the Ciherang variety has an adaptive mechanism and good production levels in saline fields. However, its production potential is not as good as that of varieties that are tolerant to salinity stress. Therefore, the colorimetric potential (green area percentage, CIVE, and GLI) in this study can be used as an early indicator of the potential productivity of rice lines, especially double haploids, when grown in saline areas.

The morphometric and colorimetric index formulas developed in this study effectively distinguished salt-tolerant double haploid rice lines from sensitive ones in hydroponic salinity tests. Their discriminative ability is supported by consistent correlations with IRRI salinity scores, conventional morphological traits, physiological parameters, tissue Na^+/K^+ content, and yield performance under saline field conditions. These indices reliably grouped known tolerant and sensitive control lines together with experimental lines in patterns consistent with biological expectations. However, since these indices were developed and validated on a limited number of genotypes under controlled conditions, broader testing across diverse genetic backgrounds, developmental stages, and imaging environments is needed. The addition of diagnostic metrics (sensitivity, specificity) and independent validation will further strengthen their utility for large-scale screening programs.

5 Conclusion

The development of criteria and selection models based on image-based phenotyping (IBP) in hydroponic salinity screening is considered effective for estimating the potential tolerance of rice lines, especially double haploid lines. This development can be performed with a combination of the best linear unbiased estimation and stress tolerance index to determine the potential of a rice line. This concept is further developed through the development of an index model based on eigenvector weighting in principal component analysis. This combination is an effective novelty in estimating the potential of selection criteria and the development of IBP tolerance models. The concept of the tolerance index model in IBP can be divided into two approaches: morphometric (geometric) and colorimetric. The morphometric (geometric) index is represented by the total area and green area, whereas the colorimetric index is represented by the percentage of green area, CIVE, and GLI. All IBP criteria showed good validation results for conventional morphological potential (IRRI tolerance score, seedling height, and shoot fresh weight), physiology (MDA, Proline, RWC, chlorophyll, and carotenoids), Na^+ and K^+ contents, and productivity in saline areas. The formulation of the two tolerance indexes are:

Morphometric (geometric) index = 0.51 green area + 0.58 total area

Colorimetric index = 0.31 green area percentage
+ 0.31 CIVE + 0.46 GLI

The interaction of the two IBP tolerance indices can map double haploid rice genotypes unequivocally, both between tolerant and sensitive traits, and potential differences to the moderate group. However, the colorimetric index roughly indicates the potential adaptability of the double-haploid rice lines in the salinity field. Therefore, the colorimetric index can be used as a reference for a double-haploid rice tolerance model in hydroponic salinity screening. However, this concept needs to be further tested with genotypes in other populations, both crossbred and mutated rice populations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

MuA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. BP: Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing. NC: Data curation, Funding acquisition, Supervision, Validation, Writing – review & editing. ID: Conceptualization, Data curation, Methodology, Resources, Supervision, Validation, Writing – review & editing. WS: Conceptualization, Formal analysis, Methodology, Software, Supervision, Validation, Writing – review & editing. SA: Conceptualization, Data curation, Methodology, Supervision, Validation, Writing – review & editing. AbB: Data curation, Supervision, Validation, Writing – review & editing. AcB: Investigation, Software, Visualization, Writing – review & editing. PK: Investigation, Visualization, Writing – review & editing. ZA: Data curation, Validation, Writing – review & editing. MaA: Funding acquisition, Supervision, Validation, Writing – review & editing. NA: Supervision, Validation, Writing – review & editing. MS: Data curation, Funding acquisition, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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