



Optimizing seedlings number and spacing to harness productivity potential of salt tolerant rice cultivars in salt affected soils

Y.P. Singh^{1*}, V.K. Mishra¹ and D. Singh²

¹Principal Scientists, ²Senior Research Fellow, ICAR-Central Soil Salinity Research Institute, Regional Research Station, Lucknow-226002, Uttar Pradesh, India *Corresponding author E-mail: ypsingh.agro@gmail.com

Received : March 9, 2020 Revised : April 18, 2020 Accepted : April 25, 2020 Published : June 30, 2020

ABSTRACT

A field experiment was conducted at Central Soil Salinity Research Institute, Regional Research Station, Shivri farm, Lucknow, Uttar Pradesh, India to optimize the number of seedlings hill⁻¹ and plant spacing for maximizing the productivity of salt tolerant rice cultivars in salt affected soils. Thirty five day old seedlings of two salt tolerant rice varieties CSR 36 and CSR 43 were transplanted using two seedlings hill⁻¹ (2 and 4) at three plant spacing (15 cm \times 15 cm, 15 cm \times 20 cm and 20 cm \times 20 cm). The results indicated that transplanting of 4 seedlings hill⁻¹ with plant to plant and row to row spacing of 15×20 cm gave the maximum values for plant height, number of productive tillers hill⁻¹, days to 50% flowering, leaf area index and days to 75% maturity. Variety CSR 36 gave the highest value of plant height, number of productive tillers hill-1 and leaf area index. On the other hand, days to 50% flowering and days to 75% maturity were significantly lesser in variety CSR 43. Dry matter content and crop growth rate of CSR 36 planted at a spacing of 15×20cm apart during three different growth periods were significantly increased with 4 seedlings hill-1. Transplanting of 4 seedlings hill-1 at a spacing of 15x20cm gave significantly higher number of grains panicle⁻¹ over 2 seedlings hill⁻¹. Variety CSR 36 planted with 4 seedlings hill⁻¹ at a spacing of 15×20cm produced significantly higher biological and grain yields over rest of the treatments evaluated in the study. From the results of the study, it could be concluded that the transplanting of salt tolerant variety CSR 36 and CSR 43 with 4 seedlings hill-1 at a spacing of 15×20 cm in salt affected soils showed better crop establishment and crop growth and resulted in higher grain yield.

Keywords: Number of seedlings hill-1, Salt tolerant varieties, Spacing

INTRODUCTION

Salt affected soils are one of the major abiotic stresses that adversely affect the overall metabolic activities and cause plant demise (Roychoudury *et al.*, 2008). The global extent of salt affected area is about 955×10⁶ ha (Szabolcs, 1994). In India 6.73 million ha land is affected with salinity/sodicity problem (Mandal *et al.*, 2009). The detrimental effects of salt stress on plant growth are associated with low osmotic potential of the soil solution (secondary drought), nutritional imbalances (nutrient disorders), specific ion effects (sodium or chlorine toxicity) or a combination of these factors

(Ashraf and Harris, 2004). Early responses during the first phase of salt stress are often a rapid decrease in cell water potential, reduction in cell division, and an expansion as well as an increase in compatible compounds such as proline, polyols, and sugars (Munns, 2008). Accumulation of exchangeable sodium in saline /sodic soils often leads to deterioration of soil structure. This results in a low infiltration rate.

Rice (*Oryza sativa* L.) is one of the most important crops in the world and is the primary stable food for over two billion people. With the rapid growth in population consuming rice and the deteriorating soil

and water quality around the globe, there is an urgent need to understand the response of this important crop towards these environmental abuses. With the ultimate goal to raise rice plant with better suitability towards changing environmental inputs, intensive efforts are on worldwide employing physiological, biochemical and molecular tools to perform this task. Abiotic stress is the main factor negatively affecting crop growth and productivity worldwide. Rice plants are relatively susceptible to salt stress (Flowers and Yeo, 1989; Gao et al., 2007). Grain yield of rice in salt affected soils is much below the potential level because farmers are using traditional varieties and following traditional management practices. The reason are modern high yielding varieties require higher prices for seeds, fertilizer, irrigation and pesticides.

The farmers having salt affected soils are generally poor, so they cannot afford higher investment on agro inputs. Hence, special attention should be given for increasing the yield per unit area through effective management practices. Among different management practices, use of appropriate number of seedlings hill⁻¹ and maintaining optimum plant spacing are important. Chowdhury *et al.* (1993) reported that number of seedlings hill⁻¹ and plant spacing are important factors which influence the plant population per unit area, availability of sunlight, competition for nutrients, photosynthesis and respiration which ultimately influence the yield contributing characters and yield of rice.

Rice is salt tolerant at germination but quite sensitive to salinity/sodicity of the rooting medium at the seedling stage (Aslam et al., 1988; Aslam et al., 1993b) and often much loss in yield have been observed because of high plant mortality at early seedling establishment stage. However, mortality of young seedlings and poor tillering of the crops are common in salt affected soils. The plants put up fewer infrastructures than in normal soils. Higher seed rate or more number of seedlings hill⁻¹ and closer spacing are advisable to counter these effects. Plant mortality varies with level of sodicity. There are certain salt tolerant varieties like CSR 10, CSR 13, CSR 23, CSR 27, CSR 36 and CSR 43 which can tolerate sodicity up to pH 9.8 (Mishra et al., 1992). High vielding salt tolerant varieties 'CSR 36' and 'CSR 43' were selected in this study. The present investigation is therefore an effort to optimize the number of seedlings hill⁻¹ and spacing to maximize the productivity of salt tolerant varieties in sodic soils.

MATERIALS AND METHODS

Selection of Varieties

Selection of varieties was made on the basis of performance of varieties in the fields and adoption rate in salt affected soils (Singh *et al.*, 2013). Variety CSR 36 released in 2005 is highly sodicity tolerant (pH 9.8) having yield potential of 4.0 t ha⁻¹. It attains plant height of 110cm and matured in 135 days. It has long slender grain. However, CSR 43 released in 2011, is short duration dwarf statured (95 cm) salt tolerant variety which can tolerate sodicity up to 9.8 and having yield potential of 4.0 t ha⁻¹ in highly sodic soils and 6.0 t ha⁻¹ in partially reclaimed sodic soils. It has short bold grain and highly preferred for puffed rice. It matures in 110 days and saves at least two irrigations when compared to CSR 36 and other traditional varieties.

Experimental details

A field experiment was conducted at Shivri farm, Central Soil Salinity Research Institute, Regional Research Station, Lucknow (26°47'45" N and 80° 46'7" E) during Kharif 2011 to optimize the number of seedlings hill⁻¹ and spacing of two salt tolerant rice varieties CSR 36 and CSR 43 in a sodic soils (pH 9.2), electrical conductivity (EC) 0.61 dS m⁻¹, organic carbon (OC) 3.30g kg⁻¹ (Table 1). The experiment was laid in split-split plot design with three replications. Numbers of seedlings (2 and 4 seedlings hill⁻¹) were allotted to the main plot, spacing (15×15cm, 15×20cm and 20×20cm) in the sub-plot and varieties viz. CSR 36 and CSR 43 in to sub-sub plot. Before initiating the experiment, soil samples were collected from 0-15 and 15-30 cm soil depth to analyze initial soil properties. The samples were air

Table 1.	Initial	properties	of the	experimental	soil
		properties	01 1110	enp er merenear	

Soil properties	Soil depth (cm)			
	0-15	15-30		
pН	9.20	9.70		
EC(dS m ⁻¹)	0.61	1.20		
OC (g kg ⁻¹)	3.30	1.20		
Ca ⁺⁺ and Mg ⁺⁺ (me L ⁻¹)	2.60	2.10		
$Na^{+}(me L^{-1})$	4.50	10.50		
K ⁺ (me L ⁻¹)	0.06	0.04		
CO ₃ (me L ⁻¹)	1.00	2.00		
HCO_3^{-} (me L ⁻¹)	4.00	6.50		
Cl ⁻ (me L ⁻¹)	2.00	2.50		
CaCO ₃ <2mm (%)	0.60	1.50		
$CaCO_3 > 2mm (\%)$	0.00	4.00		

EC: Electrical conductivity, OC: Organic carbon

dried, ground to pass through a 2.0 mm sieve. The pH and EC of the soil were determined in 1:2 soil water suspension using digital pH and conductivity meters (Jackson, 1967). Organic carbon was determined by following the method of Walkley and Black rapid titration method, modified by Walkley (1947). The concentration of Ca^{2+} and Mg^{2+} in saturation extract were estimated by an Inductivity Coupled Plasma Analyzer (Perkin Elmer), and Na⁺ and K⁺ measured by Flame Photometer. In the extract, carbonate and bi-carbonate contents were determined by titration with 0.01 N H₂SO₄ using phenolphthalein and methyl orange as indicators by following the methods given Richards (1954). For chloride estimation, the extract was titrated with N/ 100 AgNO₃ solution using potassium chromate as indicator. Free CaCO₃ content was estimated by rapid titration method using bromothymol blue and bromocresol green as indicators (Piper, 1966).

Seeds of rice varieties (CSR 36 and CSR 43) were soaked in water for 24 hours and incubated for 48 hours in wet gunny bag to assure the quick and even germination. These pre-germinated seeds were sown @ 40 kg ha⁻¹ to raise the nursery in a normal soil. Thirty five days old seedlings was transplanted in a plot size of $5 \times 5m^2$. The recommended dose of N-P-K (120-60-40 kg ha-1) and zinc sulphate @25kg ha⁻¹ were applied uniformly in the main field. Nitrogen fertilizer was applied in the form of Urea (46% N) and DAP (18% N and 46% P). One third dose of N, full dose of P, K and Zinc sulphate were applied as basal and remaining two third of N was applied in two equal splits one at active tillering and another one at panicle initiation stage. To measure the growth parameters like plant height, number of tillers hill⁻¹, number of productive tillers hill⁻¹, hills were carefully pulled before the harvest to keep the whole root system and transferred to laboratory. Plant height (cm) was determined from the base to the tip of the tallest leaf blade. Number of days from transplanting to 50% heading was recorded for each variety. After 100% heading, leaf area index was measured. Yield and yield attributing characters like length of panicle, number of grains panicle⁻¹, panicle weight, test weight, biological and grain yields were recorded. Grain yield was measured from 20 m² $(5 \times 4m^2)$ area marked in the center of the sub plot. Harvest index was calculated and grain yield was adjusted to 14% moisture content. Ten panicles were randomly collected from each sub-plot to determine 1000 grain weight and number of grains panicle⁻¹.

Three hills plot⁻¹ were uprooted randomly on 30, 60, and 90 days after transplanting (DAT) and roots were washed thoroughly and discarded. The panicles were then separated from the shoot (after heading) and dried in an oven at 70°C for 48 hours. After oven drying, the samples were weighed by using an electric balance which gave total dry matter and finally, it was expressed in g hill⁻¹. Crop growth rate (CGR) (g m⁻² month⁻¹) was determined as the increase of plant height in unit of ground area m⁻² every 30 days as the following equation:

 $CGR = g m^{-2} month^{-1}$

 $W_2 - W_1$

 $T_2 - T_1$

Where W_1 and W_2 refer to sample weight at time (months) T_1 and T_2 , respectively.

Statistical analysis: Data collected were subjected to statistical analysis of variance according to Gomez and Gomez (1984) using IRRISTAT computer program.

RESULTS AND DISCUSSION

Growth characters

Data in Table 2 showed that plant height, number of tillers hill⁻¹, days to 50% flowering, days to 75% maturity and leaf area index increased with increasing number of seedlings hill⁻¹. Transplanting of 4 seedlings hill⁻¹ gave the highest value for plant height, number of productive tillers hill⁻¹, days to 50% flowering, days to maturity and leaf area index. However, difference among these characters was not significant between 2 and 4 seedlings hill⁻¹ except leaf area index. Planting of rice seedlings at a spacing of 15×20cm recorded maximum plant height, number of productive tillers, days to 50% flowering and days to maturity but there was no significant difference in these characters. However, leaf area index was significantly higher at 15×20cm plant spacing over 15×15 and 20×20cm. Rice variety CSR 36 recorded highest value of all the growth characters than CSR 43. On the other hand, days to 50% flowering and days to maturity were significantly lesser in variety CSR 43 than CSR 36. This was however expected because CSR 36 is having high tolerance to sodicity and longer duration of growth. Higher row to row spacing allows more light penetration resulting in more number of tillers and plant height. Fig. 1 shows significant interaction

Treatments	Plant height (cm)	Number of Productive	Days to 50% flowering	Days to 75% maturity	Leaf area index
		tillers hill-1			
Seedling hill ⁻¹					
2	96.69	9.44	104.89	132.06	5.45
4	98.26	10.06	104.50	132.28	5.77
LSD _{0.05}	2.79	NS	NS	NS	0.02
Spacing (cm)					
15×15	95.68	9.35	104.42	132.00	5.02
15×20	98.90	10.08	104.83	132.33	6.68
20×20	97.85	9.81	104.83	132.17	6.01
LSD _{0.05}	NS	NS	NS	NS	0.11
Varieties					
CSR 36	107.05	10.46	112.89	143.22	5.45
CSR 43	87.90	9.04	96.50	121.11	6.13
LSD _{0.05}	4.73	2.06	1.20	1.19	0.61

Table 2. Crop growth parameters of rice varieties as affected by number of seedlings hill-¹ and spacing

NS: Non-significant

between spacing and varieties on number of productive tillers.

The effect of number of seedlings hill⁻¹ and spacing on dry matter content was significant as observed at all sampling dates. The maximum dry matter was recorded with 4 seedlings hill-1 at 90 DAT and minimum with 2 seedlings hill⁻¹ (Table 3). Total dry matter production increased with the increase of days to planting and it was also increased gradually with increasing number of seedling hill⁻¹. Highest dry matter at 30, 60 and 90DAT were obtained from 15×20cm plant spacing (Table 3). CSR 36 produced maximum dry matter at all the growth stages over CSR 43. Crop growth rates (CGR) during two growth intervals were significantly higher with 4 seedlings hill⁻¹ over 2 seedlings hill⁻¹. This can be associated to more number of tillers hill⁻¹ and dry matter content. Dry

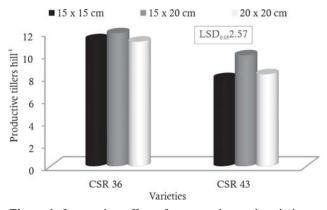


Figure 1. Interaction effect of row spacing and varieties on productive tillers.

matter content and CGR increased with increasing plant to plant and row to row spacing from 15×15 cm to 20×20 cm but there was no significant difference in these parameters between 15×20 and 20×20 cm plant spacing. CSR 36 surpassed CSR 43 in dry matter content and crop growth rate during the three intervals recorded and this might be attributed to its relatively high biomass and leaf area index.

Grain yield and its components

Data given in table 4 revealed that panicle length was significantly influenced by number of seedlings hill⁻¹ and plant spacing. Two seedlings hill⁻¹ recorded highest panicle length but it was statistically similar to that observed in 4 seedlings hill-1. Karmakar et al. (2002) reported that panicle length reduced gradually with increasing number of seedling hill⁻¹. The longest panicle (24.21cm) was found from 15×20cm plant spacing which was statistically similar to the values obtained with 15×15 and 20×20cm plant spacing. Panicle length of CSR 36 was slightly higher than that of CSR 43 but the difference in this parameter was not statistically significant. Panicle weight of CSR 36 planted with 4 seedlings hill⁻¹ at a spacing of 20×20 cm was higher than those of 15×15cm and 15×20cm spacing but the differences among them were non-significant. Number of grains panicle⁻¹ was significantly influenced by number of seedlings hill⁻¹ and spacing. Transplanting of 4 seedlings hill⁻¹ gave significantly maximum number of grains panicle⁻¹ over transplanting of 2 seedlings hill⁻¹. Reduction in vegetative growth due to less numbers of seedlings hill⁻¹ was perhaps, the reason for this reduction in the

Treatments	D	ry matter content (g hill	-1)	Crop growth ra	ate (g m ⁻² month ⁻¹)
	30DAT	60DAT	90DAT	30-60DAT	60-90DAT
Seedling hill ⁻¹					
2	9.28	41.15	52.02	35.37	12.06
4	10.97	45.39	63.69	38.20	20.31
LSD _{0.05}	0.56	1.71	2.78	2.23	7.56
Spacing(cm)					
15×15	11.25	44.48	56.80	36.88	13.67
15×20	11.33	45.13	58.18	37.52	15.91
20×20	10.81	46.21	58.59	39.29	13.75
LSD _{0.05}	0.79	1.33	0.88	0.94	NS
Varieties					
CSR 36	11.48	45.85	59.05	38.14	14.66
CSR 43	10.78	44.70	56.66	37.65	14.23
LSD _{0.05}	0.38	1.37	1.15	1.62	NS

Table 3. Dry matter content and crop growth rate as affected by number of seedlings hill-1 and spacing

DAT: Days after transplanting, NS: Non-significant

Table 4. Yield and yield attributing traits of rice cultivars as affected by number of seedlings hill-1 and spacing.

Treatments	Panicle length (cm)	Panicle weight (g)	No. of grains panicle ⁻¹	1000-grain weight(g)	Biological yield (t ha ^{.1})	Grain yield (t ha ⁻¹)
Seedling hill ⁻	1					
2	24.42	2.26	106.94	25.04	11.19	3.53
4	23.52	2.70	120.33	25.93	12.98	4.33
LSD _{0.05}	1.15	0.42	7.33	NS	NS	0.47
Spacing(cm)						
15×15	23.52	2.42	109.75	24.85	11.69	4.02
15×20	24.21	2.49	121.42	25.98	12.70	4.09
20×20	24.18	2.51	109.75	26.63	11.87	3.68
$LSD_{0.05}$	NS	NS	10.43	NS	0.73	0.28
Varieties						
CSR 36	24.13	2.55	119.50	25.48	12.10	4.05
CSR 43	23.81	2.40	110.78	25.49	12.08	3.81
LSD _{0.05}	NS	NS	7.58	NS	NS	NS

NS: Non-significant

number of grains panicle⁻¹. The maximum number of grains panicle⁻¹ was obtained from 15x20cm plant spacing which was statistically significant to 15x15 and 20x20cm spacing. Cultivar CSR 36 recorded significantly higher number of grains panicle⁻¹ than CSR 43. Interaction effect between number of seedling hill⁻¹ and spacing did not significantly influence the number of grains panicle⁻¹ (Table 4).

Weight of 1000-grains was not significantly affected by number of seedlings hill⁻¹and spacing. The maximum weight of 1000 grains was observed from 4 seedlings hill⁻¹ and minimum with 2 seedlings hill⁻¹. Results showed that the number of seedlings hill⁻¹ is not a factor for increasing or decreasing 1000grain weight. Maximum weight of 1000grains

was obtained from 20×20 cm spacing but was statistically similar to those obtained with 15×15 and 15×20 cm spacing. Cultivar CSR 43 gave higher grain weight than CSR 36 but the difference was not statistically significant (Table 4).

Grain yield was significantly influenced by number of seedlings hill⁻¹ and spacing. The significantly higher grain yield was observed from 4 seedlings hill⁻¹ as compared to 2 seedling hill⁻¹ (Table 4). Maximum tillering per unit area, largest number of grains panicle⁻¹ and the highest 1000 grain weight contributed to higher grain yield. This finding was in agreement with the findings of Prasad and Sheer (1992). Another reason may be less sensitivity of rice seedlings to salt stress during vegetative

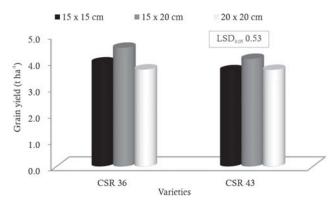


Figure 2. Interaction effect of row spacing and varieties on grain yield

(tillering) phase (Pearson, 1961; Aslam *et al.*, 1988 and 1993a). Transplanting of rice at 15×20 cm plant spacing yielded significantly higher grain yield than those of 15×15 cm and 20×20 cm spacing. Variety CSR 36 produced higher grain yield over CSR 43 but the difference between them was not statistically significant. The interaction effect of plant spacing x varieties on grain yield was significant as both the rice varieties CSR 36 and CSR 43 produced significantly higher grain yield at 15×20 cm plant spacing (Fig. 2).

CONCLUSION

From the results of the study, it could be concluded that the transplanting of salt tolerant variety CSR 36 and CSR 43 with 4 seedlings hill⁻¹ at a spacing of 15×20 cm in salt affected soils showed better crop establishment and crop growth and produced higher grain yield. It was mainly because of higher number of effective tillers, filled grain and 1000 grains weight under these treatments. However, variety CSR 36 proved superior over CSR 43 in this study. Therefore, it may be concluded that salt tolerant variety 'CSR 36' should be transplanted at a spacing of 15×20 m with 4 seedlings hill⁻¹ to harness the productivity potential of salt affected soils.

ACKNOWLEDGEMENT

The authors express their sincere thanks to European Commission-International Fund for Agricultural Development (EC-IRAD), International Rice Research Institute (IRRI), Philippines for financial support to conduct this study and to the Director, Central Soil Salinity Research Institute, Karnal, Haryana, India for the support and encouragement.

REFERENCES

- Ashraf, M. and Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 166, 3-16.
- Aslam, M., Qureshi, R.B. and Ahmad, N. (1993a). A rapid screening technique for salt tolerance in rice (*Oryza sativa* L.) *Plant Soil*, 50, 99-107.
- Aslam, M., Qureshi, R.H. and Ahmad, N. (1993b). Mechanism of salinity tolerance in rice (*Oryza sativa* L.). In: Leith H, Massoum AA (eds) *Towards the rational use of high salinity tolerant plants*. Kluwer Academic Publisher, Netherlands, pp. 135-138.
- Aslam, M., Qureshi, R.H., Ahmad, N. and Muhammad, S. (1988). Response of rice to salinity shocks at various growth stages and type of salinity in the rooting medium. *Pak. J. Agric. Sci.*, 25, 199-205.
- Chowdhury, M.J.U., Sarker, A.U., Sarkar, M.A.R. and Kashem, M.A. (1993). Effect of variety and number of seedlings hill⁻¹ on the yield and its components of late transplanted rice. *Bangladesh J. Agric. Sci.*, 20, 311-316.
- Flowers, T.J. and Yeo, A.R. (1989). Effects of salinity on plant growth and crop yield. In: Cherry, J.H. (eds) *Environmental stress in plants*, Springer Verlag, Berlin, pp 101-119.
- Gao, J.P., Chao, D.Y. and Lin, H.X. (2007). Understanding abiotic stress tolerance mechanisms: Recent studies on stress response in rice. *J. Integr. Plant Biol.*, 49, 742-750.
- Gomez, K.A. and Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research*. 2nd ed. John Wiley and Sons, New York, pp 680.
- Jackson, M.L. (1967). *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, India, pp 183-226.
- Karmakar, B., Sarkar, M.A.R., Uddin, M.R. and Biswas, M. (2002). Effect of row arrangement, number of seedlings hill⁻¹ and nitrogen rates on yield and yield components of late transplant Aman rice. *Bangladesh* J. Agric. Sci., 29, 277-278.
- Mandal, A.K., Sharma, R.C. and Singh, G. (2009). Assessment of salt affected soils in India using GIS. *Geocarto International*, 24(6), 437-456.
- Mishra, B., Singh, R.K. and Bhattacharya, R.K. (1992). CSR 10, a newly released dwarf rice for salt affected soils. *IRRN* 17: 19.
- Munns, R. (2008). Strategies for crop improvement in saline soils. In: Ashraf M et al. (eds) *Salinity and water stress: Improving crop efficiency*. Springer, Netherlands, pp 99-110.
- Pearson, C.L.A. (1961). The salt tolerance of rice. *Int. Rice Comm. News Lett.*, 10, 1-4.

- Piper, C.S. (1966). *Soil and Plant Analysis*. Hans Publishers, Bombay, pp 135.
- Prasad, K., Singh, R. and Singh, S. (1992). Effect of seedlings age and number of hill on the yield of rice in a sodic soil. *Current Agric.*, 16, 67-70.
- Richards, L.A. (1954). *Diagnosis and Improvement of Saline* and Alkali soils. USDA Agriculture Handbook 60, pp 160.
- Roychoudury, A., Basu, S., Sarkar, S.N. and Sengupta, D.N. (2008). Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. *Plant Cell Rep.*, 27, 1395-1410.
- Szabolcs, I. (1994). Prospects of soil salinity for the 21st century. In: 15th World Congress of Soil Science. Acapulco, 10–16 July 1994, pp 123-141.
- Singh, Y.P., Nayak, A.K., Sharma, D.K., Gautam, R.K., Singh, R.K., Singh, R., Mishra, V.K., Paris, T. and Ismail, A.M. (2013). Varietal selection in sodic soils of Indo-gangetic plains through farmers' participatory approaches. *Afr. J. Agric. Res.*, 8, 2849-2860.
- Walkley, A. (1947). An examination of methods for determining organic carbon and nitrogen in soils. *Agric. Sci.* (England), 25, 598-609.