

## Genetic Variability of Tea Plant on the Black Sea Coast of Caucasus

MAYA VALERIANOVNA GVASALIYA <sup>1</sup>, LIDIYA SERGEEVNA SAMARINA <sup>\*1</sup>, RAVISH CHOUDHARY <sup>2</sup>,  
JYOTI AHLAWAT <sup>3</sup> AND SHIV K YADAV<sup>2</sup>

<sup>1</sup>Federal State Budgetary Scientific Institution, Russian Research Institute of Floriculture and Subtropical Crops, Sochi, Russia

<sup>2</sup>Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi - 110012, India

<sup>3</sup>GD Goenka University, Gurugram, Haryana - 122103, India

\*samarinalidia@gmail.com

(Received : February 2019; Revised : May 2019; Accepted : June 2019)

**ABSTRACT:** Numerous environmental factors such as high irradiation, temperature shocks, pesticides and fertilizer loads have a large impact on the frequency of plant genetic variability. Tea plant is known for its tendency of hereditary genetic variability as the result of free pollination in nature within the species and between the species of the genus. In this connection, the study of spontaneous variability of tea plants on the frequency of somatic mutations and the test of chromosome aberrations was carried out. The research was conducted on industrial tea plantations located in three agro-ecological zones of humid subtropics of the Black Sea coast of the Caucasus: a coastal cluster (100 m above sea level); foothill cluster (200 m above sea level); upper cluster and low mountains (450 m above sea level). The lowest frequency of spontaneous variability was observed in the upper cluster and while the largest frequency of chromosome aberrations was observed in the foothill cluster. Morphological changes affected the size, shape, tip, edges, leaf surface, tip size and internodes. Physiological changes were represented as the anthocyanin color of the leaf and the rate of shoot formation, plastid mutations are represented by periclinal and sectorial chimeras. A karyological analysis showed the presence of a diploid ( $2n = 30$ ) chromosome set in all selected tea forms. Analysis of the frequency of the spectrum of somatic mutations has shown that half of them were plastid mutations (40.51-50.00%). The genotype played a determining role in the mutations frequency. The highest frequency of variability was found in the Kymyn population (3.95%), which showed the highest frequency of chromosomal aberrations (2.18-5.92%) depending on the growth area. The spectrum of chromosome aberrations in the Kymyn population was wider than Kolkhida cultivar. Positive correlation between the frequency of somatic mutations and the frequency of cells with aberrations was observed.

**Keywords:** *Camellia sinensis*, Spontaneous mutagenesis, Selection, Morphological descriptors, Chromosomal aberrations

Tea germplasm (*Camellia sinensis* (L.) Kuntze) is known for its tendency of hereditary genetic variability as the result of free pollination in nature within the species and between the species of the genus. Cultivation and selection of tea in the Western Caucasus has been conducted for over 80 years, as a result methodical approaches to the biodiversity conservation of tea [1, 2] and genotypes improvement [1,3] have been developed, acclimatized local cultivars were obtained [4]. Numerous environmental factors, such as high irradiation, temperature shocks, pesticides and fertilizers, affect the frequency of spontaneous genetic variability on tea plantations [5, 6]. The molecular basis of spontaneous genetic variability is the changes in the chromosome number [7], spot mutations [8], sister chromatid crosses [9], chromosome breakdowns and rearrangements [10, 11], alteration of

chloroplast and mitochondrial DNA [12], DNA methylation [13], epigenetic variability [14], histone modifications and RNA interference [15], segregation of chimeric tissues [16], as well as insertions or transfers transposable genetic elements [2]. Insertions of transposable genetic elements and retrotransposons is of particular interest and can be observed as insertional mutagens in the plant genome, and lead to a wide range of chromosomal aberrations [17]. It can be a trigger of misregulation of genes, leading to aneuploidy [14]. It has been established that tea plantations on the Black Sea coast of the Caucasus are in forms of diploid (99.8%), triploid (0.059%), tetraploid (0.024%), aneuploid (0.064%) and mixoploid (0.012%) [18]. However, many aspects and mechanisms leading to spontaneous variability remain unclear [19]. In connection with this, our research goal was to study the frequency

and spectrum of spontaneous mutations, as well as changes in the structure of chromosomes in mitosis in tea plants.

## MATERIALS AND METHODS

Plant material i.e., tea (*Camellia sinensis* (L.) Kuntze) cultivar Kolkhida; population Kymyn (Chinese variety); and the Local population (mix of Chinese, Sino-Indian and Japanese varieties) were used in this study. Experimental fields were located in three agro-ecological zones of humid subtropics of the Black Sea coast of the Caucasus: I - in a coastal cluster (100 m above sea level); II - in the foothill cluster (200 m above sea level); III - in the upper cluster (450 m above sea level). A visual observation of somatic mutations was conducted on the main valuable morphological traits (leaf size, shape, tip, serration, surface, etc.) and leaf coloration. Among the plastid mutations, periclinal chimeras (bands on leaves and apex) and sectorial chimeras (sectorial color change) were selected. Somatic mutations at each site were counted for 2 years during September, 2 to 3 weeks after the end of the harvest. Genetic variability was studied according to generally accepted methods [20 - 22].

For cytological studies, the seeds were collected in the foothill cluster in October month, germinated in a thermostat at a temperature of 27-30°C. Root tips of 1.00 cm in length were fixed in a Carnoy mixture for preparing of temporary acetocarmine samples. The karyological analysis was carried out by metaphase method. Before fixation roots were placed for 4-6 hours in bromo - naphthalene. Standard cytological protocols were used [23]. For the statistical processing of experimental data, the methods of the Fisher were used for any shares and "chi-square" test [24].

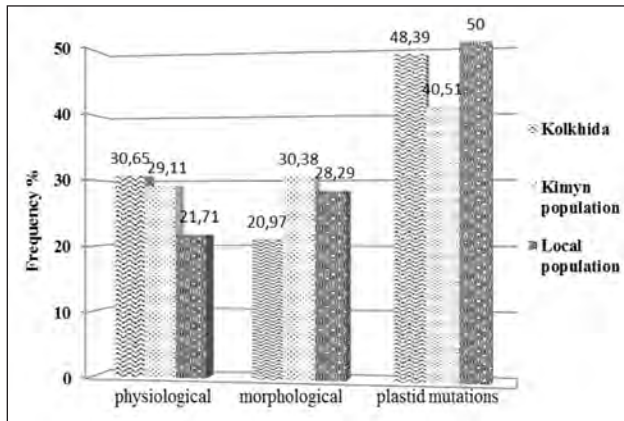
## RESULTS AND DISCUSSION

The highest frequency of plants with altered traits was observed in the Kymyn population ( $2.0 \pm 0.03 - 3.95 \pm 0.20\%$ ), comparing to Kolkhida cultivar, in which the frequency of the changes was  $1.10 \pm 0.22\% - 3.10 \pm 0.39\%$ . The local population is medium variable, the frequency of natural variability was observed such as  $1.60 \pm 0.14 - 3.50 \pm 0.17\%$  (Table 1). Statistical processing of the data by the  $\chi^2$  method showed that the difference between the varieties was statistically significant ( $P < 0.05$ ) for Kolkhida and Kymyn in I and III agro-ecological zones. Among the investigated zones, the most unfavorable in the ecological aspect was the II zone where the highest frequency of variability was observed and exceeded up to 2.0-3.0%.

The lowest frequency of spontaneous variability was observed in the Kolkhida cultivar and Local populations in zone III. The difference is statistically significant ( $P < 0.001$ ) between zones II and I for the Local population. The detected spontaneous mutations were divided into three groups: morphological, physiological and plastid. An analysis of their spectrum was made in zone II (Figure 1). Morphological changes affected the leaf size, shape, tip, edge, surface and internodes. Physiological alterations revealed as changes in the anthocyanin color of the leaf and the rate of shoot growth, plastid alterations represented by periclinal and sectorial chimeras. About half of all somatic mutations were plastid mutations (40.51-50.0%). A significant increase in the frequency of physiological mutations was observed in the Kolkhida cultivar in comparison with the morphological mutations of 30.65 - 20.97%. In populations, its frequency was relatively the same.

**Table 1.** The frequency of somatic mutations of the tea populations in three agro - ecological zones of the Black Sea coast of the Caucasus

Genotype	Number of plants observed	Mutations occasions number	Mutations %	Number of plants observed	Mutations occasions number	Mutations %			
							I zone	II zone	III zone
Kolkhida	2000	38	1.90±0.30	2000	62	3.10±0.39	2000	22	1.10±0.22
Kymyn	2000	58	2.90±0.37	10000	395	3.95±0.20	2500	50	2.00±0.03
Local Population	10000	221	2.21±0.14	10000	350	3.50±0.17	10000	160	1.60±0.14



**Figure 1.** Frequency and spectrum of tea spontaneous mutagenesis, % (II zone)

To study the genetic variability of the Kolkhida cultivar, the Kymyn population and the Local tea population, observations were conducted on the chromosome aberration test (Table 2). The highest frequency of chromosome rearrangements was observed in the Kymyn population up to  $2.18 \pm 0.20 - 5.92 \pm 0.47\%$ , depending on the growth zone. The Kolkhida cultivar and the Local population were characterized by a lower incidence of altered anaphases. In common, a large difference in the frequency of chromosome aberrations was observed in zone II, compared with other zones. Data processing by the *chi square* method showed a statistically significant difference in the Kymyn population ( $p < 0.001$ ), as well as in the Local population ( $p < 0.05$ ). There is a clear tendency to increase the frequency of chromosome rearrangements in the variety and populations of tea in the zone II, compared with other zones.

In general, it should be noted a fairly pronounced correlation was observed between the frequency of somatic mutations and the frequency of cells with

chromosome aberrations. The greatest frequency of somatic and structural mutations of chromosomes was obtained in the Kymyn population, in comparison with the Kolkhida cultivar and the Local tea population. In addition, in zone II, the frequency of somatic mutations and aberrations was greater than in plants grown in other zones. The positive correlation between the frequency of aberrations and the frequency of somatic mutations was established.

Further experiments showed that the frequency and spectrum of chromosome aberrations of the Kolkhida cultivar, the Kymyn population, and phenotype of their original forms differed significantly as well. All these forms differed in their phenotypic characteristics (leaf size, shape, color and surface). As follows from the data obtained, the frequency of aberrations of chromosomes in the mutant forms differed little from the initial varieties and the tea population. The highest frequency of anaphase with aberrations was observed in form No. 333 of the Kymyn population.



**Figure 2.** Diploid set of chromosomes in isolated forms of tea ( $2n = 30$ )

**Table 2.** Frequency of aberrations of chromosomes in tea populations, depending on the zone of growth

Genotype	Zone I			Zone II			Zone III		
	Anaphase number	Number of anaphase changes	Anaphase changes %	Anaphase number	Number of anaphase changes	Anaphase changes %	Anaphase number	Number of anaphase changes	Anaphase changes %
Kolkhida	2550	38	$1.49 \pm 0.22$	2600	78	$3.00 \pm 0.33$	2505	21	$0.84 \pm 0.17$
Local population	2501	36	$1.44 \pm 0.24$	2511	80	$3.19 \pm 0.35$	2499	19	$0.76 \pm 0.17$
Kymyn population	5580	181	$3.24 \pm 0.24$	2501	148	$5.92 \pm 0.47$	5410	118	$2.18 \pm 0.20$

Also, the spectrum of chromosome aberrations in the Kymyn population was wider than that of the Kolkhida cultivar. Kymyn encountered single and twin bridges with fragments otherwise Kolkhida had only single and paired fragments. The isolated forms from the initial Kolkhida cultivar and the Kymyn population were subjected to karyological analysis by the metaphase method. As a result, it was found that they all had a diploid set of chromosomes ( $2n = 30$ ) (Figure 2).

A highest frequency of somatic mutations observed in Kymyn population (3.95%). Analysis of the frequency of the spectrum of somatic mutations has shown that half of them are plastid mutations (40.51-50.0%). Positive correlation between the frequency of chromosome aberrations and the frequency of somatic mutations was established. The largest frequency of chromosome rearrangements were found in the Kymyn population is 2.18-5.92%, it is recommended to use it as a source material for breeding. Our results are corresponded with other researchers who concluded that among all factors influencing the genetic variability, the genotype has a dominant effect [16, 25, 26]. Other researchers showed that Chinese tea genotypes are more variable than Indian ones [27]. We also observed this in our experiments with Chinese variety (Kymyn population) which showed higher genetic variability among others. Surely, Kolkhida cultivar is expectantly showed lower genetic variability because it is more homogenous genetic material.

Spontaneous genetic variability is manifested at the morphological, physiological and biochemical and genetic levels [28]. Among the morphological characters the following types of changes are most often encountered: length, width, shape, surface (smooth, bubbly), the degree of serration of the leaf, the location of the leaves on the shoot and the length of the internodes. Physiological variations are manifested as changes in the leaf colour (light green, yellow-green, brownish-green, dark green), as well as various grade of anthocyanin colour. Extended growth period, reduced generative activity, and high yield are also among physiological variation traits. Plastid mutations are represented by sectorial and periclinal chimeras. Forms with altered photosynthetic activity, as well as improved biochemical and organoleptic parameters are of interest [29]. Somatic mutagenesis exhibits a similar spectrum of genetic variability as induced mutations – both types of mutations lead to similar changes in DNA [30]. A trigger for mutations leading to

variability can be various stress factors, including pruning, drought, solar radiation, nutritional imbalances, etc. [14]. Volatility can be caused by oxidative stress, which occurs, for example, in drought [17, 31], which is the main stress factor in tea cultivation in the world, reducing the 15-45% productivity of tea plantations [32]. Oxidative stress leads to an increase in the level of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl, peroxy and other radicals. These ROS can provoke hyper- or hypomethylation of DNA [33]; a change in the chromosome set towards polyploidy and aneuploidy, rupture of chromosome strands, chromosome rearrangements, deletions and substitutions in DNA [34], which in turn lead to mutations of somatic cells.

However, some researchers have shown that somatic mutagenesis can appeared with very low levels of morphological variability, but molecular markers, such as ISSR, can detect changes at the genetic level [35]. In general, the degree of genetic variability is difficult to determine reliably due to the involvement of multiple genes in this process. However, to fill gaps in understanding the potential, mechanisms of occurrence of spontaneous genetic variability, it is necessary to use modern tools, such as NGS-technology.

## REFERENCES

1. RYNDIN AV, TD BESEDINA, MT TUOV, VK KOZIN, ZV PRITULA, LS MALUKOVA, NV KOZLOVA AND AS TERESHKIN (2011). Inventory passport of tea plantation, methodology and regulatory documentation, Sochi.
2. SATO M, M HOSOKAWA AND M DOI (2011). Somaclonal variation is induced de novo via the tissue culture process: a study quantifying mutated cells in Saintpaulia. *PLoS ONE* 6:e23541. Retrieved from doi:10.1371/journal.pone.0023541.
3. TUOV MT, AV RYNDIN AND SV LOSHKAREVA (2007). Modern trends in tea breeding. *Subtropical Ornamental and Horticulture*, 40: 278-290.
4. TUOV MT AND MV GVASALIYA (2009). Productivity of perspective varieties and tea cultivars depending on the collector surface in Krasnodar Region. *Subtropical Ornamental and Horticulture*, 42(2): 60-70
5. GVASALIYA MV (2015). Spontaneous and induced varieties and forms of tea (*Camellia sinensis* (L.) Kuntze) in the humid subtropics of Russia and Abkhazia, the prospects for their reproduction and *in vitro* preservation: author's abstract, *Dis. Cand. Biol. Sci., Krasnodar*, pp: 24.
6. KERKADZE IG (1987). Theory and practice of spontaneous and induced mutagenesis of subtropical crops: author's abstract. *Disease Document of Biological Sciences*, Moscow, pp: 51.
7. LEVA AR, R PETRUCCELLI AND LMR RINALDI (2012). Somaclonal variation in tissue culture: a case study with olive. In: Leva AR And LMR Rinaldi (eds) Recent advances in plant *in vitro* culture, INTECH Open Access Publisher, Croatia,

- pp:123–150.
8. NGEZAHAYO F, Y DONG AND B LIU (2007). Somaclonal variation at the nucleotide sequence level in rice (*Oryza sativa* L.) as revealed by RAPD and ISSR markers and by pair wise sequence analysis. *Journal of Applied Genetics*, **48**: 329-336.
  9. BAIRU MW, AO AREMU AND JV STADEN (2011). Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulators*, **63**:147–173.
  10. ALVAREZ ME AND F NOTA (2010). Cambiagno D.A. Epigenetic control of plant immunity. *Molecular and Plant Pathology*, **11**: 563–576.
  11. TIWARI JK, P CHANDEL, S GUPTA, J GOPAL, BP SINGH AND V BHARDWAJ (2013). Analysis of genetic stability of *in vitro* propagated potato micro tubers using DNA markers. *Physiological and Molecular Biology in Plants*, **19**: 587-595.
  12. BARTOSZEWSKI G, MJ HAVEY, AZIO'KOWSKA, M D'UGOSZ AND S MALEPSZY (2007). The selection of mosaic (MSC) phenotype after passage of cucumber (*Cucumis sativus* L.) through cell culture-a method to obtain plant mitochondrial mutants. *Journal of Applied Genetics*, **48**: 1-9.
  13. LINACERO R, J RUEDA, E ESQUIVEL, A BELLIDO, A DOMINGO AND AM VAZQUEZ (2011). Genetic and epigenetic relationship in rye, *Secale cereale* L., somaclonal variation within somatic embryo-derived plants. *In Vitro Cellular Developmental Biology- Plant*, **47**: 618–628.
  14. SMULDERS M AND G DE KLERK (2011). Epigenetics in plant tissue culture. *Plant Growth Regulators*, **63**:137-146.
  15. MIGUEL C AND L MARUM (2011). An epigenetic view of plant cells cultured *in vitro* somaclonal variation and beyond. *Journal of Experimental Botany*, **62**: 3713–3725.
  16. NWAUZOMAAB AND ET JAJA (2013). A review of somaclonal variation in plantain (*Musa* spp): mechanisms and applications. *Journal of Applied Biosciences*, **67**: 5252–5260.
  17. TANURDZIC M, MW VAUGHN, H JIANG, TJ LEE, RK SLOTKIN, B SOSINSKI, WF THOMPSON, RW DOERGE AND RA MARTIENSSSEN (2008). Epigenomic consequences of immortalized plant cell suspension culture, *PLoS Biology*, **6**(12): 2880-2895.
  18. DOLIDZE KG (1986). Some questions of tea genetics. *Communication 11- Features of splitting in diploid and polyploid forms of tea during analytical crossing // Subtropicheskie Kultury*, **2**: 81-91.
  19. KRISHNA H, MALIZADEH, D SINGH, U SINGH, N CHAUHAN, M EFTEKHARI AND RK SADH (2016). Somaclonal variations and their applications in horticultural crops improvement. *Biotechnology*, **6**(1): 54.
  20. BAKHTADZE KE (1948). Biology, selection and seed production of a tea plant, Moscow, pp: 280.
  21. BAKHTADZE KE (1971). Biological basis of tea culture, Tbilisi: Metzniereba, pp: 359.
  22. KERKADZE IG (1980). Some questions of tea genetics, Communication II, Selection of tea forms with genetic markers and their selection value, *Subtropicheskie Kultury*, **2**: 36-45.
  23. PAUSHEVA ZP (1980). Workshop on plant cytology, Moscow: Kolos, pp: 304.
  24. PLOKHINSKY NA (1970). Biometrics, Moscow, MGU, pp: 367.
  25. SHEN X, J CHEN, M KANE AND R HENNY (2007). Assessment of somaclonal variation in *Dieffenbachia* plants regenerated through indirect shoot organogenesis, *Plant Cell Tissue, Organ, Culture*, **91**: 21-27.
  26. TICANA, G CAMPEANU, N CHIRU AND D IVANOVICI (2008). Using of unconventional methods for obtaining somaclonal variations, having as goal making of new potato varieties with resistance at diseases and pests. *Romanian Biotechnological Letters*, **13**: 3791-3798.
  27. DEVANAND PS, J CHEN, RJ HENNY AND CCT CHAO (2004). Assessment of genetic relationships among Philodendron cultivars using AFLP markers. *Journal of American Society for Horticulture Sciences*, **129**: 690–697.
  28. KERKADZE IG (1983). Some questions of genetics of a tea plant, Communication VIII, Somatic mutation and clonal selection of tea. *Subtropicheskie Kultury*, **1**: 42-45.
  29. KUMAR R, T BALASARAVANAN, D JAYAKUMAR, V HARIDAS AND S MARIMUTHU (2001). Physiological and biochemical features of field grown somaclonal variants. *Bulletin of UPASI Tea Research Foundation*, **54**: 73-81.
  30. CASSELLS AC, ML DEADMAN, CA BROWN AND E GRIFFIN (1998). Field resistance to late blight (*Pytophthora infestans* (Mont.) De Bary in potato (*Solanum tuberosum* L.) somaclones associated with instability and pleiotropic effects. *Euphytica*, **57**: 157-167.
  31. NIVAS SK AND L DSOUZA (2014). Genetic fidelity in micropropagated plantlets of *Anacardium occidentale* L. (Cashew) an important fruit tree. *International Journal of Science and Research*, **3**: 2142–2146.
  32. CHERUIYOT EK, LM MUMERA, WK NGETICH, A HASSANALI AND F WACHIRA (2007). Polyphenols as potential indicators for drought tolerance in tea (*Camellia sinensis* L). *Bioscience, Biotechnology and Biochemistry*, **71**:2190–2197.
  33. WACKSMAN JT (1997). DNA methylation and the association between genetic and epigenetic changes: relation to carcinogenesis. *Mutation Research*, **375**: 1–8.
  34. CZENE M AND M HARMS-RINGDAHL (1995). Detection of single-strand breaks and formamidopyrimidine-DNA glycosylase-sensitive sites in DNA of cultured human fibroblasts. *Mutation Research*, **336**:235-242.
  35. THOMAS J, D VIJAYAN, SD JOSHI, SJ LOPEZ AND RR KUMAR (2006). Genetic integrity of somaclonal variants in tea (*Camellia sinensis* (L.) O Kuntze) as revealed by inter simple sequence repeats. *Journal of Biotechnology*, **123**(2): 149-154. Retrieved from doi: 10.1016/j.jbiotec.2005.11.005.