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Research Article

Influence of land preparation on weed dynamics and growth of traditional rice landraces in the diverged location of Tamil Nadu

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Abstract
In recent years, traditional landraces are gaining importance due to its many health benefits. But the main drawback of the traditional rice variety is low productivity with increased water consumption. Therefore different rice establishment methods are to be evaluated. But weed infestation occurs in every establishment method. Hence the present study aimed to study the weed dynamics by evaluating the water-saving technologies compared with puddled transplanting using various landraces. The first field experiment was conducted during *Samba* (Aug-Sept) 2021 in the wetlands of Tamil Nadu Agricultural University, Coimbatore. The second field experiment was at the Research Station, Melalathur in Tamil Nadu during *Navara* (Nov-Jan) season 2021. The experiment was laid out in split plot design comprising four establishment methods as main plots *viz.* Aerobic rice (M_1), Puddled transplanting (M_2), Unpuddled transplanting (M_3), Direct seeded rice (M_4) with traditional landraces *viz. Karuppu kavuni* (V_1), *Mapillai samba* (V_2) and *Seeraga samba* (V_3) in sub plots. In this study, weed dynamics like weed density and weed dry weight were recorded along with plant height, leaf area index and total chlorophyll content. Weed density and weed dry weight was 87.82 % and 74.63 % higher in aerobic rice cultivation than puddled transplanting method during all the observations in both experiments. On comparing the land establishment methods, Direct seeded rice performed well with 40.61% of higher plant height, 89.38 % of higher LAI, 38.48 % of increased total chlorophyll content than Aerobic rice cultivation. The landrace *mapillai samba* showed a significantly ($p<0.05$) higher plant height of 64.09±7.14 cm, Leaf area index of 2.05±0.992 and total chlorophyll content of 2.56±0.461 mg/g of fresh leaf. Hence this study provides evidence that in the initial stage of rice cultivation, direct seeded rice and the *mapillai samba* landrace performed well than the other establishment methods and the other two landraces.

Keywords: Establishment methods, Traditional rice landraces, Total chlorophyll content, Weed dynamics

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food for half of the world's population. There is an increasing demand for rice due to increased population and urbanization of cultivable lands. According to Food and Agriculture Organization (2023), rice production during 2020-21 recorded 518.5 million tonnes and estimated to produce 525.6 million tonnes during 2021-22. In India, rice is the principal food for nearly 60 % of the population. On the other hand, the population growth is increasing (1.5 %) and by the year 2025, rice production should be enhanced to 125 million tonnes to meet the future demand. But, due to several constraints such as shrinking water resources, urbanization, increased fertilizer costs and reduced yield levels, there is an urgent need to maintain the yield level (Bhatt *et al.,* 2021).
Even though rice productivity is less in our country,

productivity increased fivefold from 1950-51 (668 kg/ha) to 2014-15 (2390 kg/ha). However in rice production, India positions second with 157.20 million tonnes in 2017 (Food and Agriculture organization, 2017). Tamil Nadu, with 7.28 million tonnes of rice production, contributed 6.47 per cent to the total production of rice to the country in 2017-18. In Tamil Nadu, paddy is the principal crop cultivated in all the districts and has a unique pattern of rice season *viz.,Kar, Kuruvai, Samba*, *Thaladi, Pishanam* and *Navarai.*

In recent years, traditional landraces are gaining importance due to the many health benefits in landraces such as *karuppu kavuni, mapillai samba* and *seeraga samba*. Kim *et al*. (2010) from American Chemical Society (ACS) reported that *karuppu kavuni* contains more antioxidants than blueberries and has 18 amino acids, zinc that counts for immunity, iron that counts for carrying oxygen, and copper, carotene, anthocyanin and several important vitamins. *Mappillai samba* rice is given to men to show their strength in lifting *ilavattakal* (Heavy spherical stones) in search of potential groom. It also improves digestion, regulates the immune system, slows down ageing process and cures mouth ulcers. It also contains about 113 metabolites (Rajagopalan *et al*., 2022). *Seeraga samba* rice contains selenium, which helps to prevent the cancer of colon and intestine. It has more fibre, which helps to remove free radicals from the colon and intestine. Due to the low palmitic acid accumulation, *Seeraga samba* helps to prevent cardiovascular diseases. It contains squalene, a triterpinoid compound with a high potential for antibacterial, anticancer, and immunostimulant activities (Ashok kumar *et al*., 2020).

But the main drawback of the traditional rice variety is low productivity due to its cultivation in drought-prone areas with less water. Water shortage critically impacts the world's food self-sufficiency and security (Ewing-chow *et al*., 2016). Therefore, it is necessary to respond to the growing demand for rice with limited resources, and it may be necessary to increase productivity per unit of surface area with less water. The new water scarcity challenges require alternative for irrigation of rice production systems that require less water than traditional flooded rice (Livsey *et al*., 2019).

For rice production in India that, eradicates puddling, reducing labour requirements at critical stages, decreasing energy consumption and promoting earlier growth and maturity of rice crops is a challenge. Puddling (wet tillage) uses up to 30 per cent of the total application of irrigation water in rice (Aslam *et al.,* 2002), hence by following un-puddled and direct seeded rice which is more water-efficient than the puddled transplanted rice. On the other hand, direct seeded rice reduces the water in rice cultivation since it eliminates the water used for nursery preparation, thereby increasing water use efficiency. Water-saving technologies like aerobic rice, unpuddled rice cultivation, Direct seeded rice and rice intensification system reduce water usage while increasing productivity. Rice landraces grow with lesser water and further following these techniques may reduce the total water usage and increase productivity.

Weeds are a major problem in every establishment method. Even though many of the rice establishment methods consume a lesser amount of water, yield loss occurs due to higher weed infestation. A definite weed flora and density must be known to control the weeds since weedicides are specifically available for broad-leaved grasses and sedges. Also, traditional landraces' weed flora and growth vary based on locations and seasons. Hence the present experiment was conducted to study the effects of different rice establishment methods on weed dynamics, plant height, Leaf Area Index (LAI) and total chlorophyll content in traditional landraces at the diverged location.

MATERIALS AND METHODS

The first field experiment was carried out at Tamil Nadu Agricultural University, wetland farms, Coimbatore, during the late *samba* (Aug-Sept) season 2021 with clay loam texture and moderately alkaline soil. The available nitrogen in the soil was low, available phosphorus was medium and available potassium was high. The second field experiment was carried out at Sugarcane Research Station, Melalathur, during *Navara* (Nov-Jan) season 2021, with clay loam texture and neutral pH soil. The available nitrogen in the soil was low, available phosphorus was medium and available potassium was high. The experiment was laid out in split plot design with four rice establishment methods *viz*. Aerobic rice (M_1), Puddled transplanting (M_2), Unpuddled transplanting (M_3), Direct seeded rice (M_4) and subplots with three traditional landraces *viz*. *Karuppu kavuni* (V_1), *Mapillai samba* (V_2) and *Seeraga samba* (V_3). The three varietal seeds were soaked and pre-germinated, then sown separately in the nursery.

Field establishment

In main field, sowing of direct seeded and aerobic rice was done initially by ploughing the field with the cultivator. The field was impounded with water for direct seeded rice, then puddled using cage wheel and rotavator. In aerobic rice, the field was ploughed with a rotavator. After the field preparation, the main and subplots were laid out with irrigation and drainage channels all around the experimental field. Buffer channels were formed with a spacing of 1 m between the vertical strips. A buffer spacing of 0.5 meter was kept between the replications. The pre germinated seeds were sown manually in both direct-seeded and aerobic rice with a spacing of 25×25 cm and 30×10 cm, respectively. The puddled

Table 1. Weed control methods for different rice establishment methods

	Puddled	Unpuddled	Aerobic	DSR
8 DAS	-	-	Pendimethalin spray	Pendimethalin spray
25 DAS/11 DAT	Pendimethalin spray	Pendimethalin spray	Hand weeding	Cono weeding
35 DAS	Cono weeding	Cono weeding	Hand weeding	Cono weeding
45 DAS	Cono weeding	Cono weeding	Hand weeding	Cono weeding

field was established using cultivator, cage wheel and rotavator, whereas the unpuddled field was established using a cultivator and rotavator. Before transplanting of seedlings, the field was irrigated in an unpuddled field. In both the establishment methods, 14 days old seedlings of traditional rice varieties were transplanted with a spacing of 25×25 cm.

Weed control measures for different establishment methods are given in Table1. Observations on individual weed density and dry matter production of weeds were recorded at 25, 35 and 45 DAS. Data on weed density and weed dry weight showed high variation. Hence they were subjected to square root transformation (√X). Weeds like *Echinochloa crusgalli, Echinochloa colona,Leptochala cinensis, Dinobara retroflexa, Cyprus difformis, Monochoria virginalis, Ammania bacifer, Bergia carpensis, Marselia quadrifolia* in location 1. *Achyranthus aspera, Boerhavia erecta, Mimosa pudica, Brachia ramose, Cyperus iria, Digitaria sanguinalis, Acalypha australis* weed species were found in the second experiment field. Recommended dose of fertilizers was given in four equal splits, *viz.,* Basal, active tillering, panicle initiation and heading stage of the crop. Observations were recorded on weed density and weed dry weight from four randomly selected quadrates of 0.25 m^2 in each plot at 25, 35 and 45 DAS. They were grouped into grasses, sedges and broad-leaved weeds and expressed as number m^{-2}. The data were subjected to square root transformation and analyzed.

Growth parameters

Growth parameters like plant height, leaf area index (LAI) and total chlorophyll content during active tillering stage were recorded.

Leaf Area Index

The leaf area was worked out by measuring leaf length and breadth as outlined by Palaniswamy and Gomez (1974) using a constant of 0.75 for the estimation. The total area of the leaf to the ground area was expressed as an index of the area of the leaf in cm^2

Leaf Area Index (LAI) = (Lx Bx K X Total number of green leaves/hill) / Spacing (cm^2) X 100 ….Eq. 1

Where,

L - Length of the third leaf from top (cm),

B - Breadth of the third leaf from top (cm), and

K - Constant (0.75).

Total chlorophyll content

To extract the chlorophyll pigments, the third leaf from the top of plant which was fully expanded was chosen. Different wavelengths in spectrophotometer were used to estimate total chlorophyll.

To calculate total chlorophyll, following formula is used, and expressed in mg/ g of fresh leaf (Yoshida, 1972).

Total chlorophyll = (20.2 x OD at 645)- (8.02 x OD at 663) / W X V mg g^{-1} …...Eq. 2

Where,

W - Weight of the leaf sample,

V - Volume of supernatant solution, and

O.D. - Optical density.

Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA) at 5% significance level using R software.

RESULTS AND DISCUSSION

Weed vegetation studies

The weed flora and dynamics varied among each establishment methods. Higher weed density was observed in aerobic rice in all the observations. Weed vegetation studies showed that the grasses were higher in unpuddled rice cultivation (M_3), sedges were higher in direct seeded rice method (M_4) and puddled transplanting method (M_2), whereas broadleaves were higher in aerobic rice (M_1) cultivation in the first season. In second location, puddled transplanting method (M_2) contained more sedges. Direct seeded rice (M_4) had a higher number of sedges and grasses than broadleaved weeds, Unpuddled transplanting method (M_3) and aerobic rice method (M_1) contained more broad leaved weeds. Jehangir *et al*. (2021) conducted experiments for two consecutive years in Kashmir and reported that sedges were higher in transplanted rice than grasses and broadleaved weeds.

Weed density and weed dry weight

In different field establishment methods at 25, 35 and 45 DAS, the weed density and weed dry weight were significantly higher with the average value of 409.3 No./m^2 and 1.83 g/m^2 respectively in aerobic rice (M_1). The lowest value was recorded in puddled transplanted method (M_2) with the average value of 49.8 No./m^2 and

0.46 g/m^{2,} respectively, in both experiments. The weed density and weed dry weight in aerobic rice (M_1) was 88 % and 74.8 %, correspondingly higher than puddled transplanted method (M_2) (Table 2 and 3). Jehangir *et al.* (2021) reported that an aerobic soil environment promoted the germination of several plant species leading to higher weed density in aerobic rice. Chauhan *et al*. (2015) experimented at International Rice Research Institute, Philippines and stated that in the puddled transplanted method, the rice seedlings' additional size difference gives them a competitive advantage over the newly emerging weeds. In the present study experiment, there was no significant difference between the different varieties.

Plant height

During active tillering, the higher plant height of 78.95±1.92 cm and 76.07±1.94 cm was recorded in the Direct seeded rice (M_4) method followed by puddled transplanting method (M_3). The lower plant height was documented in aerobic rice method (M_1) which is 40 % lesser when compared to direct seeded rice method (M_4) (Table 4). The same observation was reported by Santiago-aernas *et al*., (2020) at Asian Institute of Technology, Bangkok that direct seeded rice method produced higher plant height than other establishment methods. Kumhar *et al.* (2016) stated that due to earlier sowing of direct seeded rice allowed deep root penetration, which improved nutrient uptake and plant growth. Among the traditional landraces, *Mapillai samba* (V_2) recorded higher plant height during the active tillering stage in both experiments with the value of 65.67±7.31 cm and 62.52±6.97 cm.

LAI

LAI was found to be significantly higher in direct seeded rice method (M_4) with the value of 2.84±0.579 and 2.73±0.555 in the first and second experiments, followed by puddled transplanting method (M_2) and the lower LAI was observed in aerobic rice method (M_1) with 89 % in both the experiments. Higher LAI in direct seeded rice method (M_4) might be due to early seeding associated with suitable puddled microenvironment that promotes the expansion of leaf area for higher photo-assimilates and enhanced accumulation of dry matter (Santiago-aernas *et al*., 2020). For the landraces, LAI of *Mapillai samba* (V_2) was higher with the value of 2.10±0.991 and 2.00±0.944 followed by *Seeraga samba* (V_3) (Table 4).

Total chlorophyll

Total chlorophyll content was found to be higher in di-

Table 2. Effect of land preparation and landraces on weed density (No./m^2) and weed dry weight (g/m^2) at 25, 35 and 45 DAS in the first experiment

Treatment	25 DAS		35 DAS		45 DAS	
Establishment methods	**Weed density (No./m^2)**	**Weed dry Weight (g/m^2)**	**Weed density (No./m^2)**	**Weed dry weight (g/m^2)**	**Weed density (No./m^2)**	**Weed dry weight (g/m^2)**
M_1	17.34 (301)	0.76 (0.59)	23.80 (568)	1.38 (1.93)	13.40 (180)	1.24 (1.57)
M_2	4.16 (18)	0.30 (0.09)	11.32 (128)	0.85 (0.72)	6.66 (44)	0.36 (0.13)
M_3	12.74 (163)	0.53 (0.28)	11.97 (144)	0.82 (0.68)	10.77 (116)	0.63 (0.41)
M_4	8.77 (78)	0.37 (0.14)	10.72 (115)	0.56 (0.31)	6.46 (42)	0.36 (0.13)
S.Em	0.40	0.02	0.49	0.03	0.43	0.03
CD (P=0.05)	1.13	0.02	0.42	0.03	0.53	0.03
Varieties						
V_1	10.72 (140)	0.45 (0.23)	13.67 (211)	0.92 (0.96)	9.08 (93)	0.64 (0.57)
V_2	10.76 (139)	0.52 (0.31)	14.98 (258)	0.89 (0.88)	9.86 (106)	0.67 (0.56)
V_3	10.77 (141)	0.51 (0.28)	14.72 (247)	0.90 (0.89)	8.95 (88)	0.63 (0.54)
S.Em	2.80	0.10	3.13	0.17	1.70	0.21
CD (P=0.05)	NS	NS	NS	NS	NS	NS

Data's were subjected to square root transformation (√X). Figures in the parenthesis are means of original values. Treatment details: M_1 - Aerobic rice, M_2 - Puddled transplanting, M_3 - Unpuddled transplanting and M_4 - Direct seeded rice (M_4); V_1 - *Karuppu kavuni*, V_2 - *Mapillai samba* and V_3 - *Seeraga samba*

Table 3. Effect of land preparation and landraces on weed density and weed dry weight at 25, 35 and 45 DAS in the second experiment

Treatment	25 DAS		35 DAS		45 DAS	
Establishment methods	**Weed density (No./m^2)**	**Weed dry weight (g/m^2)**	**Weed density (No./m^2)**	**Weed dry weight (g/m^2)**	**Weed density (No./m^2)**	**Weed dry Weight (g/m^2)**
M_1	18.77 (353)	1.10 (1.22)	24.49 (601)	1.88 (3.56)	21.25 (453)	1.45 (2.13)
M_2	7.42 (56)	0.41 (0.19)	5.68 (33)	0.68 (0.46)	4.38 (20)	1.09 (1.2)
M_3	16.36 (270)	0.67 (0.46)	7.96 (64)	0.97 (0.96)	6.40 (41)	1.19 (1.43)
M_4	10.45 (109)	0.45 (0.21)	11.63 (138)	1.15 (1.33)	7.57 (59)	1.36 (1.86)
S.Em	0.51	0.03	0.35	0.03	0.31	0.05
CD (P=0.05)	1.05	0.02	1.33	0.03	1.21	0.07
Varieties						
V_1	13.51 (205)	0.63 (0.5)	12.52 (218)	1.15 (1.5)	10.46 (157)	1.28 (1.7)
V_2	12.48 (173)	0.66 (0.5)	12.39 (203)	1.20 (1.67)	9.66 (135)	1.27 (1.65)
V_3	13.76 (213)	0.69 (0.56)	12.40 (206)	1.16 (1.57)	9.59 (138)	1.26 (1.62)
S.Em	2.62	0.16	4.18	0.25	3.83	0.09
CD (P=0.05)	NS	NS	NS	NS	NS	NS

Data's were subjected to square root transformation (√X). Figures in the parenthesis are means of original value. Treatment details: M_1 - Aerobic rice, M_2 - Puddled transplanting, M_3 - Unpuddled transplanting and M_4 - Direct seeded rice; V_1 - *Karuppu kavuni*, V_2 - *Mapillai samba* and V_3 - *Seeraga samba*

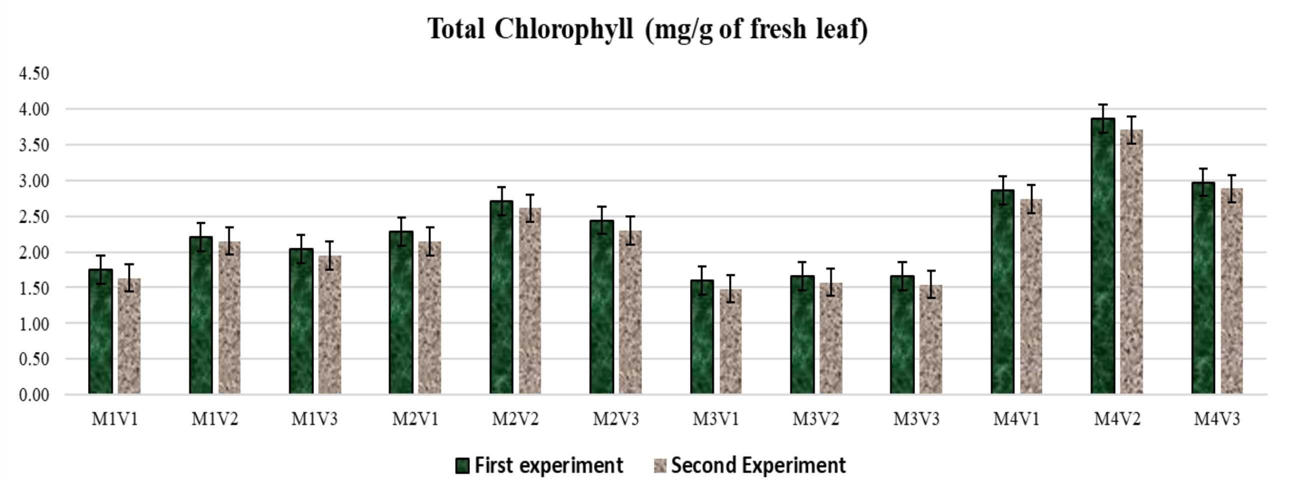


Fig. 1. *Effect of land preparation and landraces on total chlorophyll content (mg/g of fresh leaf) at active tillering in the first and second experiment.* ***Treatment details:*** *M_1 - Aerobic rice, M_2 - Puddled transplanting, M_3 - Unpuddled transplanting and M_4 - Direct seeded rice; V_1 - Karuppu kavuni, V_2 - Mapillai samba and V_3 - Seeraga samba*

rect seeded rice (M_4) with a value of 3.2±30.32 mg/g of fresh leaf and 3.11±0.30 mg/g of fresh leaf. The lowest value of 1.64±0.01 mg/g of fresh leaf and 1.53±0.02 mg/g of fresh leaf was recorded in unpuddled transplanting method (M_3) in the first and second experiments, respectively. Initially M_3 showed yellowing symptoms due to soil complexity where the soil was a bit compact, leading to anoxia (poor oxygen supply to roots); even alternate wetting and drying was followed, as also observed by Quilloy *et al*. (2021) in there, who observed that the unpuddled rice cultivation showed yellowing symptoms in rice crop. Among the landraces, visible difference was seen and it is given in Fig.2. According to Wang *et al.* (2008), the darker color denotes the higher chlorophyll as a rice mutant Chongqing 2, which is unusually dark in colour when crossed with a

Table 4. Effect of land preparation and landraces on plant height (cm) at active tillering in the first and second experiment

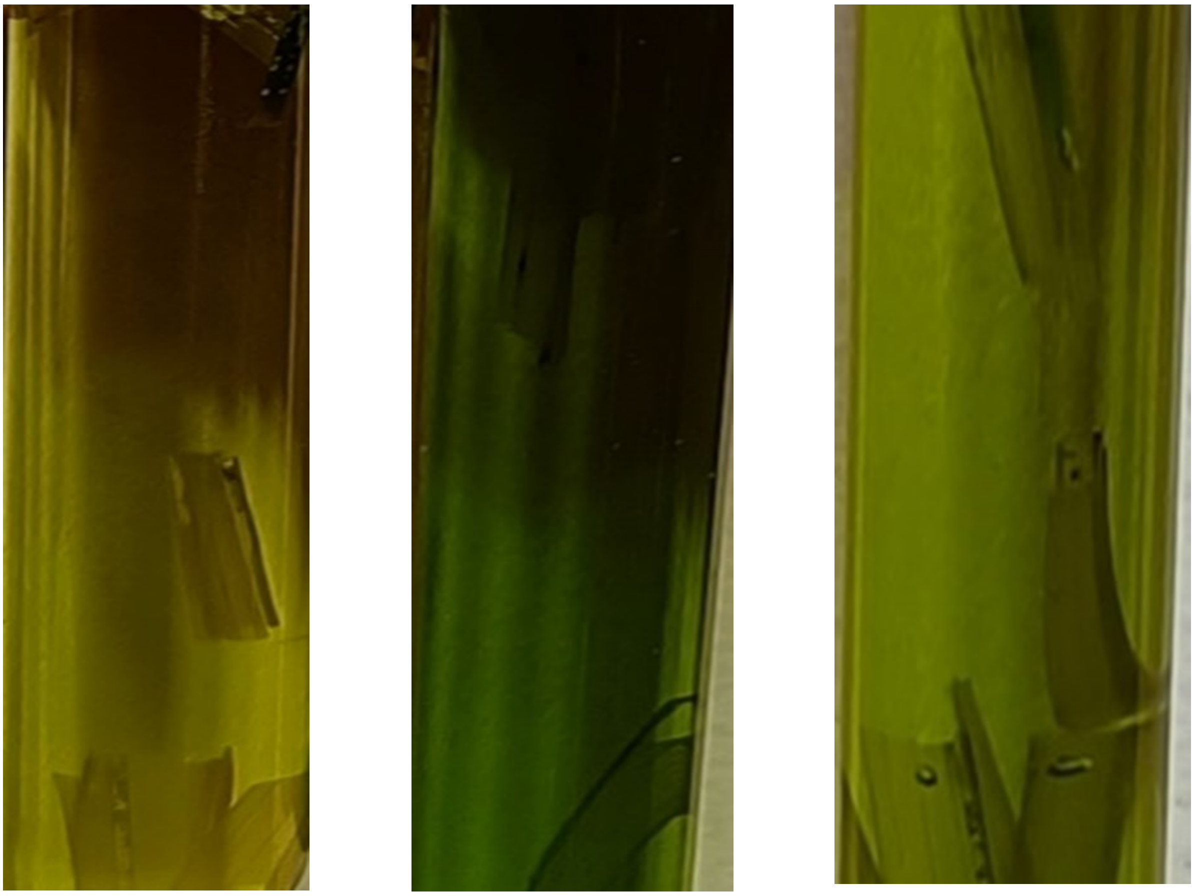
Treatments	First Experiment					Second Experiment				
	M_1	**M_2**	**M_3**	**M_4**	**Mean**	**M_1**	**M_2**	**M_3**	**M_4**	**Mean**
V_1	47.69±0.31k	68.42±0.40e	56.49±0.56h	79.02±0.54b	62.91±6.85b	45.59±0.41j	65.93±1.88e	52.29±1.21h	76.64±2.59b	60.11±6.94b
V_2	49.26±0.24j	72.63±0.33d	58.56±0.63g	82.24±1.19a	65.67±7.31a	47.91±0.43i	68.36±0.83d	54.69±1.16g	79.12±3.50a	62.52±6.97a
V_3	44.09±0.47l	65.56±0.63f	53.64±0.68i	75.57±0.97c	59.72±6.87c	41.31±0.43k	61.82±1.47f	50.98±1.25h	72.46±2.76c	56.64±6.73c
	47.01±1.52d	68.87±2.05b	56.23±1.42c	78.95±1.92a		44.94±1.93d	65.37±1.90b	52.65±1.08c	76.07±1.94a	
	M	**V**	**M at V**	**V at M**		**M**	**V**	**M at V**	**V at M**	
C.D (P=0.05)	1.90	0.55	2.10	1.10		6.43	0.74	6.54	1.49	

Values are Mean±Standard error SE. The same letter in each column are not significantly different from each other based on LSD P ≤0.05. Treatment details: M_1 - Aerobic rice, M_2 - Puddled transplanting, M_3 - Unpuddled transplanting and M_4 - Direct seeded rice; V_1 - Karuppu kavuni, V_2 - Mapillai samba and V_3 - Seeraga samba

Table 5. Effect of land preparation and landraces on LAI at active tillering in first and second experiment

Treatments	First Experiment					Second Experiment				
	M1	**M2**	**M3**	**M4**	**Mean**	**M1**	**M2**	**M3**	**M4**	**Mean**
V1	0.16±0.004h	1.28±0.048f	0.16±0.009h	2.10±0.029e	0.92±0.472c	0.15±0.004hi	1.23±0.045f	0.14±0.006i	2.03±0.012e	0.89±0.458b
V2	0.45±0.009g	3.64±0.152b	0.33±0.005gh	3.98±0.029a	2.10±0.991a	0.44±0.020g	3.43±0.112b	0.30±0.015gh	3.83±0.068a	2.00±0.944a
V3	0.29±0.021gh	3.15±0.102c	0.21±0.004h	2.44±0.117d	1.52±0.749b	0.27±0.002hi	2.97±0.029c	0.20±0.001hi	2.34±0.123d	1.45±0.710c
	0.30±0.084c	2.69±0.721b	0.23±0.050c	2.84±0.579a		0.29±0.083c	2.54±0.670b	0.22±0.047c	2.73±0.555a	
	M	**V**	**M at V**	**V at M**		**M**	**V**	**M at V**	**V at M**	
C.D (P=0.05)	0.13	0.09	0.20	0.19		0.116	0.07	0.171	0.154	

The values are Mean±Standard error SE. The same letter in each column are not significantly different from each other based on LSD P ≤0.05. Treatment details: M_1 - Aerobic rice, M_2 - Puddled transplanting, M_3 - Unpuddled transplanting and M_4 - Direct seeded rice (M4); V_1 - Karuppu kavuni, V_2 - Mapillai samba and V_3 - Seeraga samba



***a)** Karuppu kavuni showing yellowish green recorded lowest chlorophyll content*

***b)** Mapillai samba showing darker green colour denoting higher chlorophyll content*

***c)** Seeraga samba showing green color lesser chlorophyll content than Mapillai samba*

Fig. 2. *Effect of DMSO (Dimethyl sulfoxide) solvent on traditional landraces showing chloroyphyll content*

cultivar Zhenshan 97B, the chlorophyll content increased at Chongqing Agricultural Sciences Institute, China. Zhang *et al*. (2021) compared a rice mutant dark -green panicle1 (dgp1) and a wild type, resulting that the dgp1 produced higher chlorophyll a, chlorophyll b and total chlorophyll while comparing to the wild type, which is less dark green than the dgp1 grown at Jiangsu Province, China. In the present study, the higher chlorophyll content was recorded in *mapillai samba* (V_2) with the value of 2.61±0.47 and 2.51±0.45 in the first and second experiments, correspondingly followed by *seeraga samba* (V_3) 2.28±0.28 and 2.17±0.28 and the lowest was observed in *Karuppu kavuni* (V_1) 2.12±0.28 and 2.00±0.28 in the first and second experiment respectively.

Conclusion

The present study concluded that Establishment methods in rice significantly influenced weed dynamics and the growth of traditional rice landraces. The weed infestation in aerobic rice was higher with lower plant height and LAI, which are promising growth parameters that may influence final harvest yield. Active tillering of rice is one of the crucial stages where the growth parameters significantly affect yield and all the landraces tested *viz. Karuppu kavuni, Mapillai samba* and *Seeraga samba* performed well in the Direct seeded method of rice cultivation. While accounting for landraces, the visible dark green colour of *Mapillai samba* may be one reason for enhancing the plant height and LAI by accumulating more photo-assimilates. In all the treatments Direct seeded rice method, along with *Mapillai samba* landrace, recorded higher plant height, LAI and total chlorophyll content. Hence this study provides evidence that in the initial stage of rice cultivation, direct-seeded rice and the *mapillai samba* landrace performed well than the other establishment methods and the other two landraces.

Conflict of interest

The authors declare that they have no conflict of interest.

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