Jurnal Agronomi Indonesia
(Indonesian Journal of Agronomy)

ISSN 2085-2916 | e-ISSN 2337-3652
August 2025, *53*(2), 232-244
DOI: https://dx.doi.org/10.24831/jai.v53i2.63893

Research Article

Analysis of drought stress tolerance in doubled haploid lines of green super rice at the vegetative stage

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Edited by:
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Received:
24 April 2025
Accepted:
15 August 2025
Published online:
29 August 2025

Citation:
Nurhidayah, S., Purwoko, B. S., Dewi, I. S., Suwarno, W. B., Lubis, I., Hadianto, W., & Munandar, A. (2025). Analysis of drought stress tolerance in doubled haploid lines of green super rice at the vegetative stage. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy), 53*(2), 232-244. DOI: https://dx.doi.org/10.24831/jai.v53i2.63893

ABSTRACT

Current climate change has caused drought in various regions, which has decreased rice yields. Green super rice (GSR) has the characteristic of being tolerant to water limitation. GSR lines were bred through anther culture technique to obtain doubled haploid (DH) plants. This study aimed to analyze the response of DH GSR lines to drought stress at the vegetative stage and identify potential lines based on the weighted selection index. The experiment was arranged in a randomized complete block design with three replications. The materials used consisted of twenty DH lines, Inpari 42 Agritan GSR, and Inpari 18 as check varieties, Salumpikit as a drought-tolerant check, and IR 20 as a drought-sensitive check. Qualitative data were analyzed using the non-parametric Friedman test. The result showed different responses among the lines for leaf rolling, leaf drying, and recovery ability. Line SN14 exhibited moderate leaf drying and showed improvement to mild tolerance during the recovery phase. Nine lines (SN12, 14, 32, 40, 51, 57, 58, 59, and 60) were selected using a selection index based on leaf rolling, leaf drying, and recovery ability. These lines can be further tested for drought tolerance tests until the reproductive stage, and the tolerant lines could be useful for future development.

Keywords: abiotic stress; anther culture; climate change; selection index; water limitation

INTRODUCTION

Rice is a grain crop that serves as a staple food source consumed by 50% of the world's population (Ahmad et al., 2022). Indonesia ranks fourth globally as a rice producer after China, India, and Bangladesh, contributing 7% of global production, equivalent to 54.7 million tons in 2022 (FAO, 2024a). Currently, the world is facing an increase in population, where in 2024, it reached 8 billion and is estimated to increase to 9.1 billion by 2025 (FAO, 2024b). This population issue will result in an increased demand for rice.

Drought and heat are among the abiotic stresses caused by climate change and pose future threats (Sehgal et al., 2018). Water deficits due to climate change occur in most of the world's agricultural regions (Dietz et al., 2021). The dry season in Indonesia typically occurs from May to October, while the rainy season takes place from November to April. Warming sea surface temperatures (SST) in various regions have led to the El Niño phenomenon, which can delay rainfall and extend the dry season (Nurdiati et al., 2022). The decrease in rainfall due to El Niño can reach 900 mm(Firmansyah et al., 2022). Irregular rainfall patterns will lead to water scarcity in dry and semi-dry areas (El-Hashash et al., 2018). The arrival of El Niño during the rice planting season can cause long-term declines in rice production (Ismail & Chan, 2019). Rice production decreases can reach 54–58% (Lanna et al., 2021) and even 86% during El Niño events (Barrios-Perez et al., 2021).

Screening at the seedling stages is necessary to classify varieties for drought resistance (Wei et al., 2022). It can be done conventionally (Saharia et al., 2024) or by using modern technology methods, such as molecular markers (Hassan et al., 2023), and high-throughput phenotyping approaches (Kim et al., 2020). Efforts to overcome drought stress have been widely reported to produce drought-tolerant rice varieties. Several studies have reported potential lines or varieties that have good tolerance to drought stress, such as eight lines (line no 10, 34, 72, 88, 90, 104, and 108) under PEG and the wood box method (Wening & Susanto, 2017), three landraces (Seratus-Malam Boawae, Padi Putih Kuatnana, and Padi Putih Maumere) from East Nusa Tenggara were the best landraces under drought stress (Salsinha et al., 2020), the NGSR-15 line from 22 GSR lines was a drought-tolerant line at the beginning of the anthesis stage (Ahmad et al., 2022), three varieties (Inpago 9, Salumpikit, and Cisaat) were considered relatively tolerant under a limited semi-controlled field system (Susanto et al., 2022), and three upland rice lines from 11 cross combinations have good tolerance under drought stress (Mustikarini et al., 2022).

Drought stress can affect nutrient absorption, photosynthesis, and plant metabolism (Miftahudin et al., 2020). Plant adaptation mechanisms to drought stress are divided into drought escape, avoidance, and drought tolerance (Panda et al., 2021). Drought escape is the ability of a plant to complete its life cycle before experiencing a significant water deficit (Asati et al., 2022; Pamungkas et al., 2022). Drought avoidance is the ability of plants to maintain a relatively higher water content in tissues compared to the lower water content in the soil during drought stress. Drought tolerance is defined as the ability of plants to live under low water content in plant tissues (Basu et al., 2016).

GSR has the characteristics of high yield quantity and the ability to grow under stress conditions (Krishna et al., 2020) and remain stable under low input conditions (Li & Ali, 2017). The development of drought-tolerant GSR can be accelerated by using the anther culture technique. This technique can accelerate the obtaining of homozygous lines in one generation (Dewi et al., 2020), making the selection process faster and more effective. Materials used in this research are doubled haploid lines (DH) derived from anther culture and have previously undergone preliminary yield trials, advanced yield trials, and resistance tests against bacterial leaf blight disease. Based on previous research, candidate lines have high-yield potential (Nurhidayah et al., 2023) and resistance to bacterial leaf blight disease (Nurhidayah et al., 2024). Therefore, the level of line tolerance to drought stress is important for further research. This research aimed to analyze the response of DH GSR lines to drought stress in the vegetative stage and identify potential lines based on the weighted selection index.

MATERIALS AND METHODS

Research site and materials

The experiment was conducted from August to October 2023 in the greenhouse of the Center for Standard Instruments Testing of Biotechnology and Agricultural Genetic

Resources (BBPSI Biogen) at coordinates 6°34'32.2"S 106°47'06.7"E, Bogor City, West Java Province, Indonesia.

The research used 24 genotypes, i.e., 20 DH lines selected from preliminary yield trials (Nurhidayah et al., 2023), along with two check varieties (Inpari 42 Agritan GSR and Inpari 18 Agritan), a drought-sensitive check (IR 20), and a drought-tolerant check (Salumpikit). The fertilizer used in the nursery was NPK 16:16:16 at 200 kg ha^{-1}.

Research design

The study employed a randomized complete block design with genotypes as a single factor. The materials tested included 24 genotypes, each replicated three times, resulting in a total of 72 experimental units. Each experimental unit consisted of 14 seedlings. The seeds were soaked in warm water for 24 hours, then incubated in the dark for 48 hours. Each genotype was planted in rows with a spacing of 5 cm × 5 cm in a cement bench measuring 3.2 m × 0.75 m × 1 m filled with soil. Fertilizer was applied at 7 days after sowing (DAS). The plants were maintained and irrigated until they were 14 DAS. The seedlings that grew for 14 DAS were left without irrigation until the IR 20 variety, as a sensitive check, reached a score of 9. Thus, leaf rolling response assessment was carried out when the leaf of IR 20 was tightly rolled (score 9) (Table 1). The observation continued to assess the leaf drying when the IR 20 had the highest leaf drying score (score 9) (Table 2). After the scoring of the leaf drying, the plants were re-watered. After ten days of watering, a recovery ability assessment was performed for each genotype. Recovery ability is determined by calculating the percentage of plant recovery of the tested genotypes (Table 3). Those three variables were scored following the Standard Evaluation of Rice by IRRI (2014).

Drought tolerance measurement

Observation of drought tolerance consisted of leaf rolling (Table 1), leaf drying (Table 2), and recovery ability after drought stress treatment (Table 3), following the Standard Evaluation System of Rice (SES) IRRI (2014).

Table 1. Drought tolerance criteria based on leaf rolling.

Score	Criteria	Description
0	Highly tolerant	Leaves healthy
1	Tolerant	Leaves start to fold (shallow)
3	Mildly tolerant	Leaves folding (deep V-shape)
5	Moderate	Leaves are fully cupped (U-shape)
7	Mildly sensitive	Leaf margins touching (0-shape)
9	Sensitive	Leaves tightly rolled

Source: IRRI (2014)

Table 2. Drought tolerance criteria based on leaf drying.

Score	Criteria	Description
0	Highly tolerant	No symptoms
1	Tolerant	Slight tip drying
3	Mildly tolerant	Tip drying extended up to 1/4 length in most leaves
5	Moderate	One-fourth to 1/2 of all leaves are dried
7	Mildly sensitive	More than 2/3 of all leaves are dried
9	Sensitive	All plants dead

Source: IRRI (2014)

Table 3. Drought tolerance criteria based on recovery ability.

Score	Criteria	Description (plant recovered)
1	Tolerant	90-100%
3	Mildly tolerant	70-89%
5	Moderate	40-69%
7	Mildly sensitive	20-39%
9	Sensitive	0-19%

Source: IRRI (2014)

Data analysis

The scoring data on the variables of leaf rolling, leaf drying, and recovery ability were analyzed using the non-parametric Friedman test method. The Friedman test statistic with ties (genotypes with the same rank) follows the formula of Conover (1999) and Corder and Foreman (2014). The chi-square test follows Liu and Xu (2022).

The number of ranks for each treatment uses the following formula:

$$R_j = \sum_{i=1}^{b} R(X_{ij}) \qquad \ldots(1)$$

Where: R_j = sum of the rank of each genotype in each block, $R(X_{ij})$ = the rank of each genotype in each block, b = block, i = 1, 2, 3 (block), j = 1, 2, 3, ..., n (genotype).

The sum of squares of ranks and the average of adjusted ranks are used as follows:

$$A = \sum_{i=1}^{b} \sum_{j=1}^{k} [R(X_{ij})]^2 \qquad \ldots(2)$$

Where: A = sum of squares of the ranks and average ranks, i = 1, 2, 3 (block), j = 1, 2, 3, ..., n (genotype), $R(X_{ij})$ = the rank of each genotype in each block.

The corrected factor was calculated using the formula:

$$C = \frac{1}{4}\, bk\,(k+1)^2 \qquad \ldots(3)$$

Where: C = correction factor, b = block, k = number of genotypes

Two-way statistics at each rank were obtained by calculating the adjusted T_1 and T_2 values using the formula:

$$T_1 = \frac{(k-1)\left[\sum_{j=1}^{k} R_j^2 - bC\right]}{A-C} \qquad \ldots(4)$$

$$T_2 = \frac{(b-1)T_1}{b\,(k-1)T_1} \qquad \ldots(5)$$

Where: T_1, T_2= Friedman test statistics, C = correction factor, b = block, k = number of genotypes, j = 1, 2, 3, …, n (genotype), A = sum of squares of each treatment, R_j = sum of the ranks of each genotype in each block.

The chi-square test value follows Liu and Xu (2022):

$$\chi^2 = \frac{12N}{k\,(k+1)}\left[\sum_{j=1}^{k} R_j^2 - \frac{k\,(k+1)^2}{4}\right] \qquad \ldots(6)$$

Where: χ^2= chi-square test, R_j = sum of the rank of each genotype in each block, k = number of genotypes, N= number of observations

Test for significant differences between treatments using the following formula:

$$|\overline{R_b} - \overline{R_a}| > t_{1-\alpha/2}\sqrt{\frac{2(b\,A - \sum_{j=1}^{k} R_j^2}{(b-1)(k-1)}} \qquad \ldots(7)$$

Where: $\overline{R_a}$ = average rank sum of genotype a, $\overline{R_b}$ = average rank sum of genotype b, t = t table value, b = block, k = number of genotypes, A = sum of squares of each treatment, R_j = sum of the ranks of each genotype in each block.

A weighted selection index was conducted to select the best lines based on the best ranking, following Kang (2015), with the formula:

$$I = b_1x_1 + b_2x_2 + b_3x_3 + \cdots + b_nx_n \quad \ldots(8)$$

Where: I = index selection, b = the weight for each trait, i = 1, 2, 3, ..., n, where n = number of traits, x = value for each trait (leaf rolling, leaf drying, and recovery ability). Leaf rolling and leaf drying were assigned a weight of -1, while recovery ability was given a weight of -2.

The x value estimated based on the z value follows Tomé et al. (2019) with the formula:

$$Z = \frac{x_{ij} - x_j}{s_j} \quad \ldots (9)$$

Where: Z is the standardized mean of the i^{th} phenotype on the j^{th} trait, x_{ij} is the mean of the i^{th} phenotype on the j^{th} trait, $\bar{x}_j$ is the mean of the j^{th} traits, and s_j is the standard deviation of the j^{th} traits.

RESULTS AND DISCUSSION

Non-parametric analysis of Friedman's test

Non-parametric statistical analysis of the Friedman test produced different p-values for each character (Table 4). Based on the Friedman test using the chi-square, the lines affected leaf drying and recovery ability, except for leaf rolling. This test indicates a difference in ranking between the lines tested on these two characters. According to Liu and Xu (2022), the Friedman test can be adopted as a measure of conservativeness with a higher confidence level to reject the null hypothesis (H0). The anther culture technique enables the accelerated production of homozygous doubled haploid lines with desired traits. This technique is expected to speed up the selection cycle in obtaining drought-resistant lines (Usenbekov et al., 2024).

Table 4. Friedman test of drought character in DH GSR lines at the seedling stage.

Characters	$\mathcal{X}^2$-value	p-value
Leaf rolling	33.48^{ns}	0.07
Leaf drying	36.57*	0.04
Recovery ability	40.74**	0.01

Note: ns = not significant, *,** = significant based on chi-square test at 5% and 1% level, respectively.

Soil moisture content analysis

The soil moisture content after drought stress treatment is presented in Table 5. The soil moisture content after drought scoring ranged between 12.7% and 14.8%, with an average of 13.4%. According to Hamarash et al. (2024), soil moisture ranging from 10 to 20% is considered severely dry, and < 10% is considered extremely dry. Therefore, the soil conditions meet the criteria of drought stress.

Table 5. Soil water content during leaf drought scoring at 48 days after planting.

Block	Point	Moisture water content (%)
1	1	12.7
	2	13.1
	3	13.7
2	1	13.6
	2	13.3
	3	14.8
3	1	13.1
	2	13.2
	3	13.0
Minimum		12.7
Maximum		14.8
Average		13.4

Lower soil moisture values will result in a decrease in rice production. Hou et al. (2022) reported that the Hanyou 73 variety was more drought-tolerant than the hybrid rice Guangliangyou 1813 at 20-40% soil moisture levels. Salsinha et al. (2020) reported that drought stress in rice plants caused a decrease in the percentage of relative water content and the membrane stability index, but increased the proline levels. Ahmad et al. (2022) reported that GSR lines demonstrated higher plant height, increased tillers per plant, maintained grain length, and high grain yield than the check varieties under drought stress.

Response of DH GSR lines to drought stress based on leaf rolling

The leaf rolling scores are presented in Table 6. According to Jarin et al. (2024), leaf rolling is one of the early drought symptoms. The leaf rolling score in DH GSR lines ranged from 5.9 to 8.2. Based on the Friedman test ranking of leaf rolling, eight lines (40% of lines, i.e., SN11, 12, 14, 15, 40, 51, 57, and 58) differed significantly from the sensitive-drought (IR 20 variety) and did not differ from the tolerant-drought (Salumpikit) regarding leaf rolling. This indicates that those lines were better than IR 20. Most lines exhibited mild sensitivity to leaf rolling under drought stress. Lines SN11, SN14, SN57, and SN58 were more moderate to drought stress than IR 20 in the vegetative stage. This study found no lines with the criteria of mild tolerant, tolerant, or highly tolerant to leaf rolling, different from a report by Saharia et al. (2024), which found variations in rice genotypes tested for leaf rolling and leaf drying traits, ranging from sensitive to highly tolerant.

Table 6. Response of DH GSR lines to drought stress based on leaf rolling at the vegetative stage.

No	Genotype	Leaf rolling score	Criteria	Sum of rank
1	SN2	7.6	Mildly sensitive	49 [a]
2	SN3	8.2	Mildly sensitive	63 [a]
3	SN5	8.0	Mildly sensitive	56 [a]
4	SN9	7.2	Mildly sensitive	39 [aB]
5	SN11	6.0	Moderate	26 [B]
6	SN12	6.7	Mildly sensitive	28 [B]
7	SN13	7.0	Mildly sensitive	41 [a]
8	SN14	6.2	Moderate	25 [B]
9	SN15	6.8	Mildly sensitive	29 [B]
10	SN18	7.4	Mildly sensitive	44 [a]
11	SN20	7.7	Mildly sensitive	44 [a]
12	SN25	7.4	Mildly sensitive	40 [a]
13	SN28	7.4	Mildly sensitive	42 [a]
14	SN32	7.6	Mildly sensitive	53 [a]
15	SN40	6.8	Mildly sensitive	30 [B]
16	SN51	6.7	Mildly sensitive	31 [B]
17	SN57	5.9	Moderate	20 [B]
18	SN58	6.2	Moderate	23 [B]
19	SN59	7.3	Mildly sensitive	40 [a]
20	SN60	7.0	Mildly sensitive	39 [aB]
21	Inpari 42 Agritan GSR	6.8	Mildly sensitive	46 [a]
22	Inpari 18 Agritan	6.8	Mildly sensitive	27 [B]
23	Salumpikit (TC)	0.5	Tolerant	3
24	IR 20 (SC)	8.6	Sensitive	70

Note: TC = tolerant check, SC = sensitive check, numbers in column followed by lowercase letters indicate significantly lower than Salumpikit, while those followed by capital letters indicate significantly higher than IR 20 based on the LSD test at α = 0.05, LSD $_{0.05}$ = 30.53.

Mudhor et al. (2022) concluded that the morphological response to drought stress is indicated by reduced stomatal density and leaf rolling score in rice. Plants that experienced a higher leaf rolling score indicated more sensitivity to drought stress; conversely, the lower the leaf rolling score, the more tolerant the plant is. According to

Aslam et al. (2022), drought avoidance strategies in rice may include stomatal closure, cellular adaptation, and changes in root development. The leaf rolling in the tested lines was suspected to avoid water loss during drought stress. Low water potential during drought stress may change several morphological traits in leaves, such as leaf rolling, wilting, reducing leaf area, thickening leaves, stomatal closure (Yang et al., 2021), decreased germination capacity, reduced leaf area index, reduced yield components, and rice yield (Jarin et al., 2024).

Response of DH GSR lines to drought stress based on leaf drying

DH GSR lines exhibited varying responses of the leaf drying trait when subjected to drought stress in the vegetative stage (Table 7). According to the Friedman test, DH GSR lines ranged from mildly sensitive to moderate to drought stress. Based on Friedman test rankings, six lines (SN9, 13, 14, 40, 57, and 58) showed no significant difference compared to the drought-tolerant Salumpikit and were significantly better than IR 20, the drought-sensitive check. Most lines experienced changes in scoring values, with leaf rolling scores changing from 7 (mildly sensitive) to 5 (moderate) under drought conditions.

However, the leaf scores may change, resulting in lower score values or the tolerance criteria rising one level above the initial score criteria. It can be seen from the average score for leaf rolling (Table 6), which was higher than the score for leaf drying (Table 7). Changes occur from being mildly sensitive at leaf rolling to moderate at leaf drying observation. Leaf rolling and drying are effective indicators for evaluating drought tolerance in rice during the vegetative stage (Barik et al., 2019). Rice plants experiencing leaf drying can result in decreased chlorophyll content and reduced photosynthesis rates, impacting dry grain weight (Moonmoon et al., 2017).

Table 7. Response of DH GSR lines to drought stress based on leaf drying at the vegetative stage.

No	Genotype	Leaf drying score	Criteria	Sum of rank
1	SN2	6.2	Moderate	34 aB
2	SN3	7.3	Mildly sensitive	60 a
3	SN5	7.2	Mildly sensitive	54 a
4	SN9	5.7	Moderate	27 B
5	SN11	6.0	Moderate	39 aB
6	SN12	6.0	Moderate	36 aB
7	SN13	5.6	Moderate	30 B
8	SN14	5.7	Moderate	29 B
9	SN15	6.3	Moderate	45 a
10	SN18	6.6	Mildly sensitive	51 a
11	SN20	6.2	Moderate	39 aB
12	SN25	6.1	Moderate	37 aB
13	SN28	6.7	Mildly sensitive	52 a
14	SN32	6.5	Mildly sensitive	47 a
15	SN40	5.5	Moderate	20 B
16	SN51	6.2	Moderate	34 aB
17	SN57	4.9	Moderate	10 B
18	SN58	5.4	Moderate	25 B
19	SN59	6.1	Moderate	34 aB
20	SN60	6.2	Moderate	43 a
21	Inpari 42 Agritan GSR	6.5	Mildly sensitive	51 a
22	Inpari 18 Agritan	6.0	Moderate	35 aB
23	Salumpikit (TC)	1.1	Tolerant	3
24	IR 20 (SC)	9.0	Sensitive	72

Note: TC = tolerant check, SC = sensitive check, numbers followed by lowercase letters indicate significantly lower than Salumpikit, while those followed by capital letters indicate significantly higher than IR 20 based on the LSD test at α= 0.05, $LSD_{0.05}$ = 29.19.

Response of DH GSR lines to drought stress based on recovery ability

The recovery ability of DH GSR lines ranged from mildly sensitive to mildly tolerant to drought stress during the vegetative stage (Table 8). Four lines (SN12, 14, 32, and 59) and Inpari 18 showed no significant difference from the drought-tolerant check, Salumpikit. Those lines were significantly better than the drought-sensitive check IR 20 based on Friedman test rankings, with moderate to mildly tolerant criteria.

The SN14 line showed a change in criteria from moderate during drought to mildly tolerant after re-watering. However, in this study, no lines were found to be tolerant to drought based on their recovery ability. Nevertheless, the SN14 line with mild-tolerant criteria is an important finding in this research. When drought stress occurs, plants have avoidance mechanisms, and some can tolerate drought stress (Bashir et al., 2021).

Plants maintain their growth functions through photosynthetic CO_2 fixation during drought and quickly recover after re-watering in the vegetative stage. Re-watering is important in influencing final productivity (Abid et al., 2018; Bandurska, 2022). According to Abid et al. (2018), plants in moderate drought stress conditions will regain leaf water potential, membrane stability, photosynthesis, lipid peroxidation, antioxidative activity, ROS formation, and osmotic potential if re-watered. However, severely stressed plants do not fully recover in these areas.

Index selection on DH GSR lines under drought stress conditions at the vegetative stage

Drought negatively impacts plant growth parameters and reduces yield (Panda et al., 2021), making drought tolerance a crucial trait in determining rice yields (Dietz et al., 2021). According to Sujinah and Jamil (2016), morphophysiological responses can be used as indicators for selecting drought-tolerant varieties. In this context, variables like leaf rolling, leaf drying, and recovery ability are key morphological response indicators.

Table 8. Response of DH GSR lines to drought stress based on recovery ability at the vegetative stage.

No	Genotype	Recovery ability score	Criteria	Sum of rank
1	SN2	6.3	Mildly sensitive	51 a
2	SN3	7.0	Mildly sensitive	62 a
3	SN5	7.7	Mildly sensitive	65 a
4	SN9	7.0	Mildly sensitive	58 a
5	SN11	5.7	Moderate	44 a
6	SN12	4.3	Moderate	24 B
7	SN13	5.7	Moderate	44 a
8	SN14	3.7	Mildly tolerant	16 B
9	SN15	5.7	Moderate	44 a
10	SN18	5.0	Moderate	33 a
11	SN20	5.7	Moderate	42 a
12	SN25	5.7	Moderate	42 a
13	SN28	6.3	Mildly sensitive	45 a
14	SN32	4.3	Moderate	25 B
15	SN40	5.0	Moderate	33 a
16	SN51	5.0	Moderate	33 a
17	SN57	5.0	Moderate	33 a
18	SN58	5.0	Moderate	36 a
19	SN59	4.3	Moderate	24 B
20	SN60	5.0	Moderate	35 a
21	Inpari 42 Agritan GSR	5.0	Moderate	33 a
22	Inpari 18 Agritan	4.3	Moderate	25 B
23	Salumpikit (TC)	1.7	Tolerant	3
24	IR 20 (SC)	6.3	Mildly sensitive	53

Note: TC = tolerant check, SC = sensitive check, numbers in column followed by lowercase letters indicate significantly lower than Salumpikit, while those followed by capital letters indicate significantly higher than IR 20 based on the LSD at α= 0.05, $LSD_{0.05}$ = 24.38.

Selection based on these three traits is essential for identifying drought-tolerant lines. Numerous studies have employed the index selection approach to evaluate rice plants under drought stress conditions, notably those by Rahimi et al. (2017), Cui et al. (2020), Guimarães et al. (2021), Laraswati et al. (2021), and Sabouri et al. (2022).

Leaf rolling and leaf drying were assigned a weight of -1, while recovery ability was given a weight of -2. Recovery was weighted twice as much due to the potential for lines to improve in drought tolerance criteria. The negative index weighting for the three variables indicates that a smaller score reflects better plant performance or greater tolerance to drought stress (Kartina et al., 2019; Wening et al., 2019).

Water stress poses a threat that can affect all aspects of plant growth, development, and metabolism (Chada et al., 2023). Rice plants responded to drought stress through escape, avoidance, and tolerance. One mitigation effort to avoid drought is developing drought-tolerant varieties (Bhandari et al., 2023). The plant's ability to recover after drought is also one of the plant's strategies in coping with drought (Barik et al., 2019). The selection index values ranged from -5.51 to 13.77 (Table 9). The higher the index value, the greater the likelihood of possessing desirable traits, such as lower leaf rolling, lesser leaf drying, and better recovery ability compared to those with lower selection index values.

Based on the selection index values in Table 9, nine lines (45% of the tested lines) have positive selection index values, which are SN12, 14, 32, 40, 51, 57, 58, 59, and 60. These values are higher than Inpari 42 Agritan GSR and the drought-sensitive check IR20. Eleven other lines (55% of the tested lines) have negative selection index values, including SN2, 3, 5, 9, 11, 13, 15, 18, 20, 25, and 28. Lines with positive index values can be selected as potential lines for further drought stress testing at the generative stage.

Table 9. Selection index values based on leaf rolling, leaf drying, and recovery ability in DH GSR lines.

No	Genotype	Z_SLR	Z_SLD	Z_SRA	Index
1	Salumpikit (TC)	-4.20	-3.75	-2.91	13.77
2	SN14	-0.42	-0.26	-1.30	3.27
3	SN57	-0.61	-0.87	-0.22	1.93
4	SN12	-0.08	-0.03	-0.76	1.64
5	Inpari 18 Agritan	-0.02	-0.03	-0.76	1.57
6	SN58	-0.42	-0.49	-0.22	1.35
7	SN59	0.32	0.04	-0.76	1.16
8	SN40	-0.02	-0.41	-0.22	0.88
9	SN32	0.51	0.35	-0.76	0.66
10	SN51	-0.08	0.12	-0.22	0.41
11	SN60	0.12	0.12	-0.22	0.21
12	Inpari 42 Agritan GSR	-0.02	0.35	-0.22	0.12
13	SN11	-0.55	-0.03	0.31	-0.05
14	SN18	0.38	0.42	-0.22	-0.36
15	SN13	0.12	-0.34	0.31	-0.41
16	SN15	-0.02	0.20	0.31	-0.81
17	SN25	0.38	0.04	0.31	-1.05
18	SN20	0.58	0.12	0.31	-1.33
19	SN2	0.51	0.12	0.85	-2.33
20	SN28	0.38	0.50	0.85	-2.58
21	SN9	0.25	-0.26	1.39	-2.76
22	SN3	0.91	0.96	1.39	-4.64
23	IR 20 (SC)	1.18	2.25	0.85	-5.13
24	SN5	0.78	0.88	1.92	-5.51

Note: TC = tolerant check, SC = sensitive check, Z_SLR = standardization value of score leaf rolling, Z_SLD = standardization value of score leaf drying, Z_SRA = standardization value of score recovery ability.

CONCLUSIONS

The lines exhibited different responses for leaf rolling, leaf drying, and recovery ability. Nine DH GSR lines, i.e., SN12, 14, 32, 40, 51, 57, 58, 59, and 60, showed moderate to mild tolerance to drought stress in the vegetative stage. Those lines can be evaluated for their tolerance level in the generative stage.

ACKNOWLEDGEMENTS

The authors thank the Directorate General of Research and Development; Ministry of Higher Education, Science, and Technology, for funding this research through the Fundamental Research scheme (No. 006/C3/DT.05.00/PL/2025). The authors also express their gratitude to BBPSI Biogen for providing the greenhouse facilities.

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