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Physicochemical analysis of Ugandan tea (*Camellia sinensis*) germplasm reveals potential commercial green and black tea varieties

Racheal Grace Nalugo[a,†], Tadeo Kaweesi[a,†], Ronald Kawooya[a], Ephraim Nuwamanya [b], Charles Mugisa[a], Vivian Namutebi[a], Venansio Tumwine[a], Vereriano Turyahebwa[a], and Robooni Tumuhimbise [a]

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ABSTRACT

Tea (*Camellia sinensis* L.) is an important beverage consumed worldwide. In Uganda, it is the second-largest and highly prioritized export crop after coffee and provides the much-needed jobs to more than 800,000 people. Despite its importance in the country, the crop has received very limited research attention for its improvement and optimal utilization. This study was aimed at characterizing existing tea germplasm in Uganda to inform future breeding initiatives for market-preferred tea varieties. Fifty-eight advanced tea clones were randomly selected from the tea germplasm conserved at Uganda's Tea Research Center in Rwebitaba and analyzed in the laboratory for eight physicochemical descriptors. Hierarchical cluster analysis performed on the 58 clones using Cluster-R package revealed four main clusters, indicating the existence of variation for physicochemical parameters within tested germplasm. This variation can be exploited to select among and/or improve the studied germplasm genetically for quality. High fermentation rate, which is one of the key indicators for good-quality black teas, revealed 15 fast-fermenter tea clones. Clone "UTR12/12" was identified as the most rapid fermenter, fully fermenting within 30 min, which implies a good clone for black tea. The clone also had the highest polyphenol content (26.7%), higher than the high-quality clone "UTR6/8" (control). Other tea clones whose polyphenol content was within the range of the control black tea clone were: "UTR144/10" (20.9%) and 'UTR144/17, (20.66%). The identified promising black tea clones can be advanced to multi-location trials for further evaluation and selection for eventual variety release as commercial black-tea clones.

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KEYWORDS
Black tea; *camellia sinensis*; germplasm characterization; green tea; physicochemical parameters; tea quality

1 Introduction

Tea (*Camellia sinensis* L.) is the second most widely consumed beverage in the world after water (Patel et al. 2019). Its demand is influenced by briskness, liquor, aroma, and taste-quality aspects (Mukhtar and Ahmad 2000). The

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efficient utilization of the tea germplasm in a tea breeding program is dependent on profiling and characterization of tea germplasm based on different biochemical parameters (Ravichandran and Parthiban 1998). In the leading tea-producing countries, such as China, India, and Kenya, fermentation rate, coupled with biochemical markers, such as total polyphenol content, polyphenols (flavonoids and catechin), amino acids, and other physicochemical parameters, such as caffeine and crude fiber, have been extensively used to discern tea germplasm for quality aspects (Leung et al. 2001; Lopez et al. 2005). Polyphenols, the main group of chemical substances present in the tea flush, undergo oxidative changes during the making of black tea (Kottawa-Arachchi et al. 2012). According to Saravanan et al. (2005), the variation in the levels of polyphenols influences the briskness, liquor, and aroma of tea. Furthermore, the oxidation products, such as theaflavins and thearubigins, in combination with caffeine, influence tea quality (Karori et al. 2010). Variation in the levels of these essential discriminative markers for tea is influenced by numerous factors, such as genotype, climate, soil type, and management practices (Kilmartin and Hsu 2003; Ku et al. 2010; Turkmen, Sarı, and Sedat Velioglu 2009).

In Uganda, tea improvement at the Rwebitaba Tea Research Center (RTRC), established in the early 1960s, has been very slow since the 1970s. RTRC was established as a branch of Tea Research Institute of East Africa (TRIEA). Before the collapse of the East African Community due to political upheavals in 1977, TRIEA prereleased four commercial black tea clones (UTR6/8, UTR100/5, UTR31/8, and UTR108/82), which currently dominate tea production in Uganda. Since then, little progress has been made in tea genetic improvement, especially in determining the effectiveness of the physicochemical discriminative markers that influence tea quality. This partly explains why Uganda's tea quality has been low, thus fetching lower prices compared to the teas from Kenya and Rwanda (Newvision 2020). Therefore, streamlining the physicochemical profiling of physicochemical properties is a prerequisite for characterization and utilization of local tea germplasm for efficient and targeted genetic improvement of tea in Uganda. Moreover, downsizing to minimal putative markers would help save resources since characterizing the entire collection using all proposed components is costly. This could also provide a firm foundation for early-stage appraisal of the accessions conserved at the RTRC of Rwebitaba Zonal Agricultural Research and Development Institute (Rwebitaba ZARDI) in Uganda. Rwebitaba ZARDI is one of the 16 Public Agricultural Research Institutes of the National Agricultural Research Organization (NARO) in Uganda. Its mandate is to develop and disseminate tea technologies in Uganda in addition to facilitating the dissemination of appropriate technologies of other agricultural commodities to the uptake pathways (end-users/stakeholders) in the western highland agro-ecological zone of Uganda that covers Ruwenzori sub-region (Wortmann and Eledu 1999). The present study aimed at characterizing

existing tea germplasm at the RTRC to inform future breeding initiatives for market-preferred tea varieties.

2 Materials and methods

2.1 *Plant germplasm*

Fifty-eight tea clones (Table 1) were selected based on visual phenotypic traits, namely, yield (Amma 1975), leaf color (Yuchuan et al. 2022) and tolerance to drought (Mehdi, Mojtaba, and Mojtaba 2019) from the core tea germplasm collection at Rwebitaba TRC, hosted at Rwebitaba ZARDI (0°38′58.5”N 30°25′42.6”E (Location Code 6GGGJCXG+HQ)). Approximately 30 g (two leaves and a bud) were randomly picked from individual plant accessions in a clonal field trial and taken to the laboratory for physicochemical analyses. The leaf samples were carried in a container at 4°C for equilibration, followed by transfer to an ice-cooled container to avoid oxidation. Further sample handling involved storage at −20°C before processing to maintain the integrity of the samples along the analysis pipeline.

2.2 *Plant physicochemical traits assessed in the laboratory*

Data on the following eight physicochemical descriptors were collected and analyzed using the methods described below:

2.2.1 *Fermentation rate*

Fermentation rate is used as one of the key preliminary parameters for identifying high-quality tea accessions at the early stages of a tea-breeding program (Ellis and Nyirenda 1995). In this study, fermentation was assessed via a chloroform test, as described by Samaraweera and Ranaweera (1988). Briefly, the tests were carried out in a glass test tube fitted with a lid and chloroform-soaked cotton wool kept at the bottom. The second leaf of the tea shoot was hung on a wire and placed horizontally on the working table so that the leaves were nearly equidistant from the soaked wool. Fermentation was considered complete when the leaf turned bright red brown after a period of exposure to the chloroform vapor. The process was repeated twice to obtain reliable results. Each sample was scored at an interval of 30 min using the following four-point scale: 1 = green; 2 = greenish tinge; 3 = dull brown; and 4 = bright red brown. The rate at which the leaf color changed from green to bright red brown at a particular time indicated the rate of fermentation for a particular tea clone.

Table 1. Physiochemical components of selected 58 tea accessions at Rwebitaba Tea Research Center, Uganda.

Accessions Name/Code	Physiochemical components							
	Polyphenol (%)	Catechins (%)	Flavonoids (%)	Fermentation	Crude fiber (%)	Color	Brightness	Caffeine (%)
UTR 6/8	25.8 ± 0.6	3.6 ± 1.7	0.1 ± 0.0	3.1 ± 0.6	14.8 ± 1.6	1.8 ± 0.0	2.4 ± 0.8	2.4 ± 0.1
UTR 12/12	26.7 ± 1.0	8.7 ± 0.2	0.1 ± 0.0	4.0 ± 0.3	17.9 ± 2.3	1.9 ± 0.1	3.6 ± 0.4	4.3 ± 0.5
UTR 108/82	22.7 ± 6.0	7.4 ± 0.8	0.1 ± 0.0	3.3 ± 0.1	14.6 ± 1.7	1.7 ± 0.1	2.8 ± 0.3	3.4 ± 0.4
UTR 6/10	21.9 ± 2.6	5.0 ± 0.9	0.1 ± 0.0	3.4 ± 0.2	16.6 ± 0.7	1.6 ± 0.0	2.7 ± 0.2	3.8 ± 0.1
UTR 144/10	20.9 ± 0.6	6.2 ± 0.4	0.1 ± 0.0	3.0 ± 0.1	13.6 ± 1.1	1.5 ± 0.0	2.3 ± 0.4	3.1 ± 0.1
UTR 144/17	20.7 ± 1.2	3.1 ± 0.4	0.1 ± 0.0	3.7 ± 0.1	16.7 ± 1.0	1.7 ± 0.0	2.9 ± 0.5	3.9 ± 0.4
UTR 155/19	18.0 ± 0.2	4.8 ± 1.7	0.1 ± 0.0	3.2 ± 0.1	18.9 ± 1.6	1.7 ± 0.2	3.1 ± 0.8	3.3 ± 0.2
UTR 100/5	17.1 ± 0.8	5.7 ± 2.0	0.15 ± 0.0	2.6 ± 0.2	16.6 ± 2.0	1.3 ± 0.1	3.3 ± 0.6	3.8 ± 0.3
UTR 144/3	15.4 ± 1.3	3.1 ± 0.5	0.1 ± 0.0	3.8 ± 0.1	16.7 ± 1.6	1.7 ± 0.1	2.3 ± 0.3	3.8 ± 0.1
UTR 144/23	14.2 ± 0.9	7.0 ± 0.1	0.1 ± 0.0	3.2 ± 0.2	15.2 ± 0.6	1.5 ± 0.0	2.2 ± 0.3	3.5 ± 0.1
UTR 31/11	13.2 ± 0.6	5.0 ± 1.0	0.1 ± 0.0	3.7 ± 0.1	10.4 ± 2.7	1.6 ± 0.1	2.4 ± 0.6	2.8 ± 0.4
UTR 141/262	9.6 ± 5.4	5.3 ± 1.3	0.1 ± 0.0	3.2 ± 0.16	14.5 ± 0.9	1.7 ± 0.1	2.5 ± 0.2	3.3 ± 0.1
UTR 141/47	3.8 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	3.0 ± 0.00	18.5 ± 0.2	1.3 ± 0.1	0.5 ± 0.1	2.1 ± 0.0
UTR 105/3	3.7 ± 0.6	5.0 ± 0.1	0.1 ± 0.0	1.8 ± 0.4	14. 3 ± 0.4	1.4 ± 0.1	0.4 ± 0.0	0.0 ± 0.0
UTR 7/14	3.7 ± 0.2	5.8 ± 0.0	0.1 ± 0.0	2.5 ± 0.0	11.6 ± 0.5	1.7 ± 0.1	0.6 ± 0.0	0.0 ± 0.0
UTR 103/5	3.5 ± 0.1	5.7 ± 0.1	0.1 ± 0.0	2.3 ± 0.2	13.0 ± 0.4	1.6 ± 0.0	0.5 ± 0.0	0.0 ± 0.0
UTR 5/5	3.2 ± 0.1	5.5 ± 0.1	0.1 ± 0.0	2.7 ± 0.3	13.5 ± 0.3	1.8 ± 0.0	0.7 ± 0.0	0.0 ± 0.0
UTR 11/52	3.2 ± 0.0	5.0 ± 1.3	3.4 ± 0.0	2.5 ± 0.5	2.6 ± 0.1	2.4 ± 0.1	2.7 ± 0.0	7.8 ± 0.7
UTR 109/195	3.0 ± 0.4	0.2 ± 0.0	0.3 ± 0.0	2.5 ± 0.5	17.3 ± 2.2	1.4 ± 0.1	0.5 ± 0.1	2.4 ± 0.0
UTR 141/55	3.0 ± 0.7	0.2 ± 0.0	0.3 ± 0.0	2.5 ± 0.5	16.9 ± 1.6	1.4 ± 0.1	0.5 ± 0.0	2.3 ± 0.0
UTR 6/97	2.9 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	2.5 ± 0.5	14.7 ± 3.5	1.6 ± 0.0	0.7 ± 0.0	1.99 ± 0.0
UTR 12/19	2.9 ± 0.3	5.2 ± 0.1	0.1 ± 0.3	2.3 ± 0.7	13.2 ± 0.8	1.6 ± 0.1	0.5 ± 0.0	0.0 ± 0.0
UTR 109/47	2.9 ± 0.1	5.5 ± 0.1	0.1 ± 0.0	2.5 ± 0.5	12.9 ± 0.7	1.5 ± 0.1	0.6 ± 0.0	0.0 ± 0.0
UTR 2/38	2.8 ± 0.1	5.4 ± 0.8	3.2 ± 0.1	2.5 ± 0.5	2.6 ± 0.3	2.4 ± 0.2	2.6 ± 0.2	7.1 ± 0.0
UTR 109/75	2.7 ± 0.3	5.1 ± 0.1	0.1 ± 0.0	2.0 ± 1.0	12.3 ± 0.1	1.7 ± 0.1	0.5 ± 0.0	0.0 ± 0.0
UTR 109/54	2.7 ± 0.0	6.0 ± 0.0	0.1 ± 0.0	1.8 ± 0.2	12.6 ± 0.0	1.5 ± 0.1	0.5 ± 0.0	0.0 ± 0.0
UTR 139/10	2.6 ± 0.9	0.2 ± 0.0	0.3 ± 0.0	2.5 ± 0.5	15.8 ± 1.5	1.5 ± 0.0	0.6 ± 0.0	2.2 ± 0.0
UTR 108/36	2.5 ± 0.2	4.1 ± 1.0	3.7 ± 0.1	2.0 ± 0.3	2.6 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	9.2 ± 0.2
UTR 108/54	2.4 ± 0.4	5.7 ± 0.2	3.5 ± 0.3	2.0 ± 0.2	2.5 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	8.5 ± 0.6
UTR 109/72	2.4 ± 0.5	5.4 ± 0.2	0.1 ± 0.0	2.2 ± 0.1	13.9 ± 0.5	1.6 ± 0.0	0.6 ± 0.0	0.0 ± 0.0
UTR 108/62	2.3 ± 0.9	5.1 ± 0.8	3.1 ± 0.1	1.3 ± 0.3	2.0 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	7.9 ± 0.2
UTR 108/78	2.3 ± 0.4	5.4 ± 0.3	3.4 ± 0.1	2.0 ± 0.0	2.4 ± 0.0	2.3 ± 0.0	2.5 ± 0.0	8.1 ± 0.2

(*Continued*)

Table 1. (Continued).

Accessions Name/Code	Physiochemical components							
	Polyphenol (%)	Catechins (%)	Flavonoids (%)	Fermentation	Crude fiber (%)	Color	Brightness	Caffeine (%)
UTR 108/56	2.3 ± 0.1	4.6 ± 0.8	3.3 ± 0.2	1.5 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	2.3 ± 0.1	8.3 ± 0.5
UTR 108/51	2.3 ± 0.3	4.7 ± 0.3	4.0 ± 0.2	2.5 ± 0.5	2.9 ± 0.3	2.7 ± 0.2	3.0 ± 0.2	9.3 ± 0.2
UTR 109/71	2.2 ± 0.3	0.2 ± 0.0	0.3 ± 0.0	3.5 ± 0.5	16.2 ± 0.8	1.7 ± 0.1	0.8 ± 0.1	1.8 ± 0.0
UTR 108/43	2.2 ± 0.4	5.8 ± 0.4	3.5 ± 0.1	2.3 ± 0.2	2.6 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	8.3 ± 0.0
UTR 109/181	2.03 ± 0.3	0.2 ± 0.0	0.3 ± 0.0	3.0 ± 0.0	17.1 ± 1.1	1.4 ± 0.1	0.4 ± 0.0	2.2 ± 0.0
UTR 139/38	1.9 ± 0.9	0.2 ± 0.0	0.2 ± 0.0	3.5 ± 0.5	13.3 ± 1.4	1.5 ± 0.0	0.6 ± 0.0	1.2 ± 1.1
UTR 12/21	1.8 ± 0.1	4.3 ± 1.4	3.4 ± 0.2	1.5 ± 0.5	2.2 ± 0.3	2.1 ± 0.3	2.4 ± 0.2	8.5 ± 0.3
UTR 108/59	1.8 ± 0.6	4.6 ± 0.8	3.2 ± 0.3	1.5 ± 0.5	2.1 ± 0.3	2.0 ± 0.3	2.3 ± 0.3	8.0 ± 0.5
UTR 11/26	1.8 ± 0.1	6.0 ± 0.6	3.9 ± 0.1	2.2 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.9 ± 0.1	9.4 ± 0.4
UTR 1/45	1.7 ± 0.1	5.9 ± 2.5	3.1 ± 0.6	1.0 ± 0.0	1.8 ± 0.2	1.7 ± 0.2	2.1 ± 0.3	8.1 ± 1.9
UTR 11/4	1.6 ± 0.0	6.7 ± 0.4	3.4 ± 0.1	2.2 ± 0.3	2.5 ± 0.1	2.3 ± 0.1	2.6 ± 0.0	8.0 ± 0.6
UTR 108/46	1.5 ± 0.2	4.6 ± 1.2	3.1 ± 0.2	1.3 ± 0.3	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	8.0 ± 0.8
UTR 108/66	1.5 ± 0.3	4.1 ± 0.2	3.5 ± 0.3	1.8 ± 0.4	2.4 ± 0.3	2.6 ± 0.2	2.6 ± 0.3	8.8 ± 0.5
UTR 139/35	1.5 ± 1.0	0.2 ± 0.0	0.2 ± 0.0	2.5 ± 0.5	15.6 ± 0.0	1.8 ± 0.0	0.7 ± 0.1	2.5 ± 0.1
UTR 1/53	0.5 ± 0.1	6.1 ± 0.4	3.3 ± 0.2	1.0 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	2.2 ± 0.1	8.8 ± 0.5
UTR 12/50	1.5 ± 0.2	6.2 ± 0.6	3.6 ± 0.2	2.7 ± 0.6	2.8 ± 0.3	2.6 ± 0.3	2.9 ± 0.3	8.2 ± 0.4
UTR 108/41	1.3 ± 0.1	6.3 ± 0.4	3.8 ± 0.3	2.8 ± 0.4	2.9 ± 0.3	2.7 ± 0.3	3.0 ± 0.3	8.5 ± 0.5
UTR 11/56	1.2 ± 0.0	4.3 ± 0.7	3.6 ± 0.2	2.0 ± 0.5	2.5 ± 0.3	2.3 ± 0.2	2.6 ± 0.2	8.6 ± 0.2
UTR 11/25	1.2 ± 0.0	5.2 ± 0.1	3.3 ± 0.1	2.0 ± 0.0	2.4 ± 0.0	2.2 ± 0.0	2.5 ± 0.0	8.0 ± 0.3
UTR 108/63	1.2 ± 0.8	6.2 ± 1.3	3.2 ± 0.0	1.7 ± 0.3	2.2 ± 0.1	2.0 ± 0.1	2.3 ± 0.0	7.9 ± 0.3
UTR 12/13	1.2 ± 0.1	4.5 ± 0.7	3.1 ± 0.0	1.3 ± 0.3	2.0 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	7.9 ± 0.3
UTR 109/66	1.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	3.0 ± 1.0	17.2 ± 1.1	1.1 ± 0.2	0.4 ± 0.0	2.0 ± 0.1
UTR 108/68	1.1 ± 0.1	6.0 ± 0.1	3.3 ± 0.1	2.0 ± 0.5	2.3 ± 0.2	2.2 ± 0.2	2.5 ± 0.2	7.8 ± 0.1
UTR 1/44	1.0 ± 0.1	4.3 ± 1.1	3.4 ± 0.2	2.2 ± 0.4	2.5 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	7.9 ± 0.8
UTR 108/84	0.9 ± 0.1	3.5 ± 0.2	3.4 ± 0.19	1.8 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	8.4 ± 0.6
UTR 1/10	0.8 ± 0.1	6.0 ± 0.1	3.7 ± 0.1	2.0 ± 0.0	2.5 ± 0.0	2.4 ± 0.0	2.7 ± 0.0	9.1 ± 0.1
Standard Error	**3.4**	**0.16**	**0.70**	**0.62**	**3.2**	**0.29**	**0.56**	**1.15**
†LSD $_{(0.05)}$	**6.05**	**2.65**	**0.54**	**1.22**	**2.59**	**0.53**	**0.91**	**1.48**
F-values	**12.5***	**5.8***	**95.3***	**4.3***	**49.2***	**5.4****	**11.1****	**54.7****
‡CV (%)	**58.4**	**29.2**	**16.0**	**23.5**	**16.0**	**14.1**	**23.5**	**14.5**

*, and ** significant at 0.05 and 0.01 probability levels, respectively.
†$LSD_{0.05}$ = Least significant difference at 5%.
‡CV% = percentage coefficient of variation.

2.2.2 *Total polyphenols*

Total polyphenols were extracted following the method described by the International Organization for Standardization (ISO) 14,502–1, with minimal modifications. A 0.2 g tea sample was placed in an extraction tube and 5 mL of 70% methanol was added at 70°C. The extract was vortexed at 70° C for 10 min, after which, the extract was cooled and then centrifuged at 5000 rpm at room temperature for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated twice. Both extracts were pooled and the volume adjusted to 10 mL with cold 70% methanol. One mL of the extract was diluted with water to 100 mL. Polyphenols were determined by spectrophotometry using gallic acid as standard. A 1.0 mL of the diluted extract was transferred in duplicate to separate tubes containing 5.0 mL of a dilution of Folin-Ciocalteu's reagent in water (1:10). Four mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were allowed to stand at room temperature for 60 min before absorbance was measured at 765 nm against distilled and deionized water. The concentration of polyphenols in samples was derived from a standard curve of gallic acid at 20 micrograms/mL and expressed as gallic acid equivalents (GAE) in g/100 g material.

2.2.3 *Total catechins*

To each sample of ~60 mg, 7 mL of the extraction solvent was added. The mixture was vortexed followed by sonication for 90 min and centrifugation at 5000 rpm for 10 min. The supernatant was collected in vials, and a similar procedure was repeated for the pellet. The two volumes were added to one vial and thereafter filtered using 0.2 µm cellulose acetate sterile syringe filters. The filtrate was diluted in a ratio of 1:5 in the extraction solvent and thereafter the analysis was undertaken using an HPLC 1100 Agilent, with C18 column (Synergi Hydro-RP 80 R, Phenomenex, Torrance, California, USA) and UV detector (230 nm). Eluent A was acetonitril (ACN) and eluent B was 0.1% aqueous phosphoric acid; the flow rate was kept constant throughout the analysis at 1 mL min^{-1}. Chromatograms were monitored at 230 nm and identification was based on retention time in comparison with authentic standards. Quantification was performed by establishing calibration curves for each compound determined, using standards.

2.2.4 *Crude fiber content*

The method by A.O.A.C (1980) was used. For each sample, 2.0 g was weighed into a round bottomed flask. A total of 50 mL of 0.25 M sulfuric acid solution was added to each sample in the flask, and the mixture was boiled at reflux temperature (>95°C) for 30 min. The hot solution was quickly placed in a cooling bath and then centrifuged at 6000 rpm for 10 min at 4°C. The supernatant was decanted off and the residue washed with hot water. A total

of 50 mL of 0.3 M sodium hydroxide solution was added to the residues in a 50 mL Falcon tube and incubated at reflux temperature (>95°C) in the water bath for 30 min and the hot mixture was transferred to the cooling bath. Centrifugation was done at 6000 rpm for 10 min at (4°C), and the supernatant was decanted off. Repeated washing using 50 mL of hot water was done. The residue was dried to a constant weight in a hot-air oven at 105°C for 12 h, cooled in the desiccator and weighed.

$$\text{Crude fiber \%} = (\text{change in weight}/\text{initial weight}) \times 100.$$

2.2.5 *Total color and brightness of tea liquor*

Nine grams of each tea sample were used for hot-water tea extract and total color and brightness of tea liquor were determined by measuring the absorbance at 380 nm and 460 nm using UV-VIS spectrophotometer (Jenway 6305-Bibby Scientific, Stone, Staffordshire, UK) according to Roberts and Smith (1963).

2.2.6 *Flavonoids*

For determination of flavonoids, 0.5 mL of methanolic extract was generated, followed by 1.5 mL 80% methanol re-extraction. The resultant solution was added to 2.5 mL of distilled water. The resultant solution was vortexed, followed by addition of 0.15 mL of 5% $NaNO_2$ solution. To this solution, 0.3 mL of 10% AlCl3 solution was added after 5 min of incubation and the mixture was allowed to stand for 6 min. Then, 1 mL of 1 M NaOH solution was added, followed by 0.55 mL of distilled water. The resultant mixture was allowed to stand for 15 min and the absorbance measured at 510 nm (Jenway 6305-Bibby Scientific, Stone, Staffordshire, UK). The total flavonoid content was calculated from a calibration curve and the result expressed as mg rutin equivalent in dry-weight.

2.2.7 *Caffeine*

The caffeine in tea leaves of different tea clones was analyzed, as described by Jeyanthi, Seshiah, and Trisha (2014). Briefly, 0.1 g of tissue was placed in 30 mL boiled distilled water, followed by stirring the mixture for about 2 min on an electric stirrer. The sample was then filtered and left to cool on ice. To the infusion, 2 mL of dichloromethane was added, followed by separation of the dichloromethane phase from the water phase using a separating funnel. This was repeated four times and the resultant volume of dichloromethane was measured. The absorbance of the resultant solution was then measured using a spectrophotometer (Jenway 6305-Bibby Scientific, Stone, Staffordshire, UK) at 276 nm.

2.3 *Statistical analysis*

One-way analysis of variance was performed using Agricolae R package for the tea traits assessed. The differences between mean values of each physicochemical descriptor were compared using Fisher's least significant difference (LSD) at 5% probability level. Principal component analysis was performed to determine the most contributing physicochemical parameter associated with tea quality. Pearson's correlation coefficients between different physicochemical descriptors were computed using Metan R package (Olivoto and Lúcio 2020). Hierarchical cluster analysis was performed using Cluster R package to estimate and visualize the similarity among different tea genotypes based on Euclidean coefficients.

3. Results

3.1 *Variation among tea accessions based on the physicochemical parameters*

The physiochemical parameters, namely, catechin, flavonoids, caffeine, crude fiber, polyphenol, fermentation rate, brightness, and color, were used to characterize 58 tea clones at Rwebitaba TRC. The tea clones varied significantly for all the traits assessed (Table S1), forming four clusters (cluster A, B, C, and D) with differentially unique attributes (Figure 1). Cluster A was the largest cluster with 27 tea accessions, whereas cluster C was the smallest one, with 9 tea accessions. Each cluster had unique attributes that distinguished it from the others. These attributes can be used to select tea genotypes for the production of

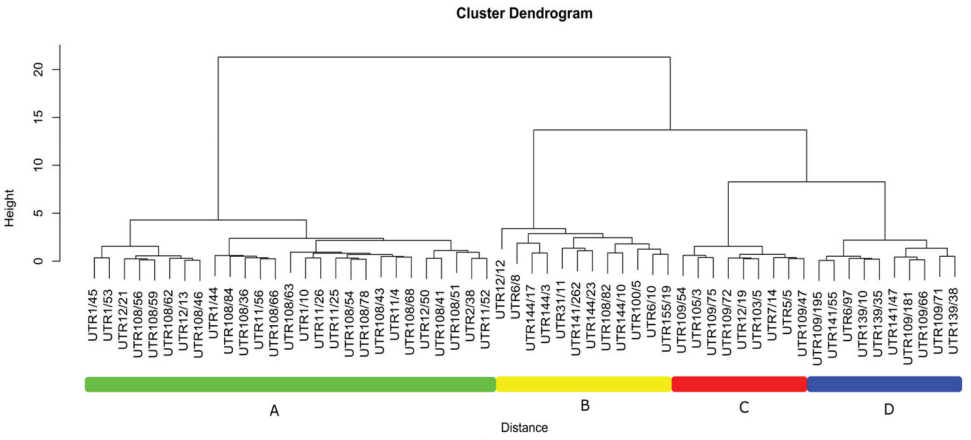

Figure 1. Hierarchical clustering of selected Ugandan tea genotypes using different physicochemical attributes of tea. Tea clones have been clustered in four clusters namely A, B, C & D based on Ward's distance to capture minimum variance between individuals of the same cluster.

different types of teas; such as black tea, green tea, oolong tea, etc. For example, cluster A contained tea accessions that are (i) slow fermenters or non-fermenter, (ii) low polyphenol content (0.5–2.3%), (iii) low crude fiber (1.8–2.7%), and (iv) high proportion of catechin (4.1–6.2%), caffeine (8.7–8.8%), and flavonoid content (3.05–3.87%). In contrast, tea accessions in cluster B exhibited very high rate of fermentation (rapid/fast fermenters) with (i) very high polyphenol content (9.6–26.7%), (ii) high catechin content (3.0–8.7%), and crude fiber (10.4–18.9%), (iii) moderate caffeine (2.4–4.3%) and very low flavonoid content (0.0–0.1%). Cluster C had very low flavonoid and caffeine, ranging from 0.0% to 0.1%, whereas cluster D had low flavonoid (0.2–0.3%) and catechin content (0.17–0.22%). Though tea accessions in cluster C and D had high crude fiber content, the distinguishing characteristic between these two clusters was that tea genotypes in cluster C had high catechin content, ranging from 5.06% to 6.03%.

In brief, the distinguishing characteristic of tea accession in different clusters included high flavonoid, catechin, and caffeine content for tea in cluster A; high polyphenol and crude fiber content in cluster B; high crude fiber and catechin content in cluster C; and high crude fiber in cluster D.

Based on the eigen values of the principal components (PCs), the first two PCs accounted for 84.1% of the total variation present in the accessions (Table 2). Eigen values of the first two PCs were greater than 1, indicating that the two PCs significantly contributed to the variation existing in the accessions studied.

The first PC accounted for 60.1% of the total variation (Table 2) and was predominantly associated with flavonoids, color, and caffeine (Table 2). Based on these three physicochemical parameters, the tea genotypes formed two major groups (Figure 2). These three physicochemical parameters influence the categorization of tea genotypes, either as green tea or black tea. For example, a total of 27 tea accessions (cluster A) had a flavonoid content ranging from 3.05% to 3.95%, whereas the remaining 31 tea accessions had flavonoid content ranging from 0.03% to 0.27% (cluster B, C, and D).

Similarly, the physicochemical parameters, including polyphenol content, brightness, and fermentation rate, accounted for the most variation associated with the second PC (Table 3). The contribution of these physicochemical parameters to the clustering of tea clones showed that these physicochemical parameters could be used as useful markers for selecting clones for quality

Table 2. Eigen values of the correlation matrix from the principal component analysis of eight physicochemical descriptors.

Principal components	Eigen values	Difference between Eigen values of different PCs	Variance (Cumulative)
PC1	4.8	2.9	60.1
PC2	1.9	1.2	84.1
PC3	0.7		94.4

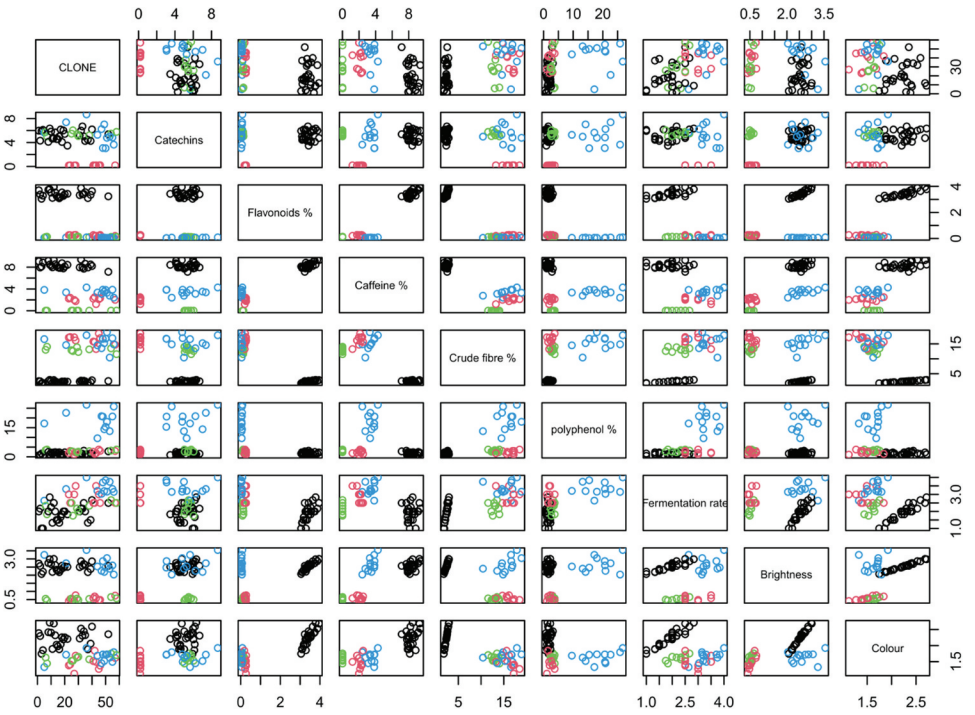

Figure 2. Pairwise comparison of physicochemical components, showing how the correlation between the selected physicochemical attributes affects the clustering of different tea clones.

Table 3. Eigen vectors for first the three principal components of the eight physicochemical descriptors.

Attribute	PC 1	PC 2	PC 3
Brightness	0.297	0.514	0.169
Caffeine	0.427	0.089	0.275
Catechin	0.211	0.394	−0.773
Color	0.403	0.119	0.228
Crude fiber	−0.443	0.104	0.070
Fermentation rate	−0.291	0.405	0.468
Flavonoids	0.448	−0.078	0.143
Polyphenol	−0.201	0.614	− 0.032

improvement. Pairwise comparison of the evaluated tea clones based on the selected physicochemical attributes revealed that flavonoid content, crude fiber, color, and brightness clearly characterized the tea clones in unique groups.

3.2 *Correlations among different physiochemical parameters*

To determine the best physicochemical parameter that can be used to characterize tea genotypes based on their quality and use, a correlation analysis among the eight parameters was done (Figure 3). There was

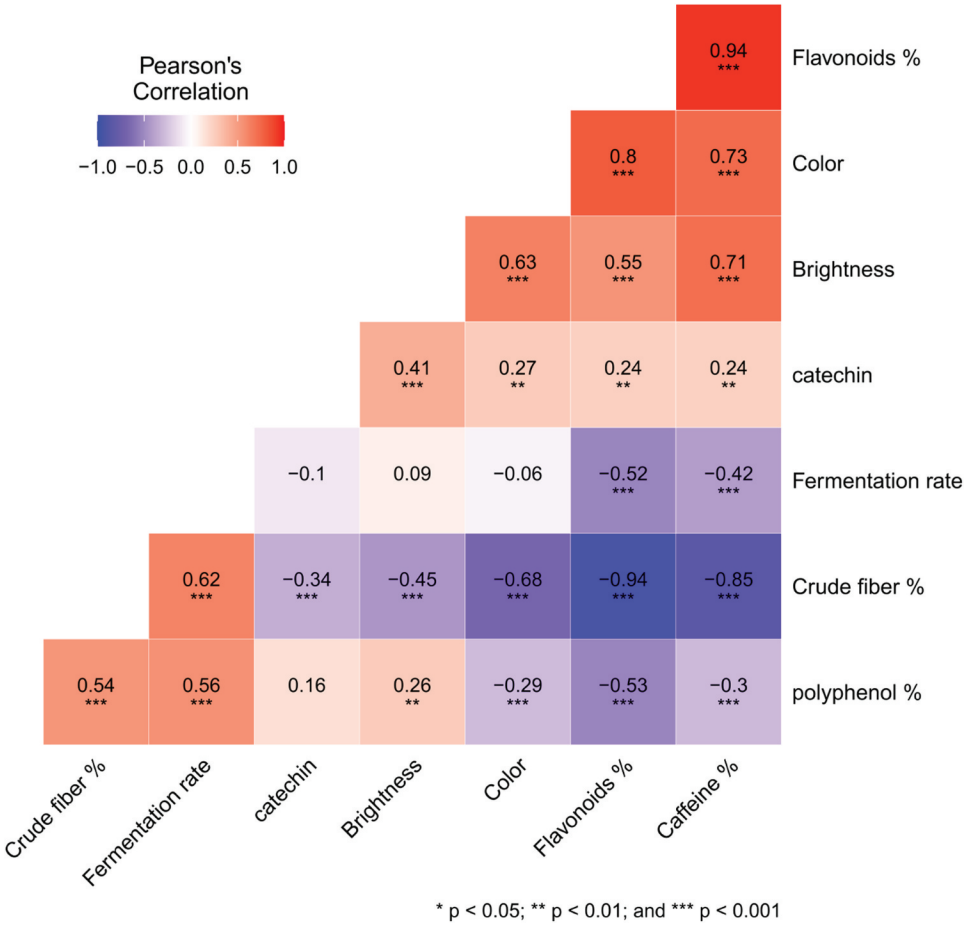

Figure 3. Pearson's correlation between different physicochemical attributes at 5%, 1% and 0.1% level of significance.

a strong positive correlation between the caffeine and flavonoid contents (R = 0.94, $P < 0.001$), color (R = 0.73, $P < 0.001$), brightness (R = 0.71, $P < 0.001$), and catechins (R = 0.24, $P < 0.05$). Therefore, caffeine can be used to predict the level of flavonoids and catechin and also give an indication on the two quality indicators, viz., color and brightness.

Fermentation rate had a positive correlation with polyphenol content (R = 0.56, P < 0.001) and crude fiber (R = 0.62, P < 0.001). Therefore, tea genotypes with high fermentation rate generally had high fiber content. On the other hand, crude fiber content had a negative correlation with the level of catechins and brightness, with correlation coefficient of 0.34 ($P < 0.001$) and 0.45 ($P < 0.001$), respectively. Fermentation rate, on the other hand, showed a negative correlation with flavonoid content, caffeine, and color.

3.3 *Selection of the best tea clones for black and green tea*

Based on fermentation rate, the 58 tea accessions were categorized into four groups: (i) rapid fermenters (0–30 min), (ii) fast fermenters (31–50 min), (iii) moderate fermenters (51–70 min), and (iv) slow or non-fermenters (71–90 min) (Table 4). Only one tea accession, UTR12/12, was identified as a rapid fermenter. This clone had the highest catechin (8.69%) and polyphenol contents (26.7%). In addition, this clone had the highest score for quality indicator brightness of 3.6. Based on current findings, only two clones UTR12/12 (26.7%) and UTR6/8 (25.8%) would be considered as high-quality clone, whereas UTR108/82 (22.6%) would be regarded as medium high quality. Other clones (UTR144/10 (20.9%), UTR144/17 (20.7), and UTR6/10 (21.9)) could be graded as medium quality. Thus, adding more evidence that these clones are best suitable for black tea-quality improvement in Uganda. UTR12/12 showed a unique pattern when compared to all the tested tea accession (Figure 4). When compared to the check tea clone for quality (UTR6/8), UTR12/12 performed better in all measured physicochemical parameters, including the tea quality indicators, color, and brightness. These results showed that tea clone UTR12/12 was a very high-quality clone.

Based on the results of the average linkage cluster analysis, the accessions studied were grouped into four main clusters (Figure 1), implying existence of considerable variation for the physicochemical markers in Ugandan accessions that can be exploited for quality improvement. In cluster B, the candidate clones UTR144/23, UTR144/10, UTR108/82, 6/10, UTR155/19, UTR144/17, UTR144/3, UTR31/11, UTR100/5, UTR6/8, UTR12/12, and UTR5/5 clustered together with the standard commercial checks clones UTR6/10, UTR6/8, UTR108/82 and UTR100/5. These accessions belonged to fast fermenters and moderate fermenter category (Table 4) and also exhibited high polyphenol content, high catechin content and crude fiber, moderate caffeine, and very low flavonoid content. These clones, therefore,

Table 4. Categorizing selected Ugandan tea genotypes based on the fermentation rate.

Fermentation rate	Accessions	Category
4 (0–30 min)	UTR12/12	Rapid fermenting
3–3.9 (31–50 min)	UTR109/181, UTR109/66, UTR109/71, UTR6/8, UTR6/10, UTR155/19, UTR144/23, UTR144/10, UTR141/47, UTR139/38, UTR31/11, UTR144/3, UTR144/17	Fast fermenting
2–2.9 (51–70 min)	UTR1/10, UTR1/44, UTR100/5, UTR103/5, UTR108/36, UTR108/41, UTR108/43, UTR108/51, UTR108/54, UTR108/68, UTR108/78, UTR108/82, UTR109/195, UTR109/47, UTR109/72, UTR109/75, UTR11/25, UTR11/26, UTR11/4, UTR1/52, UTR11/56,, UTR6/97, UTR7/14, UTR5/5, UTR2/38, UTR141/55, UTR12/50 UTR139/10, UTR139/35, UTR12/19	Moderate fermenting
1–1.9 (71–90 min)	UTR1/45, UTR1/53, UTR105/3, UTR108/46, UTR108/56, UTR108/59, UTR108/62, UTR108/63, UTR108/66, UTR108/84, UTR109/54, UTR12/13, UTR12/21	Slow fermenting

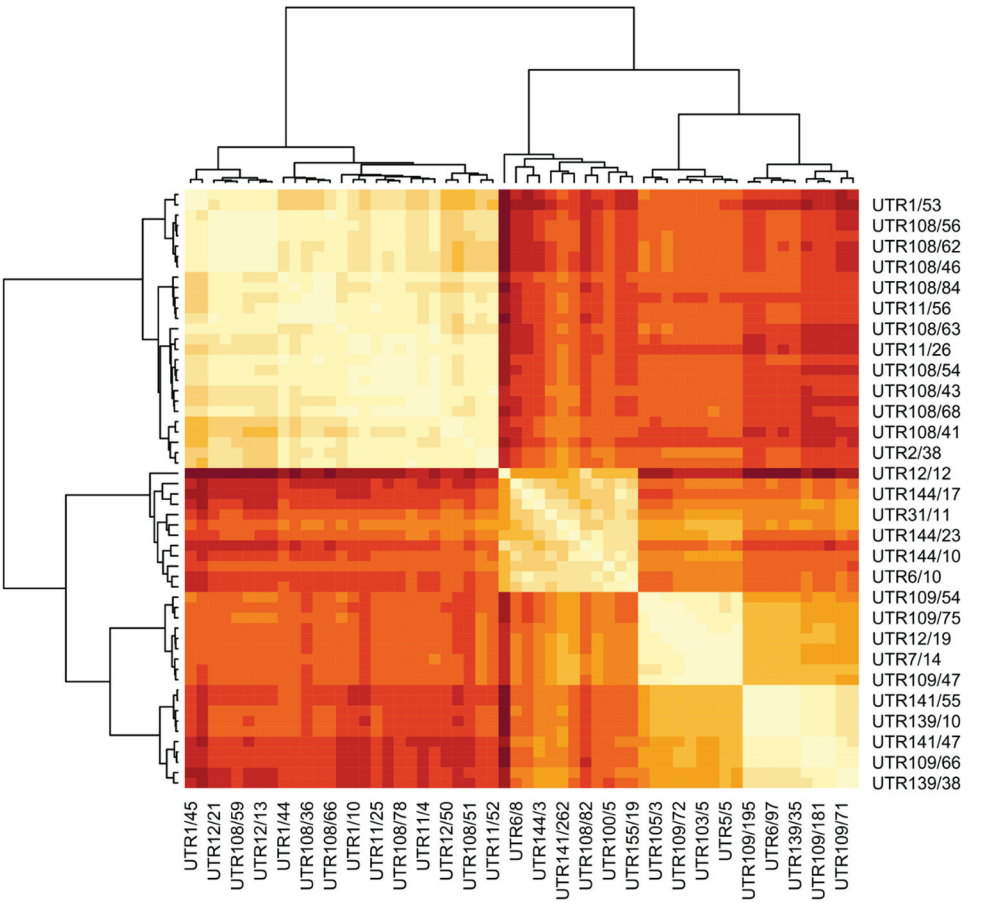

Figure 4. Heatmap showing pairwise comparison of each tea genotype. Light color shows that the compared tea accessions are similar, whereas dark color shows dissimilarity between the compared tea accessions. Based on the heatmap, UTR12/12 showed the most intense coloring, demonstrating that the tea clone was different from other tea clone in the physicochemical attributes measured.

could be advanced as commercial cultivars for black quality tea in the near future. On the contrary, a large number of tea accession from cluster A belonged to slow or non-fermenter category. A total of five tea clones (UTR12/12, UTR144/10, UTR144/17, UTR155/19, and UTR144/3) were selected as candidate tea clones for black tea, whereas nine tea accessions were selected as candidate tea clones for green tea (Table 5). The nine tea accessions selected for green tea had lower polyphenol content compared to the tea accessions selected as black tea.

The clustering of the 58 tea accessions showed that stock 108 contributed significantly to green tea accessions (cluster A), whereas stock 144 contributed to high-quality black tea (cluster B). These results showed that stocks 144, 108, 109, and 139 could be explored further as parental genotypes.

Table 5. Mean and standard error (se) of fresh leaf physicochemical parameters in different tea accessions.

	Physicochemical composition (%)							
Accessions	Polyphenol	Catechin	Flavonoids	Crude fiber	Caffeine	Color	Brightness	Fermentation
Potential black tea genotypes								
UTR 6/8	25.8 ± 0.6	3.6 ± 1.7	0.1 ± 0.0	14.8 ± 1.6	2.4 ± 0.1	1.8 ± 0.0	2.0 ± 0.8	3.1 ± 0.6
UTR 12/12	26.7 ± 1.0	8.7 ± 0.2	0.1 ± 0.0	17.9 ± 2.3	4.3 ± 0.5	1.9 ± 0.1	3.6 ± 0.4	4.0 ± 0.3
UTR 108/82	22.7 ± 6.0	7.4 ± 0.8	0.1 ± 0.0	14.6 ± 1.7	3.4 ± 0.4	1.7 ± 0.1	2.7 ± 0.3	3.3 ± 0.1
UTR 6/10	21.9 ± 2.6	5.0 ± 0.9	0.1 ± 0.0	16.6 ± 0.7	3.8 ± 0.1	1.6 ± 0.0	2.7 ± 0.2	3.4 ± 0.2
UTR 144/10	20.9 ± 0.6	6.2 ± 0.4	0.1 ± 0.0	13.5 ± 1.1	3.1 ± 0.1	1.5 ± 0.0	2.3 ± 0.4	3.0 ± 0.1
UTR 144/17	20.7 ± 1.2	3.1 ± 0.4	0.0 ± 0.0	16.7 ± 1.0	3.9 ± 0.4	1.7 ± 0.0	2.9 ± 0.5	3.7 ± 0.1
UTR 155/19	18.0 ± 0.2	4.8 ± 1.7	0.1 ± 0.0	18.9 ± 1.6	3.3 ± 0.2	1.7 ± 0.2	3.1 ± 0.8	3.2 ± 0.1
UTR 100/5	17.1 ± 0.8	5.7 ± 2.0	0.1 ± 0.0	16.6 ± 2.0	3.8 ± 0.3	1.3 ± 0.1	3.3 ± 0.6	2.6 ± 0.2
UTR 144/3	15.4 ± 1.3	3.1 ± 0.5	0.0 ± 0.0	16.7 ± 1.6	3.8 ± 0.1	1.7 ± 0.1	2.5 ± 0.3	3.8 ± 0.1
Potential green tea genotypes								
UTR 108/54	2.4 ± 0.4	5.7 ± 0.2	3.5 ± 0.3	2.5 ± 0.2	8.5 ± 0.6	2.3 ± 0.2	2.6 ± 0.2	2.0 ± 0.2
UTR 108/59	1.8 ± 0.6	4.6 ± 0.8	3.2 ± 0.3	2.1 ± 0.3	8.0 ± 0.5	2.0 ± 0.3	2.3 ± 0.3	1.5 ± 0.5
UTR 11/26	1.8 ± 0.1	6.0 ± 0.6	3.9 ± 0.1	2.7 ± 0.1	9.4 ± 0.4	2.5 ± 0.1	2.9 ± 0.1	2.2 ± 0.1
UTR 1/45	1.7 ± 0.1	5.9 ± 2.5	3.1 ± 0.6	1.8 ± 0.2	8.1 ± 1.9	1.7 ± 0.2	2.1 ± 0.3	1.0 ± 0.0
UTR 108/66	1.5 ± 0.3	4.1 ± 0.2	3.5 ± 0.3	2.4 ± 0.3	8.7 ± 0.5	2.3 ± 0.2	2.6 ± 0.3	1.8 ± 0.4
UTR 1/53	0.5 ± 0.1	6.1 ± 0.4	3.3 ± 0.2	1.9 ± 0.0	8.8 ± 0.5	1.9 ± 0.0	2.2 ± 0.1	1.0 ± 0.0
UTR 11/25	1.2 ± 0.0	5.2 ± 0.1	3.3 ± 0.1	2.4 ± 0.0	8.0 ± 0.3	2.2 ± 0.0	2.5 ± 0.0	2.0 ± 0.0
UTR 108/63	1.2 ± 0.8	6.2 ± 1.3	3.2 ± 0.0	2.2 ± 0.1	7.9 ± 0.3	2.0 ± 0.1	2.3 ± 0.0	1.7 ± 0.3
UTR 12/13	1.2 ± 0.1	4.5 ± 0.7	3.1 ± 0.0	2.0 ± 0.1	7.9 ± 0.3	1.9 ± 0.1	2.2 ± 0.1	1.3 ± 0.3

4. Discussion

This study was carried out to characterize the existing tea germplasm at RTRC in Uganda to inform future breeding initiatives for market-preferred tea varieties in Uganda. RTRC was the main tea research station for TRIEA in the 1960s, until tea research activities were abruptly stopped in the late 1970s. Therefore, characterizing tea germplasm was undertaken as a vital step for future tea improvement initiatives in Uganda. Accordingly, physicochemical parameters, viz., flavonoid content, catechins, polyphenol content, crude fiber, caffeine, fermentation rate, brightness, and color, were assessed.

The study results revealed a high degree of physicochemical diversity among the Uganda tea accessions for all the traits assessed, implying that the studied tea clones can be selected for different utilization purposes, such as green or black tea. The selection of tea genotype for a particular purpose depends on the inherent physicochemical attributes. In this study, flavonoid content, color, and content of caffeine contributed to the clustering of green tea genotypes (cluster A), whereas polyphenol content, brightness, and fermentation rate were the most important physiochemical attributes for black tea genotypes (Cluster B).

Pairwise comparison between these key physicochemical attributes revealed that some of the attributes were highly correlated with each other. This correlation can guide breeders in the selection of key physicochemical attributes that can facilitate an efficient and cost-effective selection for high-quality tea genotypes and functional characterization. For example, caffeine content had a high positive correlation with total catechin, flavonoid content, and quality indicators, such as brightness and color, whereas fermentation rate had a strong positive correlation with polyphenol. Therefore, two physicochemical attributes, caffeine content, and fermentation rate, can be used in combination to select high-quality tea clones. This confirms the report that flavanol composition and caffeine content in green leaf can be used as quality indicators of black tea (Obanda, Owuor, and Taylor 1997). Black teas are evaluated by comparing the color, strength, brightness and briskness of infusion. Of these, caffeine contributes toward briskness; therefore, the higher the amount of caffeine the better the tea infusion. This is because tea caffeine forms a self-association with theaflavin and its gallate esters forming tea cream, that contributes to the high-quality score of the tea infusion (Charlton et al. 2000).

Fermentation has been reported as the most effective criterion to screen tea germplasm, especially at the early stage of selection (Ellis and Nyirenda 1995). In this study, the effectiveness of using fermentation rate was tested. Based on this analysis, one tea clone, UTR12/12, was selected as a rapid fermenter. This clone performed better than the reference tea variety for quality (UTR6/8) and commercial tea varieties (UTR108/82) in Uganda. This clone had the highest polyphenol content, total catechin and the highest

score for brightness, therefore it was selected as a high-quality black tea clone. However, fermentation rate had a negative correlation with catechin content, color, flavonoid content and caffeine content. Rate of fermentation in tea is influenced by the action of polyphenol oxidase and peroxidase enzymes, which catalyze the oxidation of catechins to theaflavins and thearubigins (Tang et al. 2018). Results showed that the tea clones with a high amount of catechins had relatively a reduced rate of fermentation, which suggested that the higher the amount of catechin, the longer the fermentation process.

The quality of black tea is determined by the ratio of theaflavins to thearubigins in made tea. The higher the ratio, the higher the quality of made tea for black tea. This ratio is also directly related to the rate and time taken during the fermentation process by the endogenous polyphenol oxidase and peroxidase enzymes. Initially, enzymatic oxidation of catechins, especially epigallocatechin and epicatechin, produces theaflavins. However, when the fermentation process takes long, the theaflavins are degraded or converted to theanaphthoquinone that is transformed into thearubigins, which affects the theaflavin/thearubigin ratio and quality of black tea (Li et al. 2010). Thus, reliable selection of high-quality black tea genotypes requires a combination of fermentability test and other parameters, such as caffeine content, brightness, and color. The ease and low cost of the fermentation test can facilitate the early screening and selection of potential quality accessions in a tea breeding program (Karori et al. 2010).

Our results also showed that fermentation rate had a high positive correlation with the polyphenol content and crude fiber content. These are two attributes that were in very high proportions in black-tea genotypes (Cluster B). Both high polyphenol content and high polyphenol oxidase activity, which affect the rate of fermentation, have been reported as good indicators of high processing suitability of a tea clone, which result in high-quality made black tea (Zhang et al. 2020a, 2020b). Kilel et al. (2013) categorized tea genotypes into four groups based on the total polyphenol content, as high-quality teas have 24.8–27.1%; medium–high quality ones have 22.5–24.4%; medium quality teas have 19.6–22; and low-quality teas have 17.5–19.2%. Based on the polyphenol content, two tea clones (UTR 6/8 & UTR 12/12) and one tea clone (UTR 108/82) were characterized as high quality and medium–high quality black tea clones, respectively. In contrast, tea genotypes that were selected for green tea had comparatively very low polyphenol content. Low polyphenol content has been reported in high-quality green tea varieties in traditional green tea-processing countries, such as Japan and China (Patel et al. 2019).

On the other hand, some tea clones were non-fermenters or had a very low rate of fermentation. This category of tea clones were grouped in Cluster A (green tea clones). The low fermentation rate could be attributed to inherent

factors, such as low crude fiber content among the green tea genotypes. In this study, fermentation had a high positive correlation with crude fiber content; a negative correlation was also exhibited between amount of catechin and crude fiber. The catechin level in green tea, total theaflavins, brightness, color, and flavor index have been reported to decrease with an increase in crude fiber (Owuor and Obanda 1998). Our results showed that tea clones in cluster A, characterized as green tea, had low crude fiber compared to the black tea clones in cluster B. These results are consistent with the previous findings by Takeo (1966), who reported that the polyphenol oxidase activity within black tea clones was higher than that in green tea clones. High crude fiber in different tea accessions on farmers' fields may either be due to inherent physiological and genetic factors or human factors, such as coarse tea plucking (Śmiechowska and Dmowski 2006; Teshome 2019) or other management practices. Agronomic practices such as fertilizer application, which have been reported to cause an elevation in the levels of crude fiber (Venkatesan and Ganapathy 2004) and flavanoids (Hilton, Palmer, and Ellis 1973) even in the commercial clones. Therefore, care should be taken to ensure that tea genotypes with low crude fiber are chosen and management practices are implemented judiciously to maintain high green-tea quality.

Tea quality, both for green and black tea, is influenced by catechin content. Catechins in tea are responsible for flavor, bitterness, and astringency, which contribute to the quality of tea. In black tea, catechins are transformed into different forms of theaflavins, whereas they remain unchanged in green tea. All the selected tea genotypes for green tea and black tea (Table 5) had relatively high catechin content. Catechin and its derivatives, such as theaflavin, offer many health benefits (Leung et al. 2001). Catechins have been reported to provide health-promoting benefits through anti-oxidants, UV protection, and anti-microbial, anti-allergenic, anti-inflammatory, anti-viral and anti-cancer activities (Bae et al. 2020). Therefore, tea clones with a relatively high catechin content, such as UTR 11/25, UTR 108/66, UTR 108/54, and UTR 108/59, can be selected, evaluated in multi-location trials and advanced as green-tea genotypes in Uganda.

5. Conclusions

Tea genotypes evaluated in this study possessed unique physicochemical attributes that enabled the categorization of these tea clones into different functional groups. The tea genotypes were clustered in four major groups, from which potential green and black tea clones could be selected. High correlations among different physicochemical parameters revealed that a few physicochemical attributes, such as fermentation rate, caffeine content, and brightness, were good indicators of tea quality. Optimizing these parameters using a larger

number of tea genotypes should enable selection of the best set of parameters that can be used to rapidly and cost-effectively screen germplasm for potential high-quality tea genotypes. Based on the key physiochemical parameters analyzed, the clone "UTR12/12" can be considered for advanced trials and selection as a potential superior commercial clone for production of black tea.

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Disclosure statement

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