Research Paper

Recent Progress of Intra-specific Hybridization of Tea (Camellia sinensis (L.) O. Kuntze) in Sri Lanka

J.D. Kottawa-Arachchi*, M.A.B. Ranatunga and K.K. Ranaweera

Plant Breeding Division, Tea Research Institute, Talawakelle, Sri Lanka
* Corresponding Author: jeevan1188@yahoo.com, jeevank@tri.lk https://orcid.org/0000-0001-8498-0728

Abstract: Tea germplasm accessions are the most valuable material for tea breeding. However its utilization in the past was limited due to lack of available information. Recently generated information on genetic structure and metabolite profiles of the germplasm were used in selecting parents for hybridization programmes from 2012 to 2016. The main focus of the programme was to make crosses between three taxa of tea viz. Camellia sinensis var. assamica (Assam type), C. sinensis var. sinensis (China type) and C. sinensis spp. lasiocalyx (Cambod type). Pollinated buds were closely-monitored and the number of crosses, number of fruits, number of seeds and reproductive parameters were recorded. A total of 3978 crosses (2405 direct crosses and 1573 reciprocal crosses) were made and 974 seeds were obtained from 595 fruits. Percentages of fruit set and seed set varied significantly among different families. Irrespective of the inter-subspecies groups, the percentage of seed germination was above 50% and was not significantly different. Cross compatibility between taxa in terms of percentage of fruit set ranged from 4.6% to 25.6% and varied significantly. Among the inter-subspecies crosses the Assam varieties were found to be the best as female parent. The generated information would be useful for selection of parents and tea improvement programmes in the future.

Keywords: Camellia sinensis, Controlled hybridization, Cross pollination, Exotic germplasm, Germination

Introduction

The tea plant [Camellia sinensis (L.) O. Kuntze] is a woody-perennial plant of which the tender shoots are used to make the end product. The Asian countries, mainly China, India and Sri Lanka generate more than half of the world tea production. It is an important revenue source for tea producing countries both in terms of earning foreign exchange and generating employment.

The scientific approach to tea cultivation in Sri Lanka began after the establishment of the Tea Research Institute of Ceylon in 1925. The mass selection of vigorous seedlings in the nursery, based on morphological characters, followed by the establishment of seed gardens with these plants by the early planters, marked the beginning of unplanned tea breeding. This approach led to the development of several improved seed populations. This may be considered as the first step towards tea improvement prior to the official release of improved tea cultivars. Initially, introduction of new cultivars from India was
followed by selection and large scale multiplication by vegetative cuttings (Gunasekare, 2008).

The final objective of tea breeding is to improve the quality and quantity of the product. High yield, high cup quality, high resistant to biotic and abiotic stresses and, some special characteristics (such as low caffeine, high catechins, etc.) are the main tea breeding objectives in the present stage and predictable future in Sri Lankan tea industry. The scientific community is interested in exploring the health promoting constituents present in tea, namely, flavan-3-ols, flavonols, and their derivatives. Caffeine and polyphenols, the major non-nutrient components in tea, have pharmacological effects. Caffeine, the well-known stimulant, acts on the central nervous system. Scientific investigations have found that polyphenols could act as anti-oxidants after consumption, thus decreasing the risk of many diseases. With the increasing awareness of health benefit of green tea, people have become concerned about functional ingredients, such as methylate catechins, which are beneficial against allergies (Maeda-Yamamoto et al, 2001). Abiotic stresses are the principal causes of crop failure and below average yields for most crops. Abiotic stress factors include low temperatures, high salinity, or drought. The amino acid proline accumulates in many plant species in response to environmental stress such as drought, high salinity, high light etc. (Szabados and Savouré, 2010).

Hybridization is one of the main methods of obtaining genetic variation, and it is an important method of breeding new tea cultivars since the parents as well as the hybridized progeny are heterozygous and heterogeneous (Chen et al., 2007). Two parents selected for their desired characteristics are crossed to introduce genetic variation. After the initial genetic recombination all subsequent propagation steps are carried out by vegetative propagation. In tea, selection of genotypes is carried out in several steps starting from seed progeny and subsequent selections made from the vegetative propagated plants. Then the performance of these accessions is continuously evaluated ‘on station trials’ and ‘multi locational trials’. Finally accessions with favourable promising results are selected and released as new cultivars (Gunasekare et al., 2012).

Tea germplasm is the most valuable and fundamental pre-requisite for tea breeding. Considering the immense genetic diversity of tea germplasm, the future research and development of tea breeding should be focused on integrating biochemical and metabolite profiling approaches into the conventional tea breeding program (Kottawa-Arachchi et al., 2018). Furthermore, the exotic collection of tea germplasm in Sri Lanka includes wild types, cultivated types and Oolong types with unknown pedigree that were conserved in the field gene bank. Most of the accessions in the collection resemble China type and there is a high potential for making use of it for the production of green tea (Kottawa-Arachchi et al., 2017). According to the records, only 23 accessions (4% of the total germplasm) have been frequently utilized in tea breeding programmes as parents in the past two decades to develop new cultivars (Gunasekare, 2012). Germplasm activities in the recent past have shifted from collection and conservation efforts towards proper characterization, evaluation and rational utilization of tea germplasm in the current breeding program.

An understanding of morphological (Piyasundara et al., 2009), biochemical (Kottawa-Arachchi et al., 2013; 2014) and molecular (Mevan et al., 2005; Karunarathna et al., 2018) diversity among the Sri Lankan tea germplasm accessions is important if the best use is to be extracted in plant improvement programs. Meanwhile, the generated information of recent studies on floral diversity (Ranatunga et al., 2017) and metabolite profiling of tea germplasm revealed that results could be effectively used in choosing accessions of desired traits (Punyasiri et al., 2017).

All types of tea are made from *C. sinensis*, the major varieties being *C. sinensis* var. *sinensis* (L) and *C. sinensis* var. *assamica* (Masters) recognized as China type and Assam type, respectively. The morphological attributes between the Assam and China varieties being rather sharp and distinct, it was argued that these two varieties should be raised to species status, in addition to recognizing a third variety or southern form, spp. *lasiacalyx*
(Cambod type) as a subspecies of *C. assamica* (Wight, 1962). The current trend is to consider Assam, China and Cambod types as distinct variants or varieties of *C. sinensis* based on their distinctive morphological and morphometric features (Bezbaruah, 1976; Wight, 1962; 1959). Tea is highly heterogeneous, and all the above taxa freely inter-breed, resulting in a cline extending from extreme China types to those of Assam origin. Self-incompatibility and long-term allogamy make the tea plant highly heterogeneous and consequently with broad genetic variation (Chen et al., 2005).

The tea germplasm of Sri Lanka is predominantly represented by Cambod-type accessions (68%) followed by Assam types (20%) whereas availability of China type accessions is low. Besides, recent taxonomic studies revealed that the China type accessions in the exotic germplasm will immensely be useful in future tea breeding program as parental lines (Ranatunga et al., 2017).

Considering the high genetic diversity of tea germplasm, the future research and development of tea breeding should be focused on integrating biochemical and metabolite profiling approaches into the conventional tea breeding program. Therefore, the present study was focused on conducting controlled hybridization program using inter sub-specific groups as parents for developing diverse progenies for evaluation.

### Materials and Methods

Controlled hybridization programs were performed during the period, 2012-2016 using the three *Camellia* sub species viz. *C. sinensis* var. *assamica*, *C. sinensis* var. *sinensis* and *C. sinensis* subsp. *Lasiocalyx* as parents. Parental cultivars were selected based on the floral traits (style length, style column length, style arm length, stigma position, petal length, number of petals and sepals) and diversity of metabolites such as catechins, caffeine, anthocyanin and amino acids profile (Punyasiri et al. 2017). The selected accessions (Table 1) were maintained throughout the year at appropriate height in tea germplasm.

Table 1. Specific characteristics of parents selected for hybridization

| Cultivar | Characteristics |
|----------|-----------------|
| TRI 2043 | Introduction, blister blight resistant, high anthocyanin |
| TRI 3036 | TRI developed, high theanine, low proline |
| TRI 3055 | TRI developed, high yield, blister blight susceptible, high caffeine |
| CV4B1 | Estate selection, high theanine and proline |
| PBGT 41 | Exotic, high flavonols, low caffeine, low polyphenols |
| PBGT 48 | Exotic, high flavonols, low polyphenols |
| PBGT 49 | Exotic, high flavonols, low polyphenols |
| PBGT 61 | Exotic, high flavonols, low theanine and proline, low polyphenols |
| PBGT 68 | Exotic, high flavonols, low theanine and proline, low polyphenols |

Source: TRI, Sri Lanka, various documents

Hand pollination was conducted from January to April during each year. Pollinated flowers were closely monitored throughout the harvesting period and the number of crosses, number of fruits, and number of seeds were recorded. Harvested seeds were propagated in sand bed and germinated seeds were transferred to nursery bags. All TRI recommended practices were applied to raise healthy plants within the nine-month nursery period. Three reproductive biological parameters; percentage pollination success, percentage reproductive output and percentage of germination success, were also estimated.

Harvested seeds of controlled hybridization program in 2013/2014 were germinated under *in-vitro* condition. Seeds were treated in 20% (v/v) Clorox for surface sterilization and then were washed with sterile distilled water two to three times. Embryos were isolated aseptically from...
surface sterilized seeds and inoculated in vitro on Murashige and Skoog (MS) solid medium supplemented with 3 mg/L BAP, 0.5 mg/L IBA, 8 g/L Agar and 30 g/L sucrose following the previously developed method (Gunasekare and Evans, 2000). The cultures were incubated at 25 °C in 15 h photoperiod. The successfully germinated embryos were sub cultured on to the same medium and multiplied several times at two monthly intervals to increase the number of micro-shoots required for future experiments. Micro-shoots with healthy growth were transferred to the ex-vitro rooting medium (Coir dust:topsoil:sand at 1:1:1) and maintained under >80% relative humidity inside the walking type propagator. After two months, rooted micro-shoots were transferred to the nursery bags and maintained at the plant nursery for field planting. Data were analyzed by Analysis of Variance (ANOVA) using the Statistical Analysis Software (SAS version. 9.1).

Results and Discussion

The data on fruit setting and germination during the period from 2012 to 2016 are presented in Table 2. A total of 3978 crosses (2405 direct crosses and 1573 reciprocal crosses) of six families with 12 parental combinations were harvested in the hybridization programme. A total of 974 seeds were obtained from 595 fruits, of which 651 seeds were germinated.

During the hybridization programs from 2012 to 2016, a large number of artificial pollinations of different parental combinations were done. A summary of the pollinations results of various crossing families is presented in Table 3. Generally, certain degree of cross compatibility between taxa has been reported (Bezbaruah and Saikia, 1977). Percentage of fruit set (pollination success) varied significantly among 17 different cross combinations (P<0.01; CV = 16.22; 3978 crosses). The maximum percentage of fruit set was shown by the Assam x China followed by Assam x Cambod crosses. Considering the range of percentage fruit-set, direct and reciprocal cross combinations between China and Cambod varieties were found less compatible, whereas Assam x China crosses showed the highest percentage of success. Kumarihami et al. (2016) examined the self and cross compatibility among Assam and China types and fruit set was observed only in cross pollination between Assam and China types. Present study revealed that the cross compatibility between Assam and China types was higher than other combinations.

A previous study conducted in India (Bezbaruah and Saikia, 1977), reported that the Assam variety were generally more suitable as pollen sources and less suitable as female parents. But the present study revealed that, the Assam varieties as female parents showed better fruit setting abilities with the China and Cambod varieties present in Sri Lanka. The results revealed the genotypes having variable affinity to different tea varieties: C. sinensis var. assamica, C. sinensis var. sinensis, and C. sinensis spp. lasiocalyx showed dissimilarities in breeding behaviour and in reproductive ability.

Germplasm innovation is a function of pre-breeding. The method of innovation is diverse; a main important genotype innovation is hybridization, including distant hybridization. Distant hybridization is a powerful method for broadening the genetic base of new varieties (Chen et al., 2007). Based on morphological and floral traits, the exotic collection is predominantly represented by China type (72%) followed by Cambod type (20%) and Assam type (8%) (Kottawa-Arachchi et al., 2017).

The exotic tea germplasm has been used for the first time in hybridization program during 2013 and 1196 crosses were made using three divers exotic accessions as pollen parents whereas TRI 3055 was used as common female parent. Irrespective of the combinations, percentage of fruit set was below 20% in hybrids 2013 and 2014. Among the three combinations, the lowest percentage of fruit set (5.4%) was observed in TRI3055 x PBGT49 followed by TRI3055 x PBGT48 and TRI3055 x PBGT41 crosses. Interestingly, a similar trend was observed with reciprocal crosses in controlled hybridization programme in 2014.
Table 2. Summary of hybridization programs from 2012 to 2016

| Hybrid program | Combinations              | Taxa           | No. of crosses | No. of Fruits | % fruit set (PS)* | No. of Seeds | % seed set (RO)* | No. of seeds per fruit | No. of germinated seeds | % germination |
|----------------|---------------------------|----------------|---------------|---------------|------------------|--------------|------------------|-------------------------|------------------------|--------------|
| 2012           | TRI3055 x TRI 2043        | Cambod x Assam | 200           | 42            | 21.0             | 78           | 39.0             | 1.86                    | 66                     | 84.6         |
|                | TRI3055 x PBGT41          | Cambod x Cambod | 450           | 71            | 15.8             | 111          | 24.7             | 1.56                    | 79                     | 71.2         |
|                | TRI3055 x PBGT48          | Cambod x China | 396           | 48            | 12.1             | 73           | 18.4             | 1.52                    | 49                     | 67.1         |
|                | TRI3055 x PBGT49          | Cambod x China | 350           | 19            | 5.4              | 23           | 6.6              | 1.21                    | 15                     | 65.2         |
|                | TRI2043 x TRI3055         | Assam x Cambod | 555           | 124           | 22.3             | 196          | 35.3             | 1.58                    | 153                    | 78.1         |
| 2013           | TRI3055 x PBGT41          | Cambod x Cambod | 110           | 10            | 9.1              | 13           | 11.8             | 1.3                     | 10                     | 76.9         |
|                | TRI3055 x PBGT48          | Cambod x China | 92            | 5             | 5.4              | 5            | 5.4              | 1                       | 5                      | 100          |
|                | TRI3055 x PBGT49          | Cambod x China | 100           | 19            | 19.0             | 25           | 25.0             | 1.31                    | 17                     | 68.0         |
|                | PBGT41 x TRI3055          | Cambod x Cambod | 300           | 30            | 10.0             | 46           | 15.3             | 1.53                    | 38                     | 82.6         |
| 2014 (In-vitro)| PBGT48 x TRI3055          | China x Cambod | 226           | 12            | 5.3              | 24           | 10.6             | 2                       | 10                     | 41.7         |
|                | PBGT49 x TRI3055          | China x Cambod | 230           | 9             | 3.9              | 16           | 7.0              | 1.77                    | 12                     | 75.0         |
| 2015           | TRI3055 x PBGT41          | Cambod x Cambod | 132           | 23            | 17.4             | 38           | 28.8             | 1.65                    | 18                     | 47.4         |
|                | PBGT41 x TRI3055          | Cambod x Cambod | 262           | 36            | 13.7             | 59           | 22.5             | 1.63                    | 42                     | 71.2         |
|                | TRI3036 x PBGT61          | Assam x China  | 90            | 23            | 25.6             | 45           | 50.0             | 1.95                    | 24                     | 53.3         |
| 2016           | TRI3036 x PBGT68          | Assam x China  | 280           | 68            | 24.3             | 134          | 47.9             | 1.97                    | 73                     | 54.5         |
|                | CV4B1 x PBGT61            | Assam x China  | 90            | 23            | 25.6             | 41           | 45.6             | 1.78                    | 17                     | 41.5         |
|                | CV4B1 x PBGT68            | Assam x China  | 115           | 33            | 28.7             | 47           | 40.9             | 1.42                    | 23                     | 48.9         |

*PS = pollination success; RO = reproductive outputs

Utilization of *in vitro* techniques for conventional breeding program

Aborted or under developed ovaries were found very often in incompatible parents soon after anthesis and intensive abortions of fruitlets were recorded during the initial 15-20 days after pollination despite to the successful pollination (Ariyarathna *et al.*, 2011).
Table 3. Summary of the pollination results of various combinations

| Taxa           | No. of Crosses | No. of Fruits | % fruit set (PS)* | No. of Seeds | % seed set (RO)* | No. of germinated seeds | % germinated seeds |
|----------------|---------------|---------------|-------------------|--------------|------------------|-------------------------|--------------------|
| China x Cambod | 456           | 21            | 4.6c              | 40           | 8.8c             | 40                      | 58.4               |
| Assam x Cambod | 555           | 124           | 22.3a             | 196          | 35.3ab           | 153                     | 78.1               |
| Assam x China  | 575           | 147           | 25.6a             | 267          | 46.4a            | 137                     | 50.0               |
| Cambod x China | 938           | 91            | 9.7bc             | 126          | 13.4c            | 64                      | 78.1               |
| Cambod x Assam | 200           | 42            | 21.0a             | 78           | 39.0a            | 66                      | 84.6               |
| Cambod x Cambod| 1254          | 170           | 13.5b             | 267          | 21.3bc           | 139                     | 69.9               |

*PS = pollination success; RO = reproductive outputs; NP: nursery propagated, IP: In vitro propagated. Means followed by the same letter are not significantly different at P=0.01.

Tea fruit maturation requires 8-9 months after pollination, but abortion of immature fruits was also observed within 5 to 6 months after pollination. Immature embryo rescuing tissue culture strategy is now being developed to improve the success ratio of distant hybridization in the TRI Sri Lanka. The hybridization program in 2014 was focused to generate diverse progeny with the same parents used in previous year as direct crosses and reciprocal crosses. The harvested seeds with viable embryos have been tissue cultured for producing clonal progenies for further evaluations. This has been the first application of successful integration of embryo culture technique in conventional tea breeding program. As a result of in vitro propagation of embryos, the germination percentage of several combinations was above 75% (Table 2).

Establishment of hybrid progenies

A progeny was established with one hundred and twenty hybrids generated from hand crossing (both direct and reciprocal crosses) using diverse parents TRI3055 and PBGT41 in distant hybridization programs 2013 and 2014. This progeny trial would be a potential source for selecting planting materials with desirable traits such as high quality green and caffeine less tea. Recently, tea breeders in different tea growing countries have developed anthocyanin-rich purple coloured tea varieties (Joshi et al., 2015; Kerio et al., 2012). The phenolic compounds such as catechins and caffeine content of various anthocyanin-rich varieties have been compared with green tea leaves. Recent study revealed that the antioxidant values of purple coloured teas were higher than green coloured tea due to the presence of catechins and anthocyanins (Joshi et al., 2015). Therefore, tea from anthocyanin-rich cultivars can become specialty teas with high antioxidant activity. Another progeny trial established with one hundred and thirty hybrids generated by crossing two diverse parents, TRI 2043 which is characterized with high pubescence density, pigmented leaves, tolerant to blister blight disease, and TRI 3055 a non-pigmented, high yielding cultivar.

The tea plant is commonly grown in rain-fed ecosystems and thus it encounters seasonal water deficit conditions that induce loss in crop yield. Proline accumulation was significantly higher in the drought-tolerant cultivars in Kenyan germplasm and suggesting that proline concentration could be used as a marker for drought-tolerance in tea (Maritim et al., 2015). Therefore, four parental combinations were used to create diverse progeny in hybridization program 2016. Among four germplasm accession used, two cultivars (TRI 3036 and CV4B1) reported high amount of theanine and proline respectively. Other two exotic accessions (CO61 and CO68) recorded low amount of above two amino acids. Another progeny trial has been established using above combination as segregating population for important amino acids viz. theanine and proline.

The progenies generated from present controlled hybridization programs can be effectively used in selection of tea accessions with desired traits.
Integrating this information into the conventional tea improvement program to produce new tea cultivars to meet the ever-challenging environment and production of diverse products is vital for future tea breeding program.

Conclusion

Irrespective of the intra-specific, the percentage of seed germination was above 50%. Cross compatibility between taxa in terms of percentage of fruit-set ranges from 4.6% to 25.6% and varied significantly. Among the intra-specific crosses, the Assam varieties were found to be the best as female parent.

References

Ariyaratna H.A.C.K., Gunasekare M.T.K., Kottawa-Arachchige J.D., Paskaratevan R., Ranaweera K.K., Ratnayake M. and Kumara J.B.D.A.P. (2011): Morpho-physiological and phenological attributes of reproductives biology of tea (Camellia sinensis (L.) O. Kuntze) in Sri Lanka. Euphytica. 181: 203–215. https://doi.org/10.1007/s10681-011-0399-9

Bezbaruah H.P. (1976): The tea varieties in cultivation - an appraisal. Two and a Bud, 23: 13–19.

Bezbaruah H.P. and Saikia L.R. (1977): Variations in self and cross-compatibility in tea (Camellia sinensis L.) - A summary of forty years’ pollination results at Tocklai. Two and a Bud, 24, 21–26.

Chen J., Wang P., Xia Y., Xu M. and Pei S. (2007): Genetic diversity and differentiation of Camellia sinensis L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. Genet. Resour. Crop Evol. 52: 41–52. https://doi.org/10.1007/s10722-005-0285-1

Chen L., Zhou Z.X. and Yang Y.J. (2007): Genetic improvement and breeding of tea plant in China: individual selection to hybridization and molecular breeding. Euphytica, 154: 239–248.

Gunasekare M.T.K. (2012): Tea Plant (Camellia sinensis) breeding in Sri Lanka, in: Chen, L., Apostolides, Z. (Eds.), Global Tea Breeding–Achievements, Challenges and Perspectives. Springer-Verlag Berlin Heidelberg/Zhejiang press, pp. 125–176.

Gunasekare M.T.K. (2008): Planting Material, in: Zoysa, A.K.N. (Ed.), Handbook on Tea. pp. 34–49. Tea Research Institute of Sri Lanka.

Gunasekare M.T.K. and Evans P. (2000): In-vitro rooting of microshoots of tea (Camellia sinensis L.). Sri Lanka Journal if Tea Science, 66: 5–15.

Gunasekare M.T.K., Ranatunga M.A.B., Piyasundara J.H.N. and Kottawa-Arachchige J.D. (2012): Tea genetic resources in Sri Lanka: collection, conservation and appraisal. International Journal of Tea Science, 8: 51–60.

Jeganathan B., Punyasiri P.A.N., Kottawa-Arachchi J.D., Ranatunga M.A.B., Abeyesinghe I.S.B., Gunasekare M.T.K. and Bandara B.M.R. (2016): Genetic Variation of Flavonols Quercetin, Myricetin, and Kaempferol in the Sri Lankan Tea (Camellia sinensis L.) and Their Health-Promoting Aspects. International Journal of Food Science. 2016: 1–9.

Joshi R., Rana A. and Gulati A. (2015): Studies on quality of orthodox teas made from anthocyanin-rich tea clones growing in Kangra valley, India. Food Chemistry 176: 357–366. https://doi.org/10.1016/j.foodchem.2014.12.067

Kumararathna K.H.T., Mewan K.M., Weerasena O.V.D.S.J., Perera S.A.C.N., Edirisighe E.N.U. and Jayasoma AA. (2018): Understanding the genetic relationships and breeding patterns of Sri Lankan tea cultivars with genomic and EST-SSR markers. Scientia Horticulturae (Amsterdam), 240: 72–80. https://doi.org/10.1016/j.scienta.2018.05.051

Kerio L., Wachira F.N. and Rotich M.K. (2012): Characterization of anthocyanins in Kenyan teas: Extraction and identification. Food Chem.istry, 131: 31–38. https://doi.org/10.1016/j.foodchem.2011.08.005

Kottawa-Arachchi J.D., Dilrukshi S., Piyasena M. and Ranatunga M.A.B. (2017): Floral characterization of exotic tea (Camellia sinensis L) germplasm in Sri Lanka, in: Proceedings of the International Symposium on Agriculture and Environment 2017. University of Ruhuna, Sri Lanka, pp. 43–45.

Kottawa-Arachchi J.D., Gunasekare M.T.K. and Ranatunga M.A.B. (2018): Biochemical diversity of global tea [Camellia sinensis (L.) O. Kuntze] germplasm and its exploitation: a review. Genetic Resources and Crop Evolution, 1–15. https://doi.org/10.1007/s10722-018-0698-2
Kottawa-Arachchi J.D., Gunasekare M.T.K., Ranatunga M.A.B., Punyasiri P.A.N. and Jayasinghe L. (2013): Use of biochemical compounds in tea germplasm characterization and its implications in tea breeding in Sri Lanka. Journal of the National Science Foundation of Sri Lanka 41: 309–318. https://doi.org/10.4038/jnsfsr.v41i4.6252

Kottawa-Arachchi J.D., Gunasekare M.T.K., Ranatunga M.A.B., Punyasiri P.A.N., Jayasinghe L. and Karunagoda R.P. (2014): Biochemical characteristics of tea (Camellia L. spp.) germplasm accessions in Sri Lanka: Correlation between black tea quality parameters and organoleptic evaluation. International Journal of Tea Science, 10: 3–13.

Kumarihami H.M.P.C., Oh E.U., Nesumi A. and Song K.J. (2016): Comparative study on cross-compatibility between Camellia sinensis var. sinensis (China type) and C. sinensis var. assamica (Assam type) tea. African Journal of Agricultural Research, 11: 1092–1101. https://doi.org/10.5897/AJAR2015.9951

Maeda-Yamamoto M., Sano M., Matsuda N., Miyase T., Kawamoto K., Suzuki N., Yoshimura M., Tachibana H. and Hakamata K. (2001): The change of epigallocatechin-3-O-(3-O-methyl) gallate content in tea of different varieties, tea seasons of crop and processing method. Journal of the Japan Society of Food Science and Technology, 48: 64–68.

Maritim T.K., Kamunya S.M., Mireji P., Mwendia C., Muoki R.C., Cheruiyot E.K. and Wachira F.N. (2015): Physiological and biochemical response of tea [Camellia sinensis (L.) O. Kuntze] to water-deficit stress. The Journal of Horticultural Science and Biotechnology 90: 395–400.

Mevan K.M., Everard J.M.T.D., Liyanage A.C., Gunasekare M.T.K., Tirimanne T.L.S. and Karunanayake E.H. (2005): Studying genetic relationships among tea (Camellia sinensis L.) cultivars using RAPD markers. Sri Lanka Journal of Tea Science, 70: 10–17.

Piyasundara J.H.N., Gunasekare M.T.K. and Wickramasinghe I.P. (2009): Characterization of Tea (Camellia sinensis L.) Germplasm in Sri Lanka using Morphological Descriptors. Sri Lanka Journal of Tea Science, 74: 31–39.

Punyasiri P.A.N., Jeganathan B., Kottawa-Arachchi J.D., Ranatunga M.A.B., Abeysinghe I.S.B., Gunasekere M.T.K. and Bandara B.M.R. (2017): Genotypic variation in biochemical compounds of the Sri Lankan tea (Camellia sinensis L.) accessions and their relationships to quality and biotic stresses. The Journal of Horticultural Science and Biotechnology 92: 502–512. https://doi.org/10.1080/14620316.2017.1289070

Ranatunga M.A.B., Kottawa-Arachchi J.D., Gunasekere M.T.K. and Yakandawala D.M.D. (2017): Floral diversity and genetic structure of tea germplasm of Sri Lanka. International Journal of Biodiversity 2017: 1–11.

Szabados L. and Savouré A. (2010): Proline: a multifunctional amino acid. Trends in Plant Science, 15: 89–97. https://doi.org/10.1016/j.plants.2009.11.009

Wight W. (1962): Tea classification revised. Current Science, 31: 298–299.

Wight W. (1959): Nomenclature and classification of the tea plant. Nature 183: 1729–1728.