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Ankit Singh
Acharya Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh, India

AK Singh
Acharya Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh, India

AK Pandey
Acharya Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh, India

Reeshu Singh
Acharya Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh, India

Corresponding Author:
Ankit Singh
Acharya Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh, India

Assessments of different seedling ages and double transplanting of rice on root morphology, biochemical changes and nutrient uptake under rainfed condition

Ankit Singh, AK Singh, AK Pandey and Reeshu Singh

Abstract
A two-year field experiment was conducted during the kharif season of 2021-22 and 2022-23 at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh to evaluate the effect of different ages of seedling and double transplanting on the growth and yield of rice crop. Three different seedling age were taken as the treatment T_1-14 days old seedling, T_2-21 days old seedling, T_3-28 days old seedling and two popular high yielding varieties of rice V_1- DRR-44, V_2- Sahbhagi Dhan were used for the study.It was observed that different seedling ages and double transplanting substantially influenced the root morphology, biochemical activity as well as nutrient status of the rice crop. However, 21 days old seedling significantly reduced the detrimental effect of double transplanting on both the varieties by improving physiological traits which ultimately helped in obtaining higher yield. Thus, it is recommended that under double transplanting 21 days old seedlings are beneficial for the farmers to minimize yield losses under sanda cultivation.

Keywords: Rice, seedling age, double transplanting, grain yield

Introduction

Rice is one of the important rainfed crops and is a staple diet in most of the Asian countries (where >90% of the world's rice is produced) and parts of the Pacific (www.fao.org). According to the estimates of the Food and Agriculture Organization of the United Nations (UN-FAO), more than half of the global population relies on rice for calories and protein (www.fao.org). In order to feed a rapidly growing population, especially in developing nations, food production would need to almost double (www.fao.org). However, on one hand, increasing population and resultant land-use changes (especially for urbanization and other developmental activities) reduce the per-capita land availability for agriculture, whereas intensive cultivation practices reduce the quality of soil and thereby the agricultural yield, including rice production (Dubey *et al*., 2021) [1]. On the other hand, erratic weather events such as changing rainfall patterns, droughts, flooding, submergence, occurrence and prevalence of newer pests and diseases, etc., undermine the productivity of rice (Wheeler and Braun, 2013) [5]. Therefore, the adoption of climate-resilient and planet-friendly cultivation of rice is essential for ensuring food and nutritional security in India and other Asian countries (Cui *et al*., 2018; Ishfaq *et al*., 2020; Panneerselvam *et al*., 2020) [2-4].

The factors that affect the growth of rice plant can be classified as genetic and environmental. Among the external conditions temperature, moisture supply, radiant energy, composition of the atmosphere, soil aeration and soil structure, soil reaction, biotic factors and supply of mineral nutrients substances are most important for crop growth and development. Each can be a limiting factor in plant growth. Rice plants require a particular temperature for its phenological affairs such as panicle initiation, flowering, panicle exertion from flag leaf sheath and maturity and these are much influenced by the seedling age. Planting of aged seedlings generally results in yield reduction which cannot be compensated by any other means. The correct age of seedlings used for transplanting is of primary importance for uniform stand and seedling establishment, as Padalia (1981) [6] stated "half of the success of rice cultivation depends upon the seedling". Age of seedling at the time of transplanting is an important factor for uniform stand of rice and regulate its growth and yield. Transplanting of younger seedling is an important criterion. Tillering habit of rice plant greatly influenced by the age of seedling at transplanting.

The young seedling recorded better root growth and facilitated increased cell division and cell enlargement due to increased photosynthetic rate subsequently increasing the plant growth and development, ultimately resulted in increased grain yield. When seedlings stay for a longer period of time in the nursery beds, primary tiller buds on the lower nodes of the main culm become degenerated leading to reduced tiller production. Transplanting of rice seedlings at the right age, tillering and growth proceed normally and only fewer tillers are produced during vegetative period leading to poor yield if transplanting is delayed.

Double transplanting is a crop-establishment system for rice cultivation in shallow lowland areas where farmers transplant rice seedlings twice, first on secondary nursery, and then the main field. This system of rice cultivation is prevalent in certain tracts of rainfed lowland areas In this method, seeds are first thickly sown in the primary nursery and then 3 to 4 weeks old seedlings are transplanted in bunches (4–5 number) with closer spacing (7– 10 cm apart) in a secondary nursery. Subsequently after 3 to 4 weeks, rice seedlings from secondary nursery are uprooted and clones are transplanted in the main field (Das, 2006) [7]. Double transplanting of aged seedlings gives higher yield over single transplanting because it ensures optimum crop stand. Under double transplanted system, tall and aged seedling survive adverse environment better than young and single transplanted seedlings. Seedlings produced in this method is taller, healthier and easily overcome the high-water depth at the time of transplanting or immediately after transplanting Under double transplanting, seedlings have thick culm and better food reserve. The loss of nutrients especially nitrogen is greatly reduced under double transplanted system due to controlled water management. While rice is commonly cultivated by a single transplantation method, double transplantation is an alternative method adopted by very few small/medium farmers in some S.E. Asian countries (Singh *et al*., 2017; Kumar *et al*., 2019) [9, 8]. Thus validating, and upscaling these climate adaptive and innovative farm practices is critical for increasing the productivity of rainfed crops, particularly rice, under changing conditions (Dubey *et al*., 2020, 2022) [25]. A study was thus planned focused on the impact of seedling age and double transplanting on the rooting characters, nutrient uptake and enzyme activity of rice crop.

Materials and Methods

A field study was conducted at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh during the Rabi season of 2020-21. Geographically the experimental site is situated 42km away from Ayodhya on Ayodhya-Raibarielly Road between latitude of 26.47° North and longitude of 81.12° East on an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh. The weather conditions in terms of minimum and maximum temperature, relative humidity (RH), rainfall (mm) and sunshine hours were recorded during the crop season *i.e*., from June to November during 2021-22 and 2022-23. The data were collected from metrological observatory situated in the main campus of university, Kumarganj, Ayodhya. The experiment was conducted for two years in factorial randomized block design (FRBD) with three replications. Nursery were raised for the study on three different dates on a plot of size 1m^2 which were subsequently transplanted on the main field during the years 2021-22 and 2022-23. Two popular varieties of rice V_1- DRR-44, V_2-Sahbhagi Dhan were used for the study. Three different seedling age were taken as the treatment T_1-14 days old seedling, T_2-21 days old seedling, T_3-28 days old seedling. In the main field a plot size of 5 m*4 m and spacing of 20 cm*15 cm was maintained for the transplanting of seedlings. Seedlings of different age were double transplanted i.e. first the nursey was transplanted into the main field and then after 20 days the seedlings were again retrains planted. A total of five plants were taken from each plot as sample for recording the observations. Root characters like number of roots per plant, root length, root dry weight and total soluble sugar content of the roots was measured before re-transplanting. The chlorophyll 'a', chlorophyll 'b' and total chlorophyll content was estimated following the method of (Arnon 1949) [11] and expressed as mg per g fresh weight. The total carbohydrate in plant extract was estimated by the method of (Yemm and Willis 1954) [15] using anthrone reagent and expressed as mg g^{-1} dry weight. Free proline content in leaves was estimated spectro-photo metrically according to the methods of (Bates, Waldren and Teare 1973) [12] and expressed as mg g^{-1} fresh weight. Antioxidant enzyme catalase activity can be assayed calorimetrically according to method given in analytical biochemistry (Sinha 1972) [14] and expressed as Units g^{-1} fresh weight min^{-1}. Superoxide dismutase (SOD) activity in plant tissue was measured by method described by (Asada 1974) and modified by (Giannopolitis and Ries 1977) [13] and expressed in terms of enzyme unit g^{-1} fresh weight. All the biochemical activities were recorded at 45 days *i.e.* before retransplanting, 75 days *i.e.* after retransplanting and at physiological maturity. Nutrient analysis was done at the time of harvest. The nitrogen of shoot and grain was determined separately using colorimetric method of Linder (1944) [24]. Nitrogen uptake at harvest of rice crop from each plots was computed separately by multiplying percent nutrient contents in grain and straw with their respective yields (kg ha^{-1}) and dividing by 100. Nitrogen use efficiency was calculated on basis of given formula.

$$\text{Nitrogen use efficiency} = \frac{\text{Yield}}{\text{Total N fertilizer applied}} \times 100$$

The Phosphorus of shoot and grain was determined separately using colorimetric method of Linder (1944) [24] using Vanadate-molybdate reagent and The Potassium of shoot and grain was determined separately using flame photometer. The data recorded on various growth and yield attributes was subjected to statistical analysis by Fisher method of analysis of variance. Significance of various treatments was judged by comparing calculated, F value with Fisher's F value at 5 percent level.

Results and Discussion

Root morphology

Data regarding number of roots plant^{-1}, dry weight of roots plant^{-1}, to root length plant^{-1}, total soluble sugar content was recorded before re-transplanting was presented in Table 1. An interception of the data revealed that root number influenced with the age of seedling as well as double transplanting in both varieties i.e. DRR-44 and Sahbhagi Dhan. Maximum number of roots was obtained in T_3 (V_1-24.84, V_2-26.18) followed by T_2 (V_1-22.85, V_2-24.33) and T_1 (V_1-19.44, V_2-

21.74) as compared in both the varieties. Essentially, transplanting young rice seedlings reduces the transplanting shock and favours early root establishment and growth in the soil as indicated by Rasool *et al*. (2016) [26]. Maximum dry weight roots plant^{-1} was obtained in T_3 (V_1-0.044 g, V_2-0.047 g) followed by T_2 (V_1-0.031 g, V_2-0.035 g) as compared to T_1 (V_1-0.020 g, V_2-0.027 g) in both the varieties. The root length was progressively increased with age of seedlings whereas, Maximum root length plant^{-1} was obtained in T_3 (V_1-9.25cm, V_2-9.96 cm) followed by T_2 (V_1-7.77 cm, V_2-8.18cm) as compared to T_1 (V_1-7.42, V_2-8.01) in both the varieties. The perusal of data shows that total soluble sugar content was increased with increase in seedling age. However, maximum total soluble sugar content was obtained in T_3 (V_1-135.79, V_2-141.48) followed by T_2 (V_1-117.50, V_2-120.35) and T_1 (V_1-105.29, V_2-110.46) in both the varieties.

Table 1: Effect of seedling age and double transplanting on root number plant^{-1}, root dry wt. plant^{-1} (mg), root length plant^{-1}(cm) and soluble CHO content in root (mgg^{-1} dry wt.) of rice varieties

Treatments	Root number per plant before re-transplanting		Root dry wt. per plant (mg) before re-transplanting		Root length per plant (cm) before re-transplanting		Soluble CHO content in root (dry wt.mg/g) before re-transplanting	
T_1V_1	19.44		0.020		7.42		105.29	
T_2V_1	22.85		0.031		7.77		117.50	
T_3V_1	24.84		0.044		9.25		135.79	
T_1V_2	21.74		0.027		8.01		110.46	
T_2V_2	24.33		0.035		8.18		120.35	
T_3V_2	26.18		0.047		9.96		141.48	
Interaction	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Variety (V)	0.55	1.72	0.00	0.00	0.03	0.11	0.15	0.48
Treatment (T)	0.67	2.10	0.00	0.00	0.04	0.13	0.19	0.58
VxT	0.94	2.98	0.00	0.00	0.06	0.18	0.26	0.83

*Pooled data of two successive year's (2021-22 & 2022-23)

Chlorophyll content in leaf (mg g^{-1} fresh weight)

The data related to chlorophyll 'a', chlorophyll 'b' and total chlorophyll content as influenced by age of seedling and double transplanting at various crop growth stages are summarized in (Table 2). The chlorophyll 'a' content was progressively increased with plant age up to 75 DAT whereas, decrease in chlorophyll content was observed at physiological maturity in both the varieties.

Effect of age of seedlings

It is clear from the data (Table 2) that the age of seedling significantly affect the chlorophyll 'a' content of both the varieties (DRR-44 and Sahbhagi Dhan).The chlorophyll 'b' content was progressively increased with plant age up to 75 DAT whereas, decrease in chlorophyll content was observed at physiological maturity in both the varieties. It is clear from the data (Table 2) that the age of seedling significantly affect the chlorophyll 'b' content of both the varieties (DRR-44 and Sahbhagi Dhan).The total chlorophyll content was progressively increased with plant age up to 75 DAT whereas, decrease in total chlorophyll content was observed at physiological maturity in both the varieties. It is clear from the data (Table 2) that the age of seedling significantly affect the total chlorophyll content of both the varieties (DRR-44 and Sahbhagi Dhan).

Effect of double transplanting (Sanda Cultivation)

Data (Table 2) on chlorophyll 'a' content at 45 DAT shows only the response of varietal difference with respect to age of seedling in both rice varieties (DRR-44 and Sahbhagi Dhan). However, chlorophyll 'a' content ranges from (V_1-0.36-1.18) and (V_2- 0.52-1.40) respectively in both the varieties. Interaction effect of age of seedling and double transplanting on both the varieties were found non-significant during both years. Chlorophyll 'a' content increasing significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. At 75 DAT maximum chlorophyll 'a' content was obtained in T_3 (V_1-2.65, V_2-2.66) followed by T_2 (V_1-2.24, V_2-2.37) and T_1 (V_1-1.87, V_2-2.20) in both the varieties. Whereas, at physiological maturity reduction was recorded in chlorophyll 'a' content in all the treatments. So, at physiological maturity maximum chlorophyll 'a' content was obtained in T_3 (V_1-2.47, V_2-2.59) followed by T_2 (V_1-1.91, V_2-2.09) as compared to T_1 (V_1-1.44, V_2-1.54) in both the varieties under double transplanting. Data (Table 2) on chlorophyll 'b' content at 45 DAT shows only the response of varietal difference with respect to age of seedling in both rice varieties (DRR-44 and Sahbhagi Dhan). However, chlorophyll 'b' content ranges from (V_1-0.33-1.45) and (V_2- 0.62-1.69) respectively in both the varieties. Chlorophyll 'b' content increasing significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. At 75 DAT maximum chlorophyll 'b' content was obtained in T_3 (V_1-5.03, V_2-6.49) followed by T_2 (V_1-4.24, V_2-5.68) and T_1 (V_1-2.68, V_2-3.12) in both the varieties. Whereas, at physiological maturity reduction was recorded in chlorophyll 'b' content in all the treatments. So, at physiological maturity maximum chlorophyll 'b' content was obtained in T_3 (V_1-3.90, V_2-4.16) followed by T_2 (V_1-3.05, V_2-3.35) as compared to T_1 (V_1-2.21, V_2-2.37) in both the varieties under double transplanting. Data (Table 2) on total chlorophyll content at 45 DAT shows only the response of varietal difference with respect to age of seedling in both rice varieties (DRR-44 and Sahbhagi Dhan). However, total chlorophyll content ranges from (V_1-0.69-2.63) and (V_2- 1.13-3.09) respectively in both the varieties. Interaction effect of age of seedling and double transplanting on both the varieties were found non-significant during both years. Total chlorophyll content increasing significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. At 75 DAT maximum total chlorophyll content was obtained in T_3 (V_1-7.69, V_2-9.15) followed by T_2 (V_1-6.48, V_2-8.05) and T_1 (V_1-4.55, V_2-5.32)in both the varieties. Whereas, at physiological maturity reduction was recorded in total chlorophyll content in all the treatments. So, at physiological maturity maximum total chlorophyll content was obtained in T_3 (V_1-6.37, V_2-6.75) followed by T_2 (V_1-

4.96, V_2-5.44) as compared to T_1 (V_1-3.65, V_2-3.90) in both the varieties under double transplanting. Similarly, high chlorophyll contents might also contribute to higher photosynthetic rate and significant positive correlation between chlorophyll content and photosynthesis rate was reported in earlier findings (Shubhra *et al*. 2006) [17].

Table 2: Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content as influenced by age of seedling and double transplanting at various crop growth stages

Treatment Varieties	Chlorophyll 'a' content before re-transplanting at 45 days			chlorophyll 'a' content at 75 days			Chlorophyll 'a' content at physiological maturity			Chlorophyll 'b' content before re-transplanting at 45 days			chlorophyll 'b' content at 75 days			Chlorophyll 'b' content at physiological maturity			Total chlorophyll content before re-transplanting at 45 days			Total chlorophyll content at 75 days			Total chlorophyll content at physiological maturity		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
DRR-44	0.36	0.74	1.18	1.87	2.24	2.65	1.44	1.91	2.47	0.33	1.11	1.45	2.68	4.24	5.03	2.21	3.05	5.03	0.69	1.85	2.63	4.55	6.48	7.69	3.65	4.96	6.37
Sahbhagi Dhan	0.52	0.86	1.40	2.20	2.37	2.66	1.54	2.09	2.59	0.62	1.32	1.69	3.12	5.68	6.49	2.37	3.35	4.16	1.13	2.18	3.09	5.32	8.05	9.15	3.90	5.44	6.75
Interaction	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T
SEm±	-	-	-	0.016	0.019	0.027	0.009	0.011	0.015	0.013	0.016	0.023	0.013	0.016	0.022	0.009	0.011	0.016	-	0.041	0.058	0.020	0.024	0.034	0.013	0.016	0.023
CD at 5%	NS	NS	NS	0.050	0.061	0.086	0.028	0.034	0.048	0.041	0.051	0.072	0.041	0.050	0.017	0.029	0.035	0.050	NS	0.129	0.183	0.062	0.076	0.107	0.042	0.051	0.073

Proline and total soluble sugar content

The data presented in (Table 3) represents the proline content and total soluble sugar content influenced by different age of seedling and double transplanting at different crop growth stages. A perusal of data showed that that proline content progressively increased with the increase of plant age in all the treatments.

Effect of age of seedling

It is clear from the data (Table 3) that the age of seedling significantly affect the proline content of both the varieties (DRR-44 and Sahbhagi Dhan).A critical examination of data in (Table 3) reveals that age of seedling influenced total soluble sugar in of both the varieties (DRR-44 and Sahbhagi Dhan).

Effect of double transplanting (Sanda Cultivation)

Data (Table 3) on proline content at 45 DAT shows only the response of varietal difference with respect to age of seedling in both rice varieties (DRR-44 and Sahbhagi Dhan). However, proline content ranges from (V_1-50.67-52.14) and (V_2- 51.78-52.44) respectively in both the varieties. Proline content increasing significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. At 75 DAT maximum proline content was obtained in T_1 (V_1-101.66, V_2-104.60) followed by T_3 (V_1-101.33, V_2-103.57) and T_2 (V_1-100.61, V_2-103.54) in both the varieties. Whereas, at physiological maturity reduction was recorded in proline content in all the treatments. So, at physiological maturity maximum proline content was obtained in T_1 (V_1-148.61, V_2-150.61) followed by T_3 (V_1-144.40, V_2-146.13) and T_2 (V_1-141.05, V_2-144.03) in both the varieties under double transplanting. According to (Dubey *et al*. 1997) increase in proline levels is considered to help the cell in osmo-protection as well as in regulating their redox potential, scavenging hydroxyl radicals and protection against denaturation of various macro molecules. Proline is thought to play adaptive roles in mediating osmotic adjustment and protecting sub cellular structure in stressed plants (Ashraf and Foolad 2007) [18]. Data (Table 3) on total soluble sugar at 45 DAT shows only the response of varietal difference in both rice varieties (DRR-44 and Sahbhagi Dhan). However, total soluble sugar ranges from (71.33) to (112.00) and (83.00) to (114.33) respectively in both the varieties.

Double transplanting was found effective in increasing total soluble sugar significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. Maximum total soluble sugar was obtained at 75 DAT in T_2 (V_1-141.33, V_2-144.33) followed by T_3 (V_1-125.67, V_2-132.67) and T_1 (V_1-100.61, V_2-103.54) in both the varieties. So, at physiological maturity maximum total soluble sugar was obtained in T_2 (V_1-158.33, V_2-163.00) followed by T_3 (V_1-142.67, V_2-151.00) as compared to T_1 (V_1-134.00, V_2-140.00) in both the varieties under double transplanting.

Table 3: Proline and total soluble sugar content

Treatments	Proline content before re-transplanting at 45 days		Proline content at 75 days		Proline content at Physiological maturity		Soluble CHO content before re-transplanting at 45 days		Soluble CHO content at 75 days		Soluble CHO content at physiological maturity		Soluble CHO content in grain	
T_1V_1	52.14		148.61 (64.91)		101.66 (-46.18)		71.33		103.33 (30.96)		134.00 (22.88)		36.17 (36.83)	
T_2V_1	51.11		141.05 (63.76)		100.61 (-40.19)		96.00		141.33 (32.07)		158.33 (10.73)		49.47 (19.28)	
T_3V_1	50.67		144.40 (64.91)		101.33 (-42.50)		112.00		125.67 (10.87)		142.67 (11.91)		43.98 (25.19)	
T_1V_2	52.44		150.61 (65.18)		104.60 (-43.98)		83.00		113.00 (26.54)		140.00 (19.28)		39.55 (32.20)	
T_2V_2	51.78		144.03 (64.05)		103.54 (-39.10)		102.67		144.33 (28.86)		163.00 (11.45)		50.52 (18.48)	
T_3V_2	51.93		146.13 (64.46)		103.57 (-41.09)		114.33		132.67 (13.82)		151.00 (12.13)		46.43 (22.82)	
Interactions	SEm±	CD at 5%	SEm±	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	CD at 5%	SEm±	CD at 5%
Variety (V)	0.33	1.03	0.50	0.91	2.87	1.60	5.03	0.91	2.87	0.32	1.00	1.58	0.64	2.02
Treatment (T)	0.40	1.27	0.61	1.12	3.52	1.96	6.16	1.11	3.51	0.39	1.23	1.93	0.79	2.48
VxT	0.57	1.79	0.87	1.58	4.98	2.76	8.71	1.58	4.97	0.55	1.74	2.73	1.11	3.51

*Pooled data of two successive year's (2021-22 & 2022-23)

Note-T_1- 14 day's old seedling, T_2- 21 day's old seedling and T_3- 28 day's old seedling.

Parenthesis indicate percent increase/decrease

Antioxidant Enzymes Activities

Data recorded on superoxide dismutase activities and catalase activities at different crop growth stages are presented in (Table 4). The perusal of data shows that superoxide dismutase activity was affected due to age of seedling and double transplanting. SOD activity increased gradually at all stages of plant growth in both the genotypes. Highest SOD activity was recorded at physiological maturity followed by 75 DAT stage and lowest activity was recorded at 45 DAT stage. In order to limit oxidative damage under stress condition plants have developed a series of detoxification systems that break down the highly toxic reactive oxygen species (Larkin dale *et al*. 2002) [19]. The perusal of data shows that catalase activity was affected due to age of seedling and double transplanting.

Effect of age of seedling

The accumulation of superoxide dismutase activity differed significantly among varieties for various planting times (Table 4) at all the stages of crop growth. Both DRR-44 and Sahbhagi Dhan had accumulated the highest superoxide dismutase activity which can be related to sharp rise in temperature at flag leaf stage.CAT activity increased gradually at all stages of plant growth in both the genotypes. Highest CAT activity was recorded at physiological maturity followed by 75 DAT stage and lowest activity was recorded at 45 DAT stage. The accumulation of catalase activity differed significantly among varieties for various planting times (Table 4) at all the stages of crop growth. Both DRR-44 and Sahbhagi Dhan had accumulated the highest catalase activity which can be related to sharp rise in temperature at flag leaf stage.

Effect of double transplanting (Sanda Cultivation)

Data (Table 4) on superoxide dismutase activity at 45 DAT shows only the response of varietal difference in both rice varieties (DRR-44 and Sahbhagi Dhan). However, superoxide dismutase activity ranges from (96.88) to (120.35) and (100.55) to (124.92) respectively in both the varieties. Double transplanting was found effective in increasing superoxide dismutase activity significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. Maximum superoxide dismutase activity was obtained at 75 DAT in T_3 (V_1-147.80, V_2-157.30) followed by T_2 (V_1-140.40, V_2-152.32) and T_1 (V_1-126.29, V_2-133.55)in both the varieties. So, at physiological maturity maximum superoxide dismutase activity was obtained in T_3 (V_1-181.78, V_2-191.51)followed by T_2 (V_1-175.67, V_2-180.16) as compared to T_1 (V_1-164.83, V_2-171.71) in both the varieties under double transplanting. Similar results were also reported by (Edreva and Yordanov 1998) [21] they concluded that, these reductions in enzyme activities could be attributed to antioxidants direct effects on scavenge ROS (O^{-2}), hydrogen peroxide (H_2O_2) and singlet oxygen and /or preventing the enhancement of the mentioned activated oxygen species in tomato plants. Data (Table 4) on catalase activity at 45 DAT shows only the response of varietal difference in both rice varieties (DRR-44 and Sahbhagi Dhan). However, catalase activity ranges from (56.94) to (67.61) and (61.42) to (68.23) respectively in both the varieties. Double transplanting was found effective in increasing catalase activity significantly at 75 DAT and at physiological maturity of observations in both the varieties i.e. DRR-44 and Sahbhagi Dhan. Maximum catalase activity was obtained at 75 DAT in T_3 (V_1-119.03, V_2-129.14) followed by T_2 (V_1-112.66, V_2-120.11) and T_1 (V_1-103.54, V_2-110.06) in both the varieties. So, at physiological maturity maximum catalase activity was obtained in T_3 (V_1-171.46, V_2-172.60) followed by T_2 (V_1-167.58, V_2-168.85) as compared to T_1 (V_1-145.39, V_2-154.78) in both the varieties under double transplanting. Our findings also supported by (Nayyar *et al.* 2005) [20] reported that antioxidants play an important role scavenging of free radical. It could be concluded that, these reduction in enzyme activities could be attributed to antioxidants direct effects on scavenge ROS (O^{-2}), hydrogen peroxide (H_2O_2) and singlet oxygen (O^{-2}) and /or preventing the enhancement of the mentioned activated oxygen species in pigeon pea crop.

Table 4: Superoxide dismutase activities and catalase activities at different crop growth stages

Treatments	SOD activity before re-transplanting at 45 days		SOD activity at 75 days		SOD activity at Physiological maturity		Catalase activity before re-transplanting at 45 days		Catalase activity at 75 days		Catalase activity at Physiological maturity	
T_1V_1	96.88		126.29 (23.29)		164.83 (23.38)		56.94		103.54 (45.01)		145.39 (28.78)	
T_2V_1	114.29		140.40 (18.60)		175.67 (20.07)		62.97		112.66 (44.11)		167.58 (32.77)	
T_3V_1	120.35		147.80 (18.57)		181.78 (18.69)		67.61		119.03 (43.20)		171.46 (30.57)	
T_1V_2	100.55		133.55 (24.71)		171.71 (22.22)		61.42		110.06 (44.19)		154.78 (28.89)	
T_2V_2	119.20		152.32 (21.74)		180.16 (15.45)		63.84		120.11 (46.85)		168.85 (28.86)	
T_3V_2	124.92		157.30 (20.58)		191.51 (17.86)		68.23		129.14 (47.17)		172.60 (25.17)	
Interaction	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Variety	0.53	1.67	0.30	0.93	0.58	1.84	0.46	1.46	0.23	0.73	0.53	1.68
Treatment	0.65	2.05	0.36	1.14	0.72	2.25	0.57	1.78	0.28	0.89	0.65	2.05
VxT	0.92	2.90	0.51	1.61	1.01	3.19	0.80	2.52	0.40	1.26	0.92	2.90

Note-T_1- 14 day's old seedling, T_2- 21 day's old seedling and T_3- 28 day's old seedling. The values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (p>0.05)

*Pooled data of two successive year's (2021-22 & 2022-23)

Parenthesis indicate percent increase/decrease

Table 5: nitrogen content in grain and straw, Nitrogen Uptake (kgha^{-1}) & Nitrogen Use Efficiency (%)

Treatment Varieties	Nitrogen content in grain (%)			Nitrogen content in straw (%)			Nitrogen uptake (kgha^{-1})			Nitrogen use efficiency (%)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
DRR-44	1.51	1.64	1.57	0.41	0.49	0.45	4.23	4.96	4.66	44.63	50.96	48.72
Sahbhagi Dhan	1.52	1.68	1.62	0.44	0.53	0.50	4.55	5.07	4.83	46.82	51.25	49.52
Interaction	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T
SEm±	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.11	0.13	0.18
CD at 5%	0.02	0.02	0.03	0.02	0.02	0.03	0.03	0.04	0.06	0.34	0.41	0.58

Note-T_1- 14 day's old seedling, T_2- 21 day's old seedling and T_3- 28 day's old seedling. The values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (p>0.05)

*Pooled data of two successive year's (2021-22 & 2022-23)

Parenthesis indicate percent increase/decrease

Nutrient Status

Data on nitrogen content in grain and straw, Nitrogen Uptake (kgha^{-1}) & Nitrogen Use Efficiency (%) is presented in (Table 5). It is marked from the data that maximum N content grain was showed in T_2 (1.64%) followed by T_3 (1.57%) and T_1 (1.51%) in DRR-44 similarly in Sahbhagi Dhan maximum N content was noted in T_2 (1.68%) followed by T_3 (1.62%) and T_1 (1.52%).Whereas, maximum straw N content was found in T_2 (0.49%) followed by T_3 (0.45%) and T_1 (0.41%) in DRR-44 similarly in Sahbhagi Dhan maximum N content was noted in T_2 (0.53%) followed by T_3 (0.50%) and T_1 (0.44%). Moreover, significant interaction was found in both the grain and straw of rice varieties for N content. Our finding also supported by Kabat and Satapathy (2013) [22] conducted a field experiment during wet season of 2007 and 2008 on sandy loam soil having pH 6.61 with rice hybrid 'Rajalaxmi' at Bhanjanagar, Odisha with different seedling ages, recorded significantly higher nitrogen concentration in grain and straw in 21 days old seedling than other seedling ages. Data on nitrogen uptake in shoot is presented in table (Table 5). Obvious from the data that maximum N uptake was recorded in T_2 (4.96 kg/ha) followed by T_3 (4.66 kg/ha) and T_1 (4.23 kg/ha) in DRR-44 likewise in Sahbhagi Dhan 5.07 kgha^{-1}, 4.83 kgha^{-1} and 4.55 kgha^{-1} in T_2, T_3 and T_1 respectively. Whereas, Data on nitrogen use efficiency is presented in table 6. It is marked from the data that maximum N use efficiency was showed in T_2 (50.96%) followed by T_3 (48.72%) and T_1 (44.63%) in DRR-44 similarly in Sahbhagi Dhan maximum N use efficiency was noted in T2 (51.25%) followed by T_3 (49.52%) and T_1 (46.82%). This result confirms the finding of Rasool *et al*. (2016) [26] who obtained higher NU for 35 days seedlings than those of 45-day old. As a result of increased NU with young seedlings over old ones, the physiological efficiency (PE; being inverse function of NU) was higher for aged seedlings as compared to young ones while the recovery efficiency (RE) was higher with the young seedlings than with old ones. Salem *et al*. (2011) reported high NU and RE for young seedlings.

Data on phosphorus content and potassium content in grain and straw is presented in table (Table 6). It is marked from the data that maximum P content grain was showed in T_2 (0.14%) followed by T_3 (0.10%) and T_1 (0.07%) in DRR-44 similarly in Sahbhagi Dhan maximum P content was noted in T_2 (0.16%) followed by T_3 (0.14%) and T_1 (0.11%). Whereas, maximum straw P content was found in T_2 (0.11%) followed by T_3 (0.09%) and T1 (0.05%) in DRR-44 similarly in Sahbhagi Dhan maximum P content was noted in T_2 (0.14%) followed by T_3 (0.11%) and T_1 (0.09%). Moreover, significant interaction was found in both the grain and straw of rice varieties for P content. Similarly, significantly higher nutrient uptake (NPK) values by grain, straw and total uptake were recorded by 10 days old seedlings than 8 and14 days old seedlings. These results are in close conformity with the findings of Hussain *et al*. (2012) [23]. Data associated to 'K' content in grain and straw is presented in table (Table 6). It is marked from the data that maximum K content grain was showed in T_2 (0.57%) followed by T_3 (0.54%) and T1 (0.50%) in DRR-44 similarly in Sahbhagi Dhan maximum K content was noted in T_2 (0.58%) followed by T_3 (0.56%) and T_1 (0.51%).Whereas, maximum straw K content was found in T_2 (1.64%) followed by T3 (1.63%) and T_1 (1.60%) in DRR-44 similarly in Sahbhagi Dhan maximum K content was noted in T_2 (1.70%) followed by T3 (1.66%) and T_1 (1.62%). Moreover, significant interaction was found in both the grain and straw of rice varieties for K content. Similarly, significantly higher nutrient uptake (NPK) values by grain, straw and total uptake were recorded by 10 days old seedlings than 8 and14 days old seedlings. These results are in close conformity with the findings of Hussain *et al*. (2012) [23].

Table 6: Data on nitrogen use efficiency is presented

Treatment Varieties	Potassium content in grain (%)			Potassium content in straw (%)			Phosphorus content in grain (%)			Phosphorus content in straw (%)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
DRR-44	0.50	0.57	0.54	1.60	1.64	1.63	0.07	0.14	0.10	0.05	0.11	0.09
Sahbhagi Dhan	0.51	0.58	0.56	1.62	1.70	1.66	0.11	0.16	0.14	0.09	0.14	0.11
Interaction	V	T	V×T	V	T	V×T	V	T	V×T	V	T	V×T
SEm±	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01
CD at 5%	0.02	0.02	0.03	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.03

Note-T_1- 14 day's old seedling, T_2- 21 day's old seedling and T_3- 28 day's old seedling. The values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (p>0.05)

*Pooled data of two successive year's (2021-22 & 2022-23)

Parenthesis indicate percent increase/decrease

Conclusion

Present study revealed that seedling age and double transplanting (sanda cultivation) at vegetative stage or reproductive stage severely decreased the plant growth and development. More atrocious decrease was observed in T_1 (14 days old seedling) as compare to other seedling ages (T_2- 21 days old seedling & T_3- 28 days old seedling) respectively in both the varieties. Age of seedling and double transplanting significantly partial the chlorophyll content of the both the varieties. Moreover, Sahbhagi Dhan showed more depression in comparison with DRR-44. Analysis of variance clearly indicates that 21 days old seedling successfully ameliorates the adverse effect of seedling age as well as double transplanting. Similarly, significant positive correlation was obtained among growth attributes and chlorophyll content (r=0.34), (r=0.57) and (r=0.68) respectively.

Assimilate accumulation like total soluble sugar content is the paramount importance for plant growth and development. Present study indicated that total soluble sugar content gradually increased with the advancement of crop stages. But as per varietal concern (Sahbhagi Dhan) partial enhancement was observed up to (11.45%-19.28%), while (DRR-44) (10.73%-22.88%).

However, 21 days old seedling effectively reduces the detrimental effect of age of seedling and double transplanting stress on both the varieties. Maximum total soluble sugar content was obtained with T_2 (21 days old seedling) at par with T_3 (28 days old seedling). Strong positive correlation was observed between plant height and total soluble sugar content (r=0.90).

Similarly, proline content was higher in case Sahbhagi Dhan compared to DRR-44 but minimum damage in proline content noted in 21 days old seedling. Maximum proline content was observed in T1 (14 days old seedling) in both the varieties. However, Minimum proline content was obtained with T_2 (21 days old seedling).

On the basis of results obtained it may be concluded that the enzyme activities like SOD and catalase activity in leaves are the key component of plant adaptation to double transplanting as well as age of seedling. But, it in adverse condition the activity of these enzymes is increased. Our results also indicate that under T_2 (28 days old seedling) the activity of SOD and catalase activity in leaves gradually increased in both the varieties. However in case of 28 days old seedling the activities of SOD and catalase activity in leaves are potentially increased to protect the plant from oxidative damage. Similarly, all other seedling ages help in increasing the activity of SOD and catalase activity in leaves to reduce plant mortality at detrimental stress. On the basis of above study it can be concluded that 21 days old seedling is was able to ammeleorate the effect of double transplanting and was able to achieve high yield. It is thus recommended that under double transplanting 21 days old seedlings are beneficial for the farmers to minimize yield losses under sanda cultivation.

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