



Research Article

Assessment of Somaclonal Variation for Growth, Yield, and Grain Traits in Second-Generation Rice Somaclones Derived from the Aromatic Landrace Ranisalute

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ARTICLE INFO	ABSTRACT
Article history Received: 31 July 2026 Accepted: 24 September 2026 Published: 30 September 2026 Keywords Ranisalute, landrace, somaclone, cluster, correlation Correspondence R. N. Remme ✉: nusratremme@at.ku.ac.bd OPEN ACCESS	Somaclonal variation is considered an effective technique to introduce genetic variability in crop plants and selecting superior genotypes with desirable agronomic traits. A field experiment was conducted to evaluate twenty-eight (28) rice somaclones (2nd generation) developed from Ranisalute, a popular aromatic landrace of the Khulna district, during <i>Aman</i> season (August-January, 2022) at Professor Dr. Purnendu Gain Field Laboratory of Agrotechnology Discipline, Khulna University following a Randomized Complete Block Design (RCBD) with three replications. The objective was to identify morphological variations among the somaclones and to select promising yield contributing somaclone/s compared to the parental genotype. Analysis of variance revealed that variations exist in different parameters except for secondary branches hill⁻¹, and panicle length among the somaclones. But in comparison with control using Dunnett test, most of the somaclones were statistically similar for all the parameters. Among the evaluated genotypes, RSC-2021-16 showed maximum number of total tillers hill⁻¹ and effective tillers hill⁻¹, maximum grain length, highest 1000-grain weight and maximum grain weight hill⁻¹. RSC-2021-17 recorded the highest number of grain panicle⁻¹, filled grains panicle⁻¹ and maximum leaf area. RSC-2021-27 produced the lowest grain breadth. Cluster analysis grouped the genotypes into four major clusters, where Cluster II and Cluster IV exhibited the highest inter-cluster distance, indicating greater genetic divergence and potential for heterosis and transgressive segregation. Correlation analysis revealed strong positive associations of effective tillers hill⁻¹, grain length, and 1000-grain weight with grain weight hill⁻¹ and harvest index, suggesting their usefulness as indirect selection criteria for yield improvement. The study recommends further investigation of the selected somaclones from Cluster II and Cluster IV, along with superior genotype RSC-2021-16 from Cluster I in the third generation, including their evaluation of genetic stability and quality-related traits.
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Introduction

Rice is the world's most significant crop, providing a key source of nutrition to half of the world's population (Fukagawa and Ziska, 2019). Over 90% of the world's rice is cultivated and consumed in Asia (Samal et al., 2022). Bangladesh has been the world's third-largest producer of rice and has maintained its position as the top producer for the past seven years (Food Outlook, 2025). In 2023-2024, Bangladesh produced a total of 40.697 million metric tons of rice (BBS, 2024).

Rice landraces are traditional cultivars developed and maintained by farmers over generations. Although generally less productive than modern varieties, they

are valuable sources of genetic diversity, particularly for genes associated with tolerance to biotic and abiotic stresses (Azeez et al., 2018; Greveniotis et al., 2019). Many landraces also possess desirable traits, including high iron and zinc content, unique aroma and taste, and superior cooking quality (Islam et al., 2016; Dwivedi et al., 2019). However, the widespread adoption of high-yielding varieties has led to the decline and genetic erosion of many Bangladeshi rice landraces, resulting in the loss of valuable genetic resources for future rice improvement and adaptation to diverse agroecological conditions. In the Khulna district, despite the dominance of modern high-yielding varieties in rice cultivation, several indigenous cultivars, such as

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Ranisalute, Jotaibalam, Chiniatap, Hoglapata, Bashful, Balam, and Khoijhuri, are still grown by farmers.

Among them, Ranisalute is a coarse-grained, popular, aromatic landrace that commands a high market price in Bangladesh. This rice cultivar requires relatively little attention and cost to cultivate because it is tolerant of most biotic and abiotic stresses and requires only half the doses of HYV fertilizers. However, its major limitation is low yield (about 3 t ha⁻¹). Therefore, improving the yield potential of Ranisalute could increase its adoption and value in the coastal region of Bangladesh.

Most rice breeding programs use landraces as genetic resources to transfer unique genes into HYVs (Brar and Khush, 2018), but efforts to develop HYVs from landraces are essentially nonexistent. There is a pressing need to produce healthy foods using eco-friendly agricultural practices while keeping farmers financially sustainable. As a result, promising rice landraces should be prioritized for study to enhance yield *via* plant breeding approaches.

Nowadays, various tissue culture techniques are being applied for rice improvement in different countries (Chen et al., 2025). Somaclonal variation, also known as *in vitro* mutation, refers to variation observed in regenerated plants using plant tissue culture from somatic cells (Bhojwani and Dantu, 2013). In the year 2021, some rice somaclones (SC₁) were developed in the Plant Breeding and Biotechnology Laboratory of the Agrotechnology Discipline, Khulna University, using dehusked Ranisalute seeds, and they were planted in pots under a controlled environment. Visual observation revealed significant morphological differences among the somaclones.

The major objective of this investigation is to evaluate second-generation (SC₂) rice somaclones developed from the local aromatic rice cultivar Ranisalute in order to identify promising genotypes with improved yield and grain quality traits while maintaining desirable parental characteristics. The study specifically aims to assess the extent of somaclonal variation in agro-morphological traits among the somaclones, to identify superior genotypes based on yield-contributing and grain-related characters, to classify the somaclones into distinct groups based on morphological diversity using appropriate statistical approaches, and to analyze the association among growth, yield, and grain quality traits for determining effective indirect selection criteria. In addition, the study aimed to identify promising somaclones and genetically diverse groups suitable for future breeding programs.

Materials and Methods

Experimental site and duration

The research was carried out at Professor Dr. Purnendu Gain Field Laboratory, Agrotechnology Discipline, Khulna University, Khulna, from August 2022 to January 2023.

Geographical location and climate of the experimental site

The experimental site was located at 22°47' North latitude, 89°34' East longitude, and 9 m elevation. Khulna experiences a tropical monsoon climate with distinct rainy and dry seasons. Rainfall is most abundant between May and September. The amount of rainfall is, however, significantly less than in monsoon climate zones. The annual average rainfall of the Khulna area is 1,878.4 mm. The year-round climate is pleasant but during the wet season, temperatures may rise a few degrees. The annual average temperature of the Khulna area is 26.3°C. During the growing season of this crop, the average temperature was 25.92°C and the average rainfall was 137.94 mm.

Soil Condition

The soils in the study area were usually clay loams. The soil properties were pretested before growing the rice crop, which consists of 16% sand, 36% silt, 51% clay, and 2.4% organic matter. The pH of the soil was 7.6.

Plant Material

Ranisalute is a vulnerable local rice cultivar, popular in the southern coastal region of Khulna, Bangladesh. It is valued for its aroma and grain quality but has a relatively low yield potential of approximately 3 t ha⁻¹. Somaclones were developed from Ranisalute seeds in the Plant Breeding and Biotechnology Laboratory, Agrotechnology Discipline, Khulna University, Bangladesh. The seeds were manually dehusked and surface sterilized by immersing in 70% ethanol for 1 minute, then in 0.2% HgCl₂ for 15 minutes followed by 4-5 rinsing with autoclaved distilled water. The sterilized seeds were cultured onto the MS medium containing 2.0 mg L⁻¹ 2,4-D. The cultures were kept in dark for four weeks at 25±1°C. Then they were transferred to the light of 3000 lux under 16 hours photoperiod maintaining the same temperature regime for another two weeks. Callus induction was noticed within two weeks of inoculation of cultures. After six weeks of inoculating seeds, calli were transferred into the plant regeneration medium (MS + 3 mg L⁻¹ BAP) and were kept in a growth room maintaining a light intensity 3000 lux under 16 hours photoperiod at 25±1°C temperature. Within one-month greenish plantlets

were obtained from the plated calli. About 100 regenerated plants (called somaclones) were transferred to a small pot containing semi-decomposed rice husk supplemented with vermicompost after removing agar attached with roots and treated with Bavistin @ 0.1%. Well hardened somaclonal plants were acclimatized and grown to maturity under net-house conditions. Seeds were collected separately from the first generation, and twenty-eight rice somaclones (designated as RSC-2021-1...28) and parental cultivar Ranisalute (designated as RANI) were used as the study material.

Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with 3 replications. Each plot size was 4 m², having 9 plants plot⁻¹.

Crop establishment and management

The seedbed was prepared by plowing and cross-plowing four times, followed by laddering. Before sowing, the seeds were treated with Vitavax at a rate of 2-3 g kg⁻¹ for 24 hours to protect the seeds from seed-borne and soil-borne fungal pathogens, followed by a 24-hour soak in distilled water. The seeds were then removed from the water, covered with moist gauge cloth, and left at room temperature for 48 hours to germinate. The germinated seeds were manually broadcasted into the wet seedbed.

Twenty-five (25) days old seedlings were transplanted into the main field. There was a total of 87 plots for 29 genotypes (28 somaclones and 1 control). The plot size was 4 m², and in each plot, there were 9 plants. Row-to-row distance in each plot was 50 cm, and the plant-to-plant distance was also 50 cm within a row. Single plant hill⁻¹ was planted.

The following fertilizer doses were used: Urea: 150 kg ha⁻¹, TSP: 52 kg ha⁻¹, MoP: 82 kg ha⁻¹ and Gypsum: 60 kg ha⁻¹. Weeding was done by hand to ensure optimal plant development. The first weeding was done at 20 DAT, and the second weeding was done at 50 DAT. No pesticides were applied in the field. When the grains achieved 90% maturity and turned a golden color, three randomly selected plants from each plot were individually uprooted to collect data on growth and yield-contributing attributes.

Data collection

Data on the following growth and yield contributing parameters were taken into consideration for further analysis viz.

Plant height (cm) was measured from the base of the plant to the tip of the tallest panicle. Primary branch panicle⁻¹ was measured from five randomly selected panicles and the average was computed. Secondary branch panicle⁻¹ was noted from those primary branches, and the average was computed. Straw weight hill⁻¹ was measured by drying and weighing the straw using an electronic balance. Leaf area (cm²) was calculated by measuring the leaf width at the base, middle, and apex of the leaf, and the average was multiplied with leaf length. Panicle length (cm) was measured from five randomly selected panicles from each plant, and the average was computed. Tiller number hill⁻¹ was noted from each plant along with both effective tillers hill⁻¹ and non-effective tillers hill⁻¹. Grain panicle⁻¹ was recorded from the five panicles chosen from each plant the average was computed along with both filled grain panicle⁻¹ and unfilled grain panicle⁻¹. Grain length (mm) and grain breadth (mm) The grain's length were measured using a digital slide caliper in millimeters (mm). 1000 grain weight was recorded by counting thousand grains randomly from each sample and weighed with a 4-digit electric balance in grams (g). Grain weight hill⁻¹ was recorded using a 4-digit electric balance in grams (g). It was assured that the grains retained 14% moisture at that time. The harvest index is expressed as the percent and calculated by the ratio of the economic yield and biological yield.

$$\text{Harvest Index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Grain yield and straw yield were all together regarded as biological yield. It is calculated with the following formula: Biological yield (t ha⁻¹) = Grain yield (t ha⁻¹) + Straw yield (t ha⁻¹).

Statistical Analysis

Analysis of variance, Dunnett Test, Cluster analysis based on Ward's agglomerative method, and Pearson correlation analysis were performed using the computer software package 'R' (version 4.2.3).

Results

Variation in growth and yield parameters

There was significant variation among the rice genotypes for most of the traits, excluding secondary branches panicle⁻¹ and panicle length, in the analysis of variance. In general, the somaclones' agronomic parameters were statistically similar to those of RANI. No significant variation was observed in the Dunnett test among the somaclones for SEB, PL, NEFT, UFGPP, LA, and HI traits in comparison with RANI (Table 1, Table 2.1, and Table 2.2).

The plant height of most somaclones was similar to that of RANI. But some somaclones were significantly different viz. RSC-2021-2, RSC-2021-3, RSC-2021-5, RSC-2021-10, RSC-2021-12, RSC-2021-13, RSC-2021-19, RSC-2021-23, RSC-2021-25, RSC-2021-26, RSC-2021-27, and RSC-2021-28. Primary branches panicle⁻¹ were also similar to those of RANI, except for RSC-2021-15 and RSC-2021-27. SW of RSC-2021-4, RSC-2021-15, RSC-2021-17, RSC-2021-20, and RSC-2021-27 differed significantly from RANI (Table 1). Total tillers hill⁻¹ and effective tillers hill⁻¹ of RSC-2021-16 were significantly different from RANI. Grain panicle⁻¹ of RSC-2021-15 and RSC-2021-28 was significantly different from RANI. Filled grain panicle⁻¹ of RSC-2021-28 varied significantly

from RANI. Grain length of RSC-2021-1, RSC-2021-8, RSC-2021-13, RSC-2021-16, RSC-2021-20, RSC-2021-25, RSC-2021-26, and RSC-2021-28 was significantly different from RANI (Table 2.1). Grain breadth of RSC-2021-23, RSC-2021-24, and RSC-2021-27 varied significantly from RANI. 1000 grain weight of RSC-2021-1, RSC-2021-9, RSC-2021-16, and RSC-2021-27 were significantly different from RANI. Grain weight hill⁻¹ of RSC-2021-2, RSC-2021-3, RSC-2021-15, RSC-2021-16, RSC-2021-17, RSC-2021-20, RSC-2021-21, RSC-2021-22, RSC-2021-24, RSC-2021-25, RSC-2021-26, RSC-2021-27, and RSC-2021-28 varied significantly from RANI (Table 2.2).

Table 1. Comparison of growth parameters of somaclones and control.

Rice Genotype	Agronomic trait $\pm$ SD				
	PH	PRB	SEB	SW	LA
RANI (Control)	121.57 $\pm$ 1.65	7.33 $\pm$ 0.58	10.67 $\pm$ 1.15	75.64 $\pm$ 4.54	19.74 $\pm$ 2.21
RSC-2021-1	117.52 $\pm$ 4.80	7.00 $\pm$ 0.00	10.00 $\pm$ 0.00	72.42 $\pm$ 2.32	18.63 $\pm$ 0.68
RSC-2021-2	112.24 $\pm$ 3.14*	7.00 $\pm$ 0.00	10.00 $\pm$ 0.00	66.56 $\pm$ 5.07	18.20 $\pm$ 0.53
RSC-2021-3	110.41 $\pm$ 2.01**	6.67 $\pm$ 0.58	11.00 $\pm$ 1.00	72.71 $\pm$ 3.47	19.60 $\pm$ 1.09
RSC-2021-4	115.16 $\pm$ 3.35	7.00 $\pm$ 0.00	11.00 $\pm$ 0.00	62.34 $\pm$ 5.33**	19.80 $\pm$ 0.77
RSC-2021-5	111.65 $\pm$ 3.52*	6.67 $\pm$ 0.58	9.33 $\pm$ 2.08	66.90 $\pm$ 2.60	19.31 $\pm$ 0.66
RSC-2021-6	115.06 $\pm$ 3.20	6.33 $\pm$ 0.58	10.00 $\pm$ 1.73	75.72 $\pm$ 1.23	19.10 $\pm$ 0.33
RSC-2021-7	113.64 $\pm$ 4.41	6.67 $\pm$ 0.58	9.33 $\pm$ 2.08	71.31 $\pm$ 0.90	19.96 $\pm$ 0.31
RSC-2021-8	114.51 $\pm$ 3.34	7.00 $\pm$ 0.00	9.33 $\pm$ 0.58	73.08 $\pm$ 4.50	19.51 $\pm$ 1.28
RSC-2021-9	117.70 $\pm$ 3.75	6.67 $\pm$ 0.58	10.33 $\pm$ 1.15	74.97 $\pm$ 3.15	19.06 $\pm$ 0.34
RSC-2021-10	111.27 $\pm$ 3.53*	6.67 $\pm$ 0.58	10.00 $\pm$ 0.00	75.88 $\pm$ 2.32	18.46 $\pm$ 0.71
RSC-2021-11	116.13 $\pm$ 4.97	7.00 $\pm$ 0.00	10.00 $\pm$ 1.00	72.05 $\pm$ 2.23	19.78 $\pm$ 1.22
RSC-2021-12	108.09 $\pm$ 3.64***	6.67 $\pm$ 0.58	10.00 $\pm$ 2.00	68.06 $\pm$ 6.31	18.31 $\pm$ 2.43
RSC-2021-13	110.22 $\pm$ 4.35**	7.00 $\pm$ 0.00	10.33 $\pm$ 0.58	75.90 $\pm$ 1.47	18.95 $\pm$ 0.25
RSC-2021-14	117.37 $\pm$ 3.75	7.33 $\pm$ 0.58	10.67 $\pm$ 1.15	75.14 $\pm$ 2.56	17.38 $\pm$ 0.94
RSC-2021-15	119.50 $\pm$ 3.23	6.00 $\pm$ 0.00*	9.33 $\pm$ 0.58	50.91 $\pm$ 3.80***	20.22 $\pm$ 0.59
RSC-2021-16	117.62 $\pm$ 2.75	6.67 $\pm$ 0.58	9.00 $\pm$ 1.73	78.64 $\pm$ 0.76	19.44 $\pm$ 0.58
RSC-2021-17	121.48 $\pm$ 3.36	7.00 $\pm$ 0.00	8.67 $\pm$ 1.15	65.59 $\pm$ 6.03*	21.22 $\pm$ 0.67
RSC-2021-18	121.24 $\pm$ 2.39	7.00 $\pm$ 0.00	10.00 $\pm$ 0.00	78.52 $\pm$ 3.97	19.95 $\pm$ 0.76
RSC-2021-19	108.14 $\pm$ 3.80***	6.67 $\pm$ 0.58	9.00 $\pm$ 2.00	67.11 $\pm$ 3.56	19.89 $\pm$ 0.98
RSC-2021-20	115.45 $\pm$ 2.16	6.67 $\pm$ 0.58	8.33 $\pm$ 0.58	56.34 $\pm$ 7.75***	17.93 $\pm$ 1.04
RSC-2021-21	119.68 $\pm$ 4.87	7.00 $\pm$ 0.00	11.00 $\pm$ 1.73	70.54 $\pm$ 5.12	19.72 $\pm$ 2.10
RSC-2021-22	116.36 $\pm$ 4.03	7.33 $\pm$ 0.58	11.00 $\pm$ 1.00	69.12 $\pm$ 2.53	18.92 $\pm$ 0.48
RSC-2021-23	111.08 $\pm$ 2.23*	6.67 $\pm$ 0.58	10.00 $\pm$ 1.73	67.24 $\pm$ 4.37	20.53 $\pm$ 0.65
RSC-2021-24	114.68 $\pm$ 6.31	6.67 $\pm$ 0.58	10.00 $\pm$ 1.00	73.49 $\pm$ 5.07	19.67 $\pm$ 0.85
RSC-2021-25	111.04 $\pm$ 2.32*	7.00 $\pm$ 0.00	10.67 $\pm$ 0.58	73.52 $\pm$ 2.46	18.97 $\pm$ 0.83
RSC-2021-26	109.05 $\pm$ 1.39**	6.67 $\pm$ 0.58	9.67 $\pm$ 0.58	68.44 $\pm$ 2.82	20.21 $\pm$ 0.53
RSC-2021-27	109.87 $\pm$ 4.56**	6.00 $\pm$ 0.00**	9.00 $\pm$ 0.00	63.33 $\pm$ 1.49**	17.83 $\pm$ 0.98
RSC-2021-28	107.91 $\pm$ 1.44***	6.33 $\pm$ 0.58	9.00 $\pm$ 1.00	67.03 $\pm$ 3.96	18.37 $\pm$ 1.13

PH= plant height; PRB= primary branches panicle⁻¹; SEB= secondary branches panicle⁻¹; SW= straw weight hill⁻¹; LA= leaf area; * P < 0.05, ** P < 0.01, *** P < 0.001 (Dunnett's multiple comparison tests against 'RANI')

Table 2.1. Comparison of yield parameters of somaclones and control.

Rice genotype	Agronomic trait $\pm$ SD					
	TT	EFT	NEFT	PL	GPP	FGPP
RANI (control)	27.33 $\pm$ 2.52	22.00 $\pm$ 3.00	5.33 $\pm$ 0.58	23.85 $\pm$ 0.86	75.67 $\pm$ 1.53	69.67 $\pm$ 1.53
RSC-2021-1	27.00 $\pm$ 1.00	22.67 $\pm$ 0.58	4.67 $\pm$ 0.58	23.67 $\pm$ 0.79	76.33 $\pm$ 3.21	70.33 $\pm$ 3.51
RSC-2021-2	31.00 $\pm$ 2.65	26.00 $\pm$ 2.00	5.00 $\pm$ 1.00	23.06 $\pm$ 1.02	75.67 $\pm$ 3.21	70.67 $\pm$ 3.21
RSC-2021-3	21.00 $\pm$ 1.00	16.67 $\pm$ 1.53	4.33 $\pm$ 1.15	23.18 $\pm$ 0.63	71.33 $\pm$ 4.62	66.33 $\pm$ 4.62
RSC-2021-4	22.33 $\pm$ 2.31	18.33 $\pm$ 3.21	3.33 $\pm$ 0.58	24.07 $\pm$ 0.81	75.00 $\pm$ 2.00	69.33 $\pm$ 2.08
RSC-2021-5	24.00 $\pm$ 2.64	20.00 $\pm$ 2.00	4.00 $\pm$ 1.00	22.37 $\pm$ 1.23	66.67 $\pm$ 6.51	60.33 $\pm$ 6.50
RSC-2021-6	27.67 $\pm$ 2.08	24.33 $\pm$ 1.52	3.33 $\pm$ 0.58	23.09 $\pm$ 1.00	78.33 $\pm$ 2.08	73.33 $\pm$ 3.05
RSC-2021-7	31.33 $\pm$ 4.16	27.33 $\pm$ 3.21	4.00 $\pm$ 1.00	22.77 $\pm$ 0.91	75.67 $\pm$ 5.51	69.67 $\pm$ 5.68
RSC-2021-8	31.67 $\pm$ 2.52	27.00 $\pm$ 2.00	4.67 $\pm$ 2.08	22.14 $\pm$ 1.34	73.00 $\pm$ 3.61	66.33 $\pm$ 5.13
RSC-2021-9	34.67 $\pm$ 2.89	27.33 $\pm$ 1.53	7.33 $\pm$ 3.05	23.37 $\pm$ 1.81	73.67 $\pm$ 4.51	67.33 $\pm$ 4.04
RSC-2021-10	31.33 $\pm$ 4.04	24.33 $\pm$ 3.51	7.67 $\pm$ 4.04	22.52 $\pm$ 0.46	70.67 $\pm$ 6.35	65.00 $\pm$ 7.00
RSC-2021-11	29.33 $\pm$ 3.21	25.33 $\pm$ 2.31	4.67 $\pm$ 0.58	23.14 $\pm$ 0.38	72.00 $\pm$ 2.00	65.67 $\pm$ 1.53
RSC-2021-12	26.00 $\pm$ 2.65	21.00 $\pm$ 1.73	5.00 $\pm$ 1.00	22.90 $\pm$ 2.2	75.67 $\pm$ 5.51	70.33 $\pm$ 4.93
RSC-2021-13	24.67 $\pm$ 2.52	21.00 $\pm$ 2.00	4.00 $\pm$ 0.00	24.00 $\pm$ 1.52	78.33 $\pm$ 5.03	72.00 $\pm$ 4.58
RSC-2021-14	28.33 $\pm$ 4.16	25.33 $\pm$ 2.52	3.00 $\pm$ 1.73	24.3 $\pm$ 0.40	80.00 $\pm$ 2.64	75.00 $\pm$ 3.60
RSC-2021-15	24.67 $\pm$ 2.52	21.67 $\pm$ 2.31	3.00 $\pm$ 1.00	22.09 $\pm$ 0.22	64.33 $\pm$ 1.53*	58.00 $\pm$ 3.00
RSC-2021-16	36.33 $\pm$ 3.51*	29.33 $\pm$ 5.13*	7.00 $\pm$ 3.46	22.93 $\pm$ 1.29	70.67 $\pm$ 4.93	63.33 $\pm$ 7.23
RSC-2021-17	27.00 $\pm$ 1.00	23.33 $\pm$ 2.08	3.67 $\pm$ 1.52	23.83 $\pm$ 1.37	83.67 $\pm$ 3.51	79.33 $\pm$ 4.04
RSC-2021-18	27.33 $\pm$ 2.51	24.67 $\pm$ 1.15	2.67 $\pm$ 1.52	23.12 $\pm$ 0.09	73.67 $\pm$ 2.51	69.00 $\pm$ 3.00
RSC-2021-19	30.00 $\pm$ 2.00	26.00 $\pm$ 2.00	4.00 $\pm$ 0.00	22.36 $\pm$ 1.33	75.67 $\pm$ 4.04	70.67 $\pm$ 4.51
RSC-2021-20	21.33 $\pm$ 3.21	18.33 $\pm$ 2.52	3.00 $\pm$ 1.00	23.33 $\pm$ 1.13	79.67 $\pm$ 1.53	74.67 $\pm$ 1.53
RSC-2021-21	21.00 $\pm$ 1.00	18.00 $\pm$ 1.00	3.00 $\pm$ 1.00	23.13 $\pm$ 1.24	78.67 $\pm$ 3.05	74.33 $\pm$ 3.21
RSC-2021-22	27.67 $\pm$ 2.51	21.33 $\pm$ 0.58	6.33 $\pm$ 2.08	22.91 $\pm$ 1.51	71.67 $\pm$ 6.66	65.33 $\pm$ 8.08
RSC-2021-23	27.33 $\pm$ 4.93	19.67 $\pm$ 3.05	7.67 $\pm$ 2.08	22.16 $\pm$ 1.86	70.33 $\pm$ 5.20	64.00 $\pm$ 7.00
RSC-2021-24	32.33 $\pm$ 3.05	26.33 $\pm$ 2.52	6.33 $\pm$ 1.53	23.22 $\pm$ 1.44	70.67 $\pm$ 5.50	63.00 $\pm$ 7.55
RSC-2021-25	34.67 $\pm$ 5.03	28.67 $\pm$ 3.51	6.33 $\pm$ 2.31	23.39 $\pm$ 0.44	68.67 $\pm$ 2.08	62.67 $\pm$ 2.08
RSC-2021-26	32.67 $\pm$ 5.86	26.00 $\pm$ 5.57	6.33 $\pm$ 1.52	23.33 $\pm$ 0.19	72.67 $\pm$ 1.53	65.67 $\pm$ 0.58
RSC-2021-27	25.33 $\pm$ 5.13	21.00 $\pm$ 5.29	4.33 $\pm$ 0.58	22.02 $\pm$ 0.54	67.67 $\pm$ 2.52	60.67 $\pm$ 4.51
RSC-2021-28	28.00 $\pm$ 3.00	23.33 $\pm$ 2.08	4.67 $\pm$ 1.15	22.09 $\pm$ 0.60	62.33 $\pm$ 8.96*	55.00 $\pm$ 8.66**

TT= total tillers hill⁻¹; EFT= effective tillers hill⁻¹; NEFT = non-effective tillers hill⁻¹; PL= panicle length; GPP= grain panicle⁻¹; FGPP = filled grain panicle⁻¹; * P < 0.05, ** P < 0.01, *** P < 0.001 (Dunnett's multiple comparison tests against 'RANI')

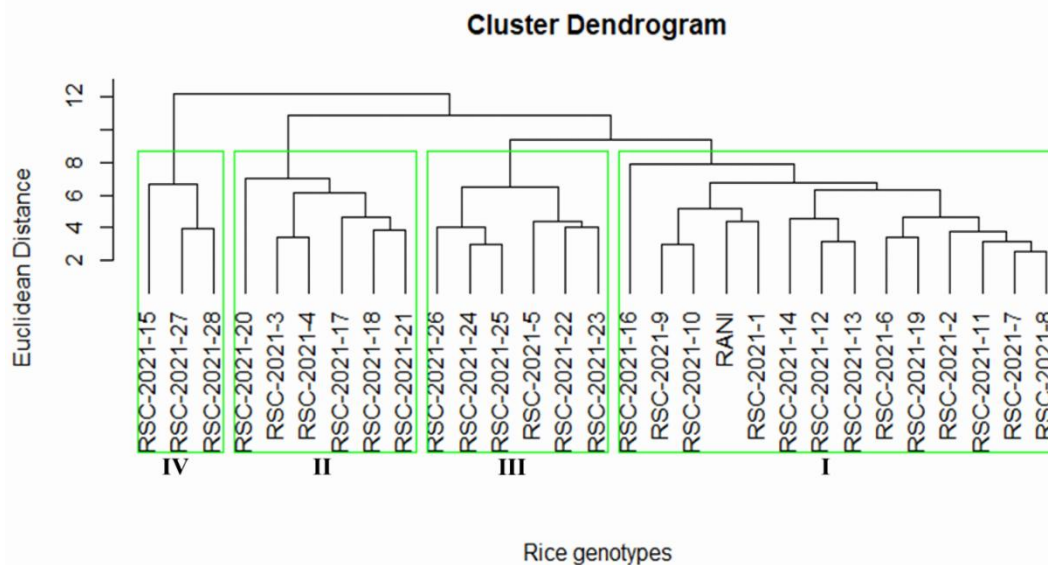
Cluster analysis

The most often used approach for classifying genotypes into homogenous groups is cluster analysis. In the current study, cluster analysis using Ward's agglomerative clustering method was performed among 29 genotypes (control and 28 somaclones) of Ranisalute rice cultivar and classified into 4 clusters (Figure 1). Cluster I had the highest number of genotypes (14) followed by Cluster II and III, which had the second largest number of genotypes (6). Cluster IV had the lowest number of genotypes (3).

Table 2.2. Comparison of yield parameters of somaclones and control.

Rice Genotype	Agronomic trait $\pm$ SD					
	UFGPP	GL	GB	TGW	GWPH	HI
RANI	5.67 $\pm$ 0.58	8.39 $\pm$ 0.01	2.38 $\pm$ 0.03	32.56 $\pm$ 1.16	45.35 $\pm$ 3.24	37.48 $\pm$ 1.26
RSC-2021-1	6.00 $\pm$ 1.00	8.26 $\pm$ 0.01*	2.28 $\pm$ 0.07	27.92 $\pm$ 0.82***	40.52 $\pm$ 1.95	35.89 $\pm$ 1.84
RSC-2021-2	5.00 $\pm$ 0.00	8.34 $\pm$ 0.01	2.38 $\pm$ 0.03	31.61 $\pm$ 0.35	40.83 $\pm$ 2.90*	38.04 $\pm$ 2.15
RSC-2021-3	4.67 $\pm$ 0.58	8.47 $\pm$ 0.02	2.32 $\pm$ 0.06	31.89 $\pm$ 1.13	39.41 $\pm$ 1.78***	35.16 $\pm$ 1.50
RSC-2021-4	6.00 $\pm$ 1.00	8.38 $\pm$ 0.01	2.32 $\pm$ 0.04	29.76 $\pm$ 0.79	35.23 $\pm$ 1.55*	36.15 $\pm$ 1.08
RSC-2021-5	6.33 $\pm$ 0.58	8.29 $\pm$ 0.01	2.46 $\pm$ 0.01	30.90 $\pm$ 1.01	39.14 $\pm$ 0.38	36.92 $\pm$ 0.67
RSC-2021-6	5.33 $\pm$ 0.58	8.36 $\pm$ 0.03	2.38 $\pm$ 0.01	31.91 $\pm$ 0.54	45.34 $\pm$ 0.87	37.45 $\pm$ 0.51
RSC-2021-7	6.00 $\pm$ 1.73	8.38 $\pm$ 0.04	2.46 $\pm$ 0.02	31.88 $\pm$ 0.32	42.31 $\pm$ 0.91	37.24 $\pm$ 0.77
RSC-2021-8	6.33 $\pm$ 1.15	8.53 $\pm$ 0.03*	2.43 $\pm$ 0.01	32.40 $\pm$ 0.63	46.78 $\pm$ 0.97	39.06 $\pm$ 1.19
RSC-2021-9	6.33 $\pm$ 0.58	8.40 $\pm$ 0.12	2.36 $\pm$ 0.06	29.40 $\pm$ 0.57*	48.25 $\pm$ 0.78	39.18 $\pm$ 1.37
RSC-2021-10	6.00 $\pm$ 1.00	8.31 $\pm$ 0.01	2.33 $\pm$ 0.08	30.62 $\pm$ 0.65	47.14 $\pm$ 1.90	38.32 $\pm$ 0.21
RSC-2021-11	6.33 $\pm$ 1.53	8.45 $\pm$ 0.03	2.32 $\pm$ 0.05	33.29 $\pm$ 0.73	46.16 $\pm$ 3.49	39.03 $\pm$ 1.63
RSC-2021-12	5.33 $\pm$ 1.15	8.44 $\pm$ 0.04	2.29 $\pm$ 0.03	31.38 $\pm$ 1.38	46.10 $\pm$ 2.553	40.43 $\pm$ 1.02
RSC-2021-13	6.33 $\pm$ 1.15	8.52 $\pm$ 0.04*	2.35 $\pm$ 0.03	32.13 $\pm$ 1.13	47.70 $\pm$ 1.51	38.59 $\pm$ 0.70
RSC-2021-14	5.00 $\pm$ 1.00	8.50 $\pm$ 0.02	2.40 $\pm$ 0.03	32.85 $\pm$ 0.23	46.96 $\pm$ 3.96	38.44 $\pm$ 2.81
RSC-2021-15	6.33 $\pm$ 1.52	8.35 $\pm$ 0.04	2.45 $\pm$ 0.05	30.14 $\pm$ 1.73	34.32 $\pm$ 2.02***	40.28 $\pm$ 0.37
RSC-2021-16	7.33 $\pm$ 2.31	8.68 $\pm$ 0.02***	2.30 $\pm$ 0.06	36.57 $\pm$ 0.98**	53.23 $\pm$ 0.82***	40.37 $\pm$ 0.60
RSC-2021-17	4.33 $\pm$ 0.58	8.38 $\pm$ 0.01	2.37 $\pm$ 0.03	31.30 $\pm$ 1.53	37.85 $\pm$ 1.20**	36.67 $\pm$ 2.20
RSC-2021-18	4.33 $\pm$ 0.58	8.38 $\pm$ 0.01	2.38 $\pm$ 0.01	31.04 $\pm$ 0.60	43.77 $\pm$ 0.38	35.82 $\pm$ 1.18
RSC-2021-19	5.00 $\pm$ 1.00	8.43 $\pm$ 0.03	2.34 $\pm$ 0.04	32.54 $\pm$ 0.91	42.01 $\pm$ 0.69	38.52 $\pm$ 1.59
RSC-2021-20	5.00 $\pm$ 0.00	8.58 $\pm$ 0.01***	2.36 $\pm$ 0.03	33.96 $\pm$ 1.32	33.70 $\pm$ 0.44***	37.60 $\pm$ 1.63
RSC-2021-21	4.33 $\pm$ 0.58	8.47 $\pm$ 0.11	2.44 $\pm$ 0.02	32.36 $\pm$ 0.85	39.53 $\pm$ 1.86*	35.97 $\pm$ 1.54
RSC-2021-22	7.00 $\pm$ 1.00	8.51 $\pm$ 0.01	2.45 $\pm$ 0.03	30.50 $\pm$ 0.62	39.93 $\pm$ 3.97*	36.57 $\pm$ 1.35
RSC-2021-23	6.67 $\pm$ 1.15	8.46 $\pm$ 0.02	2.53 $\pm$ 0.02***	32.28 $\pm$ 0.37	44.55 $\pm$ 1.21	39.88 $\pm$ 0.83
RSC-2021-24	7.67 $\pm$ 2.08	8.38 $\pm$ 0.12	2.51 $\pm$ 0.01**	32.02 $\pm$ 1.55	37.18 $\pm$ 3.04***	33.60 $\pm$ 1.29
RSC-2021-25	5.67 $\pm$ 0.58	8.57 $\pm$ 0.03***	2.42 $\pm$ 0.02	31.73 $\pm$ 1.71	37.81 $\pm$ 1.05**	33.97 $\pm$ 0.47
RSC-2021-26	7.67 $\pm$ 1.53	8.27 $\pm$ 0.04*	2.40 $\pm$ 0.04	30.89 $\pm$ 1.76	37.74 $\pm$ 2.58**	35.53 $\pm$ 0.82
RSC-2021-27	7.00 $\pm$ 2.00	8.32 $\pm$ 0.04	2.22 $\pm$ 0.02***	26.31 $\pm$ 3.40***	34.66 $\pm$ 2.19***	35.36 $\pm$ 1.07
RSC-2021-28	7.00 $\pm$ 0.00	8.23 $\pm$ 0.02**	2.33 $\pm$ 0.03	31.21 $\pm$ 1.79	37.99 $\pm$ 3.44**	36.16 $\pm$ 1.32

UFGPP= unfilled grain panicle⁻¹; GL= grain length; GB= grain breadth; TGW= 1000 grain weight; GWPH= grain weight hill⁻¹; HI= harvest index; * P < 0.05, ** P < 0.01, *** P < 0.001 (Dunnett's multiple comparison tests against 'RANI')

**Fig. 1:** Cluster dendrogram representing the relationship among the rice genotypes

The mean values, and the cluster means are shown in Table 3. Cluster II contained eight parameters with the highest mean values, viz. plant height, panicle length, primary branches panicle⁻¹, secondary branches panicle⁻¹, grain panicle⁻¹, filled grain panicle⁻¹, grain length, and leaf area, indicating good performance in most of the growth and yield-related traits. Cluster III had the

highest mean value of six traits, viz. total tillers hill⁻¹, non-effective tillers hill⁻¹, primary branches panicle⁻¹, secondary branches panicle⁻¹, unfilled grain panicle⁻¹, and grain breadth, indicating good performance in most of the growth traits. Cluster I had the highest mean

values for five traits, viz., effective tillers hill⁻¹, 1000 grain weight, grain weight hill⁻¹, straw weight hill⁻¹, and harvest index, indicating good performance across most yield traits. Cluster IV had the highest mean value of unfilled grain panicle⁻¹, indicating poor performance.

Table 3. Cluster means for agronomic traits of rice genotypes.

Parameters	Cluster			
	I	II	III	IV
Plant height (cm)	114.36	117.24	<i>112.31</i>	112.4
Total tillers hill ⁻¹	29.75	23.28	29.80	26.10
Effective tillers hill ⁻¹	24.71	<i>20.00</i>	23.66	22.00
Noneffective tillers hill ⁻¹	4.86	<i>3.50</i>	6.00	4.00
Panicle length (cm)	23.15	23.44	22.90	22.07
Primary branches panicle ⁻¹	6.93	7.00	7.00	<i>6.00</i>
Secondary branches panicle ⁻¹	9.78	10.00	10.00	<i>9.00</i>
Grain panicle ⁻¹	75.21	77.17	70.17	<i>64.67</i>
Filled grain panicle ⁻¹	69.28	72.00	63.33	<i>58.00</i>
Unfilled grain panicle ⁻¹	5.78	<i>4.67</i>	6.67	6.67
Grain length (mm)	8.43	8.44	8.41	<i>8.30</i>
Grain breadth (mm)	2.36	2.36	2.46	<i>2.33</i>
1000 grain weight (g)	31.93	31.72	31.39	<i>29.22</i>
Grain weight hill ⁻¹ (g)	45.62	38.24	39.39	<i>35.66</i>
Straw weight hill ⁻¹ (g)	73.03	67.67	69.78	<i>60.42</i>
Leaf area (cm ²)	19.03	19.70	19.60	<i>18.80</i>
Harvest Index (%)	38.43	36.23	<i>36.08</i>	<i>37.27</i>

Bold= highest value, Italic= lowest value

The highest inter-cluster distance was observed between Cluster II and Cluster IV (7.63), indicating the greatest diversity among the genotypes in those

clusters. The lowest inter-cluster distance was between Cluster II and Cluster III (6.21), indicating the lowest diversity among the genotypes of these clusters (Table 4).

Table 4. Inter-cluster distances of rice genotypes

Cluster	I	II	III	IV
I	-	5.76	5.37	7.19
II	5.76	-	<i>6.21</i>	7.63
III	5.37	<i>6.21</i>	-	6.42
IV	7.19	7.63	6.42	-

Bold= highest value, Italic= lowest value

Correlation analysis of the parameters

Correlation quantifies how closely two variables differ from one another on a scale of relationship strength. The key to selection is to correlate knowledge of the direction and magnitude of the relationship between yield and its components. Most of the traits denoted a positive relationship among them. Grain weight hill⁻¹ denoted a highly significant and positive relation with total tillers hill⁻¹ (0.42), effective tillers hill⁻¹ (0.45), straw weight hill⁻¹ (0.65), grain length (0.32), 1000 grain weight (0.37), and harvest index (0.57) and a negative relation with grain breadth (-0.08). The highest positive correlation (0.98) was recorded between grain panicle⁻¹ and filled grain panicle⁻¹. On the contrary, the highest negative correlation (-0.55) was recorded between filled grain panicle⁻¹ and unfilled grain panicle⁻¹.

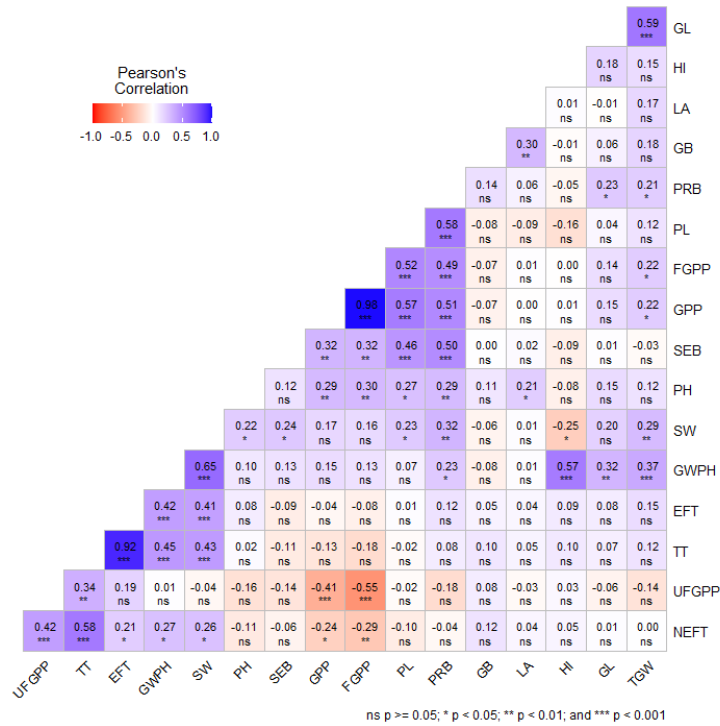


Fig. 2: Correlation between different growth and yield parameters

Discussion

Variation in growth and yield parameters

The large extent of variation present among somaclones for most of the traits validates the effectiveness of somaclonal variation in generating phenotypic diversity in Ranisalute rice (Larkin and Scowcroft, 1981; Jain, 2001; Henry, 1998). This finding is consistent with earlier studies that tissue culture derived populations can provide a resourceful source of variation for crop improvement (Hussain et al., 2012; Song et al., 2016; Joshi and Rao, 2009). Nonetheless, the lack of much variation for secondary branch number indicates this is a relatively stable character and not as readily disrupted by somaclonal factors. This is in agreement with Liu JinBo et al. (2013) reported the phenomenon of overdominance, a form of genetic control that acts as a stabilizing force against mutational change, to account for dominance in secondary branch number in rice. In contrast to the above parameters, primary branch number exhibited significant variability among somaclones and is consistent with an additive genetic effects trait that responds more acutely to locus level changes by tissue culture.

The wide variation in plant height among the somaclones have been reported for various tissue culture derived rice populations (Zong-Xiu et al., 1983; Araújo and Prabhu, 2002; Ngezahayo et al., 2007).

Reduced plant height of somaclone populations by as much as 11.5–39.5 percent in rice somaclones is a well-documented phenomenon attributed to the mutagenic effect of synthetic phytohormones and osmotic agents on loci controlling height (Zong-Xiu et al., 1983). From an agronomic point of view, shorter plant height has no disadvantage. It is linked with increased lodging resistance, better nitrogen uses efficiency and greater harvest index under intensive management conditions (Khush, 1997).

The straws weight hill^{-1} showed high variation among the somaclones have been reported for various tissue culture derived rice populations (Al et al., 2022; Matías et al., 2019). This is agronomically relevant as grain yield is positively correlated with straw yield, and straw biomass serves as an indicator of the overall photosynthetic productivity and source strength of the crop canopy (Matías et al., 2019). The significant differences in leaf area of the somaclones are consistent with the findings obtained by El Banna et al. (2008) who reported similar variation among gamma radiation induced rice mutants. A high leaf area can be directly related to a high light interception and photosynthetic activity leading to an increase in biomass accumulation and ultimately grain yield (El Banna et al., 2008; Chen et al., 2020).

The non-significant difference in the panicle length among somaclones was earlier reported by Chowdhury et al. (2023) also observed non-significant range of panicle length in rice mutant populations. This finding indicates that panicle length probably has a high genetic determinacy compared to the level of somaclonal disturbance (Chowdhury et al., 2023; Jain, 2001). As expected, the large variation in maximum and effective tiller number follows a general phenotype alteration of rice growth characteristics often seen following tissue culture events during an *in vitro* regeneration (Zong-Xiu et al., 1983; Chatterjee & Gupta, 1997; Oladosu et al., 2014; Joshi and Rao, 2009; Adkins et al., 1990). The variability of tillers is also influenced due to the polygenic nature of its inheritance as various polygenes interact with each other to yield genotype specific ontogenetic responses towards lateral shoots (Ashrafuzzaman et al., 2009; Zhao et al., 2020). In particular, the number of effective tillers hill⁻¹ is important because it directly determines the number of yields bearing panicles per plant unit (Ren et al., 2021). The increase in tiller numbers, a common observation of somaclonal variation, has also been observed by Zong-Xiu et al. (1983) and numerous others have since shown a similar increase in tissue culture derived rice populations (Adkins et al., 1990; Joshi and Rao, 2009; Chatterjee & Gupta, 1997).

Grain filling is a key process affecting yield and quality of rice grains (Chen et al., 2020; Kato and Katsura, 2010). Considerable variability of grain number panicle⁻¹ and filled grain number panicle⁻¹ indicates that somaclonal variation in these traits (Wang et al., 1995; Joshi and Rao, 2009; Gunasekaran et al., 2023). The filled grain percentage is also sensitive to salinity stress at reproductive stage (Khatun et al., 1995) and the water management practices during grain filling (Kato and Katsura, 2010; Gunasekaran et al., 2023). These results emphasize that somaclones should be evaluated under field conditions to assess the impact of certain environmental factors during grain filling performance.

Grain morphology is a quantitative trait that encompasses grain length, breadth and thickness (Xing and Zhang, 2010; Li et al., 2011), where each of three individual components is associated closely with overall grain size, a major target through natural selection and artificial breeding in rice. Despite a polygenic basis for grain length, the high degree of variation between somaclones is consistent with documented changes in grain shape caused by tissue culture (Kang-Le et al., 1989; Chatterjee & Gupta, 1997; Elsherbiny et al., 2024). This finding is especially relevant to enhancing aromatic landraces like Ranisalute, where elongated grain phenotype determines market price and consumer preference. This also confirms previous

results obtained on rice mutant lines showing large residual variation in grain breadth (Elsherbiny et al., 2024). The extent of 1000-grain weight variation is consistent with the extreme variability observed for this trait among rice somaclones and mutants (Chauhan et al., 1993; Bagheri et al., 2022; Elsherbiny et al., 2024), which is explained by diversities in gene networks that regulate grain sink strength but vary among genotypes based on genetic background (Li et al., 2018).

The large variation in grain weight hill⁻¹ corroborates documented mutations accelerating yield per plant unit in numerous rice mutant and somaclone studies (Chowdhury et al., 2023; Chatterjee & Gupta, 1997; Oladosu et al., 2014). Although the harvest index variation was significant only at five percent level, these results concur with data on harvest index of some genetically diverse rice genotypes reported by Jayasudha and Sharma (2010). This character is controlled by both genetic and environmental factors including their interaction, in addition to soil mineral availability (Amanullah and Inamullah, 2016). Together, these findings indicate the potential for identifying high yielding somaclones within this Ranisalute somaclonal population.

Cluster Analysis

Ward's agglomerative hierarchical cluster analysis clearly segregated the 29 genotypes into four major clusters which implies that somaclonal variation has expanded Ranisalute genetic base beyond its parental constitution (Larkin and Scowcroft, 1981; Jain, 2001). Genetic diversity analysis using quantitative traits to divide the genotypes into clusters is a conventional method that has been extensively utilized for population structure characterization and guiding parents selection in plant breeding programs (Franco et al., 2001; Ghaderi et al., 1980; Faysal et al., 2022). The clustering together of the parent cultivar RANI with other somaclones in the same cluster indicates that these specific somaclones have preserved a genetic background which is relatively close to the parent. The other three clusters also indicate a gradual genetic divergence and were all generated through tissue culture regeneration. This specific four-cluster resolution pattern is also in line with the multi-cluster partitioning reported in other rice co-macronutrient mutants and somaclone populations (Joshi and Rao, 2009; Oladosu et al., 2014).

Cluster mean analysis indicated that Cluster II was good for most of the grain yield and quality parameters, in which it produced highest means for grain panicle⁻¹, filled grain panicle⁻¹, grain length, panicle length, leaf area and average performance in 1000-grain weight,

grain weight hill⁻¹ and harvest index. This confirms that the combination of favorable alleles at multiple loci co-occurs in certain genotypic clusters which outperform other clusters consistently for yield related traits, a general observation reported across rice genetic diversity studies (Franco et al., 2001; Faysal et al., 2022; Gyawali et al., 2018). The interaction approach can also group genotypes according to their joint responses to environments (Lin, 1982), as this makes the interpretation of multi-trait performance data across very different germplasm easier.

Cluster II and Cluster IV had the highest inter-cluster distance being the two most distantly related groups in the same population. Extensive inter-cluster genetic distance is one of the best predictors for heterosis (Lin, 1982; Ghaderi et al., 1980) and transgressive segregation in progeny populations (Franco et al., 2001; Faysal et al., 2022). Based on the same results, it can then be suggested to perform in the coming breeding cycle crosses between genotypes from Cluster II and Cluster IV for greater chances of obtaining superior progenies.

Correlation Analysis

Correlation analysis is an important precursor for indirect selection strategies in crop improvement programs, as association between pairs of trait indicates nature, direction and magnitude of that association (Ahmadikhah et al., 2008; Chen and Popovich, 2002; Digby, 1983). Grain yield is a complex quantitatively inherited trait, which is affected simultaneously by genes and environmental factors, so directly improve grain yields using single trait selection is inherently difficult (Ahmadikhah et al., 2008). Breeders can indirectly select traits that are positively and consistently correlated with yield in large somaclonal populations (Gyawali et al., 2018; Oladosu et al., 2014). The strong significant positive relationships of total and effective tillers hill⁻¹ with straw weight and grain weight hill⁻¹ indicate that selection for more tillering capacity will indirectly improve grain yield in this somaclonal population (Ren et al., 2021; Ashrafuzzaman et al., 2009; Zhao et al., 2020).

The strong positive correlation of panicle length with primary branch number, secondary branch number, grain number panicle⁻¹ and filled grain number panicle⁻¹ shows that structural connection exists among panicle length and primary/secondary branches development as well as the spikelet bearing capacity. Thus, selection for longer panicles indirectly favors higher grain density per unit. A near perfect relationship between grain number panicle⁻¹ and filled grain number panicle⁻¹

confirms that an excess of grain number panicle⁻¹ translates to large filled grain number which in turn leads to more grain weight hill⁻¹ (Kato and Katsura, 2010; Oladosu et al., 2014; Gunasekaran et al., 2023). This finding demonstrates that selection based on higher grain number panicle⁻¹ is sufficient and filled grain percentage can be neglected and does not need to be treated independently in this population.

These highly significant positive relationships of grain length and 1000-grain weight as well as by further downstream association with grain-weight hill⁻¹ and harvest index, confirm the usefulness for yield improvement of compound indirect selection criteria based on morpho-physiological traits associated with grains which would simultaneously improve grain quality (Li et al., 2011; Xing and Zhang, 2010; Gyawali et al., 2018; Oladosu et al., 2014). This outcome is significant for further enhancement of the Ranisalute line, which is notably characterized by its elongated grain length. The strong positive correlation of straw weight hill⁻¹ and grain weight hill⁻¹ likely confirms Matías et al. (2019) and provides further evidence for the strong relationship between vegetative biomass production and assimilate available grains during reproductive growth (Al et al., 2022). The high positive correlation between grains per panicle and filled grains per panicle indicates a strong dependency between these traits, while the negative correlation between filled and unfilled grains suggests a trade-off between grain filling and sterility. These findings emphasize the importance of improving grain filling efficiency to enhance yield. Overall, traits such as effective tillers, total tillers, straw weight, and harvest index can be considered reliable selection criteria for yield improvement, supporting their use in breeding programs.

A limitation of the present study is that only the phenotypic variations among the SC₂ somaclones were evaluated, while their genetic stability and degree of homozygosity were not assessed. These aspects will be further investigated through the evaluation of promising somaclones in subsequent generations, together with appropriate heritability and molecular analyses. In addition, important quality-related traits, including aroma, fineness and physicochemical properties were not evaluated in the present study which will be addressed in a separate study.

Conclusion

Somaclonal variation was found to be an effective method to broaden genetic base of this aromatic rice landrace since most of the agro-morphological characters evaluated exhibited high level of genotypic variance in second generation Ranisalute somaclones.

Most somaclones stayed statistically similar to the parent cultivar, showing that there was no overall loss of the parental phenotype. Among the evaluated genotypes, RSC-2021-16 showed superior performance with the highest number of total tillers hill⁻¹, effective tillers hill⁻¹, grain length, 1000-grain weight, and grain weight hill⁻¹. RSC-2021-17 produced the highest numbers of grains panicle⁻¹ and filled grains panicle⁻¹. Correlation analysis identified effective tillers hill⁻¹, grain length, and 1000-grain weight as important traits for indirect selection of higher yield due to their strong positive association with grain weight hill⁻¹ and harvest index. Cluster analysis revealed considerable genetic divergence among the somaclones, with Cluster II and Cluster IV showing the greatest inter-cluster distance. Therefore, promising somaclones from these clusters, along with the superior genotype RSC-2021-16 from Cluster I, are recommended for evaluation in the third generation. Further studies on their genetic stability and quality-related traits like aroma and cooking quality are also suggested.

Author's contribution

RNR and MMI designed the experiments. MTA conducted the experiments. RNR, and MTA analyzed the data. RNR, MTA and MMI contributed to the writing of the manuscript.

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