International Journal of Research Studies in Science, Engineering and Technology
Volume 6, Issue 10, 2019, PP 27-33
ISSN : 2349-476X

Genetic Variability Estimation of Tea (Camellia Sinensis (L.) O. Kuntze) Clones Based on Biochemical Markers in Southwestern Ethiopia

Mohammedsani Zakir[1*], Kassaye Tolessa[2], Melaku Addisu[3]

[1,3] Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia
[2]Ethiopian Institute of Agricultural Research, Addis Abeba, Ethiopia

***Corresponding Author:** Mohammedsani Zakir, Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia, Email: mohammedsani641@gmail.com*

ABSTRACT

Information on genetic variability for biochemical characters is a prerequisite for improvement the quality of tea. Thirteen introduced tea clones characterized with objective of assessing tea clones based on biochemical characters at Melko and Gera research stations. The study was conducted during 2017/18 cropping season on experimental plots laid down in RCBD with three replications. Data recorded on biochemical traits such as ascorbic acid, beta-carotene, chlorophyll content, fresh leaf moisture content, total anti-oxidant, total polyphenol and photosynthesis efficiency. Analysis of variance showed significant ($P<0.01$) variation among the clones for all biochemical traits. Estimates of genetic variance indicated high phenotypic and genotypic coefficients of variation for chlorophyll content. The phenotypic coefficient of variation (PCV) for all the characters was slightly higher than genotypic coefficient of variation (GCV), which signified the presence of environmental influence to some degree in the phenotypic expression of characters. All traits considered in the study exhibited high heritability. Genetic gain (GAM) that expected from selecting the top 5% of the genotypes as percentage of the mean, varied from 5.51% for fresh leaf moisture content to 49.31% for chlorophyll content. Accordingly, the high GAM recorded for chlorophyll content (49.31%). Therefore, this implies greater effectiveness of selection and improvement to be expected in future tea breeding program.

Keywords: *Clones, genotypic variation, heritability, genetic advance, polyphenol, tea*

INTRODUCTION

Tea, made from the fresh leaves of the tea plant [*Camellia Sinensis* (L.) O. Kuntze] is one of the most popular healthy beverages consumed next to water in the world (Kottawa-Arachchi *et al*., 2013). The tea plant belonging to family the aceae is a small evergreen, perennial and develops normally as tall as 15 m. At the present commercial tea population includes three species, viz China type (*Camellia sinensis* (L.) O. Kuntze), Assam type (*Camellia assamica (*M.) Wight), Cambod type (*Camellia ssp. Lasiocalyx planch.* ex. Watt Weight) and their subordinates (Smith and Barua, 2011). World tea production (black, green, instant and other) come to 5,954,091 tons while top five producers were China (2,414,802 tons) 40.56%, India (1,252,174 tons) 21.03%, Kenya (473,000 tons) 7.94%, Sri Lanka (349,308 tons) 5.87% and Turkey (243,000 tons) 4.08%. Ethiopia existed at 21th in area production 9,727ha and 23th in production from 47 tea delivering nations by producing (10,806 tons) 0.18% (FAOSTAT, 2016).

Tea producing countries such as China, Japan, India and Kenya have used biochemical constituents like total catechins and their fractions, total polyphenols, chlorophylls, carotenoids and caffeine in the fresh leaf as discriminative markers for characterizing their tea germplasm to evaluate diversity and genetic potential warehoused in the germplasm (Sabhapondit *et al*., 2012). The commercial tea clones under production in Ethiopia were last for the long period of time without detail characterization and there was no clear information for tea clones on biochemical attributes which in fact is crucial to design conservation strategy and efficient exploitation of the tea clones as parent for crossing, expanding the genetic base of the existing tea clones and as cutting source for further tea production in Ethiopia.

MATERIALS AND METHODS

Description of the Study Site

The experiment was conducted at Jimma Agricultural Research Centers (JARC) Melko and Gera during 2017/2018. Melko is located at 7°46′ N and 36° E Latitude and Longitude, respectively with altitude of 1750 m a.s.l., average of last five year temperature was minimum11.7°c and maximum 25.9°c, rain fall of 1511.7 mm, 68.4% relative humidity, wind speed at 1m 2.448 km/hrs, monthly mean soil temperature at 5cm 24.9°c and 73.95 hrs average annual sun shine. Melko characterized by Eutric Nitosol (reddish brown) with a pH of around 5.2 (Simegn *et al.*, 2016).

Gera is located at 7 °7′ N and 36° E Latitude and Longitude, respectively with altitude 1940 m a.s.l., average of last five year temperature was minimum 11.1°c and maximum 23.9°c, rain fall of 1558.9 mm, 71.7% relative humidity, wind speed at 1m 1.92 km/hrs, monthly mean soil temperature at 5 cm 22.46°c and 61.76 hrs average annual sun shine. Gera, station also characterized by red soil, which was loam type and quite fertile (Solomon *et al*., 2014).

Experimental Materials

The experiment was superimposed on thirteen introduced Assam type tea clones that collected from different tea farms (Wushwush, Gumero and Chewaka) and JARC and established at Melko and Gera research stations (Table 1).

Table1. *Description of tea clones used for the study*

Serial no.	country of introduction	tea clones	sources of tea clones
1	Kenya	Mlk-2	JARC
2	India	L6	Gummaro
3	Kenya	Mlk-1	JARC
4	India	B9	Gummaro
5	Kenya	11/56	Wushwush
6	India	Chai	Gummaro
7	Kenya	S-15/10	Chewaka
8	Kenya	FNF	Wushwush
9	India	BB-35	Gummaro
10	India	SR-18	Gummaro
11	Kenya	11/4	Wushwush
12	Kenya	6/8	Wushwush
13	Kenya	31/11	Chewaka

Source: *Jimma Agricultural Research Center (JARC, 2005)*

Experimental Design, Field Management and Sample Preparation

The experiment was superimposed on the effectively settled tea plantation in 2005 at Gera and Melko research station using RCBD with three replications. Twelve years old tea bushes medium pruned with shears at 50 cm height from ground level in December 2017 according to Ahmad *et al.* (2014). After these treatments, tea plants brought back to the normal plucking cycle or shoot replacement cycle; in the spring (pre-monsoon) season during the middle May green tea sample was prepared from shoots plucked at the position of 2/3 of the inter node between the 2nd and the 3rd leaf measured from the 2nd leaf (IPGRI, 1997).

The shoot harvested and put in the water proof bag and brought immediately to the shade until take to the drying home which was aerated type and put in the dry container prepared from wire mesh and timber. Close follow up and turning was undertaken for even drying of green tea leaf until it was ready for milling. The dried leaf was milled in the laboratory for further analysis of traits like beta-carotene, total polyphenol, total anti-oxidant and ascorbic acid. The biochemical analysis was undertaken at Jimma University post harvest laboratory.

DATA COLLECTED

Moisture Content

Fresh tea leaf moisture content determined according to the Chinese national standard GB8304-87. In detail, every sample was heated in a constant temperature oven at 103°c for 4 hrs and weighed before and after the heating by an electronic balance. Then, wet based moisture content was estimated by the following formula.

Moisture Content = M1-M2/M1*100

Where, M1 = mass of sample before drying

M2 = mass of sample after drying

Total Polyphenol Content

The samples were extracted following method described by Maruf *et al.* (2011). Ten gram of ground tea were mixed with 100 ml methanol and the mixture were homogenized for 1 min in a homogenizer (PLTYRON®2500E, Switzerland) and kept in a water bath at 20^{o}c for 60 min. The samples were then centrifuged at 2500 rpm for 15 min and the supernatants were kept for the analysis (1st extraction). The residues re-extracted in the same conditions and the supernatants taken for analysis (2nd extraction). Both supernatants (1st and 2nd extractions) were mixed and the combined methanolic extracts were evaporated in the conventional oven at 40^{o}c to dryness and the extracted samples were stored at 4^{o}c.

After samples were extracted total polyphenol contents were determined according to methods of Maruf *et al.* (2011) which involves the reduction of folin-ciocalteau reagent by phenolic compounds, with an associated formation of a blue complex. Ten ml of the extracts was added with 2 ml of 2 N folin-ciocalteau reagents. Immediately, 2 ml of 7.5% sodium carbonate solution was added. Then, the mixture was incubated for 30 min at 37^{o}c and the absorbance reading was taken at 765 nm using UV-spectrophotometer, T80 China. Gallic acid used as a standard and the measurement compared to a standard curve prepared with gallic acid solution. The total polyphenol contents expressed as mg of gallic acid equivalents per gram of sample (mg GAE/g sample). All the samples were prepared in triplicate for each analysis and the mean value of absorbance obtained at 765 nm.

To draw the calibration curve gallic acid stock solution prepared by accurately weigh 0.5 g gallic acid into 10 ml volumetric flask, dissolved in 10 ml absolute methanol and the solution made up to 100 ml with 80% of the same solvent. From the stock solution 0, 2, 3, 4, 5, 6, 7 ml added in to 100 ml flask and diluted to give 0, 15, 30, 60, 90, 120 and 150 mg/l of gallic acid in methanol. Then 0.5 ml of each sample introduced into test tubes and mixed with 2 N Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes covered with aluminum foil and allowed to stand for 30 minutes at room temperature and the absorbance read at 765 nm using UV- spectrophotometer, T80 China.

Ascorbic Acid (Vitamin C)

Vitamin C was determined using method described by Sowa and Kondo (2003). One percent starch indicator solution was prepared in a beaker by adding 0.5 g soluble starch to 50 ml distilled water near boiling. The mixture was mixed well and allowed to cool before use. Iodine solution was prepared in a beaker by dissolving 5 g potassium iodide and 0.268 g potassium iodate in 200 ml of distilled water. Thirty milliliters of 3 M sulfuric acid added to the solution and the solution diluted to final volume of 500 ml with distilled water. Finally, the solution labeled as iodine solution. Vitamin C standard solution was prepared in a beaker by dissolving 0.250 g ascorbic acid in 100 ml distilled water and diluted to 250 ml with distilled water in a volumetric flask. Finally, the flask labeled as vitamin C standard solution. Vitamin C of the sample determined by titrating 10 ml of the extract with iodine solution until the end point reached. Ten milliliters of the sample taken to volumetric flask and 10 drops of 1% starch solution added and shaken properly until the end point reached. The endpoint of the titration is the first permanent trace of a dark blue-black color due to the starch-iodine complex that persists after 20 seconds of swirling the solution. The volume of Iodine solution used up for titration recorded by subtracting the starting volume from final volume. The titration repeated with further extracts of sample solution until the results agreed with 0.1 ml and the average used for final calculation. Vitamin C standard was titrated against iodine solution by applying the same procedure for sample titration and the volume of iodine solution used up for titration recorded by subtracting the starting volume from final volume. Milliliter of titrate used for each volumetric flask was calculated by taking the measurements obtained and average them (Average volume = total volume/number of trials). The volume of titrate required for the standard calculated and recorded. Finally, the amounts of ascorbic acid (mg /100g) found in the tea samples estimated from the volume of titrate required for standard solution.

Ascorbic acid =Ascorbic acid in sample (mg) x Volume made up (ml) x 100

Beta-Carotene

Extraction and determination of total beta-carotene content was followed the method

described by Park *(*1987). Briefly, 1 g of sample flour mixed with 1 g $CaCl_2.2H_2O$ and 50 ml extraction solvent (50% hexane, 25% acetone and 25% ethanol) and gently shaken for 30 min. After adding 15 ml of distilled water, the solution was shaken frequently again for a further 15 min. The organic phase, containing the beta-carotene separated from the water phase, using a separation funnel and filtered using what man filter paper number one. The extraction procedure carried out under subdued light to avoid degradation of carotenoids and the extracted samples were stored for analysis.

After sample was extracted beta-carotene was estimated from absorbance read at 450 nm using UV-spectrophotometer, T80 China and compared with beta-carotene standard. Pure beta-carotene standard (Sigma Aldrich) was used as a standard and the measurement was compared to a standard solution. To draw the calibration curve, beta-carotene standard stock solution prepared by accurately weigh 0.01 g beta-carotene standard and dissolved in 20 ml solvent, which was similar to extraction solvent used to extract samples (50 % hexane, 25 % acetone and 25 % ethanol) and made the volume to 100 ml using the same solvent. From the stock solution 0, 2, 3, 4 and 5 ml added in to 100 ml flask and diluted to give 0, 0.1, 0.2, 0.4, and 0.8 mg/l of beta-carotene standard in the same solvent. Then 0.5 ml of each sample introduced into test tubes and covered with aluminum foil and the absorbance read at 450 nm using (UV-spectrophotometer, T80 China). All samples were prepared in triplicate for each analysis and the mean value of absorbance obtained at 450 nm.

Total Anti-Oxidant

The samples were extracted following the method described by Lu and Foo (2000). Ten gram of ground tea were mixed with 100 ml methanol and the mixture was homogenized for 1 min in a homogenizer (PLTYRON®2500E, Switzerland) and kept in a water bath at 20^{o}c for 60 min. The samples were then centrifuged at 2500 rpm for 15 min and the supernatants were kept for the analysis (1st extraction). The residues re-extracted in the same conditions and the supernatants taken for analysis (2nd extraction). Both supernatants (1st and 2nd extractions) were mixed together and the combined methanolic extracts were evaporated in the conventional oven at 40^{o}c to dryness and the extracted samples were stored at 4^{o}c for further use. After sample was extracted total anti-oxidant contents were determined according to methods of Lu and Foo (2000) which involves DPPH assay (2, 2-diphenyl-1-picryl hydrazyl) or free radical scavenging assay. Percentage of free radical scavenging activity calculated from the absorbance of the solvent extract, methanol and standard solution (equation 3). For IC50 value the solvent extracts of the sample was taken in the following concentration range i.e., 200, 400, 600, 800, 1000 µl in each test tube and the volume was made up to 1 ml with the solvent and 2 ml of 0.1 mM DPPH was added to the tubes. The mixtures were shaken well and incubated at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution then measured by UV-spectrophotometer, T80 China at 517 nm. All the experiments performed in triplicate and the mean taken.

Radical scavenging activity (%) $= \left(\frac{Ac - At/As}{Ac}\right)$ X100

Where: Ac-Absorbance of control

At- Absorbance of test solution

As- Absorbance of standard Solution

The IC50 value, defined as the amount of the sample to scavenge 50% of the DPPH radicals, calculated from percentage of radical scavenging activity results by plotting the graph of DPPH free radical scavenging activity verses concentration of the sample.

Statistical Analysis

Analysis of variance of the traits was computed using SAS 9.3 computer program (SAS, 2014. Homogeneity test for error variances of the locations made before combined analysis and error variances of each location found homogenous for all considered traits. The combined analysis was estimated using RCB design. Least significant difference (LSD) at P= 0.05 and 0.01 was employed to identify clones that are significantly different from each other. Phenotypic and genotypic variance and coefficient of variability were analyzed according to Burton and Devane (1953). Broad sense heritability, genetic advance and genetic advance as percent of the mean were computed

according to Falconer (1989) and Johnson *et al.* (1955).

RESULTS AND DISCUSSION

Highly significant (P<0.01) differences among the tea clones were observed for all biochemical traits over-location, indicating the existence of substantial amount of variability among the genotypes tested which shows the possibility to select best and exploit through selection. There was significant (p<0.05) variation on location by tea clones interaction for chlorophyll content, total polyphenol, beta-carotene and ascorbic acid, indicates the difference in performance of the tea clones for those traits over-locations. However, non-significant interaction was observed between location and tea clones for fresh leaf moisture content, total ant-oxidant and photosynthetic efficiency (Table 2).

Table2. *Analysis of variance (mean squares) for biochemical traits over-location*

Variables	loc(df=1)	rep(loc)(df=2)	trt (df=12)	loc*trt(df=24)	error(df=48)	cv
CC	10.48**	1.82	188.76**	2.78**	1.12	4.59
FLMC	0.19	2.09	16.19**	1.46	1.27	2.01
TPP	10.20**	0.04	26.39**	0.73**	0.04	0.81
TAO	1.49	0.20	20.76**	1.61	1.21	4.58
BC	0.45**	0.009	3.51**	0.06**	0.006	0.92
AA	0.001	0.0004	0.02**	0.004**	0.0007	4.09
PE	0.02**	0.001*	0.002**	0.0004	0.0005	5.15

AA=ascorbic acid (mg/100g), BC=beta-carotene (mg/g), CC=chlorophyll content, CV=coefficient of variance, FLMC=fresh leaf moisture content (%), LSD=least significant difference, PE=photosynthesis efficiency (µmol/m^{2}s), TAO=total anti-oxidant (IC50) and TPP=total polyphenol (mg GAE/g)

Range and Mean Values for Biochemical Traits

The wider mean range was observed for chlorophyll content 11.09-32.63 for B9 and 11/4 tea clones whereas; the lower mean range was recorded for photosynthetic efficiency that was between 0.69-0.75 µmol/m^{2}s for B9 and 6/8 tea clones (Table 8). The range of other important traits in tea were indicated as total polyphenol 24.17-30.82 mg GAE/g of sample for L6 and 6/8, beta-carotene 7.42-10.13 mg/100g for Chai and SR-18, total anti-oxidant 21.17-26.93 IC50 for S-15/10 and 6/8 and ascorbic acid 0.92-1.12 mg/100g for L6 and Mlk2, respectively. In case of anti-oxidant the lower IC50 value indicate the high anti-oxidant value; this implies S-15/10 clones have high performance in absorbing 50% DPPH in minimum concentration than 6/8. The result was in conformity with Pereira *et al.* (2014) who reported 18.79-45.10 IC50 for green to black tea. Somanchi *et al.* (2017) indicated different tea types ascorbic acid that ranged <3-178 mg/100g this was partly in line with this study. Based on beta-carotene this study harmonized with Kottur *et al.* (2010) that reported beta-carotenoids range from 0.06 to 0.21 mg/g of sample. The result also coincided with Rahman *et al.* (2013) that reported amount of ascorbic acid 0.98 to 6.44 mg/100g and total polyphenol 18.23 to 30.88 mgGAE/g of sample respectively. Kottur *et al.* (2010) also reported 22.86-32.10mg GAE/g of sample of total polyphenol over different season. Fresh leaf moisture content was ranged from 53.55 to 57.78% for Mlk1 and 11/4, respectively. This was partly in line with Xiaoli *et al.* (2012) that indicated fresh tea leaves, partially processed tea and manufactured tea moisture content values in the range of 3.15% -71.40%.

Generally considering the yield and biochemical performance tea clones like 6/8, 31/11 and Mlk2 (Appendix Table 6 and 7) can be used as parent for crossing and recommended as source of cutting for further tea production by farms and out growers.

Genotypic and Phenotypic Coefficient of Variation

Burton and Devane (1953) classified PCV and GCV values as high (>20%), medium (10-20%) and low (<10%). Accordingly, high PCV and GCV value was exhibited for chlorophyll content, while the rest traits were exhibited low GCV and PCV (Table 8). Genotypic coefficient of variation found lower than phenotypic coefficient of variation in small numerical difference for all characters studied, which signifies the presence of small environmental influence in phenotypic expression of characters.

In case of traits like beta-carotene and total polyphenol the phenotypic expression is almost due to genotypic differences and the effect of

environment was very low that indicates lower environmental influence on the variation (Table 8).

Table3. *Estimate of variances for biochemical traits*

Variables	range		Mean	σ^2g	σ^2p	H^2%	GCV%	PCV %	GA	GAM%
	min	max								
CC	11.09	32.63	23.12	30.9	31.46	98.53	24.08	24.26	11.40	49.31
FLMC	53.55	57.78	55.96	2.46	2.70	90.98	2.80	2.94	3.08	5.51
TPP	24.13	30.82	27.30	4.28	4.40	97.23	7.58	7.68	4.21	15.41
TAO	21.17	26.93	24.03	3.19	3.46	92.24	7.43	7.74	3.54	14.73
BC	7.42	10.13	8.69	0.58	0.59	98.29	8.73	8.80	1.55	17.85
AA	0.92	1.12	0.88	0.003	0.003	80.00	4.97	5.55	0.10	9.16
PE	0.69	0.75	0.72	0.0004	0.0005	86.67	2.89	3.11	0.04	5.55

AA=ascorbic acid, BC=beta-carotene, CC=chlorophyll content, FLMC=fresh leaf moisture content, GA=genetic advance, GAM=genetic advance as percent of mean, GCV=genotypic coefficient of variation, σ^2g=genotypic variance, H^2=heritability, σ^2p=phenotypic variance, PCV=phenotypic coefficient variation, PE=photosynthesis efficiency, TAO=total anti-oxidant and TPP=total polyphenol

Heritability and Genetic Advance

High heritability recorded for all biochemical characters recorded in this study like chlorophyll content (98.53%), beta-carotene (98.29%), total polyphenol (97.23%), total anti-oxidant (92.24%), fresh leaf moisture content (90.98%), photosynthetic efficiency (86.67%) and ascorbic acid (80%), respectively. The highest heritability, which obtained was not from high genotypic variance, but from the very little difference between genotypic and phenotypic variances, although both were low except for chlorophyll content. The finding was in line with Kalpande *et al*. (2018) that reported high heritability for all traits. The estimated high heritability for these traits, were suggesting the greater effectiveness of selection and low effect of environment on phenotypic expression of the characters.

Genetic gain (GAM) as percentage of the mean, varied from 5.51% for fresh leaf moisture content to 49.31% for chlorophyll content (Table 8). Accordingly, the high GAM recorded only for chlorophyll content (49.31%). The characters that exhibited moderate level of genetic advance as percent of means are beta-carotene (17.85%), total polyphenol (15.41%) and total anti-oxidant (14.73%). The other traits had low level of GA as percent of mean (<10 percent). This low estimates genetic advance as percent of mean arises from low estimates of phenotypic variability; therefore, selection based on those traits with a high level GAM will result in improvement of the performance of the tea clones than those traits. In the current study, high heritability coupled with high genetic advance as percent of mean was observed for chlorophyll content. This indicates the lesser influence of environment in expression of the characters, prevalence of additive gene action in their inheritance and high chance of improvement through direct selection.

On the other way beta-carotene, total polyphenol and total anti-oxidant manifested high heritability coupled with moderate genetic advance as percent of mean implies the low range of genetic variability present in the population restricted the scope for their improvement. Besides, high heritability with low genetic advance recorded for fresh leaf moisture content, photosynthetic efficiency and ascorbic acid implying less influence of environment and prevalence of non-additive gene action for which simple selection will be less effective thus, heterosis breeding recommended for the improvement of such traits.

Summary and Conclusion

The analysis of variance revealed the presence of significant differences among the tested tea clones for all biochemical traits, indicating the presence of variability that can be exploited through selection and hybridization to tea quality. High heritability coupled with high genetic advance as percent of mean was observed for chlorophyll content. This indicates the lesser influence of environment in expression of the characters, prevalence of additive gene action in their inheritance and high chance of improvement through direct selection. High heritability coupled with moderate genetic advance as percent of mean for beta-carotene, total polyphenol and total anti-oxidant manifested the low range of genetic variability present in the population guide to improvement through both selection and hybridization. Besides, high heritability with low genetic advance recorded for fresh leaf moisture content, photosynthetic efficiency and ascorbic acid implying less influence of environment and

prevalence of non-additive gene action for which simple selection will be less effective thus, heterosis breeding recommended for the improvement of such traits.

CONCLUSION

In conclusion, the present study exhibited the presence of considerable genetic diversity for several biochemical traits among tea clones that must be exploited to improve the quality of this valuable crop. However, the diversity observed in this study must be confirmed using molecular markers like SSR and SNP for further utilization of these clones in the tea breeding program of Ethiopia.

REFERENCES

[1] Ahmad, F., F.S. Hamid, S. Sarwar, S. Waheed, S. Aslam, U. Islam, S. Hussain, N. Ahmad, I. Ali, 2014. Effect of different pruning times on the yield of tea (*camellia sinensis L.)* under the climatic conditions of Mansehra Pakistan. *Sarhad Jour. of Agric.*, **30**:305-309

[2] Burton, G.W., E. de Vane, 1953. Estimating heritability in tall festuca (*Festuca arudinacea)* from replicated clonal material. *Agron. J.*, **45**:478-481

[3] Falconer. D.S., 1989. Introduction to Quantitative Genetics. Longman Scientific and Technical. Jhony Wiley and Sons, Inc. Newyork. 438p

[4] FAOSTAT, 2016. Food and Agriculture Organization of the United Nations. Tea, production quantity (tons) - for all countries, *Fact fish*, http://www.factfish.com/statistic/tea

[5] IPGRI, 1997. Descriptors for Tea (*Camellia sinensis*). International Plant Genetic Resources Institute, Rome, Italy

[6] Johnson, H.W., H.F. Robinson, R.F. Comstock, 1955. Estimates of genetic and environmental variability in Soya bean. *Agron. J.* **47**:314-318

[7] Kalpande, H.V., A.W. More, R.L. Aundhekar, R.R. Dhutmal, 2018. Genetic Variability, Heritability and Genetic Advance in Sweet Grain (Hurda) sorghum [*Sorghum bicolor* (L.) Moench]; *Intern. Jour. of Curr. Microb. and App Sci*., **6**:400-405

[8] Kottawa-Arachchi, J.D., M.T.K. Gunasekare, M.A.B. Ranatunga, P.A.N. Punyasiri, L. Jayasinghe, 2013**.** Use of biochemical compounds in tea germplasm characterization and its implications in tea breeding in Sri Lanka; *J.Natn. Sci. Found. Sri.*,41:309-318

[9] Sabhapondit S., Karak T., Bhuyan L.P., Goswami B.C., Hazarika M., 2012. Diversity of catechin in Northeast Indian tea cultivars. *The Scientific World Journal* 425893. DOI: http://dx.doi.org/10.1100/2012/485193

[10] SAS, 2014. Statistical analysis System (Version 9.3) SAS Institute Cary, NC. USA

[11] Simegn, K., A. Sentayehu, A. Melaku, 2016. Genetic Diversity Studies on Yield and Its Related Traits in Korarima (*Aframomum corrorima* (Braun) Jansen)) Germplasms; *Jour. of Bio. Agri. and Healthc.*, **6**:1-7

[12] Smith, A., S.C. Barua, 2011. Characterization of some tea (*Camellia sinensis* (L) O. Kuntze) genetic resources on the basis of young shoot characters. Department of Tea Husbandry and Technology, Assam, Agricultural University, Jorhat. *Two and a Bud*., **58**:103-108

[13] Solomon, T., B. Gezahegn, D. Abnet, G. Biyensa, T. Wondimu, S. Meseret, A. Teshome, 2014. Participatory Rural Appraisal Report: Gera Woreda, West Oromia Region; Cascape Working Paper; capacity building for scaling up of evidence based best practices in agricultural production in Ethiopia; 20p

Citation: *Mohammedsani Zakir, Kassaye Tolessa, Melaku Addisu, "Genetic Variability Estimation of Tea (Camellia Sinensis (L.) O. Kuntze) Clones Based on Biochemical Markers in Southwestern Ethiopia", International Journal of Research Studies in Science, Engineering and Technology, vol. 6, no.10, pp. 27-33, 2019.*