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Research Article

Performance Evaluation of Newly Developed Tea (*Camellia sinensis* L.) Cultivars in Response to Different Levels of Nitrogen Fertilizer

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Abstract

Background and Objective: The necessity of assessing responses of tea cultivars to variable nitrogen (N) application has been emphasized to ensure the selection of suitable cultivars with improved N utilization. This study was conducted in the central highlands of Sri Lanka to determine the effects of N rates (0, 120, 240 and 360 kg N/ha/year) on yield, yield components, leaf N content and Agronomic Nitrogen Use Efficiency (AgNUE) of newly developed tea cultivars (VP 80/05, VP 80/89, VP 80/208 and VP 80/272).

Materials and Methods: This experiment was carried out in a split-plot design, where cultivar as the main factor and N rates as the subfactor with three replications. Cultivar variation was significant for all the traits studied and interestingly, VP 80/272 was superior in all traits, followed by VP 80/89. However, shoot dry weight showed no significant increment in varying N applications.

Results: There was no significant interaction between cultivars and N rates on the traits studied. The highest performance in AgNUE was recorded in cultivar VP 80/89. Cultivar studied could be categorized into two main clusters, one with VP 80/272 and VP 80/89, which showed better efficiency in N utilization. Importantly, cultivar variation in N response was detected. **Conclusion:** Therefore, exploiting this type of screening technique in the tea breeding program will enable plant breeders to select cultivars that show a consistent yield and efficient N fertilizer usage.

Key words: Tea, cultivar difference, nitrogen rate, nitrogen use efficiency, tea breeding, yield, yield components

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Successful selection of high-yielding tea cultivars or genotypes through effective use of germplasm and fixing desirable character combinations is a primary objective of a tea breeding programme¹. Therefore, newly developed tea cultivars are initially evaluated for yield attributes and the high-yielding tea cultivars are then screened for other important characteristics such as made tea quality, pests and diseases resistance and drought tolerance. Finally, plant progenies with favourable promising results are selected and released as new cultivars².

Yield, however, is influenced by factors like soil type, altitude, weather fluctuations, agronomic inputs, cultivar, age, stage of pruning and management practices^{3,4}. The quantity of harvest depends on tea yield components described as the number of shoots per unit area (shoot density), shoot replacement rate and average shoot weight. It has been reported that the shoot replacement rate highly depends on seasonal/weather fluctuations and, as a result, causes yield variations. Shoot density and shoot weight have been reported as clonal characters which determine clonal yield differences⁵. Therefore, it is apparent that the yield components that significantly contribute to the potential productivity of tea are affected by various factors such as genetic (inherent) characteristics, management and environmental conditions⁶. Generally, tea yield represents a complex quantitative character and depends upon several yield-related traits. Thus, a better understanding of the interrelationship between yield and yield-related traits and their importance while selecting high-yielding tea cultivars in tea breeding programmes is critical⁷.

Since tea is a perennial leafy crop grown as a monoculture, the application of fertilizer plays a vital role in its economic production⁸ and it requires substantially higher inorganic nitrogen (N) compared to most other crops. It is also widely reported that N fertilization affects the growth, yield and quality components of tea and its influence has generally been more pronounced than that of the other fertilizers⁹. The recommendation for N fertilizers varies from country to country. In Sri Lanka, it ranges from 90-400 kg N/ha/year, the actual rate dependent on potential yield¹⁰. However, it is challenging to optimize N fertilizer applications because of the dynamic nature of available nitrogen in soils. Therefore, N applications should be carefully managed, especially in tea, to maximize marketable yield while minimizing environmental effects¹¹. However, average nitrogen use efficiency (NUE) in tea fields has been estimated as very low and less than half of the applied nitrogen is recovered in the green leaf. It has been

reported NUE is around 30% in the tea field of China⁹. As a result, the negative environmental effects of excess N are well documented¹².

NUE is defined as the cultivar's capacity to receive N from the soil and/or incorporate or utilize it in production¹³. It comprises three key components: The Nitrogen Uptake Efficiency (NUpE), the Nitrogen Utilization Efficiency (NUE) in producing biomass and the Nitrogen Harvest Index (NHI). Both NUpE and NHI can be merged into one component: The utilization efficiency for harvestable products¹¹. These components are controlled by genetic and physiological factors and environmental factors¹³. Further, recent studies have reported that the NUE of tea varies depending on cultivars and locations^{6,14,15}. Hence, tea breeders can optimize the NUE of tea fields by producing tea cultivars with a high Agronomic Nitrogen Use Efficiency (AgNUE), i.e., tea cultivars that produce a large quantity of harvestable crop per unit N supplied through fertilizer¹¹. This explains how screening and selecting tea cultivars for consistent N response and high NUE may minimize tea production's economic and environmental costs while also increasing yield and other desired features¹⁶.

The main goal of this project was to examine the effects of variable N application on yield, yield components, leaf nitrogen content and AgNUE of new tea cultivars, highlighting the need to adopt this screening technique to ensure the selection of varieties with consistent yield, as well as improved nitrogen use efficiency.

MATERIALS AND METHODS

Study location: The study was carried out at the experimental field, Tea Research Institute of Sri Lanka (TRISL), Field No.13NC, St. Coombs Estate, Talawakelle (lat. 6° 80'N, long. 80° 40'E and alt. 1382 m amsl) from January, 2016 to December, 2018. This location is categorized under the WU2 agro-ecological region with 2250 mm average annual rainfall, 14.2°C minimum and 22.8°C maximum annual temperature. The soil of this experimental field belongs to Red Yellow Podzolic great soil group¹⁷ and the soil series is Mattakelle¹⁸. Soil samples were taken from the experimental field (In a depth of 0-15 cm) before planting and analyzed for chemical characteristics, including soil mineral N levels (Soil and Plant Nutrition Laboratory, Tea Research Institute of Sri Lanka) (Table 1). The sufficiency levels of the tested soil characters for the tea plant are also presented in Table 1^{18,19}.

Experimental treatments and design: The experiment was conducted as a split-plot in a randomized complete block design arrangement with three replicates. Twenty treatments included five main plots of cultivar and four N rates as

Table 1: Soil chemical characteristics of the trial field at 0-15cm depth

	pH	Organic carbon (%)	Total N (%)	Available plant nutrients (ppm)			
				P	K	Mg	S
Level in trial field	4.92	3.59	0.34	32	240	93	103
Sufficiency level ^{18,19}	4.5-5.5	3-4	-	≥20	≥100	≥60	≥40

subplots. In main plots, new tea cultivars, namely VP 80/5, VP 80/89, VP 80/208 and VP 80/272, were grown and commercially available TRI 2025 was grown as a control cultivar (These cultivars were planted in main plots and maintained under standard management practices until mature to proceed with the experiment). Urea N fertilizer treatments were selected as control and three rates [$N_1 = 0$, $N_2 = 120$, $N_3 = 240$ and $N_4 = 360$ kg N/ha/year]. Treatments were applied to the subplots after maturing the tea bushes along with Eppawala Rock Phosphate (ERP) 35 kg P_2O_5 /ha/year and muriate of potash (MOP) 120 kg K_2O /ha/year in four splits per year.

Each plot is comprised of 20 bushes at a spacing of 1.2×0.6 m. Plots were demarcated by guard rows of TRI 2043 cultivar and lock and spill trenches, which were maintained around plots to reduce the runoff of fertilizers and contamination of different treatments. Other management practices followed standard practice for the region of the experimental field.

Yield records: Yield records were maintained throughout three years. Harvesting was done by hand plucking and tender shoots containing bud, 1st and 2nd succulent leaves and at times 3rd succulent leaves may have harvested at 7-10 days intervals and green leaf weights were expressed to dry matter yield (kg/ha/year).

Yield components: Yield components were determined by plucking the mature harvestable shoots (two leaves and a bud) captured within a 0.3×0.3 m² quadrant randomly placed three times onto the plucking tables of each plot at every plucking round. The collected leaf samples were separated, counted and weighed as active shoots with a growing terminal bud (one leaf and bud, two leaves and bud, three leaves and bud) and dormant shoots (*banji* shoots). The shoots were then oven-dried overnight at 80°C and weighed⁷.

Leaf N content: The mother leaves were sampled from each plot for two years at the end of each year. The collected leaves were oven-dried overnight at 80°C, ground into a fine powder using a leaf grinder. Ground leaf samples (0.2 g) were digested for an hour in concentrated H_2SO_4 with 0.5 g of catalyst (Na_2SO_4 : $CuSO_4$: Se). After cooling, the contents were

quantitatively transferred to Kjeldahl tubes and distilled using the Kjeldahl distillation unit (Behr distillation unit S_4). Then, total N contents were determined by titrating the distillates against 0.1 N HCl²⁰.

Agronomic nitrogen use efficiency (AgNUE): NUE can be quantified in several ways. In this study, NUE was based on agronomic efficiency and defined as the additional amount of economic made tea yield per unit nutrient applied (kg made tea yield $kg^{-1}N$) (Equation 1)¹³. Residual N content in the soil that could affect the calculated AgNUE¹⁶ was not included in the calculation:

$$AgNUE = \frac{Yield\ at\ N_i\ (kg\ ha^{-1}\ year^{-1}) - Yield\ at\ N_0\ (kg\ ha^{-1}\ year^{-1})}{N_i\ (kg\ ha^{-1}\ year^{-1})} \quad (1)$$

Where:

N_i = i^{th} N rate

N_0 = Zero N rate

Statistical analysis: The data collected were subjected to Analysis of Variance (ANOVA) using RStudio (version 3.5.0) statistical package, as a split-plot design, with cultivar as the main factor and N treatments as the sub factor. Mean separation was obtained by least significant difference (LSD) at $p < 0.05$. Complete linkage cluster analysis was also performed using the RStudio (version 3.5.0) statistical package, considering the data of all the tested traits to classify the differences in response to N rates across the cultivars.

RESULTS

Annual yields: The mean comparison for the effects of cultivar and N rates on first- and second-year annual yields of a pruning cycle is presented in Table 2. Overall, the application of N increased yields significantly ($p \leq 0.001$) over the control treatment (0 kg N/ha/year). However, with rising N rates, yields did not rise significantly. At 240 and 360 kg N/ha/year N rates the annual yields were statistically insignificant. There were also variations ($p \leq 0.001$) in yield between cultivars, in the order VP 80/05 < VP 80/208 < VP 80/89 < TRI 2025 < VP 80/272. The cultivar \times N rate

Table 2: Mean comparison for the effects of cultivar and nitrogen rates on annual yield

Factors	Year 1 (kg/ha/year)	Year 2 (kg/ha/year)
Cultivar		
VP 80/05	2321 ± 56 ^b	2736 ± 80 ^d
VP 80/89	2632 ± 79 ^a	3049 ± 102 ^b
VP 80/208	2451 ± 68 ^b	2908 ± 103 ^c
VP 80/272	2712 ± 68 ^a	3203 ± 81 ^a
TRI 2025	2654 ± 61 ^a	3054 ± 68 ^b
Nitrogen rates		
N ₁	2378 ± 66 ^c	2662 ± 45 ^c
N ₂	2526 ± 70 ^b	3006 ± 62 ^b
N ₃	2637 ± 52 ^{ab}	3122 ± 74 ^a
N ₄	2675 ± 65 ^a	3169 ± 93 ^a
CV (%)	7.45	5.08
Cultivar		
LSD	158.64	126.73
Nitrogen rates		
LSD	141.89	113.35

N₁ = 0, N₂ = 120, N₃ = 240, N₄ = 360 kg N ha⁻¹ year⁻¹, values represent Mean ± SE and means followed by the same letters are not significantly different

Table 3: Mean comparison for the effects of cultivar and nitrogen rates on Sd, SFW and SDW

Factors	Shoot density (shoots/m ²)	Shoot fresh weight (mg/shoot)	Shoot dry weight (mg/shoot)
Cultivar			
VP 80/05	222 ± 5 ^c	732 ± 19 ^d	181 ± 5 ^c
VP 80/89	248 ± 11 ^b	798 ± 16 ^b	198 ± 4 ^b
VP 80/208	233 ± 12 ^{bc}	575 ± 10 ^e	139 ± 3 ^d
VP 80/272	308 ± 10 ^a	895 ± 18 ^a	210 ± 5 ^a
TRI 2025	249 ± 12 ^b	769 ± 23 ^c	188 ± 5 ^{bc}
Nitrogen rates			
N ₁	214 ± 11 ^c	689 ± 27 ^d	178 ± 6 ^a
N ₂	251 ± 11 ^b	741 ± 26 ^c	181 ± 7 ^a
N ₃	262 ± 9 ^{ab}	770 ± 29 ^b	185 ± 8 ^a
N ₄	281 ± 9 ^a	816 ± 35 ^a	189 ± 8 ^a
CV (%)	10.33	4.72	7.18
Cultivar			
LSD	21.71	29.65	10.97
Nitrogen rates			
LSD	19.42	26.52	NS

N₁ = 0, N₂ = 120, N₃ = 240, N₄ = 360 kg N/ha/year, NS: Not significant, values represent Mean ± SE and means followed by the same letters are not significantly different

interactions were not significant. However, there were still variations in cultivar yield response to the applied N rates (Fig. 1). Yields of all cultivars increased with the application of N fertilizer up to 360 kg N/ha/year but yield responses of VP 80/208 and VP 80/05 were lower than those of the other three cultivars. The yield of VP 80/89 increased rapidly with the application of N fertilizer over the control, but above this rate, the increments were insignificant. The best yield at each N rate was recorded in VP 80/272.

Yield components: In this study, the total number of active plus *banji* shoots within one square meter area of the plucking table was calculated as shoot density (Sd) and it showed significant ($p \leq 0.001$) variations due to cultivars and N rates. The cultivar × N rate interaction was not significant (Table 3). The Sd increased with the N rate. At the same

time, the highest mean Sd was recorded in VP 80/272 (308 shoots m⁻²) and the lowest in VP 80/05 (222 shoots m⁻²). The cultivar Sd ranking showed a similar ranking to annual yield and confirmed that the Sd contributes strongly to the annual yield variation among cultivars.

The shoot fresh weight (SFW) was determined by dividing the total fresh weights of active and *banji* shoots by the total harvested shoots. As shown in Table 3, the effect of cultivars on SFW was significant ($p \leq 0.001$), in the order VP 80/208 < VP 80/05 < TRI2025 < VP 80/89 < VP 80/272, with the highest and lowest values, recorded as 895 mg/shoots and 575 mg/shoots, respectively. Meanwhile, the effect of N rates on SFW was also significant ($p \leq 0.001$), but the effect of interaction between cultivar and N rate on SFW was not significant.

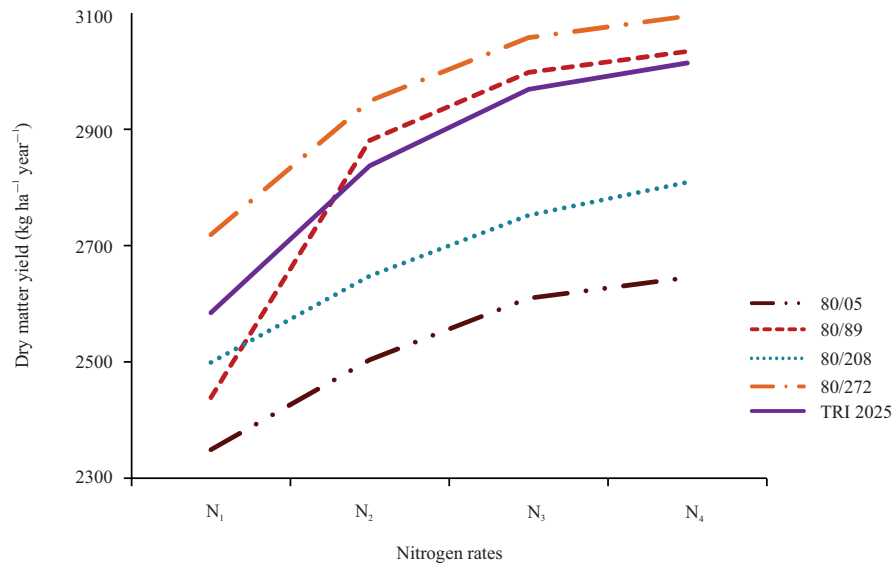


Fig. 1: Average annual dry matter yield of tested accessions/cultivar with varied nitrogen rates

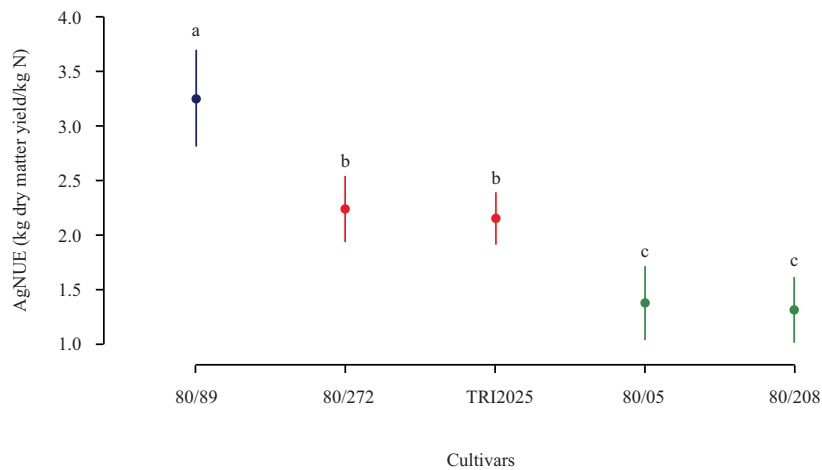


Fig. 2: AgNUE groups and SE of tested accessions/cultivar LSD ($p < 0.001$) = 0.61

The shoot dry weight (SDW) was determined by dividing the total dry weights of active and *banji* shoots by the total number of shoots harvested. It is evident from Table 3 that maximum SDW (210 mg/shoots) was produced by VP 80/272 while minimum SDW (139 mg/shoots) was recorded in VP 80/208, at the same time, the SDW among the different cultivars was highly significant ($p < 0.001$). Because the cultivar VP 80/272 has the highest SFW, the SDW is likewise the highest in VP 80/272. However, the response of SDW to N rates and the interaction between cultivar x N rate was not significant.

Leaf nitrogen content: Table 4 data show that the N content of the mother leaf was statistically significant by

cultivars ($p \leq 0.05$) and N rates ($p \leq 0.001$) while it is not significantly affected by interaction. The cultivar variation of N contents manifested the highest value at VP 80/272 (3.29%), followed by VP 80/89 (3.21%) and the lowest value at VP 80/05 (3.00%). In general, VP 80/272 cultivar had the highest leaf N contents at every N rate, probably because of the high N uptake ability from the soil. The N content of the mother leaf was sensitive to the N rate and it was increased with the increasing rate.

Agronomic nitrogen use efficiency (AgNUE): Variation in AgNUE appeared to result from differences among cultivars ($p \leq 0.001$) and rate of N ($p \leq 0.001$). However, their interaction was not significant. Figure 2 shows that VP 80/89

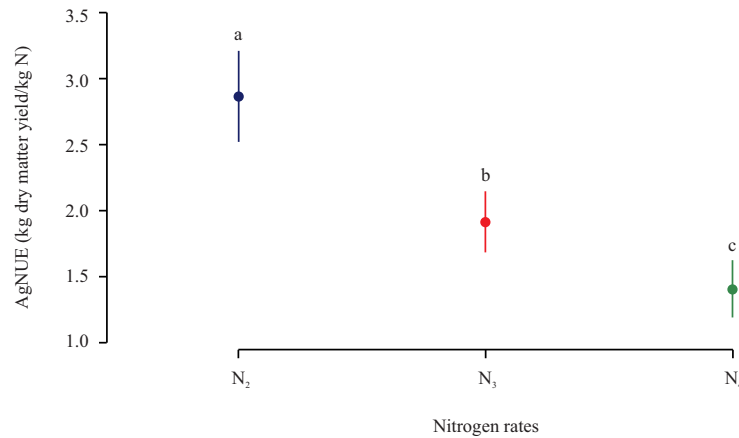


Fig. 3: AgNUE groups and SE of different nitrogen rates LSD ($p < 0.001$) = 0.48

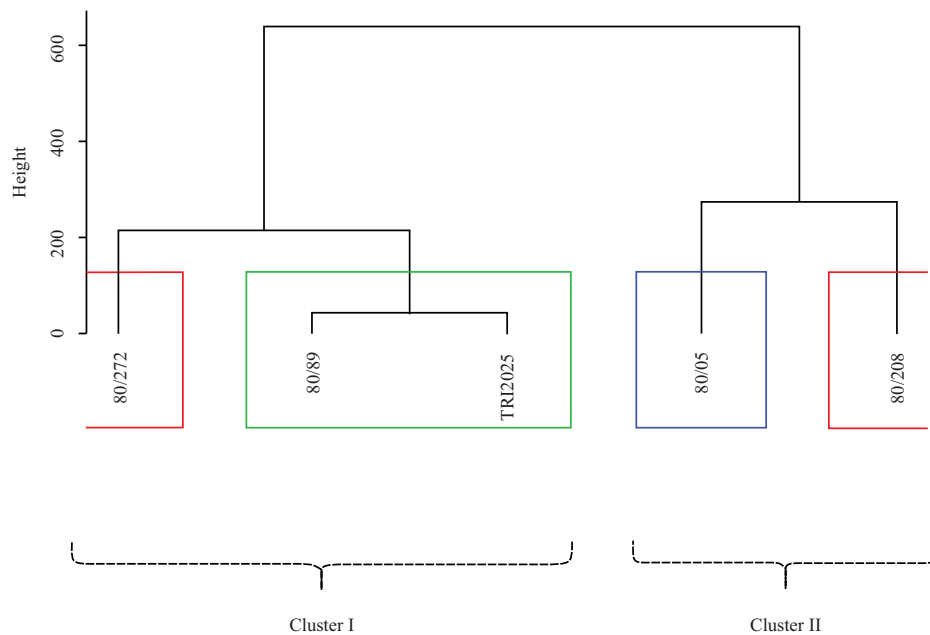


Fig. 4: Dendrogram constructed from complete linkage cluster analysis based on tested parameter

recorded the highest AgNUE (3.25 kg dry matter yield/kg N), which produced a high tea yield per unit of nitrogen supply. The AgNUE of VP 80/272 (2.24 kg dry matter yield/kg N) and TRI 2025 (2.14 kg dry matter yield/kg N) were statistically similar. The AgNUE of VP 80/05 (1.37 kg dry matter yield/kg N) and VP 80/208 (1.31 kg dry matter yield/kg N) were also statistically similar. Among the N application, supplying N by 120 kg N/ha/year produced the maximum crop yield per N unit increase, being observed the most incredible agronomic efficiency which reached 2.87 kg dry matter yield/kg N whereas low agronomic efficiency 1.41 kg

matter yield/kg N was observed at 360 kg N/ha/year (Fig. 3).

Complete linkage cluster analysis: The cultivar studied could be categorized into two main clusters based on their performances (Fig. 4). Cluster I comprised VP 80/272, VP 80/89 and the control cultivar TRI 2025, a well-known high yielding and widespread commercially cultivated cultivar. In further clustering, VP 80/272 was split into a new cluster because of distinguishing performance better than TRI 2025. Meanwhile, the performance of VP 80/89 was on par with TRI

Table 4: Mean comparison for the effects of cultivar and nitrogen rates on leaf nitrogen (%)

Cultivar	VP 80/05	VP 80/89	VP 80/208	VP 80/272	TRI 2025	Nitrogen rate mean
Trt						
N ₁	2.70	3.02	2.92	3.07	2.96	2.93±0.06 ^c
N ₂	2.89	3.10	3.14	3.28	3.14	3.11±0.07 ^b
N ₃	3.10	3.27	3.20	3.39	3.21	3.23±0.07 ^{ab}
N ₄	3.29	3.45	3.24	3.43	3.34	3.35±0.09 ^a
Cultivar Mean	3.00±0.09 ^c	3.21±0.10 ^{ab}	3.13±0.10 ^{bc}	3.29±0.09 ^a	3.16±0.09 ^{ab}	
CV (%)	6.08					
Cultivar						
LSD (p<0.05)	0.16					
Nitrogen rates						
LSD (p<0.001)	0.14					
Cul:Trt						
LSD	NS					

N₁ = 0, N₂ = 120, N₃ = 240, N₄ = 360 kg N/ha/year, mean values represent Mean ± SE and means followed by the same letters are not significantly different

2025. On the other hand, VP 80/05 and VP 80/208 were in cluster II, where the overall performances were lower than TRI2025.

DISCUSSION

In this study, we observed that the N application significantly increases the yield, probably because it is present in the essential substances in the plant, which contributed to the maximum vegetative growth of tea^{8,21}. However, under this field condition, N availability satisfied the plant requirement for growth and development at 240 kg N/ha/year, enabling the plants to produce a higher annual yield. As a result, the increment of N beyond this level did not result in a substantial yield. Thus, to optimise cultivar N fertilizer response, it is required to investigate further the benefits of matching supply and demand, taking into consideration not just productivity but also the risk of N loss. In Russia, the highest tea yields were obtained with the application of 300 kg N as compared to 450-600 kg N/ha/year⁸. However, yields of seedling tea in Kenya increased with the application of N fertilizer up to 470 kg N/ha/year¹⁴. The probable reason for the variation in yield between cultivars under the same experimental condition might be that different cultivars have different yield potential due to genetic make-up differences. The genetic make-up determines the productivity of the cultivars. Therefore, we can assume that the cultivar VP 80/272 has good genetic makeup compared to the control cultivar TRI2025. Although the Cultivar x N rate interactions were significant, there was a slight variation in cultivar yield response to the nitrogen application rate. The phenomenon elucidated the cultivar evaluations and

selections need to incorporate the evaluations of the responses to N fertilizers. Because specific cultivars may generate a high yield on soil low in N, while others may be able to maximise the usage of applied N and maximum economic crop yield^{11,13}, thus, N fertilizer should not be applied universally to all cultivars to optimize economic crop yield. Meanwhile, evaluating the yield response of new cultivars to diverse doses of N is vital in developing cultivars with high NUE.

The Sd, the number of shoots per unit area of the bush, is a significant factor determining tea yield. It has a broader variation depending on the cultivar, weather and management conditions²². Both active and *banji* shoots are accountable for the Sd²³ and it is essential to select high-yielding cultivars because tea yield was highly correlated with Sd^{1,7}. The results of Sd, which confirmed that the Sd contributes enormously to the annual yield variation among cultivars, are in close agreement with the finding of Neranjana *et al.*⁷, who reported the high positive correlation (0.75, p<0.0001) between Sd and yield based on the study of 11 different cultivars in Sri Lanka. A similar study conducted with 34 diverse tea cultivars in India also revealed a positive correlation (0.670, p<0.01) between Sd and yield¹. The SFW is another important yield component because it has a significant direct effect on tea yield⁷. Furthermore, SFW has a high correlation to SDW¹. As the marketable product of tea is the dried leaves, the dry matter content of tea shoots is of great importance to tea manufacturers. The SDW has a significantly high correlation to the dry matter yield of tea^{1,7}. In Sri Lanka, the mean dry weight of a harvested shoot has been reported to be in the range of 130-250 mg²². This study recorded the highest SFW and SDW in VP 80/272, which has comparatively large leaves and packable shoots. The

morphology differences which are governed by genetic variations, are highly influenced on the yield components and ultimately on yield. Further, high rates of N improved SFW but not SDW¹⁴. Thus, an increase in N rate beyond the optimum level does not provide any economic return in tea production.

The N content of the mother leaf was sensitive to the N rate and it was increased with the increasing rate. Sitienei *et al.*¹⁵ and Tabu *et al.*²⁴ also observed similar N rates on a single tea cultivar in one location. However, literature on the N content of "harvestable shoot" has reported less sensitivity to increasing rates of N¹⁴. An increase in N supply improves growth up to a point beyond which N absorbed is not used to grow shoots but rather is accumulated as soluble compounds in the mother leaves²⁵.

Agronomic Efficiency (AE) is the additional yield obtained per unit of the applied nutrient and it is affected by the ability of the plant to take up the nutrient and the ability to utilize the nutrient to yield. Hence, Agronomic Nitrogen Use Efficiency (AgNUE) is useful to differentiate tea cultivars for their ability to absorb and utilize N. Estimates of AgNUE were only where external N was applied. Though tea may realize yields without applying N fertilizer, the yields are commercially meagre and unsustainable¹⁴. Higher AgNUE by tea cultivars could reduce N fertilizer input costs, decrease the rate of N losses and enhance crop yields¹³. There is a genetic variability for N absorption and utilization efficiency among studied tea cultivars. A cultivar with low AgNUE should receive lower N fertilizer rates, while a cultivar with high AgNUE should receive higher N fertilizer rates¹⁴ and the best performing cultivars at high N fertilization input are not necessarily the best ones when the supply of N is lower¹². Further, if a unit of N fertilizer does not increase the yield enough to pay for its cost, its application will not be economical and will not return profit even after a constant increase in the yield.

CONCLUSION

In conclusion, we identified genetic variation for yield, yield-related traits, leaf N content and AgNUE among a selection of newly developed tea cultivars grown in central highlands climatic conditions of Sri Lanka. We selected two cultivars, VP 80/272 and VP 80/89, having significant improvement towards efficient use of applied N compared to the TRI 2025 control cultivar. Meanwhile, the variety VP 80/89 is more suitable to achieve a comparatively better crop yield with a low N application rate. The results suggest the potential use of these traits for evaluating the cultivar and nitrogen rate interactions and exploiting this type of screening technique in

the tea breeding program will enable plant breeders to select cultivars that show a consistent yield and efficient usage of N fertilizer.

SIGNIFICANCE STATEMENT

We specifically looked at how varying N affected yield, yield components, leaf nitrogen concentration and AgNUE in novel tea cultivars. The findings of the study underscore the necessity of using this sort of screening approach to assure the selection of tea cultivars with consistent yields and increased nitrogen utilization, opening up a new knowledge that has practical advantages for growers and the environment.

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