

# **Assessment of Biochemical Parameters and Yield Performance in Rice under Different Crop Establishment Methods**

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## **ABSTRACT**

Rice (*Oryza sativa L.*), a staple food for half of the global population, is cultivated using various methods, with transplanting being conventional in many Asian countries. However, challenges such as high-water consumption, labour intensiveness, and environmental degradation have prompted the exploration of alternative methods. In this study, we assessed the impact of various crop establishment methods (CE) i.e. conventional puddled transplanting, direct drill seeding on flatbed (DSR), and direct seeding on raised beds (FIRB) on five stress-tolerant rice varieties (V) i.e. DRR 42, DRR 44, Sukha Dhan 5, Sukha Dhan 6 and Sarjoo 52 by analysing biochemical parameters i.e. total sugar content, starch content, MDA content, SOD content and yield outcomes. Our findings reveal significant variations in biochemical parameters and yield across different CE and V combinations. Notably, FIRB consistently outperformed other CEs, indicating its potential for enhancing stress tolerance and yield. Similarly, DRR 44 exhibited superior performance across most growth stages. Our study highlights the potential advantages of FIRB method in mitigating water wastage and addressing the limitations of conventional transplanting practices.

*Keywords: Crop Establishment Methods, Direct drill seeding on flatbed (DSR), Direct seeding on the raised bed (FIRB), Puddled transplanting, Stress-Tolerant Rice Varieties*

## **1. INTRODUCTION**

Rice (*Oryza sativa L.*), stands as the primary cereal crop globally, serving as a vital staple food for the world's population. *Oryza sativa L.*, commonly known as rice, can be divided into two main groups: aerobic and anaerobic rice. Various methods are employed for its cultivation, including drilling of soaked seeds in water, drilling on beds, drilling on beds and furrow, planting on beds and furrow, transplanting on beds, line transplanting in well-puddled soil, conventional transplanting, and parachute transplanting etc. [1].

Conventionally, rice cultivation, particularly in Asian countries, relies heavily on the transplanting method. Notably, transplanted rice with proper spacing (20×20cm) demonstrates superior yields among different cultivation systems. This method facilitates significant nitrogen fixation owing to the presence of aquatic nitrogen-fixing bacteria, thereby contributing to increased yields [2]. Additionally, transplanted rice exhibits lower weed populations compared to alternative cropping methods [3]. However, despite its advantages, transplanted rice poses challenges, especially with growing populations and depleting water levels. Key issue with transplanting, is its high-water consumption, requiring up to 150 cm of water [4]. Transplanting also demands substantial labour and is time-consuming and hence, costly [5]. Puddling, commonly associated with transplanting, form hard pans in the soil, reducing water losses through percolation [6]. However, these hard pans detrimentally affect

soil quality, leading to decreased yields in crop rotations such as the rice-wheat system. Moreover, puddled rice fields contribute significantly to environmental pollution, with approximately 65% of applied nitrogen lost due to various factors like volatilization, denitrification, leaching, and runoff [7]. In light of these challenges, scientists are exploring alternative cultivation methods such as direct seeding and bed planting to mitigate water wastage and address the limitations of transplanted rice.

Direct Seeded Rice (DSR) involves sowing rice directly in non-puddled and unsaturated soil, making it suitable for upland areas and aerobic rice cultivation [8]. DSR has the potential to achieve yields comparable to transplanting while reducing water usage by 44% [9]. Moreover, nitrogen use efficiency increases to 80% under DSR [10]. However, weed management remains a significant challenge in DSR, impacting its economic viability [11;12]. Its alternative, furrow-irrigated raised bed (FIRB) method entails planting crops in ridges or beds, offering several advantages such as high-water use efficiency, effective weed control, reduced lodging instances, and improved light penetration in the canopy [13]. Researchers have observed significant reductions in water usage and increased nitrogen uptake in this method compared to flatbed planting [14;15;16]. Additionally, FIRB promotes grain protein content and enhances various high-yielding attributes. It also improves soil porosity and water-holding capacity, contributing to increased crop productivity [17].

In our study, we assessed how different crop establishment methods impact several stress-tolerant rice varieties by analysing biochemical parameters and yield outcomes. Through this study, we sought to unveil the potential advantages of alternative cultivation techniques like DSR and FIRB. Our aim was to contribute to the enhancement of rice productivity while simultaneously tackling pressing challenges such as water scarcity and environmental issues associated with conventional transplanting practices.

## 2. MATERIAL AND METHODS

### 2.1 Experimentation

The experiment took place at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during the kharif season of 2018-19. A set of five Stress-Tolerant Rice Varieties (STRVs) was obtained from the Department of Agronomy, Institute of Agricultural Sciences, BHU, Varanasi. Details regarding the crop establishment methods (CE) and varieties (V) are presented in Table 1. The parameters were recorded at three stages (S) of crop growth i.e. active tillering, 50 % flowering and grain filling. Experiment was performed under rainfed stress conditions. Rice sowing using conventional puddled transplanting, direct drill seeding on flatbed (DSR), and direct seeding on raised beds (FIRB) occurred on last week of June, 2018. During this period, there was recorded rainfall of 8.0 mm, with weekly minimum and maximum temperatures averaging 27.9°C and 35.5°C, respectively. Transplanting of conventionally sown rice crops commenced in the first week of August. Each plot measured 4.5x4 m, bordered by 0.5 m, with replication borders extending 1.0 m, resulting in a total field area of 77.5x17.2 m.

**Table 1. Description of Crop Establishment Methods, Varieties, and Stages utilized in the experiment.**

| S. No.                                | Description           | Symbol |
|---------------------------------------|-----------------------|--------|
| <b>Crop establishment methods(CE)</b> |                       |        |
| 1                                     | Puddled transplanting | CE1    |

|                      |   |     |
|----------------------|---|-----|
| 2                    | Direct drill seeding on flatbed [DSR]   | CE2 |
| 3                    | Direct seeding on the raised bed [FIRB] | CE3 |
| <b>Varieties (V)</b> |   |     |
| 1                    | DRR 42                                  | V1  |
| 2                    | DRR 44                                  | V2  |
| 3                    | Sukha Dhan 5                            | V3  |
| 4                    | Sukha Dhan 6                            | V4  |
| 5                    | Sarjoo 52                               | V5  |

## 2.2 Measurement of biochemical parameters and yield

Total Soluble Sugar Content (TSS, mg g<sup>-1</sup> fresh weight of leaves) and Starch Content (mg g<sup>-1</sup> fresh weight of leaves) was evaluated in fully expanded flag leaves across three growth stages: active tillering, 50% flowering, and grain filling by Anthrone method as detailed by Dubois et al. [18]. For TSS determination, 100 mg leaf samples underwent homogenization in 5 mL of 80% ethanol, followed by centrifugation at 4000 g for 15 minutes. The resulting supernatant was collected and subjected to two additional extractions, then adjusted to a final volume of 50 mL with 80% ethanol. A portion of this extract was mixed with distilled water and 5 mL Anthrone reagent, followed by boiling, cooling, and measurement of absorbance at 620 nm. The concentration of soluble sugars was determined using a standard curve prepared with graded concentrations of glucose.

For starch estimation, the residue obtained from the TSS extraction process was utilized. This residue was treated with 6.5 mL of 52% Perchloric acid, followed by centrifugation at 10,000 g for 10 minutes. The resulting supernatant was collected and adjusted to a final volume of 20 mL with distilled water. A portion of this extract was mixed with Anthrone reagent, placed in boiling water bath for 10 minutes, cooled, and then subjected to absorbance measurement at 620 nm. Starch content was calculated using a standard curve.

Malondialdehyde (MDA) content, serving as an indicator of lipid peroxidation, was determined according to the method given by Hodges et al. [19]. First fully expanded leaves from plants at active tillering, 50% flowering and grain filling stage were homogenized in 0.1% TCA. Following centrifugation, the supernatant was collected, mixed with 0.5% TBA solution, subjected to heating at 95°C for 30 min, cooling, and subsequent absorbance measurement at 532 nm. The value for nonspecific absorption at 600nm was subtracted. MDA content was calculated using following formula:

$$\text{MDA } (\mu \text{ moles g}^{-1} \text{ F.W.}) = [(A_{532} - A_{600}) / 155] \times 10^6$$

Superoxide Dismutase (SOD) activity was assayed in the first fully expanded flag leaf at 50% flowering and grain filling stages, following the methodology established by Dhindsa et al. [20]. Enzyme extract was prepared from frozen leaf samples and combined with a reaction mixture. After incubation and illumination, absorbance was measured at 560 nm. SOD activity was then calculated and expressed as per gram of fresh weight.

$$\text{Enzyme Unit (EU)} = \frac{\text{Enzyme light} - [\text{Enzyme light} - \text{Enzyme dark}]}{\text{Enzyme light} / 2}$$

Upon reaching physiological maturity, all plants from a plot were harvested, air-dried, and then manually threshed. The seeds collected after threshing were weighed using an electronic balance, and the weight was recorded as the seed yield in kg per plot basis.

### **2.3 Statistical analysis**

The experimental design adopted a split-plot layout with three replications, where three CEs were designated as main plots and five rice varieties as sub-plots. To test the significance of the treatments and period of sampling, two-way analysis of variance (ANOVA) was performed for biochemical parameters. Means were separated by least significant differences at  $P= 0.05$  level using Duncan's Multiple Range Test (DMRT) with web based statistical analysis platform STAR NEBULA (Statistical Tool for Agricultural Research, International Rice Research Institute).

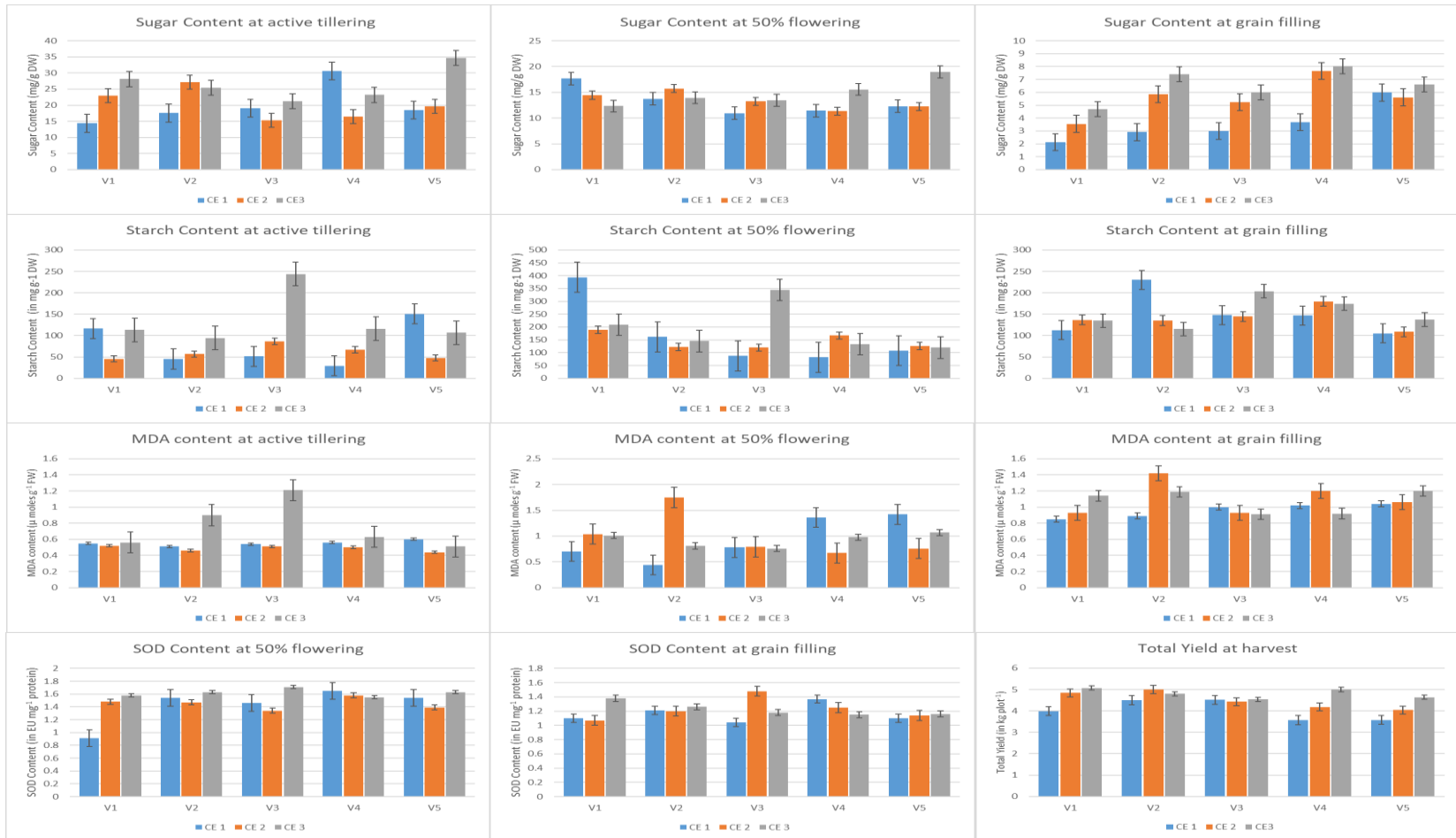
### **3. RESULTS AND DISCUSSION**

ANOVA results for biochemical parameters and yield studied in five STRVs across three CEs at different growth stages under split plot design are shown in Table 2 and 5. All the studied traits were found significantly influenced at  $p<0.05$  by CE, V and their interaction CE  $\times$  V. The effect of different CEs and V on biochemical parameters and yield at different growth stages is shown in the graphical manner in Figure 1.

**Table 2. ANOVA results for biochemical parameters studied in five stress-tolerant rice varieties (V) across three crop establishment methods(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 x V | CE               | V      | CE x V | CE               | V     | CE x V |
| Sugar Content                    | SE(m) | 0.46                | 0.62  | 1.07   | 0.37             | 0.31   | 0.54   | 0.07             | 0.13  | 0.22   |
|                                  | CD    | 1.82*               | 1.80* | 3.12*  | 1.47*            | 0.92*  | 1.59*  | 0.28*            | 0.37* | 0.64*  |
|                                  | CV    | 8.07                | 8.31  | -      | 10.49            | 6.82   | -      | 5.38             | 7.23  | -      |
| Starch Content                   | SE(m) | 1.59                | 1.69  | 2.93   | 3.56             | 3.68   | 6.37   | 4.21             | 2.66  | 4.61   |
|                                  | CD    | 6.25*               | 4.93* | 8.55*  | 13.98*           | 10.74* | 18.61* | 16.53*           | 7.76* | 13.44* |
|                                  | CV    | 6.75                | 5.55  | -      | 8.25             | 6.61   | -      | 11.06            | 5.41  | -      |
| MDA content                      | SE(m) | 0.01                | 0.01  | 0.03   | 0.03             | 0.02   | 0.04   | 0.02             | 0.04  | 0.06   |
|                                  | CD    | 0.04*               | 0.04* | 0.07*  | 0.10*            | 0.07*  | 0.12*  | 0.08*            | 0.11* | 0.18*  |
|                                  | CV    | 6.05                | 7.33  | -      | 10.17            | 7.50   | -      | 7.86             | 10.44 | -      |
| SOD Content                      | SE(m) | -                   | -     | -      | 0.02             | 0.03   | 0.04   | 0.04             | 0.03  | 0.05   |
|                                  | CD    | -                   | -     | -      | 0.08*            | 0.07*  | 0.13*  | 0.14*            | 0.09* | 0.16*  |
|                                  | CV    | -                   | -     | -      | 5.57             | 5.09   | -      | 11.80            | 7.65  | -      |

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 crop establishment methods(CE)and stress-tolerant rice varieties (V)on biochemical parameters and yield studied at different growth stages.**

### 3.1 Impact of different crop establishment methods on total soluble sugar content in stress-tolerant rice varieties

In the context of higher plants, total soluble sugars, including sucrose, serve as essential carbon reservoirs and function as signalling molecules for genes involved in photosynthesis [21;22]. Sugar signalling also plays a pivotal role in regulating stomatal conductance [23]. The findings presented in Table 3 underscore the consistent superiority of CE3 across all growth stages, while most varieties show suboptimal performance at CE1. Conversely, Table 4 reveals V5 performed the best across all growth stages. Specifically, during the active tillering stage, all varieties exhibit significantly higher total sugar content at CE3, except for V4. At stage S2, varieties V3, V4, and V5 demonstrate superior performance at CE3. By stage S3, V5 excels at CE1, while V4 stands out at both CE2 and CE3. Shehab et al. [24] suggests that the accumulation of soluble sugars enhances a crop's tolerance to drought stress. This accumulation involves various organic and inorganic solutes such as sucrose, proline, and glycine betaine, facilitating improved water absorption from dry soil and thereby aiding stress tolerance. It can be inferred that the increased sugar content observed in CE3 and V4 may contribute to enhanced tolerance in rainfed stress-prone areas.

**Table 3. Mean of crop establishment methods(CE) for all the studied biochemical parameters at different growth stages.**

| Stages of observation<br>CEs              | At active tillering |       |        | At 50% flowering |        |        | At grain filling |        |        | Total mean |        |        |
|---|---------------------|-------|--------|------------------|--------|--------|------------------|--------|--------|------------|--------|--------|
|   | CE 1                | CE 2  | CE3    | CE 1             | CE 2   | CE3    | CE 1             | CE 2   | CE3    | CE 1       | CE 2   | CE3    |
| Sugar Content [mg g <sup>-1</sup> DW]     | 20.03               | 20.32 | 26.54  | 13.22            | 13.40  | 14.85  | 3.54             | 5.59   | 6.54   | 12.26      | 13.10  | 15.98  |
| Starch Content [mg g <sup>-1</sup> DW]    | 78.60               | 60.50 | 134.80 | 166.40           | 144.70 | 190.20 | 148.40           | 140.90 | 153.10 | 131.13     | 115.37 | 159.37 |
| MDA content [μ moles g <sup>-1</sup> FW]  | 0.55                | 0.49  | 0.76   | 0.94             | 1.00   | 0.93   | 0.96             | 1.11   | 1.07   | 0.82       | 0.87   | 0.92   |
| SOD Content [EU mg <sup>-1</sup> protein] | -                   | -     | -      | 1.42             | 1.45   | 1.62   | 1.16             | 1.23   | 1.23   | 1.29       | 1.34   | 1.43   |

### 3.2 Impact of different crop establishment methods on starch content in stress-tolerant rice varieties

Table 3 reveals that CE3 exhibited the highest starch content among nearly all the CEs, while CE2 displayed the lowest. In Table 4, it is evident that V3 consistently outperformed other varieties across various growth stages. Specifically, at S1, V3 demonstrated the highest starch content, whereas V2 exhibited the lowest. At S2, V1 exhibited the highest starch content among all CEs at both CE1 and CE2, while

V3 excelled at CE3. Singh et al. [25] conducted a study in maize, reporting an increase in starch content by 3.50% and 3.19% in FIRB over conventional practices. Starch serves as a stored form of photo-assimilate in plants. According to Stitt and Zeeman [26], starch undergoes degradation and conversion into sugars during the night, which are then utilized for growth and metabolism. Therefore, the proper accumulation of starch during the day and its optimal degradation into sugars at night are crucial components of growth [27;28;29]. The high starch content observed at CE3 indicates robust crop growth.

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

| Stages of observation | Varieties | Sugar Content [mg g <sup>-1</sup> DW] | Starch Content [mg g <sup>-1</sup> DW] | MDA content [μ moles g <sup>-1</sup> FW] | SOD Content [EU mg <sup>-1</sup> protein] |
|-----------------------|-----------|---------------------------------------|--|--|---|
| At active tillering   | V1        | 21.81                                 | 91.70                                  | 0.54                                     | -   |
|                       | V2        | 23.39                                 | 65.40                                  | 0.62                                     | -   |
|                       | V3        | 18.57                                 | 127.00                                 | 0.75                                     | -   |
|                       | V4        | 23.43                                 | 70.60                                  | 0.56                                     | -   |
|                       | V5        | 24.27                                 | 101.70                                 | 0.52                                     | -   |
| At 50% flowering      | V1        | 14.82                                 | 264.10                                 | 0.92                                     | 1.32                                      |
|                       | V2        | 14.48                                 | 142.70                                 | 1.00                                     | 1.54                                      |
|                       | V3        | 12.54                                 | 184.00                                 | 0.78                                     | 1.50                                      |
|                       | V4        | 12.78                                 | 126.90                                 | 1.00                                     | 1.59                                      |
|                       | V5        | 14.51                                 | 117.80                                 | 1.08                                     | 1.52                                      |
| At grain filling      | V1        | 3.45                                  | 127.90                                 | 0.97                                     | 1.18                                      |
|                       | V2        | 5.39                                  | 160.20                                 | 1.17                                     | 1.22                                      |
|                       | V3        | 4.74                                  | 165.40                                 | 0.95                                     | 1.23                                      |
|                       | V4        | 6.45                                  | 166.90                                 | 1.05                                     | 1.26                                      |
|                       | V5        | 6.07                                  | 117.00                                 | 1.10                                     | 1.14                                      |
| Total mean            | V1        | 13.36                                 | 161.23                                 | 0.81                                     | 1.25                                      |
|                       | V2        | 14.42                                 | 122.77                                 | 0.93                                     | 1.38                                      |
|                       | V3        | 11.95                                 | 158.80                                 | 0.83                                     | 1.37                                      |
|                       | V4        | 14.22                                 | 121.47                                 | 0.87                                     | 1.43                                      |
|                       | V5        | 14.95                                 | 112.17                                 | 0.90                                     | 1.33                                      |

### 3.3 Impact of different crop establishment methods on MDA content in stress-tolerant rice varieties

The findings from Table 3 indicate that both CE2 and CE3 performed equally well across all growth stages. CE3 exhibited superior performance at S1, while CE2 outperformed at S2 and S3. Meanwhile, Table 4 reveals that V2 consistently performed overall the best across most growth stages, with V3 excelling at S1 and S3, and V5 exhibiting the highest MDA

content at S2 among the varieties. MDA, a byproduct of the decomposition of polyunsaturated fatty acid hydroperoxides [30], serves as an indicator of lipid peroxidation, highlighting stress-induced oxidative damage. Numerous studies have documented the increase in MDA content under drought stress conditions. Gong et al. [31] observed elevated MDA levels in water-stressed wheat compared to controls, a trend similarly reported by Lima et al. [32] in Coffea and Sofo et al. [33] in olive trees. Baisak et al. [34] noted that lipid peroxidation tends to increase significantly under severe water stress conditions rather than mild stress in wheat. The rise in MDA content observed in CE3 suggests a heightened stress tolerance level, both in CE3 itself and in variety V2.

### 3.4 Impact of different crop establishment methods on SOD content in stress-tolerant rice varieties

V4 and CE3 exhibited the highest SOD content at both growth stages, indicating their resilience to stresses (Table 3 and 4). Notably, there was no significant difference in SOD content observed for V4 across all three CE conditions. Among the CE treatments, V4 showed the highest SOD content at both CE1 and CE2, while V3 excelled at CE3. Conversely, notably low SOD content was detected in V1 at CE1, V3 at CE2, and V4 at CE3. The findings from Table 3 highlight CE1 as the least favourable condition, while Table 4 reveals V1 as the poorest-performing variety. SOD catalyses the dismutation of superoxide into oxygen and hydrogen peroxide, according to Peltzer et al. [35]. Under adverse environmental conditions, such as those induced by Wise and Naylor [36], plants may overproduce reactive oxygen species (ROS). These ROS, by-products of various degenerative reactions, can impair regular metabolism by damaging cellular components [37]. Consequently, plants accelerate the production of oxidative stress protectors and accumulate protective solutes [38]. SOD work in tandem to convert toxic  $O_2^-$  and  $H_2O_2$  into water and molecular oxygen [39;40;41]. Conversely, Quartacci and Navari-Izzo [42] and Baisak et al. [34] have asserted that water-stressed conditions lead to a reduction in SOD content in plants compared to control situations. Similarly, Chowdhury and Choudhuri [43] and Zhang and Kirkham [44] observed a notable decrease in SOD content under water-stressed plants.

**Table 5. ANOVA results and mean of crop establishment methods (CE) and stress-tolerant rice varieties (V) for yield.**

|              | ANOVA |       |        | Total mean |      |      |      |      |     |      |      |
|--------------|-------|-------|--------|------------|------|------|------|------|-----|------|------|
|              | CE    | V     | CE x V | CE 1       | CE 2 | CE3  | V1   | V2   | V3  | V4   | V5   |
| <b>SE(m)</b> | 0.26  | 0.18  | 0.32   | 4.03       | 4.5  | 4.81 | 4.63 | 4.77 | 4.5 | 4.25 | 4.09 |
| <b>CD</b>    | 1.00* | 0.54* | 0.93*  |            |      |      |      |      |     |      |      |
| <b>CV</b>    | 22.28 | 12.45 | -      |            |      |      |      |      |     |      |      |

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

### 3.5 Impact of different crop establishment methods on yield in stress-tolerant rice varieties

The yield of rice in the current experiment exhibited significant variation among varieties and crop establishment methods. CE3 consistently outperformed others at all growth stages, whereas CE1 consistently showed the poorest performance (Table 5). Similarly, among all varieties, V2 yielded the highest, while V5 yielded the lowest (Table 5). Singh et al. [45] conducted an experiment that also demonstrated an increase in yield under the FIRB system. They observed enhancements in all yield attributes, including grains per spike, spike length, spikelets per spike, productive tillers per square meter, and test weight. Fahong et al. [46] proposed that the prolonged greenness of leaves contributes to an increase in the rate of grain filling, thereby enhancing crop yield. Moreover, the highest sustainability yield index (SYI) was reported with the FIRB method by Lopez et al. [47]. A similar study by Singh et al. [25] in maize revealed a significant increase in grain yield under FIRB, with improvements of 61.79% and 59.68% over conventional practices during 2010 and 2011, respectively.

## 4. CONCLUSION

The study demonstrated significant effects of different crop establishment methods like conventional puddled transplanting, DSR and FIRB on five stress-tolerant rice varieties. Both CE and V exerted significant variations, along with their interaction, on the studied traits. Total soluble sugars exhibited consistent superiority at CE3 and V5 across growth stages, emphasizing their importance in drought-stress mitigation. Starch content, vital for plant growth and metabolism, showed a noteworthy increase in CE3, indicating its potential for robust crop development under stress-prone environments. MDA content, indicative of oxidative damage, showed elevated levels observed in CE3, suggesting heightened stress tolerance. V4 and CE3 exhibiting superior SOD content, essential for ROS detoxification, reflected resilience to stresses. Rice yield, a crucial determinant of agricultural productivity, displayed significant variations across CE and V conditions. CE3 i.e. FIRB emerged as the most favourable establishment system, while V2 which is DRR 44 exhibited the highest yield potential. These findings emphasize the importance of strategic selection of sowing methods and genotypes to enhance crop resilience and productivity. It's high time to address water scarcity by embracing alternative rice sowing methods like FIRB, ensuring sustainable agriculture amidst environmental stresses and aiding global food security.

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