Indian J. Plant Genet. Resour. 28(2): 256-259 (2015)
DOI 10.5958/0976-1926.2015.00031.5

SHORT COMMUNICATION

Screening of *Coffea arabica* Germplasm for Caffeine Level

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(Received: 28 August 2014; Revised: 02 January 2015; Accepted: 23 March 2015)

A total of 76 collections of coffee bean (*Coffea arabica*) are being maintained in the germplasm bank at Coffee Research Sub Station in North Kodagu, Karnataka, India. Following evaluation of caffeine level in 34 collections, a wide variability ranging from 1.18% (*Misan Taferikala*) to 2.07% (*Sumatra*) with an overall mean value of 1.84%, was observed.

Key Words: Caffeine, *Coffea arabica*, Germplasm bank

Coffee is a popular beverage consumed worldwide. Although more than 80 coffee species have been identified worldwide, only two species *viz*., *Coffea arabica* (arabica coffee) and *C. canephora* (robusta coffee) are economically important. India grows both arabica and robusta coffee almost in equal proportions mainly in the southern states of Karnataka, Kerala and Tamil Nadu covering 4 lakh ha. The annual production is around 3.1 lakh MT with 4.5% share in global production. Regarding processing methods, 75% of arabica coffee is processed by wet method and 80 to 85% of the robusta produce is processed by dry method. In wet method, only ripe fruits are harvested and pulped by passing through a pulping machine to remove the fruit skin. Later, the pulped bean along with mucilage is kept overnight for natural fermentation and then the beans are washed with water. The washed beans are soaked in water for four to six h and then sun dried for 6 or 7 days by spreading them evenly on drying yards. In certain cases, the pulped beans with the mucilage are immediately passed through aqua washers to remove mucilage by friction and thus skipping the natural fermentation step. The coffee thus prepared is called parchment coffee. In dry method, the ripened or un-ripened mature green fruits are sun dried for 12 to 15 days on drying yards. Coffee obtained by this method is called cherry coffee which is inferior in quality to parchment and fetches less price (Anonymous, 2000).

Arabica and robusta coffee have different chemical composition including the caffeine level. The stimulating effect of coffee, one of the most popular beverages of the world, is due to caffeine (1, 3, 7- trimethylxanthine). It is an alkaloid synthesized from purines and is found in coffee seeds. Caffeine-containing beverages are popular due to their effect on decreasing fatigue and increasing mental acuity following their intake in moderation. However, several medical conditions including hypertension and arrhythmias call for medical professionals to recommend caffeine-less (or) caffeine-free diets. In an effort to abstain from caffeine, many people substitute decaffeinated coffee in place of caffeinated coffee. Over the years, there has been an increasing demand for decaffeinated coffee and currently, it accounts for 10% of total coffee consumption (Silvarolla *et al*., 2004; Mazzafera *et al*., 2009).

There are problems associated with the artificial decaffeination methods and hence development of varieties with low caffeine is the best option. However, breeding for low caffeine requires variability for caffeine either in cultivated or in wild species. With an increasing interest of consumers for low caffeine coffee, there is a need to extensively evaluate the coffee germplasm available in the germplasm bank, established at Coffee Germplasm Research Sub Station (CRSS), North Kodagu, Karnataka for caffeine level as most of the collections

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are yet to be characterized for biochemical constituents. It was, therefore planned to screen all the 76 arabica collections in two phases. In the first phase, only 34 collections representing different origins were tested for caffeine levels. The most promising collection from this screening exercise can be exploited as a potential genetic stock in a commercial breeding programme for producing low caffeine level coffee beans.

Thirty four *C. arabica* collections having a designated accession number (Table 1) were analysed for caffeine level. These collections are part of exotic germplasm introduced from different parts of the world including the wild Ethiopian land races collected during an FAO-sponsored expedition to Ethiopia in 1964 (Narasimmaswamy, 1965). These collections were originally introduced in Central Coffee Research Institute (CCRI), Chikmagalur, Karnataka and from these, a sub collection was established at Coffee Research Sub Station (CRSS), North Kodagu in 1967. In CRSS, these collections were established in contiguous plot and each collection was planted in two continuous rows with 15 to 20 plants in each row. These arabica collections were maintained following the agronomic practices adopted at the estate level. The coffee samples for caffeine estimation were prepared during the harvest season of 2011-12.

Mature coffee fruits were handpicked from all the plants in each collection. From this, 12 kg of fruits were pulped, naturally fermented overnight, washed and dried under sun till it reached the desired moisture level of 10%. The raw coffee samples thus obtained were hulled to obtain the coffee beans. The coffee beans were cleaned to remove the defective beans and a representative sample

Table 1. Caffeine content of various progenies from *Coffea arabica* germplasm bank

S. No.	Source of material	Accession No.	Name of progeny	Caffeine content range	% dry basis Mean ± SD
1	Congo	S. 1502	Bourbon Mayagese	1.63 – 1.70	1.66 ± 0.05
2	Congo	S. 1504	Mysore	1.77 – 1.95	1.86 ± 0.13
3	Congo	S. 1695	Antigua	1.94 – 1.97	1.95 ± 0.02
4	Congo	S. 1698	Local Bronze de Mulungu	1.89 – 1.91	1.90 ± 0.01
5	Ethiopia	S. 1576	S. 26 Enneria	1.17 – 1.53	1.35 ± 0.25
6	France	S. 1659	Kouti	1.64 – 1.84	1.74 ± 0.14
7	France	S. 1661	Nkourgan	1.86 – 1.99	1.92 ± 0.09
8	France	S. 1662	Foumban	2.03 – 2.08	2.00 ± 0.04
9	France	S. 1663	Reunion	1.83 – 1.88	1.85 ± 0.04
10	France	S. 1667	Mazeron	1.81 – 1.82	1.81 ± 0.01
11	FAO, Ethiopia	S. 1497	S.12 Kaffa	1.58 – 1.86	1.73 ± 0.21
12	FAO, Ethiopia	S. 1768	Murta	1.82 – 2.00	1.91 ± 0.13
13	FAO, Ethiopia	S. 1783	Babaca Kaffa	1.29 – 1.48	1.38 ± 0.13
14	FAO, Ethiopia	S. 1784	Misan Taferikala	1.14 – 1.22	1.18 ± 0.06
15	FAO, Ethiopia	S. 1785	Ainamba Kaffa	1.93 – 2.00	1.96 ± 0.05
16	Guinea	S. 1656	Salvador	1.88 – 1.96	1.92 ± 0.06
17	Guatemala	S. 1467	Sumatra	2.06 – 2.09	2.07 ± 0.02
18	Guatemala	S. 1468	Surinam	1.94 – 2.03	1.98 ± 0.06
19	Guatemala	S. 1469	San Ramon	1.70 – 2.02	1.86 ± 0.23
20	Kenya	S. 1587	Rume Sudan	1.73 – 1.99	1.86 ± 0.18
21	Kenya	S. 1591	Dilla (Melville)	1.97 – 2.00	1.98 ± 0.02
22	Kenya	S. 1593	Gimma Galla Sidamo	1.77 – 2.06	1.91 ± 0.21
23	Maryland, USA	S. 1490	Bourbon Amarelho	1.87 – 1.91	1.89 ± 0.03
24	Maryland, USA	S. 1492	Caturra Amarelho	1.83 – 2.07	1.95 ± 0.17
25	Portugal	S. 2126	S6 Cioccie (113/1)	1.80 – 1.82	1.81 ± 0.01
26	Portugal	S. 2127	S6 Cioccie (113/2)	1.95 – 1.96	1.95 ± 0.01
27	Portugal	S. 2129	S4 Agaro (110/2)	1.97 – 1.98	1.97 ± 0.01
28	Portugal	S. 1582	S. 16 Wollamo	1.46 – 1.67	1.56 ± 0.15
29	Portugal	S. 1699	Mbrizi de Mulungu	2.01 – 2.02	2.01 ± 0.01
30	Portugal	S. 1726	Cundina Marca	1.94 – 2.03	1.98 ± 0.06
31	Tanzania	S. 1483	KP 423 Kents	2.04 – 2.08	2.06 ± 0.03
32	Tanzania	S. 1741	Burbok Sudan	1.75 – 1.80	1.77 ± 0.04
33	Tanzania	S. 1743	Geisha	1.79 – 1.88	1.83 ± 0.06
34	USDA, USA	S. 1494	Munda Nova	1.85 – 1.93	1.89 ± 0.06

of 100 g was drawn for both moisture and caffeine estimation. The moisture content in coffee beans was estimated by oven dry method adopting the procedure set out in the ISO 6673 (2003). The caffeine level in coffee beans was determined by high performance liquid chromatography following extraction detailed in ISO 20481 (2008). The caffeine level in coffee bean was expressed as percentage by mass (g/100g) on dry matter basis (% db). All the results were expressed as mean ± SD of three replications.

In the present study, 34 collections from the arabica germplasm bank were tested for caffeine level. The analytical data revealed that there were significant differences in the caffeine level among these collections ranging from 1.18% (*Misan Taferikala*) to 2.07% (*Sumatra*) with a mean caffeine content of 1.84% (Table 1).

Similar to the present study, Silvarolla *et al*. (2000) also analyzed 99 arabica collections conserved in the germplasm bank, at the experimental center of Instituto Agronômico de Campinas in Brazil, for caffeine level. The results indicated that the caffeine level ranged between 0.46 and 2.82% (mean 1.18%) in 68 collections from Kaffa region, and it was between 0.42 and 2.90% (mean 1.10%) in 22 collections from Illubabor region. The caffeine content in five collections from Gojjam region was in the range of 0.98-1.99 (mean 1.10%). Further, the arabica collection obtained from Giesha, Eritrea, Harar and Shoa regions had a mean caffeine content of 0.93, 0.95, 1.18 and 1.42%, respectively. Regarding caffeine content of arabica collections of different origins it is clear from the present study that collections from Ethiopia exhibited the lowest mean caffeine content of 1.59% and those from Guatemala recorded the highest mean caffeine content of 1.97% (Table 2).

In the present study, the mean caffeine value (1.84%) of the arabica collections was much higher than that the (1.2%) reported in *C. arabica* varieties by Mazzafera and Carvalho (1992) and Silvarolla *et al*. (2000). This is probably due to limited number of progenies examined in the present study. Nevertheless, Silvarolla *et al*. (2000) reported caffeine value as high as 2.9% in certain arabica coffees collected from Illubabor region of Ethiopia. Some cultivars like *C. arabica* cv. *Laurina* and species like *C. salvatrix* have low caffeine content of 0.6% and 0.71%, respectively. Recently, an Ethiopian *C. arabica* cultivar has been reported to have close to zero caffeine content (Silvarolla *et al*., 2004). This plant was obtained from the Tropical Agricultural Research and Higher Education Center (CATIE), Turrialba, Costa Rica, coffee collection and deposited at CATIE after the FAO 1964-1965 coffee mission to Ethiopia.

With respect to 34 collections analyzed in the present study, there is no correlation based on geographical origin of the collections. However, they can be classified into two groups based on caffeine levels *i.e*. collections with less than 2% caffeine level and collections with more than 2% caffeine level. Based on the results of the present study coupled with similar observations reported by others, it can be inferred that there exists a greater scope for identifying the variability for low caffeine coffees in natural population that can be exploited for commercial cultivation directly or it can be used in breeding programmes.

The available options for obtaining decaffeinated arabica coffee beans include (a) industrial extraction, (b) selection and breeding and (c) genetic modification. As of now, major portion of decaffeinated coffee beans is obtained by industrial decaffeination. Nevertheless,

Table 2. Mean caffeine content of *Coffea arabica* progenies of different origin

S. No.	Origin	Number of progenies	Caffeine content range	% dry basis Mean ± SD
1	Congo	4	1.66 – 1.90	1.85 ± 0.13
2	Ethiopia	6	1.18 – 1.96	1.59 ± 0.32
3	France	5	1.74 – 2.00	1.88 ± 0.13
4	Guinea	1	1.88 – 1.96	1.92 ± 0.06
5	Guatemala	3	1.86 – 2.07	1.97 ± 0.14
6	Kenya	3	1.86 – 1.98	1.92 ± 0.14
7	Maryland, USA	2	1.85 – 1.95	1.93 ± 0.10
8	Portugal	6	1.56 – 2.01	1.81 ± 0.17
9	Tanzania	3	1.77 – 2.06	1.89 ± 0.14
10	USDA, USA	1	1.85 – 1.93	1.89 ± 0.06

the size of the market for each type of decaffeination process in future will depend on the price and also health concerns related to the way the decaffeinated coffee beans are obtained. Hence, identifying a low caffeine coffee cultivar from the *C. arabica* genebank through screening for variability will be advantageous over the other options considering the apprehension over the artificial decaffeination methods and also the cost-effectiveness. In this direction, it is contemplated to continue the screening process for the remaining collections available in the *C. arabica* germplasm bank in search of low caffeine coffees.

Acknowledgements

Authors express their sincere thanks to Dr Jayarama, Director of Research, Central Coffee Research Institute, Chikmagalur, Dr N Ramamurthy, Deputy Director, (Research), Coffee Research Sub Station, North Kodagu and Dr K Basavaraj, Divisional Head (Coffee Quality) for providing all the facilities for undertaking this study.

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