RESEARCH ARTICLE

Utilization of within location non-replicated multi-locational yield trials and GGE biplot methods for testing adaptability of tea [(*Camellia sinensis* (L.) O. Kuntze)] over diverse environments

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Abstract

Selection of tea [*Camellia sinensis* (L.) O. Kuntze] cultivars with wide adaptability over diverse environments is an important criteria to get prior information to recommend the cultivar for a particular region. A study was carried out to identify the most adaptable and stable tea genotypes for recommendation in the Up-country tea growing region in Sri Lanka by evaluating several potential tea genotypes for their wide adaptability and stability over diverse environments based on the analysis of GE interaction using within location non-replicated multi-environmental yield trials and GGE biplot methods. Six promising tea genotypes with desirable agronomic traits selected based on preliminary yield trials were evaluated along with a standard check in large scale within location non-replicated multi-locational yield trials at six locations (tea estates) in the Up-country tea growing region in Sri Lanka. The crop yields were recorded by harvesting green leaves in every seven days for three years covering six seasons (1st pruning cycle). Yield data were analyzed by within location non-replicated multi-locational yield trials and GGE biplot analysis. The genotype 272 was identified as the most adaptable and stable genotype followed by 582 and rest of the genotypes showed poor adaptability in the test region.

Keywords: Adaptability, *Camellia sinensis*, diverse environments, GGE biplot, within location non-replicated multi-locational trials

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze], the second most consumed beverage in the world, is a rich source of polyphenolic compounds. Tea plant is a woody-perennial of which the tender shootsare used to make the end product (Banerjee 1992). The Asian countries mainly India, China and Sri Lanka contribute to more than half of the world tea production so that the tea trade has become an important business for those countries. The development and sustainability of the tea industry relies mainly on the success of tea improvement programs.

Tea improvement program, in general, is a multi-stage procedure including multi-location testing with a span of over 20-25 years (Gunasekare 2008). Plants can experience abiotic stresses resulting from the shortage of an essential resource or from climatic extremes (Grando and Ceccarelli 2009). Therefore, the genotypes are initially evaluated for yield attributes and the high yielding genotypes are then screened for other important characteristics such as made tea quality, pest and disease resistance and drought tolerance (Kulasegaram 1978).

Tea in Sri Lanka is grown under widely varying climatic conditions, wide range of elevations (0–1800 AMSL) and hence, considerable cultivar differences in response to environmental changes have been identified. Therefore, the same genotypes can have different phenotypes, based on the environmental influence. At the last stage of tea

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Table 1. The data on pest and disease resistance and quality parameters of promising tea genotypes

Genotype	Parents	BB	SHB	P	TF(%)	TR(%)	TPP(%)	Qlt.
15 (TRI5001)	777 op	R	MS	MT	1.1	15.6	28.5	H
89 (TRI 5002)	777 op	R	S	MT	1.1	16.8	24.4	H
210 (Breeding Line)	2025 op	S	S	MT	0.9	14.8	25.4	L
272 (TRI 5003)	243 op	R	MS	HT	1.0	19.6	25.4	M
497 (Breeding Line)	2025 op	MR	S	S	1.0	17.2	26.2	L
582 (TRI 5004)	2043 op	R	MS	MT	0.9	16.8	23.8	L
DT1	ES	R	S	R	1.2	18.4	24.9	H

OP = Open pollinated; ES = Estate selection; BB = Blister blight; SHB = Shot-hole borer; P = Pratylenchus loosi; TF = theaflavins; TR: thearubigins; TPP = total poly-phenols; Qlt: black tea quality; R = resistant; MR = moderately resistant; M= Moderately susceptible; S = Susceptible; HT = Highly tolerant; MT = Moderately tolerant; H High quality; M = Medium quality and L = Low quality

cultivar evaluation, green leaves are harvested in seven days intervals for at least one pruning cycle, *i.e.,* three years. The period between two consecutive pruning events is called a pruning cycle (Wijeratne 2008). When different genotypes exhibit differential responses to different environments, a genotype x environment (GE) interaction is said to occur (Acquaah 2007). Plant breeders are not only interested in developing high-yielding cultivars but also interested in developing cultivars with wide adaptability and stability over locations, seasons and years so that GE interaction should be properly analyzed. Thus, for the identification of new genotypes for commercial release, it is necessary to test different genotypes in different environments over several years to evaluate their adaptability and stability.

A variety of statistical methods such as regression method (Eberhart and Russell 1966), variance component method (Abeysiriw ardena et al. 1991; Anputhas et al. 2011), additive mean effect and multiplicative interaction effect (AMMI) and genotype and genotype x environment interaction (GGE) biplot analysis have been proposed for adaptability and stability analysis of genotypes. Lin and Binns (1988) calculated the measure of varietal superiority as the distance of the varietal response (variety yield) from the maximum response (maximum variety yield in the trial) averaged over all environments. Abeysiriwardena (2001) proposed an efficient and simple method to analyze on-farm multi-locational yield trials with few genotypes and minimum replicates. The studies carried out earlier (Wickramaratne 1981; Wachira et al. 1990; Wachira et al. 2002; Murti et al. 2014) used above indicated statistical models to determine the implications of GE interaction in tea breeding and selection. However, all these methods require within location replication in yield trials. Within location replication in multi-locational, large scale commercial yield trials in farmers' fields even with few genotypes is practically difficult, cumbersome and comparatively more expensive. Employing of a method to conduct within location non-replicated multi-locational yield trials and analysis of data from such trials would be very useful and ideal to be used in tea variety testing program. Abeysiriwardena and Samita

(2016) had proposed such an efficient and simple method for testing adaptability of crop varieties. In addition, the GGE biplot method is considered as an attractive method to identify superior genotypes with wide adaptability and stability because it provides effective overview of the genotype main effect plus genotype x environment interaction (GGE) effect (Yan2015). This method has been used for the evaluation of stability and adaptability of various annual crop species such as rice (Samonte et al. 2005), sugarcane (Glaz and Kang 2008), soybean (Dalló et al. 2019) and cowpea (Cruz et al. 2020) in multi-environment trials. Recently, Reddy et al. (2022) also used GGE biplot analysis to identify the ideal cultivars and testing locations of pearl millet (*Pennisetum glaucum* L.R. Br.) for peninsular India. Keeping in view the above, the present study was carried out to identify the most adaptable and stable tea genotypes for recommendation in the Up-country tea growing region in Sri Lanka by evaluating several potential tea genotypes for their wide adaptability and stability over diverse environments based on the analysis of GE interaction using within location non-replicated multi-environmental yield trials and GGE biplot methods.

Materials and methods

Plant material, experimental conditions, and evaluations

Six promising tea genotypes with desirable agronomic traits, which have been selected based on preliminary yield trials were finally tested in large scale (about 150–250 plants per plot) non-replicated multi-locational commercial yield trials at six locations namely, Lamelier Division, St Coombs Estate (LM), St. Coombs Estate (SC), Dayagama West Estate (DG), Glasgow Estate (GG), Mattakelle Estate (MK) and Pedro Estate (PD) to represent diverse environments in the Up country tea growing region in Sri Lanka. Agro-climatic and meteorological data of tested locations are presented in Supplementary Table S1 and S2. A recommended high quality made tea producing cultivar DT1 was used as the standard check for the yield comparison. The data on pest and disease resistance and quality parameters of promising

tea genotypes recorded are given in Table 1. The criteria of selection of lands were based on agro-ecological zones in Sri Lanka (Panabokke and Kannangara 1975). Tea genotypes in each trial were grown under the estate management practices.

The crop yields were recorded by harvesting shoots (two leaves and a bud) in every 7 day interval for three years (1st pruning cycle). Generally, the pruning cycle for Up-country tea growing region is three year period, depending on the growth rate. The green leaf yield was converted into made tea yield and location yield collected over three years was measured in terms of kg/hectare/year. There are two seasons, namely, Wet (May to October in the same year) and Dry (November to April in the following year) based on two tropical monsoons in the Up-country tea growing region within a year (Department of Meteorology 2019). Thus, yield records over six seasons spreading over three years were available in each location. Each season included six months and made tea yield averaged over all locations per each season was expressed in terms of kg/hectare/month.

Statistical analysis

Adaptability and stability of the test genotypesacross locations and seasons was evaluated by the method described by Abeysiriwardena and Samita (2016) using within location non-replicated multi-locational on-farm yield trials. There were six locations and six seasons over three years covering one pruning cycle. Two separate analyses were performed for locations and seasons. The statistical model for the analysis is given below.

$$d_{ij} = \mu + V_i + L(V)_{ij}$$

Where d_{ij} = yield deviation of the ith variety in the jth location or season from the maximum variety made tea yield in that location or season, μ = grand mean of the plot yield deviations, V_i is the effect of the ith variety, $L(V)_{ji}$ is the effect of the jth location or season within i[th] variety and i = 1….p and j = 1…n. In the method, two adaptability parameters *viz*., mean deviation across locations or seasons (d_i) and the

variance of deviations across locations or seasons or the stability parameter (s_i^2) for each variety were computed as same as in the reference method.

If the V_i term was significant, mean separation was performed using Duncan's Multiple Range Test (DMRT). The stability parameters were tested for homogeneity of variances using Bartlett's test (Bartlett 1947). If the stability parameters were found to be heterogeneous, separation of variances was performed using F tests to detect comparatively what variances are lower or higher than the others. Genotypes were selected simultaneously for lower $d_i$and non-significant s_i^2.

The statistical theory of GGE methodology has been explained in detail by Yan and Tinker (2006). The Multi-locational trial (MLT) analysis was carried out at a network of sixdifferent locations. The MLT data was analyzed without scaling ('Scaling 0' option) to generate a tester centered (centering 2) GGE biplot. For genotype evaluation, genotype-focused singular value partitioning (SVP = 1) was used using the 'Mean versus stability' option of GGE biplot software, while for location evaluation, environment-focused singular value partitioning (SVP = 2) was employed using 'Relation among testers' option. 'Which-won-where' option was used to identify which genotype was the winner in a given set of environment and to identify mega-environments. Statistical analyses were performed using GGE biplot software Version 4.1 (Yan and Tinker 2006).

Results and discussion

Average made tea yield over seasons (kg/ha/year) at 6 locations and average made tea yield over six locations (kg/ha/month) in six consecutive seasons of seven tea genotypes in the Up-country tea growing region of Sri Lanka are presented in Tables 2 and 3, respectively. Location means varied from 2207 kg/ha the lowest to 4136 kg/ha the highest so that adequate yield variation across locations has been achieved. Similarly season means varied from 135 kg/ha the lowest to 286kg/ha the highest so that adequate

Table 2. Average made tea yield over seasons (kg/ha/year) at six locations

Genotype	Locations[†]					
	LM	SC	DG	GG	MK	PD
15 (TRI 5001)	2418	1944	3701	2275	2669	2989
89 (TRI 5002)	2894	1956	5310	2439	2255	2078
210 (Breeding Line)	2604	2095	4273	2641	2248	3281
272 (TRI 5003)	3112	2714	4588	3098	3565	3621
497 Breeding Line)	3185	2167	3292	2236	2536	3555
582 (TRI 5004)	2346	2345	4495	3039	3023	3071
DT1	2385	2231	3296	2514	2685	2243
Location mean	2706	2207	4136	2606	2712	2977

[†]LM = Lamilier Division, St. Coombs Estate; SC = St. Coombs Estate; DG = Diyagama West Estate; GG = Glasgo Estate; MK = Mattakelle Estate and PD = Pedro Estate

Table 3. Average made tea yield over locations (kg/ha/month) in six seasons.

Genotype	Seasons†					
	SW1	NE1	SW2	NE2	SW3	NE3
15 (TRI 5001)	137	203	171	155	248	269
89 (TRI 5002)	103	187	184	190	297	324
210 (Breeding Line)	131	216	181	179	264	282
272 (TRI 5003)	164	267	294	183	306	330
497 (Breeding Line)	134	199	177	155	238	244
582 (TRI 5004)	159	255	250	199	255	306
DT1	116	182	157	140	227	249
Seasons mean	135	216	202	172	262	286

† SW = South-west monsoon and inter-monsoon (May to October); NE = North-east monsoon and inter-monsoon (November to April); 1-3 numbers indicate the year of harvesting

Table 4. Mean deviations (d) and the stability parameters (s_i^2) across locations and over seasons for six tea genotypes and the standard check (DT1) tested in six locations over six seasons in the Up-country tea growing region, Sri Lanka

Genotype	Across locations†			Over seasons†		
	d_i	Si^2	AR#	d_i	Si^2	AR#
15 (TRI 5001)	916[b]	122841[a]	3	63[cd]	1052[b]	3
89 (TRI 5002)	760[b]	344704[a]	3	46[bc]	1952[b]	3
210 (Breeding Line)	725[b]	139930[a]	3	51[c]	1032[b]	3
272 (TRI 5003)	133[a]	84295[a]	1	3[a]	43[a]	1
497 (Breeding Line)	754[b]	553705[a]	3	69[cd]	958[b]	3
582 (TRI 5004)	529[ab]	85023[a]	2	23[ab]	431[b]	2
DT1	1023[b]	332473[a]	3	81[d]	955[b]	4

†Values with the same letter within a column are not significantly different (p < 0.05),
AR – Adaptability Rank – lower the rank higher the adaptability

yield variation across seasons has also been achieved. With regards to made tea yield, the mean values of genotypes over different environments and seasons are graphically present in Figs. 1 and 2, respectively.

Within location non-replicated multi-locational yield analysis

Mean deviation (d) and the stability parameter (s^2) across locations for the genotype deviations from the maximum response calculated for the each genotype by the method of Abeysiriwardena and Samita (2016) for seven tested genotypes are presented in Table 4. The genotype giving the highest yield in all locations would be the best or the most adaptable genotype in the test. Such a genotype would have zero 'd' and zero S^2. Thus, genotypes are selected simultaneously for comparatively lower d and s^2. While stability parameters (s^2) were found to be homogeneous, genotypic mean deviations (d) across locations was found to be significant at 5% probability level so that genotypes could be evaluated for their adaptability across locations only using the parameter d. The genotype 272 showed the significantly lower d and the d of the genotype 582 was not significantly different from that of genotype 272. However,

d of genotype 582 was also found to be not significantly different from that of rest of the genotypes with substantially higher d values which were found to be not significantly different from each other. Thus the genotype 272 could be identified as the most adaptable genotype across locations with the adaptability rank 1 and this was followed by genotype 582 with the adaptability rank 2 in the test. All the other genotypes in the test showed poor performance with adaptability rank 3 in relation to their adaptability across locations.

Mean deviation (d) and the stability parameter (s^2) over seasons for seven tested genotypes showed much explanatory adaptability ranking than that of across locations (Table 4). Stability parameters (s^2) were found to be heterogeneous and genotype 272 recorded the lowest variance based on the F test at 5% probability level. Variances of all the other genotypes were not significantly different from each other. Genotypes were found to be significant for d at 5% probability level and the results of mean separation test is shown in Table 4. Genotypes were selected simultaneously for comparatively lower d and s^2 and the most adaptable genotype was found to be 272 with adaptability rank 1 followed by 582 with adaptability rank 2
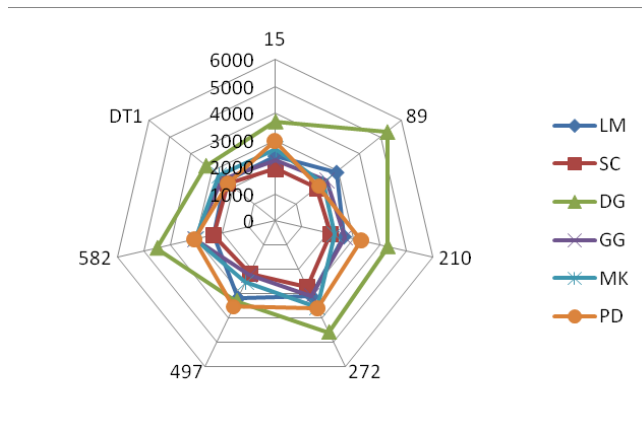
Fig. 1. Radar showing mean performance (kg/ha/year) of genotypes over the environments

Fig. 2. Radar showing mean performance (kg/ha/month) of genotypes over the seasons

and the least adaptable genotype was found to be DT1 with adaptability rank 4 while rest of the genotypes recorded the adaptability rank 3 over seasons.

When overall adaptability both across locations and over seasons is considered, the genotype 272 could be identified as the highest performing genotype in the test and it was followed by genotype 582. The variety DT1 was identified as the least adaptable genotype and rest of the genotypes were found to be intermediate in their overall adaptability across locations and over seasons.

Genotype-by-environment interaction effect

Under genotype evaluation, much work has been devoted in developing stability indices to quantify and select against GE interactions. Some procedures can be performed to attenuate the inconsistency of the superiority of the genotypes (Cruz et al. 2020). Although many stability indices have been proposed by various researchers, graphical interpretation of genetic main effect (G) and genotype-by-environment interaction effect (GE) is the most preferable method (GGE). A biplot is a scatter plot that approximates and graphically displays a two-way data Table. The term GGE emphasizes the understanding that G and GE are the two sources of variation that are relevant to genotype evaluation and test environment evaluation (Yan and Tinker 2005).

Performance and stability of genotypes were visualized graphically through GGE biplot. This can be evaluated by average environment coordination (AEC) method (Yan 2002). Fig. 3 is the environment-vector view of the GGE biplot for the data in Table 2. The biplot is based on an environment-centered (centering = 2) G by E Table without any scaling (scaling = 0), and it is environment-metric preserving (SVP = 2). The first two principal components (PCs) of biplots explained 79.2% of the total GGE variation of made tea average yield over apruning cycle (Fig. 3).

Discrimination power and the representativeness of the test environments is presented in Fig. 4. The average environment has the average coordinates of all test
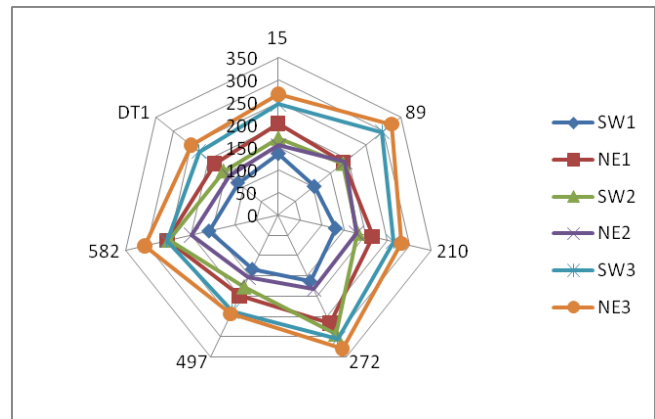
environments, and Average Environment Axis (AEA) is the line that passes through the average environment and the biplot origin (Yan and Tinker 2006). A test environment that has a smaller angle with the AEA is the most representative of other test environments. Thus, location Glasgow was the most representative whereas locations Dayagama and Pedro were the least representative but the most discriminating locations. The location LM displayed the lowest values of discrimination to the genotypes studied, followed by the locations St. Coombs and Glasgow. Discriminating but non-representative test locations of Dayagama and Pedro are useful for both selecting specifically adapted genotypes and culling unstable genotypes. Ajay et al. (2021) and Lalet al. (2021) studied discriminative power vs. representativeness of different environments for P treatments using GGE-biplot analysis separately for Spanish and Virginia groups in peanut and reported that lengths of environmental vectors are proportional to the standard deviation of genotype yield in a corresponding treatment.

The ideal test environment should be the most discriminating and the most representative of the target environment. An ideal test environment which is the center of the concentric circle is presented in Fig. 5. The closest test environment to the ideal environment is the best whereas furthest environment is the poorest for selecting genotypes adapted to the whole region (Yan and Tinker 2006). None of the locations are very close to the ideal test environment but locations Glasgow and Mattakelle showed considerable representation for selecting genotypes with general adaptability.In future, it is suggested to increase the number of test locations to represent wide range of environments in the Up-country tea growing region.

In the present study, the stability and yielding performance of seven tea genotypes were evaluated using average environment coordination (AEC) method as shown in Fig. 6. The single-arrowed line is the average environment axis (AEA); it points to higher mean yield across locations. Thus, 272 (TRI 5003) had the highest mean yield, followed by
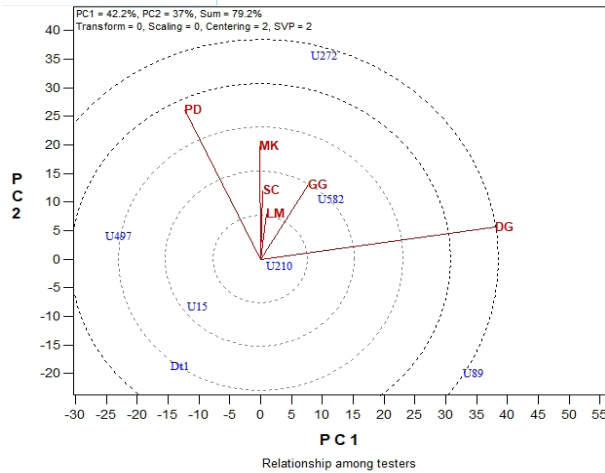
Fig. 3. GGE biplot of the discrimination and representativeness among the environments of seven genotypes of tea for the made tea yield (kg ha-1) in the Up-country tea growing region, Sri Lanka (* letter U denotes the Up-country tea growing region).

582 whereas 210 had a mean yield similar to the grand mean; and DT1 had the lowest mean yield. Genotype 272 (TRI 5003) was the most stable genotype whereas 89 (TRI 5002) and 497 a breeding line were the least stable genotypes. Locally adapted standard check DT1 was low yielding but highly stable. The results confirmed that 272 (TRI 5003) is a high yielding and highly stable genotype across the test locations. The GGE biplot approach in the analysis of the interaction between genotypes and environment is useful to identify desirable tea genotypes in the present study. Though, GGE biplot procedure has been used in stability studies of annual crops such as maize (Fan et al. 2007), barley (Dehghani et al. 2006), lentil (Sabaghnia et al. 2008), lupin (Atnaf et al. 2018), sorghum (Rakshit et al. 2012), peanut (Lal et al. 2021) and pearl millet (Reddy et al. 2022), it has been used in limited occasions for perennials.

Which-won-where graph is constructed first by joining the farthest genotypes forming a polygon. Subsequently perpendicular lines are drawn from the origin of the biplot to each side of the polygon, separating the biplot into several sectors (Yan and Tinker 2006). The genotypes that were farthest from the GGE biplot origin, 272(TRI 5003), 89 (TRI 5002), 497, a breeding line and DT1 served as corners of a polygon (Fig. 7). The genotype at the vertex of the polygon performs best in the environment falling within the sectors. The genotype 272 (TRI 5003) was the highest performer followed by and 582 in all test locations except location Dayagama. The genotype 89 was the best performer in location Dayagama indicating its specific adaptability. Therefore, 272 (TRI 5003) and 582 (TRI 5004) can be considered as stable genotypes with general adaptability in Up-country tea growing region. Reddy et al. (2022) reported that 'Which-won-where' study partitioned the testing locations into two mega-environments: first
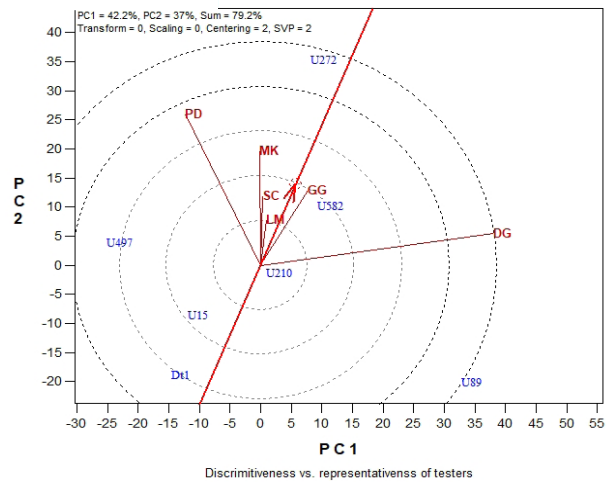
Fig. 4. The discrimination and representativeness view of the GGE biplot to show the discriminating ability and representativeness of the test environments.

with four locations with 86M01 as winning genotype and second encompassed three locations with KSB as the winning genotype of pearl millet hybrids. A few locations were identified for initial testing of genotypes. Further, they found that location attributed higher proportion of the variation for eight traits, while genotype and genotype × environment interaction accounted for less variability for grain and dry fodder yields. Majority of the testing locations were highly correlated with one location, which is the most discriminative and representative location.

The most ideal genotype, 272 (TRI 5003) performed the best at all tested locations except in Dayagama (Fig. 8). The above result suggests crossover GE interaction, i.e. order of genotypes based on their performance varied depending on the testing environment. The crossovers due to GE interaction have been observed in different crops
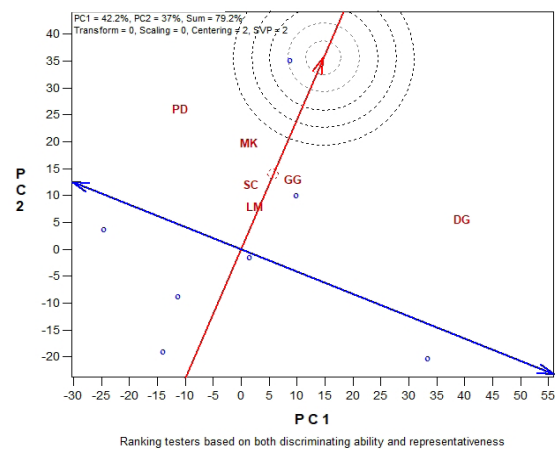
Fig. 5. The discrimination and representativeness view of the GGE biplot to rank test environments relative to an ideal test environment
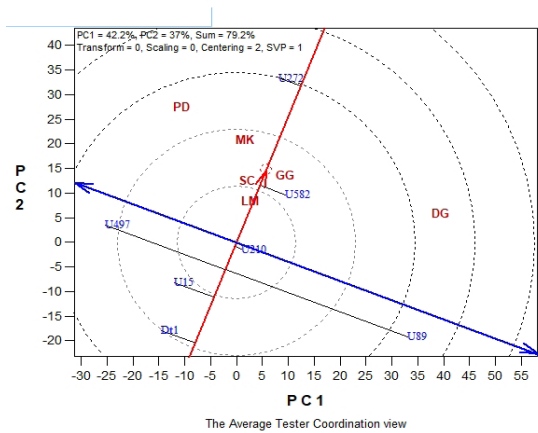
Fig. 6. GGE biplot of the average values versus genotypic stability among the environments of sevengenotypes of tea for the made tea (kg/ha/year)
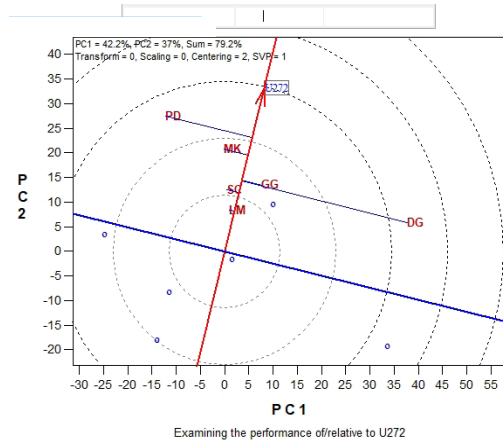

Fig. 7. The which-won-where view of the GGE biplot based on yield data

Fig. 8. Ranking of environments based on the performance of highest yielding genotype 272

Authors' contribution

Conceptualization of research (JDK, MABR, DSZA); Designing of the experiments (MABR, DSZA); Contribution of experimental materials (JDK, MABR); Execution of field/lab experiments and data collection (JDK, MABR); Analysis of data and interpretation (JDK, MABR, DSZA); Preparation of the manuscript (JDK, MABAR,DSZA).

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(Dehghani et al. 2006; Rakshit et al. 2012) as well as in tea. Furthermore, Murti et al. (2014) reported significant effect of cropping season, rainfall, temperature and soil type on yield contributing to the GE interaction.

The method that uses within location non-replicated, multi-locational large-scale yield trials appeared very efficient and effective for evaluating tea genotypes for their general adaptability over diverse environments. The biplot analysis suggested to increase the number of test locations to represent wide range of environments in the Up-country tea growing region. The genotype 272 (TRI 5003) was identified as the most adaptable genotype, followed by 582 in the Up-country tea growing region by the both methods. Rest of the varieties in the test were found to be poor in their overall adaptability while the genotype DT1 was identified as the least adaptable genotype in the Up-country tea growing region.

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Supplementary Table 1. Agro-climatic characteristics of test environments

Location	Latitude	Longitude	Altitude	Agro-ecological region*	Soil series
Lamilier	6.9274N	80.6926E	1336	WU2	Mattakelle
St. Coombs	6.9102N	80.7018E	1425	WU2	Mattakelle
Mattakelle	6.9279N	80.7097E	1483	WU2	Mattakelle
Glasgow	6.8686N	80.7259E	1452	WU2	Mattakelle
Dayagama West	6.8519N	80.7629E	1493	WU2	Mattakelle
Pedro	6.9734N	80.7901E	1961	WU3	NuwaraEliya

*WU: Wet zone-up country

Supplementary Table 2. Meteorological data of last 10 years in UW2 and UW3

Location	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
WU2												
Min. Temp. (°C)	14.5	14.4	15	16.1	17	16.5	16.2	16	15.8	15.7	15.5	15.2
Max. Temp. (°C)	21.1	21.8	22.7	22.5	22.2	21.4	21.2	21.2	21.3	21.2	22	22
Rainfall (mm)	85	158	121	273	290	112	128	135	182	656	416	228
Sunshine hours	7.1	7.4	7.9	8.3	9.1	10	9.7	8.2	9.5	7	5.6	4.6
WU3												
Min. Temp. (°C)	15.4	15.4	15.9	16.9	17.9	17.7	17.4	17.1	16.8	16.6	16.3	16
Max. Temp. (°C)	21.3	22.3	23.6	23.7	23.8	23.3	23.1	23.1	23.1	22.5	21.6	21.2
Rainfall (mm)	130	108	120	245	217	179	186	193	232	374	331	235
Sunshine hours	5.9	7	8	7.1	6.3	6.3	6.3	6.3	6.6	6	5.7	5.4

*WU: Wet zone-up country