Review Article

Chapter 2

REVIEW OF TEA (*CAMELLIA SINENSIS*) BREEDING AND SELECTION IN SOUTHERN AFRICA

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ABSTRACT

Historically tea seeds were imported into Southern Africa that belonged to either the *sinensis* or *assamica* varieties. Later on vegetatively propagated hybrid cultivars selected from F_1 progeny raised from a deliberate hybridization programme between selected parents with desirable characteristics on the basis of quality and yield related parameters have been used in establishing tea plantations. New high throughput screening methods including biochemical and molecular markers are being investigated to improve the selection process and eliminate genotypes with poor quality potential before the expensive mini manufacture stage. A call is made for the establishment of a Global Tea Research Project to address common problems.

Keywords: Tea, *Camellia sinensis*, yield, quality, HPLC chromatography, capillary electrophoresis, leaf color, chlorophyll fluorescence, elite mother bushes, cultivars, breeding and selection, early prediction.

Introduction

The first tea planted in Africa was in 1850 in the Durban Botanical Garden on the eastern shore of South Africa near the port of Durban. This tea was China type. In 1877 Sir Liege Hulett imported Indian hybrid seeds and planted it on the Kearsney Estate near Durban. He encouraged others to follow suite and by 1900, 472 tons of made tea was being produced in South Africa. In a parallel development, tea seeds were imported from the Kew Botanic Gardens in England to Malawi (then Nyasaland) in 1876. This tea was all China type (*Camellia sinensis var. sinensis*) that is, on one hand, characterized by low yield due to small leaf and light shoots and low/moderate quality due to low levels of catechins and slow or poor fermentation potential and aroma but tolerant to adverse climatic conditions, on the other hand. This was later followed by the importation of seed of Assam type (*Camellia sinensis var. assamica*) that is generally characterized by large heavy shoots and fast fermentation. Initially planting in Malawi was with China type tea seeds. Later, in the early 1930s when Indian hybrid seeds were imported into Malawi and several other African countries including Tanzania, Kenya and Uganda.

In order to improve yield and quality of the crop in Southern Africa the Tea Research Foundation of Central Africa (TRFCA) was established in Malawi in 1933, initially as a tea research station under the Department of Agriculture before gaining autonomy in 1966. The TRFCA has an active plant breeding and selection programme aimed at solving tea production problems such as drought, extreme temperatures, pests and diseases, low yield and low quality, under local growing conditions in order to increase productivity and maximize total value and income from the crop.

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I. Breeding strategy

Tea production in the Southern Africa region is largely limited by climatic factors, particularly low and unevenly distributed rainfall and large fluctuation in temperatures, resulting in three distinct seasons. These seasons are the warm wet season that is the main growing season running from mid November to mid April, followed by the cool wet season from mid April to mid August when minimum night temperatures limit shoot extension and development, finally the hot dry season from mid August to mid November when soil moisture stress and high saturated vapor pressure deficit (SVPD) limit shoot growth. These factors equally affect both yield and quality. This confines the main growing season to the rainy season from mid November to mid April when growing conditions are optimum. The thrust has therefore been to look for vegetatively propagated cultivars that are drought tolerant, have a low base temperature and are high yielding and that would thrive under these harsh conditions and make high quality teas in the main growing season.

Furthermore, with the current increase in labor shortage problem, particularly in Zimbabwe and South Africa, it has also become imperative to find cultivars that are suitable for mechanical harvesting. In addition, the breeding programme has also taken steps to start breeding for health benefit teas with high levels of antioxidants and low levels of caffeine as a value adding strategy for the crop. There has been no systematic breeding for disease resistance/ tolerance because the region does not experience any serious problem from insect pests and diseases probably as a result of the absolute phytosanitary embargo on tea plant material into African countries since 1951 that has helped to prevent the spread of diseases (Ellis, 1983).

I (a) Source of variation (gene pool)

Initially the established seedling fields provided the gene pool from which elite mother bushes for VP (Vegetatively Propagated) cultivars were selected. This started in the mid 1950s and selection was based on drought tolerance and rate of recovery from prune. Some of the field selections were made at Swazi (now Nsuwadzi) research station in Malawi, e.g. SFS150 and SFS204 have become very popular cultivars in the region, each now covering over 100 hectares. In recognition of the limited natural variation for specific attributes expected of seedling tea population, a deliberate hybridization programme to create variable F_1 progeny populations for specific attributes from which to select elite mother bushes for bulking up vegetatively, started in 1960 at the TRFCA (Ellis and Nyirenda, 1995). Some of the released VP cultivars along with some of the field selections have also been used as parents in deliberate crossings to create variations for specific attributes.

I (b) Selection of elite mother bushes

Selection of elite mother bushes for quality attributes is based on fast fermentation using the chloroform test. Other attributes considered when selecting mother bushes include growth vigor, shoot size and tolerance to major pests and diseases. The chloroform test is very inexpensive (about USD 1/ genotype) and is used to classify plants as "slow", "medium" or "fast" fermenters (Sanderson, 1963). Using this test, the "slow" fermenters are eliminated from the selection programme in year 4. This occurs in the nursery plants, thus eliminating them from expensive field trials (Table 1).

Over the years there have been changes in the weather pattern (characterized by short rainy

Table 1: Breeding and selection programme for new cultivars at the Tea Research Foundation in Central Africa (Malawi)

Place	Year	Activity	Number of genotypes	Number of plants per genotype
SeedGarden	0	Controlled crosses of **flowers** in seed garden	5000	5000
Nursery	1	Germinate **viable seeds** in pots	3750	3750
Nursery	2,3	Select **seedlings** on the basis of plant vigor. Take single cuttings for vegetative propagation	2500	2500
Nursery	4	Select **seedlings** on the basis of rooting ability and nursery performance. Eliminate slow fermenters (chloroform test) Take 30-50 cuttings from selected **seedlings**	350	350
Field	5	Plant **cultivars** in 2 x 8 = 16 bush observation plots in field Mini-manufacture for organoleptic assessment by expert tea taster	150	2400
Field	6,7,8	Select **cultivars** on field performance and organoleptic quality	20	2400
Field	9	Establish **cultivars** in replicated field trials of 5 x 6 = 30 bushes per plot replicated five times, on several research stations	20	3000 per station
Field	10	Early release of **cultivars** to estates	5	as required
Field	11,12,13,14	Evaluation of **cultivars** on all traits for yield and quality	5	10,000 to 100,000
Industry	15	Possible release of 1-2 new **cultivars** to industry	0-3	as required

season and prolonged drought conditions), life style of people (leading to decreased desire to work on farms) and consumers' needs or taste (going for specific quality). These changes have strongly impacted on the breeding and selection strategy and establishment of the relevant selection criteria for elite cultivars. For example, the two main buyers of Malawi tea at Limbe auctions look for two distinct types of tea. One of them looking for red coppery teas and the other, looking for yellow teas. Similarly, growers with irrigation facilities can cope with drought susceptible cultivars whereas drought tolerance is crucial to growers without irrigation. Selection of these attributes requires establishment of rapid and reliable selection criteria and methods.

I (c) Assessment of made tea quality

(i) Organoleptic assessment

The most expensive part of the selection process is the organoleptic tea quality assessment by expert tea tasters. This can only be done when about 200 grams of fresh shoots can be obtained from each cultivar. This occurs in year 8 when the plants selected in year 5 for field trials are 3 years old and produce enough leaves (Table 1). At this stage, up to 150 cultivars have to be individually mini-manufactured into black tea. This is done so that tea tasters with expertise in teas from Southern Africa can perform organoleptic evaluations for tea quality. This number of 150 cultivars is a bottleneck that cannot be opened any wider due to limits on available land for field trials, the capacity of the mini-manufacture facility and the work load on the tea tasters. Thus, it is imperative to eliminate cultivars with poor quality potential before this stage. Tea quality is a complex trait and it is impossible to find one or two markers that will predict it perfectly. However, a few strong markers may be quite useful at this stage of the selection programme. These markers may be suitable physical, morphological, biochemical or genetic markers. Such markers are needed to help the plant breeder decide which cultivars should be rejected before costly field trials and mini-manufacture is undertaken. Since the plants from year 5, 6, 7 and 8 are in field trials at the

same time, with 2400 plants from each year, a total of 10,000 plants are in the field. These require 1 hectare of land. It is important to realize that for cultivar selection only semi-quantitative tests are required. Normally only two, three or four levels of classification are required. Thus only "present/ absent" or "low/medium/high" or "low/medium/high/ excellent" classification criteria are necessary.

(ii) Total theaflavins (Flavognost test)

Hilton and Ellis (1972) reported the earliest correlation between a biochemical constituent and the value of tea. Several commercial packets of tea were purchased in London super-markets and analyzed for theaflavins with the flavognost test at the TRFCA. Since then, several attempts to find correlation between value and theaflavins in other countries have failed (Owuor and Obanda, 1995). This is because different countries produce tea with different attributes. For example, Southern Africa produces plain but very brisk red coppery teas that are characterized by high levels of theaflavins, whereas, other regions like Darjeeling in India and high altitude areas in Sri Lanka and Kenya, produce aromatic teas. Theaflavins are not very important in aromatic teas.

(iii) Individual progeny theaflavins – analysis by Capillary electrophoresis (CE)

By the early 1990s, CE became available for analyzing made tea. Then theaflavin that was measured by the flavognost reagent could be measured by CE. It was soon discovered that there are four major theaflavins. We developed capillary electrophoresis chromatographic methods and found good correlations between individual theaflavins and value (Wright *et al*, 2002). These correlations are better than with the total theaflavins measured by the flavognost reagent. However, to obtain theaflavins in the tea sample, mini-manufacture is still required. Thus, biochemical markers in the green leaf that could be correlated with the value of the final product are being evaluated.

(iv) Catechins analysis by High performance liquid chromatography (HPLC)

TRFCA undertook the search for catechins and value by selecting twenty "Good" and twenty "Poor" (GAP) cultivars. "Good" and "Poor" were based on valuations of these cultivars on several prior occasions by expert tea tasters. The leaves were harvested at the TRFCA, steamed and dried. They were analyzed at the University of Pretoria (Wright *et al*, 2000). The results showed that the concentration of two minor catechins, namely epicatechin (EC) and epicatechin gallate (ECg) could be used to cluster the forty cultivars into two clusters that matched our classification of "Good" and "Poor". With this knowledge, young seedlings in the nursery can be analyzed during year 4. This can eliminate some "mother plants" in the nursery and prevent costly experiments in the field. This EC+ECg test has not yet been implemented into the breeding programme due to limited funding. It costs about USD 50 per genotype.

The HPLC and capillary electrophoresis methods require costly equipment and skilled operators. These are not readily available at the research organizations, where the green leaf is. Thus, simpler, affordable methods have to be found that are suitable for field use.

II. Limitations of current selection criteria

II (a) Mini-manufacture

This is limited by the age of the bushes when adequate leaf can be harvested and number of

cultivars that can be mini-manufactured and tasted by expert tea tasters per season. There are also high electricity costs involved. Being a final confirmatory quality assessment, only short-listed potentially high quality selections should undergo this test. This can only be achieved when rapid, inexpensive screening methods are in place to eliminate poor quality genotypes in the early stages of selection.

II (b) Time consuming protocols

Most of the current selection methods can only start once the bushes have attained a particular stage. This is responsible for the long selection cycle making the programme expensive. The early methods of catechin analysis had to be abandoned because they were expensive and time consuming.

II (c) Cost of assessment

The current biochemical methods are expensive due to the need for special equipment and expertise. The limited funding to most research organizations makes it impossible to analyze all samples going through the breeding and selection process. For example, the fresh leaf catechins can be measured in a single two and a bud shoot, but cost about USD 101 per sample. The flavognost costs only about USD 8 per sample but can only be done after mini-manufacture that costs USD 50 per sample, plus 50g fresh shoots costing another USD 10, for a total cost of USD 68. The catechin test is quite attractive due to the possibility of doing this test early (single bush, year 4) in the selection process.

II (d) Need for theaflavin analysis

It is interesting to note that most of the strong predictors are theaflavin related. Thus, HPLC analysis and mini-manufacture are needed, for which 50-500 grams fresh leaf material is required. This makes theaflavin based predictors very expensive. The most affordable tests are those based on the color of the reflected light from the surfaces of fresh leaf or the leaf before and after the chloroform test. It is also interesting to note the parameters that are weak predictors of valuation. The polyphenols, measured as milligrams per gram dry leaves, "Total polyphenols (mg/g DL)" as measured by the Folin Ciocalteu test on fresh leaf showed a weak correlation with the valuation of tea. This suggests that the anti-oxidants are not correlated to Tea tasters' valuation. Neither is "Caffeine (umol/g DL)" or "EGCg (umol/g DL)". Thus, cultivars that have a low valuation need to find other markets. Since EGCg and anti-oxidants are finding new applications in the confectionary, food, cosmetic and pharmaceutical industries, new market opportunities are increasing for cultivars with high EGCg and "Total polyphenols (mg/g DL)".

III. New (potential) selection criteria for tea quality

The TRFCA constantly investigates new tests of tea quality. The tests should be either less expensive than mini-manufacture, or show stronger correlation than the current tests e.g. the EC+ECg test. Ideally they should be of the less expensive category and suitable for low-technology field conditions. The HunterLab colorimeter has been tested to measure the color of reflected light from leaves but the results are not yet good enough (correlation with tea value is weak), as discussed below.

III (a) Improved chloroform test

Essentially this simplifies to the color of the single leaf after 80 minutes in chloroform vapor. Up to now, the chloroform test has only been used to classify genotypes as "slow", "medium" or "fast" fermenters. Now that some buyers look for specific

color of the infusion, the test can be used to predict color of the infusion as there is marked variation in color of the fermented leaf in chloroform vapor, ranging from light brown to dark brown.

III (b) Catechins in fresh leaf

HPLC was used to measure several catechins in micro moles per gram dry leaf (DL). These were catechin, epi-catechin (EC (μmol/g DL)), epi-catechin gallate (ECg (μmol/g DL), epi-gallocatechin (EGC (μmol/g DL)) and epi-gallocatechin gallate (EGCg (μmol/g DL)). Epi-catechin had the highest r-square = 0.39. Previously Ellis and Nyirenda (1995) had suggested that the ratio of the simple- to the gallo-catechins might be a good predictor of value. This was expected since the simple catechins are the limiting factor in the production of theaflavins. This ratio had a low r-square = 0.18. However, the sum of the simple catechins, namely catechin + epi-catechin + epi-catechin gallate had a r-square = 0.38. Thus, it is as good as the epi-catechin. The sum of the individual catechins (flavan-3-ols) or (SIF) had an r-square = 0.37. This makes HPLC analysis of the fresh leaf catechins as good a predictor of valuation as the flavognost test (r-square = 0.37). The advantage of the SIF test is that it requires a single leaf, thus it can be performed at the nursery stage before expensive field trials are undertaken.

III (c) Chlorophyll fluorescence

Measurement of the chlorophyll fluorescence of the dark adapted leaf, using a plant efficiency analyzer, showed that minimum fluorescence (FO) on the second and third leaves of a tea shoot and maximum fluorescence (FM) on the third leaf were negatively correlated to the tasters total score, theaflavin (TF) and valuation (Mphangwe and Nyirenda, 1997). This work requires confirmatory tests on materials grown under different management conditions as well as monitoring the changes in chlorophyll fluorescence with age of the bushes. Once these are established the method can be used in the early stages of selection by selecting against high values of chlorophyll fluorescence.

IV. Future strategy

The bottle-neck in the screening for quality of tea worldwide seems to be the mini-manufacture of tea samples for organoleptic assessment by expert tea tasters. It is impossible to train experts quickly. Thus, it is important that their valuable time is not wasted on inferior genotypes. High throughput methods for eliminating inferior genotypes at the single "mother bush" stage must be sought so that only genotypes with good quality (briskness or aroma) potential are advanced into field trials.

To fully achieve these objectives a global tea research project with the following goals is recomemded:

1. The free exchange of well-researched cultivars for use as parents.
2. To fund novel scientific research to discover biochemical/genetic markers for quality selection criteria e.g. HPLC, capillary electrophoresis, GC-MS, gene arrays, DNA probes, mono-clonal antibodies for each of the theaflavins, aroma precursors, aroma releasing enzymes, etc.
3. To develop low technology methods based on the above scientific discoveries e.g. leaf color measurements with color charts and dip-sticks with anti-bodies for theaflavins.
4. To fund outreach programmes to train research staff in the tea research institutes to use the low technology methods for selection of elite mother bushes.

5. To develop fast grafting techniques suitable for tea bushes.
6. To develop methods for storage of fresh leaf for winter manufacture.
7. Genotype x Environment trials need to be done to select the adaptable and stable clone(s).

The breeding should be left to local tea research institutes. They will select bushes with high yield in their respective regions of interest. The local yield parameters will be for diseases, pests, soil conditions, temperature and drought.

Conclusion

Conventional tea breeding and selection requires 15 years. This is a very long-term process, as in other fruit trees. Inexpensive selection criteria are required to eliminate genotypes with low potential as early as possible. Such selection criteria will not shorten the selection process; they only make it more efficient. Thus, instead of starting with only 5000 flowers, one can start with two or three times this amount and use selection criteria to advance only the best 150 genotypes beyond the 4th year. Since such criteria will be common to many tea producing countries, it would benefit all tea research institutes to collaborate more in the future.

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