Physiological Responses of Stress Tolerant Rice Varieties across Different Cropping Systems under Rainfed Stress

ABSTRACT

Rice, sustaining half of the world's population, is traditionally cultivated through transplanting, particularly in Asia. However, challenges like excessive water use, labour demands, and environmental stresses like drought prompt the search for alternatives. Our study evaluates the impact of various crop establishment methods (CEs) – conventional puddled transplanting, direct drill seeding on flatbeds (DSR), and direct seeding on raised beds (FIRB) – on five stress-tolerant rice varieties (V): DRR 42, DRR 44, Sukha Dhan 5, Sukha Dhan 6, and Sarjoo 52. The key physiological parameters like Relative Water Content, Membrane Stability Index, and Chlorophyll content were analysed across different CE and V combinations. Notably, FIRB consistently surpasses other methods, suggesting its potential in bolstering stress tolerance and yield. Among the five varieties, Sukha Dhan 5 (V3) displays the highest RWC, Sarjoo 52 (V5) in MSI, and DRR 44 (V2) demonstrates superior chlorophyll content. These varieties underscore their pivotal role in maintaining plant water status, facilitating robust photosynthesis, and enhancing stress resilience, thereby ensuring stable yields. Our findings underscore FIRB's promise in curbing water waste and mitigating drawbacks associated with conventional transplanting practices.

Keywords: Cropping Systems, Direct drill seeding on flatbed (DSR), Direct seeding on the raised bed (FIRB), Puddled transplanting, Stress-Tolerant Rice Varieties

1. **INTRODUCTION**

Rice, a vital staple food, is cultivated in over 95 countries worldwide, providing approximately 20% of the global dietary energy supply, with Asia alone accounting for 92% of production and consumption [1;2]. India ranks as the world's second-largest producer of rice [3]. However, only a fraction of rice cultivation in India is irrigated [4]. Traditional methods like transplanting in puddled soil dominate, especially in regions like North Western India [5;6]. While offering benefits such as higher yields and effective weed control, these methods are water-intensive, laborious and environmentally concerning due to soil degradation [7]. Hence, there is a growing need for alternative methods to address water scarcity and environmental issues while ensuring food security.

Dry direct seeding of rice (DSR) involves planting crop in non-puddled, unsaturated soil. Kumar et al. [8] stated its efficacy in reducing water losses and labour costs. The yield of rice from direct-seeded crops is comparable to or higher than that from transplanted rice systems [9;10]. Additionally, Bajpai et al. [11] suggest that minimal tillage in rice achieves results similar to conventional puddling while reducing transplanting expenses and preserving soil structure. DSR offers the potential to decrease water usage by 44% without compromising yield [12]. DSR maximizes water utilization, making it suitable for regions with limited water resources

[13]. This method also sustains rapid growth under low irrigation and fertilizer conditions, contributing to its economic viability [14]. Moreover, aerobic rice cultivation promotes tiller emergence and enhances the rhizosphere environment [15;16]. Selecting large, deep-rooted cultivars ensures the stability of continuous aerobic rice production, with additional benefits observed when rotating aerobic rice with upland crops, particularly soybeans [17].

The furrow-irrigated raised bed (FIRB) cropping method is cropping in ridges or beds, a technique widely adopted for wheat globally and increasingly so for rice and vegetables in the Indo-Gangetic plains. Singh et al. [18] stated, higher tiller density, longer ears, and increased test weight, resulting in greater rice yields in FIRB when compared to conventional methods. Coventry et al. [19] revealed significantly higher yields with this method compared to direct-seeded rice. Water savings of 18% to 50% have been observed with FIRB [20;21], attributed to increased biomass and border effects [22]. Microclimate and rhizosphere conditions contribute to its high-yield characteristics [23], while reduced soil bulk density benefits crop growth [24]. Additionally, bed planting reduces water usage and weed control costs [25]. Adoption of this method has boosted wheat production in regions like North-western Mexico [26]. Hobbs et al. [27] highlight benefits like efficient water use, weed control, and lesser lodging, with FIRB allowing better light penetration and reduced seed rates [28].

While there is growing recognition of the need for alternative cultivation methods to enhance rice productivity and address environmental challenges, there remains a gap in understanding the specific benefits and limitations of these methods, particularly in the context of physiological traits and stress-tolerant rice varieties. In our study, we aimed to evaluate the impact of different cropping systems on stress-tolerant rice varieties by assessing various physiological parameters. By exploring alternative cultivation techniques such as DSR and FIRB methods, we aimed to uncover their potential advantages. Our goal was to contribute to increasing rice productivity while addressing pressing issues like water scarcity and environmental concerns associated with traditional transplanting practices.

2. MATERIAL AND METHODS

2.1 Experimentation

Sowing was done at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University in the Northern Gangetic Alluvial Plains, India, having a subtropical climate. Experiment was conducted during the kharif season of 2018-19 under rainfed stress condition. A set of five Stress-Tolerant Rice Varieties (STRVs) was obtained from the Department of Agronomy, Institute of Agricultural Sciences, BHU, Varanasi. The sowing methods were conventional puddled transplanting, direct drill seeding on flatbed (DSR), and direct seeding on raised beds (FIRB), all conducted on first week of June 2018, amidst 8.0 mm of recorded rainfall. Weekly minimum and maximum temperatures stood at 27.9 and 35.5ºC, respectively, with relative humidity at 77% in the morning and 57% in the evening. Weekly evaporation and sunshine hours were measured at 4.4 mm and 6.4 hours, respectively. Subsequently, conventional rice transplanting occurred in the first week of August, coinciding with 26.6 mm of rainfall and weekly minimum and maximum temperatures of 24.7 and 31.8ºC. The weekly evaporation rate and sunshine hours were recorded at 2.8 mm and 4.8 hours, respectively, with morning and evening relative humidity at 92% and 77%, respectively.

2.2 Design and layout

The experiment utilized a split-plot design with three replications, as outlined in Table 1. Three different cropping systems were assigned as the main plots: Puddled transplanting represented by CE1, DSR as CE2, and FIRB denoted as CE3. Five STRVs were allocated as sub-plots, with DRR 42, DRR 44, Sukha Dhan 5, Sukha Dhan 6, and Sarjoo 52 labelled as V1, V2, V3, V4, and V5, respectively. Observations were made at three growth stages: Active tillering stage (S1), 50% Flowering (S2), and Grain filling stage (S3).

Table 1. Description of the experiment.

Design	-	Split Plot
No. of main plots	-	3 (Cropping Systems)
No. of sub-plots	-	5 (Varieties)
Replications	-	3
Total number of plots	-	3 x 5 x 3 = 45
Area of field	-	77.5 m x 17.2 m
Area of plot	-	4.5 m x 4 m
Plot Border	-	0.5 m
Replication border	-	1.0 m

2.3 Measurement of physiological parameters and yield

The relative water content (RWC) was assessed following the procedure outlined by Weatherley [29]. Initially, 100 mg of leaf material was selected and immersed in double distilled water in a Petri dish for a duration of two hours to ensure the leaf tissue reached turgidity. After this period, the turgid masses of the leaf materials were measured by gently blotting excess water and placing the tissues between two filter papers. Subsequently, the leaf material was transferred to a butter paper bag and subjected to drying in a hot air oven set at 65ºC for 24 hours, after which their dry masses were recorded. The RWC was then calculated using the appropriate formula.

$$\text{RWC (\%)} = \frac{[\text{Fresh mass} - \text{Dry mass}]}{[\text{Turgid mass} - \text{Dry mass}]} \times 100$$

The membrane stability index (MSI) was determined following the protocol outlined by Sairam [30], which involved measuring the electrical conductivity of leaf leachates. Initially, the MSI was assessed by placing two identically sized leaf discs into separate standard test tubes containing 10 mL of double distilled water. One set of test tubes was then placed in a water bath set to 40ºC for 30 minutes, while another set was submerged in boiling water at 100ºC for 15 minutes. Subsequently, the electrical conductivities (EC1 at 40ºC and EC2 at 100ºC) of the respective leachates were measured using a conductivity meter (Systronics conductivity meter, 306). The membrane thermo-stability was then calculated using the provided formulae.

$$\text{MSI} = 1 - \frac{\text{EC 1}}{\text{EC 2}} \times 100$$

The chlorophyll content in leaf samples was determined following the method outlined by Hiscox and Israelstam [31]. Initially, the uppermost fully expanded fresh leaf samples were selected, thoroughly washed, and then cut into small sections. Subsequently, 50mg of leaf sample was placed into test tubes containing 5mL of Dimethyl sulfoxide (DMSO), and the tubes were placed in a hot air oven set to 72°C for 2 hours. After cooling, the final volume of the supernatant was adjusted to 10 mL using DMSO. For the blank, 10 mL of DMSO was taken in a test tube without the leaf sample. Absorbance readings were taken at 663 nm and 645 nm, respectively, using a spectrophotometer (SSC-177 Scanning Mini Spectrophotometer) and the chlorophyll content was recorded. The Chlorophyll content was determined by using formula given by Arnon [32].

$$\text{Chlorophyll } a \text{ content (mg/g FM)} = \frac{[12.7 \times A_{663} - 2.69 \times A_{645}] \times \text{Volume of sample}}{1000 \times \text{Mass of sample}}$$

$$\text{Chlorophyll } b \text{ content (mg/g FM)} = \frac{[22.9 \times A_{645} - 4.68 \times A_{663}] \times \text{Volume of sample}}{1000 \times \text{Mass of sample}}$$

$$\text{Total chlorophyll content (mg/g FM)} = \frac{[20.2 \times A_{645} + 8.02 \times A_{663}] \times \text{Volume of sample}}{1000 \times \text{Mass of sample}}$$

where, A_{645}, A_{663} are the absorbance readings at 645 and 663 nm wavelengths, respectively and FM is fresh mass of the sample.

2.3 Statistical analysis

A split-plot layout with three replications was employed in the experimental design, featuring three CEs as main plots and five rice varieties as sub-plots. To assess the significance of treatments and sampling periods, a two-way analysis of variance (ANOVA) was conducted for all the parameters. Mean differences were determined using Duncan's Multiple Range Test (DMRT) at the significance level of p= 0.05. The statistical analysis was performed utilizing the web-based platform STAR NEBULA (Statistical Tool for Agricultural Research, International Rice Research Institute).

3. RESULTS AND DISCUSSION

The ANOVA results for the physiological parameters studied in five STRVs across three CEs at various growth stages are presented in Table 2. It was observed that all the studied traits were significantly influenced ($p < 0.05$) by CE, V, and their interaction CE × V. The impact of different CEs and V on physiological parameters at different growth stages is visually depicted in Figure

Table 2. ANOVA results for physiological parameters studied in five stress-tolerant rice varieties (V) across three Cropping Systems (CE) at different growth stages under split plot design.

Stages of observation/Parameters		At active tillering			At 50% flowering			At grain filling		
		CE	**V**	**CE × V**	**CE**	**V**	**CE × V**	**CE**	**V**	**CE × V**
Relative Water Content	**SE(m)**	0.97	1.40	2.42	0.96	1.58	2.73	0.88	1.28	2.22
	CD	3.81*	4.08*	7.07*	3.79*	4.6*	7.97*	3.46*	3.73*	6.47*
	CV	4.60	5.13	-	4.27	5.41	-	4.55	5.12	-
Membrane Stability Index	**SE(m)**	1.43	1.70	2.94	1.29	2.09	3.61	1.01	1.46	2.53
	CD	5.6*	4.96*	8.6*	5.06*	6.09*	10.55*	3.96*	4.27*	7.39*
	CV	9.33	8.62	-	9.43	11.83	-	6.90	7.74	-
Chlorophyll *a* content	**SE(m)**	0.00	0.00	0.01	0.03	0.04	0.07	0.01	0.06	0.10
	CD	0.008*	0.01*	0.017*	0.111*	0.122*	0.212*	0.03*	0.171*	0.295*
	CV	4.26	5.40	-	4.06	4.66	-	2.00	11.68	-
Chlorophyll *b* content	**SE(m)**	0.01	0.01	0.01	0.02	0.02	0.04	0.02	0.04	0.08
	CD	0.03*	0.02*	0.035*	0.06*	0.069*	0.12*	0.091*	0.128*	0.221*
	CV	30.61	21.23	-	5.72	6.87	-	15.69	23.02	-
Total chlorophyll content	**SE(m)**	0.01	0.01	0.01	0.02	0.04	0.07	0.02	0.09	0.16
	CD	0.03*	0.02*	0.03*	0.08*	0.11*	0.19*	0.09*	0.27*	0.46*
	CV	11.12	5.94	-	2.05	3.04	-	4.34	13.30	-

SE(m) - Standard Error for the mean, CD - Critical Difference, CV - Coefficient of Variation, * represents significance level at $p<0.05$

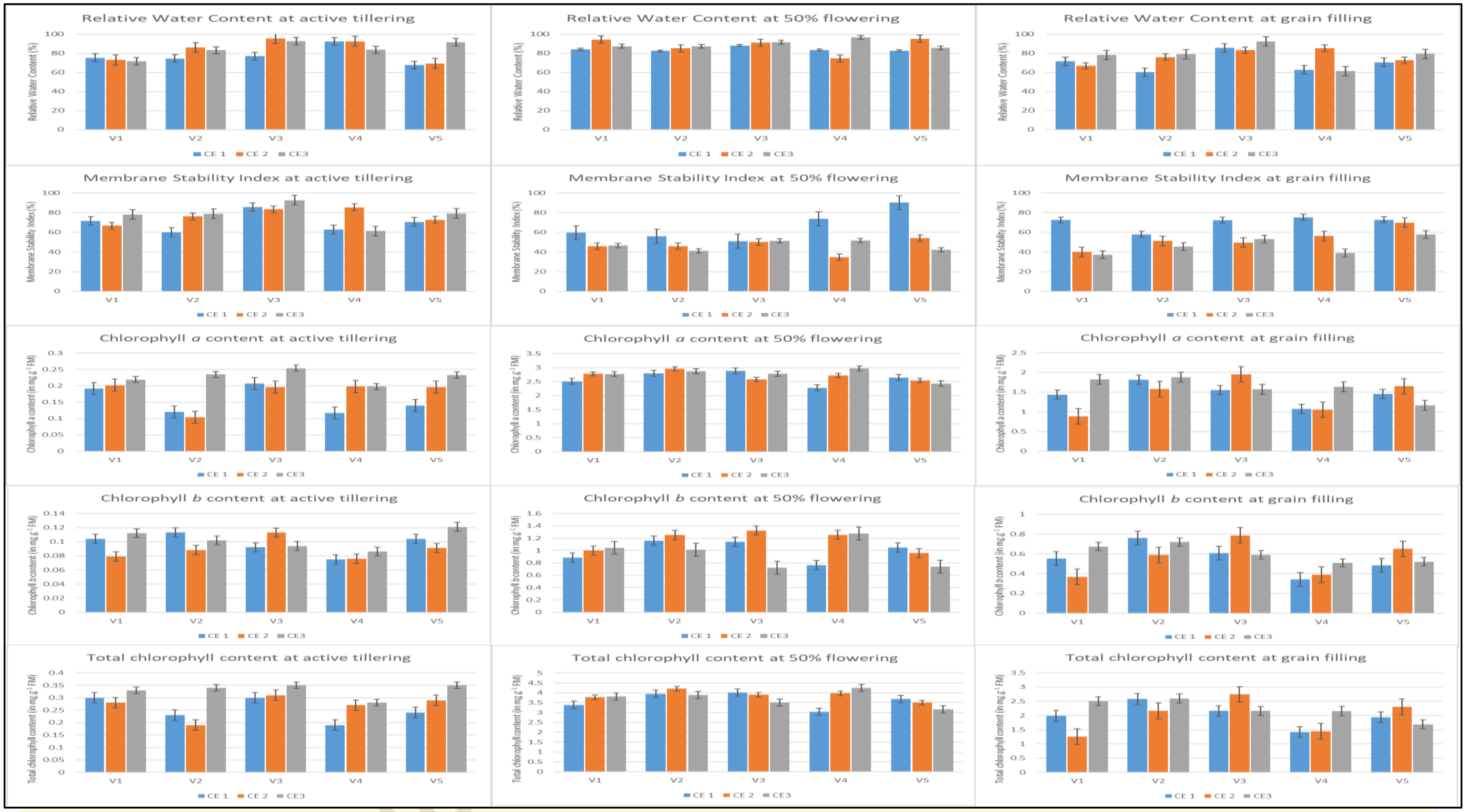

Figure 1. Effect of different Cropping Systems (CE) and stress-tolerant rice varieties (V) on physiological parameters studied at different growth stages. Bars represent Standard Error. Abbreviations; V1 - DRR 42, V2 - DRR 44, V3 - Sukha Dhan 5, V4 - Sukha Dhan 6, V5 - Sarjoo 52, CE1 - puddled transplanting, CE2 - direct drill seeding on flatbed (DSR), and CE3 - direct seeding on raised beds (FIRB).

3.1 Impact of different Cropping Systems on relative water content in stress-tolerant rice varieties

The RWC analysis revealed notable variations among the different growth stages and CEs examined in this study. CE3 exhibited significantly higher RWC content across all growth stages, while CE1 displayed the lowest levels [Table 3]. Highest RWC observed at 50% maturity, indicating a critical phase in rice development. Interestingly, CE3 × V3 consistently outperformed other combinations in terms of RWC content throughout the experiment. During the active tillering stage, V4 at CE1 and V3 at both CE2 and CE3 exhibited the highest RWC content, while V5 at CE1 and V1 and V5 at CE2 displayed significantly lower RWC levels. At 50% flowering, all varieties were relatively equivalent at CE1, while V1 and V5 at CE2 and V4 at CE3 demonstrated the highest RWC content among the CEs. However, at maturity, V3 consistently exhibited the highest RWC content across all CEs, with V2 and V4 at CE1, V1 at CE2, and V4 at CE3 displaying the lowest levels. Further analysis focusing on individual varieties revealed V3 consistently demonstrating the highest RWC levels across all three stages [Table 4]. Notably, during the active tillering stage, nearly all varieties, except V4, displayed optimal performance at CE3. Similarly, at 50% flowering, V2 and V3 exhibited comparable RWC levels across all CEs. However, at maturity, all varieties, except V4, showcased their best RWC performance at CE3, with CE1 consistently displaying the lowest RWC levels among all varieties.

RWC serves as a crucial indicator of plant water status due to its direct correlation with cell volume and the balance between water absorption and transpiration. Osmoregulation plays a pivotal role in maintaining cell turgor pressure, ensuring normal water absorption and metabolic processes [33]. Reduced RWC levels can lead to diminished plant vigour, as observed in various plant species [34]. Studies have indicated that RWC serves as an index for assessing stress intensity, with higher RWC content contributing to enhanced drought resistance in crops like wheat [35, 36]. Additionally, water deficit conditions may lead to damage to cellular components such as polyribosomes, resulting in protein breakdown [37]. Thus, maintaining optimal RWC levels is essential for sustaining high yields under drought stress conditions.

Table 3. Mean of Cropping Systems (CE) for all the studied physiological parameters at different growth stages.

Stages of observation	At active tillering			At 50% flowering			At grain filling			Total mean		
CEs	CE 1	CE 2	CE3	CE 1	CE 2	CE3	CE 1	CE 2	CE3	CE 1	CE 2	CE3
Relative Water Content [%]	77.34	83.31	84.59	84.23	88.12	89.71	70.10	76.84	78.02	77.22	82.76	84.11
Membrane Stability Index [%]	59.04	62.83	55.60	66.06	46.13	46.54	70.16	53.29	46.48	65.09	54.08	49.54
Chlorophyll *a* content [mg g^{-1} FM]	0.16	0.18	0.23	2.62	2.71	2.76	1.47	1.42	1.62	1.41	1.44	1.54
Chlorophyll *b* content [mg g^{-1} FM]	0.10	0.09	0.10	1.00	1.16	0.96	0.55	0.56	0.60	0.55	0.60	0.56
Total chlorophyll content [mg g^{-1} FM]	0.25	0.27	0.33	3.62	3.87	3.72	2.02	1.98	2.22	1.96	2.04	2.09

Here, FM is fresh mass of the sample.

3.2 Impact of different Cropping Systems on Membrane Stability Index in stress-tolerant rice varieties

Table 3 indicates that CE1 demonstrated the highest MSI among most CEs, while CE3 showed the lowest. In Table 4, V5 consistently outperformed other varieties across different growth stages. During the active tillering stage, MSI content was highest in CE2 among CEs and in V1 among varieties. At the 50% flowering stage, V5 exhibited the highest MSI content among varieties, while CE1 had the highest among CEs. Similarly, at maturity, CE1 had the highest MSI content among CEs, and V5 among varieties. Notably, CE1 displayed higher MSI content in the last two growth stages. Tyagi et al. [38] emphasized the significant role of MSI in rice drought tolerance. Sairam et al. [39] associated membrane stability with water and high-temperature stress tolerance in various crops. Several studies have shown a direct correlation between MSI and field performance under high-temperature stress [40].

Table 4. Mean of stress-tolerant rice varieties (V) for all the studied physiological parameters at different growth stages.

Stages of observation	Varieties	Relative Water Content [%]	Membrane Stability Index [%]	Chlorophyll *a* content [mg g^{-1} FM]	Chlorophyll *b* content [mg g^{-1} FM]	Total chlorophyll content [mg g^{-1} FM]
At active tillering	V1	73.27	63.27	0.20	0.10	0.30
	V2	81.29	62.04	0.15	0.10	0.25
	V3	88.40	57.44	0.22	0.10	0.32
	V4	89.55	60.50	0.17	0.08	0.25
	V5	76.20	52.54	0.19	0.11	0.30
At 50% flowering	V1	88.68	50.65	2.68	0.98	3.66
	V2	84.96	47.66	2.88	1.14	4.01
	V3	90.27	50.73	2.75	1.06	3.81
	V4	84.95	53.30	2.65	1.10	3.75
	V5	87.88	62.20	2.54	0.92	3.46
At grain filling	V1	72.13	49.81	1.38	0.53	1.91
	V2	71.69	51.54	1.76	0.69	2.45
	V3	87.15	58.29	1.69	0.66	2.35
	V4	69.79	56.84	1.25	0.41	1.67
	V5	74.18	66.74	1.42	0.55	1.97
Total mean	V1	78.03	54.58	1.42	0.54	1.96
	V2	79.31	53.75	1.60	0.64	2.24
	V3	88.61	55.49	1.55	0.61	2.16
	V4	81.43	56.88	1.36	0.53	1.89
	V5	79.42	60.49	1.38	0.52	1.91

3.3 Impact of different Cropping Systems on Chlorophyll content in stress-tolerant rice varieties

CE3 demonstrated the highest Chlorophyll *a* content among almost all the CEs, whereas CE1 exhibited the lowest [Table 3]. V2 consistently outperformed other varieties across various growth stages [Table 4]. At the active tillering stage, CE3 had the highest chlorophyll *a* content, while V3 stood out among varieties. At CE2, all varieties except V2 performed equally well. Among CEs, the highest chlorophyll *a* content was observed in V1 and V3 at CE1, and V3 at CE3. Conversely, significantly low chlorophyll *a* content was found in variety V2 and V4 at CE1 and V4 at CE3. At the 50% flowering stage, CE3 and V2 exhibited the highest chlorophyll *a* content among Cropping Systems. Similarly, at the grain filling stage, the highest chlorophyll *a* content was observed in CE3 and V2.

Table 3 indicates that CE2 displayed the highest chlorophyll ‘b’ content among nearly all the CEs, while CE1 showed the lowest. In Table 4, it is evident that V2 consistently outperformed other varieties across various growth stages. At the active tillering stage, CE3 and V5 had the highest chlorophyll *b* content. During the 50% flowering stage, CE2 and V2 had the highest chlorophyll *b* content among CEs. Similarly, at the grain filling stage, CE3 and V2 exhibited the highest chlorophyll *b* content.

Table 3 illustrates that CE3 showed the highest total chlorophyll content among almost all the CEs, while CE1 displayed the lowest. In Table 4, V2 consistently outperformed other varieties across various growth stages. At the active tillering stage, CE3 had the highest total chlorophyll content, while among varieties, V3 had the highest. At the 50% flowering stage, CE2 had the highest total chlorophyll content among CEs, whereas V2 showed the highest among varieties. At the grain filling stage, V2 exhibited the highest total chlorophyll content among varieties, while CE3 had the highest among CEs. However, V4 at CE1 and V1 and V4 at CE2, as well as V5 at CE3, showed significantly low total chlorophyll content.

Research by Kura-Hotta et al. [41] on rice seedlings suggested that low chlorophyll content leads to the inactivation of photosynthesis. Moreover, increased resistance to various stresses is commonly observed with an increase in chlorophyll content in plants [42]. In a study by Ihsan et al. [43] on wheat, findings revealed that chlorophyll contents in the FIRB method were significantly greater than in DSR planting, potentially due to higher nitrogen uptake under the furrow-irrigated raised bed method. Another reason for increased chlorophyll content could be better sunlight absorption under the FIRB method. The difference in chlorophyll content between DSR planting and the FIRB method was more significant at later growth stages. According to Fahong et al. [44], the longer the duration of greenness of leaves due to higher chlorophyll content, the longer the rate of grain filling and consequently, the higher the yield.

4. CONCLUSION

The study demonstrated significant effects of different cropping systems like conventional puddled transplanting, DSR and FIRB on physiological parameters like RWC, MSI and chlorophyll content in five stress-tolerant rice varieties. Our findings reveal significant variations in RWC, MSI, and chlorophyll content across different growth stages and crop establishment methods. Both CE and V exerted significant variations, along with their interaction, on the studied traits. Notably, FIRB method consistently exhibited superior performance in RWC and chlorophyll content, indicating its potential in promoting water retention and photosynthetic efficiency. Among five STRVs, Sukha Dhan 5 (V3) showed highest RWC, which emphasizes its critical role in maintaining plant water status and stress tolerance, with implications for drought mitigation strategies. Additionally, Sarjoo 52 (V5) outperformed in the MSI which serves as a valuable indicator of plant resilience under environmental stressors, resulting in yield stability. Furthermore, DRR 44 (V2) showed best chlorophyll content which elucidates its pivotal role in photosynthesis and stress response mechanisms. The significant disparities in chlorophyll content between different cropping systems underscore the importance of optimizing cultivation practices to maximize photosynthetic efficiency and yield potential. Overall, our research contributes valuable insights into the complex interplay between crop establishment methods, physiological traits, and stress tolerance in rice cultivation. By elucidating the specific advantages and limitations of alternative cultivation techniques such as DSR and FIRB methods, we aim to inform sustainable agricultural practices and enhance food security in the face of evolving environmental challenges.

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