



**PRELIMINARY SCREENING OF SUITABLE CLONES FOR MANUFACTURING GREEN TEA ON THE BASIS OF TOTAL CATECHIN ANALYSIS**

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**ARTICLE INFO**

**Article History:**

Received 12<sup>th</sup> December, 2018

Received in revised form 23<sup>rd</sup>

January, 2019

Accepted 7<sup>th</sup> February, 2019

Published online 28<sup>th</sup> March, 2019

**ABSTRACT**

In India, the existing clones and variety of tea plants have been developed for manufacturing of black tea (CTC & Orthodox Tea). But till now no clone & variety of tea have been identified exclusively for manufacturing of Green Tea (steamed/ roasted) So one humble attempt was made to identify tea clones from the existing released clones for preference of manufacturing Green Tea on the basis of their taste & their Polyphenol: Amino Acid ratio. Selected ten clones representing the China Jat, Assam Jat, Cambod Variety and Industry released Clones of Assam on the basis of their availability and potential quality on CTC & Orthodox Tea were analyzed with their morphological character, chemical analysis and green tea taste by tea taster indicates that TV-1,TV-7,P126 have the best potentiality for manufacturing green tea.

**Key words:**

tea (CTC & Orthodox Tea).

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**INTRODUCTION**

Demand for green tea is growing in both domestic and international markets. The tea clones and seed stocks presently grown by the tea industry in N E India are suitable for black tea (CTC/orthodox).The breeding criteria to develop green tea varieties are different from that of black tea varieties. Generally the small tea leaf variety, also called China *Jat*, is best for making green tea, while the Assam *Jat*, or big leaf variety, is best processed as black teas. Total catechin-Theanine ratio is a good indicator of taste of green tea. The high ratio of Total catechin to Theanine causes a strong and bitter taste. Whereas tea clones with low Total catechin-Theanine ratio are expected to yield green tea with good taste. Unlike black tea, green tea does not undergo the oxidation (fermentation) process which makes the green leaves to turn brown. Because this step is skipped in the manufacturing of green tea, the chlorophyll remains in the tea leaves which make green tea green. Some of our existing clones that are commonly used for orthodox black tea manufacturing may have potentiality to yield good green tea.

Green tea production in Assam is at its infancy stages. A few tea processing factories are currently producing it. This is because Assam and may other Indian tea producing states predominantly process black tea (CTC&Orthodox Tea) as their major export market product. As a result, the green teas

that exist in the market are thought not to be as good as the ones which are imported into the country. Research was carried out to determine the suitability of ten selected clones from Tea Research Association of India (TRA) in the production of green tea by assaying for bio-chemical components including Theanine and Total catechins, gallic acid .Two leaves and a bud were obtained from bushes of the selected clones and processed into un-aerated teas before the assays. The results showed that several TRA clones contained high quantities of total polyphenols and total catechins and catechins. Clones which exhibited, low Total catechins were TV-17,TV-1 and TV-7 and clones which exhibited high Theanine were S3A3,P126,TV-17 and TV-7

**Objective of Investigation**

Evaluation of the chemical constituents (Total Catechin and Theanine content) of the green tea manufactured from the selected clones for their liquor characters.

**MATERIALS AND METHODS**

The China hybrid TV-7, AC hybrid(Assam x China) TV-1,TV-17,TV-31,Assam type TV-21,Cambod type TV-9,TV-23,TV-26,Industry released clones S<sub>3</sub>A<sub>3</sub> and P<sub>126</sub> were evaluated for their potentiality to release as clones for Green tea.

The Green tea manufactured from the selected planting materials was evaluated for the content of Total catechin, caffeine and Theanine using HPLC (Agilent Technology 1260 infinity) following standard protocol.

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The transmission color difference of liquor of the Green teas manufactured from different entry in different season was measured by using Colorimeter.

**Table 1** Collection details of tea samples

Area	Place of collection	Clones/Variety	Month & year of collection
Assam	Borbhetta T.E.,Jorhat	TV-1	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-7	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-9	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-17	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-21	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-23	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-26	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	TV-31	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	P126	May, August & October 2018
Assam	Borbhetta T.E.,Jorhat	S3A3	May, August & October 2018

Extraction of Total catechins : Extraction of Total catechins were done according to the procedure by ISO14502-2-2005E (2005). Ground tea samples (0.2g) were weighed into graduated extraction tubes, 5ml of 70% hot methanol/water (MeOH) added, stoppered and mixed under vortex (Rotamixer, Huck and Tucker, England). Incubation was done in a water bath at 70°C for 10 min with vortexing after 5 and 10min respectively, cooling to room temperatures and centrifugation (3000rpm) for 10min them followed. A second extraction was done on the residue using 5ml of 70% hot methanol/water, the extracts were combined and made up to 10ml with cold methanol/water (70%).

Determination of Catechins HPLC analysis was done according to the procedure by ISO14502-2-2005E, (2005). One (1) ml of sample was pipetted into a test tube and diluted to 5ml with stabilizing solution (10% v/v acetonitrile with 500µg/ml of EDTA and 500µg/ml ascorbic acid), filtered and loaded into 2ml vials. A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C6, 25cm x 4.6 i.d. column fitted with e Rheodyne precolumn filter (model 7335) was used for determination at 278nm. Gradient elution was employed using the following solvent systems: Mobile phase A (9:2:89 v/v/v Acetonitrile: Acetic acid: EDTA) and mobile phase B (80:2:18 v/v/v

Catechin identification was done by comparing the retention times of samples with those of standards under similar conditions. The standards used included; +C, (-)-EGC, (-)EC, (-)-EGCG and (-)-ECG. Total catechins were expressed as a percentage by mass on a sample dry matter basis and given as a summation of individual catechins as ;

$$\% \text{ Total Catechin} = [(\% \text{EGC}) + (\% +\text{C}) + (\% \text{EC}) + (\% \text{EGCG}) + (\% \text{ECG})]$$

**Determination of Theanine**

Theanine is estimated in HPLC by extracting from finely powdered sample by brewing with hot water for 5 min using a magnetic stirrer. After filtration of the brew with filter paper the brew is allowed to cool. Cooled extract is filtered with 0.45µm membrane filter and 20 µl is injected into a 4.6 mm

HPLC reverse phase C18 column with 1.0 ml flow rate using water as eluent and UV detection at 210 nm. Theanine is detected by comparing with Theanine standard through a previously prepared calibration curve (Draft International Standard, ISO/DIS 11287)

**RESULTS AND DISCUSSION**

*Analysis of Catechin profile*

**No of samples:** 10 different cultivars

**Sample type:** Green leaf received were deactivated and dried for analysis

**Table 2**

Sample	Caffeine	EGC	+C	EC	EGCG	ECG	Total Catechin
TV1	4.32	2.80	1.17	1.17	7.04	3.63	15.82
TV7	4.74	2.27	0.89	0.83	8.41	3.18	15.58
TV9	6.16	2.84	0.45	0.73	12.46	3.00	19.46
TV17	4.71	2.24	0.82	1.50	7.08	3.76	15.40
S.3A/3	4.16	7.37	0.59	2.55	10.99	4.43	25.92
P 126	4.38	5.79	0.67	1.66	10.57	2.83	21.53
TV23	3.63	4.75	0.74	2.13	10.35	4.50	22.46
TV26	4.04	4.11	0.55	1.03	11.55	2.80	20.03
TV31	3.98	2.44	0.95	0.71	10.80	3.45	18.36

*(The values of EGC, +C, EC, EGCG, ECG and Total Catechin are in % dry mass basis)*

EGC –Epigallo catechin, +C – Catechin, EC – Epicatechin, EGCG – Epigallo catechin gallate & ECG – Epicatechin gallate

Table 2 shows that among the 10 different cultivars analyzed Total Catechin was found to be lowest in TV-17(15.40),followed by TV-7(15.58),TV-1(15.82),TV-31(18.36),TV-9(19.46),TV-26(20.03),P<sub>126</sub> (21.53),TV-23(22.46),TV-21(22.52),S<sub>3</sub>A<sub>3</sub>(25.92)

**Total Catechin to Theanine Ratio**

**Table 3**

Tea clones	Total catechin	Theanine	Catechin: theanine
TV 1	15.82	0.56	28.3
TV 7	15.58	1.15	13.5
TV 9	19.46	0.95	20.5
TV 17	15.40	1.15	13.4
TV 21	22.52	0.82	27.5
TV 23	22.46	0.77	29.2
TV 26	20.03	1.04	19.3
TV 31	18.36	0.93	19.7
P 126	21.53	1.17	18.4
S3A3	25.92	1.83	14.2

Table 3 shows that as per ratio of total Catechin to theanine it was lowest in TV 17 (13.4) followed by TV 7 (13.5), S3A3 (14.2), P126 (18.4), TV 26 (19.3), TV 31 (19.7), TV 9(20.5), TV 21(27.5), TV 1 (28.3), TV 23 (29.2)

**CONCLUSION AND RECOMMENDATIONS**

The investigation on 10 different clones of N.E.India has shown that the chemical composition have varied composition some have exhibited higher Total catechin and some have higher Theanine which implies that the clones with lower Total Catechin to Theanine ratio would have lesser bitterness and astringency and thus suitable for Green Tea processing. Individually each assayed tea exhibited a unique combination of biochemical compounds which will uniquely affect the taste of the made teas this is subject for assessment through sensory

evaluation. Green tea products generally cost much more than black tea products.

**Recommended Clones for Green tea:** TV-7, TV-17, S3A3. P126

**Acknowledgements:** The authors wish to thank the Director of Tocklai Tea Research Institute, TRA, Jorhat, Assam, India and Head of the Department Tea Husbandry & Technology, Assam Agricultural University, Jorhat, Assam, India for providing the necessary resources needed to conduct the research.

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**How to cite this article:**

Bhupen Deka *et al* (2019) 'Preliminary Screening of Suitable Clones for Manufacturing Green tea on the Basis of Total Catechin Analysis', *International Journal of Current Advanced Research*, 08(03), pp.17980-17983.  
DOI: <http://dx.doi.org/10.24327/ijcar.2019.17983.3427>

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