Genetic diversity and correlation of bean caffeine content with cup quality and green bean physical characteristics in coffee (*Coffea arabica* L.)

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Abstract

BACKGROUND: Although people began to consume coffee for its stimulating effect, the demand for decaffeinated coffee is increasing and now accounts for 10% of the total amount of coffee consumed in the world. Forty-two arabica coffee genotypes originating from Ethiopia were tested to assess caffeine content variability among them, and the correlation of caffeine content with cup quality and green bean physical characteristics.

RESULTS: Green bean caffeine content was measured using high-performance liquid chromatography, while cup quality was determined by professional coffee tasters. Caffeine content ranged from 9.1 to 13.2 g kg^{-1} on dry mass basis (d.m.b.). Six genotypes – AD0291, AD0591, AD2491, AD2691, AD2791 and AD2891 – had a caffeine content of less than 10.0 g kg^{-1}. Caffeine content showed negative and statistically significant correlations with cup quality attributes. Correlations between caffeine content and green bean physical characteristics were non-significant.

CONCLUSIONS: Simultaneous selection for low caffeine content and good cup quality is possible. Some accessions had low caffeine content, and may serve as a source of desirable genes for variety development of types with relatively low caffeine content.

Keywords: caffeine; *Coffea arabica* L.; cup quality; Ethiopian coffee; green beans

INTRODUCTION

Coffee, one of the most important non-alcoholic beverage crops, is grown in over 80 countries in the tropical and subtropical regions of the world,[1,2] exported in different forms to more than 165 nations,[3] and provides a livelihood for some 25 million coffee-farming families around the world.[4] It is the second most important commodity in the global trade, rated after petroleum products.[2,3] The monthly coffee price averages declined during the late 1990s, reaching a minimum in September 2001 and stayed low until 2004, partly due to a collapse of the International Coffee Agreement, and the expansion of Brazilian coffee plantations and Vietnam's entry into the market in 1994. There has been a price rebound since then.[5] Although people began to consume coffee for its stimulating effect, caused by an alkaloid known as caffeine (1,3,7-trimethylxanthine), the demand for decaffeinated coffee is increasing. Currently, decaffeinated coffee accounts for 10% of the total amount of coffee consumed in the world.[6,7] Consequently, manufacturers of instant coffee have devised processes for the artificial removal of caffeine from coffee. However, the process of decaffeination is expensive and modulates the content of flavor compounds and precursors.[7,8] Thus a coffee naturally low in caffeine should be of considerable commercial importance, and development of coffee varieties with such characteristics seems vital.

Caffeine content variability has been observed among different varieties and species of coffee.[9,10] Similarly, caffeine content variability was observed in different parts of a coffee plant. The highest levels are found in beans, flowers, and leaves,[11] and young tissues were shown to contain more caffeine than mature or old tissues.[12] The contribution of the rootstock to the caffeine content of coffee beans was also studied and the results indicated that the caffeine content of the scion is not influenced by that of the rootstock genotype.[13]

One might imagine that coffee species with lower caffeine content in the beans would have commercial value. However, species with this characteristic are low yielding and produce a poor-quality beverage.[10] Efforts were made to transfer the caffeine-free characteristic from wild coffee species to arabica and robusta

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coffee. However, this effort failed since interspecific hybrids had sterile flowers, low productivity, pollen grains with low viability, and abnormal meiotic behavior. Beans from some interspecific hybrids even showed higher caffeine contents than their parental lines.[10] Biotechnological attempts have also been made to prevent the expression of genes that encode key enzymes in the caffeine biosynthesis pathway. This effort resulted in the development of a transgenic *Coffea canephora* Pierre plant, with 50–70% reduction of caffeine content in the leaves.[7] However, non-significant correlations were observed among the caffeine contents of roots, stem, leaves, and beans in several *C. arabica* cultivars.[13] According to the current attitude of society towards genetically modified organisms, it is unlikely that consumers will accept such a product in the near future.

A different option to obtain a low-caffeine coffee is to assess and utilize the variability of caffeine content within *C. arabica.* A number of investigators have observed caffeine content variability among *C. arabica* accessions.[8–10] Green bean caffeine content variability ranging from 0.62% to 1.21% d.m.b. was observed among nine *C. arabica* cultivars.[10] Similarly, caffeine content variability ranging from 0.96% to 1.62% d.m.b. was reported among 38 *C. arabica* accessions.[9] *Coffea arabica* is indigenous to Ethiopia and natural variation exists for various characteristics.[11,12] Recently, three naturally decaffeinated arabica coffee trees were identified from 300 accessions that were collected in Ethiopia.[2]

Although the demand for decaffeinated coffee is increasing and results of previous studies indicated the presence of caffeine content variability among some *C. arabica* accessions collected from Ethiopia, the caffeine content of arabica coffee genotypes currently growing in Ethiopia is unknown. The present study was conducted to determine the caffeine content of different coffee genotypes and to evaluate the association of caffeine content with cup quality and different green bean physical characteristics.

MATERIALS AND METHODS

Genotypes and description of the trial site

Forty-two *C. arabica* genotypes collected from two coffee-producing regions of Ethiopia were used (Table 1). Each genotype was represented by five trees and they were five years old during this investigation. Genotypes were maintained at the Finoteselam coffee trial site, Ethiopia, and all management practices were implemented as recommended. The trial site is located at latitude 10°67′N, longitude 37°11′E, and at an elevation of 1850 m above sea level. It has an average annual rainfall of 950 mm and an average annual temperature of 20.8 °C. Its soil has a pH of 6.5 and contains 3.02% organic carbon, 5.21% organic matter, 0.13% total nitrogen, and 10.11 ppm available phosphorus. The cropping season was conducive to coffee production. Since there were no insect pest and disease outbreaks, pesticides and fungicides were not applied.

Table 1. Mean caffeine content of 42 *Coffea arabica* genotypes at Finoteselam, Ethiopia

No.	Accession number	Collection region	Caffeine content (g kg^{-1} d.m.b.)
1–11	AD0191-1191	Southwest	9.3–12.5
12	AD1291	Northwest	12.3
13–15	AD1391-1591	Southwest	11.4–12.7
16–22	AD1691-2291	Northwest	10.8–13.2
23	AD2391	Southwest	11.7
24–27	AD2491-2791	Northwest	9.1–10.1
28	AD2891	Southwest	9.7
29	AD2991	Northwest	11.3
30–31	AD3091	Southwest	11.2–11.6
32	AD3291	Northwest	10.4
33	AD3391	Southwest	11.0
34–42	AD3491-4291	Northwest	10.2–11.1

Sample preparation

Healthy and red-ripe berries were harvested by hand in bulk from five trees of each genotype and processed according to the dry processing method (berries were dried under the sun on a cement floor for three weeks and dehulled by pounding with a pestle in a mortar). Mechanically undamaged beans were used for cup quality, green bean physical characteristics, and caffeine content analyses.

Evaluation of cup quality and green bean physical characteristics

From each genotype 600 g dry processed green beans were submitted to the Coffee Cup Quality Evaluation and Standardization Center in Addis Ababa, Ethiopia, for evaluation of cup quality and green bean physical characteristics. Both cup quality and green bean physical characteristics of each genotype were assessed by a team of five professional coffee tasters of the center. The assessed characteristics were acidity, body, flavor, and overall standard of the brew and bean size, shape, uniformity, and weight, respectively. Cup quality parameters were scored from coffee made from roasted and ground beans. Beans were roasted in the traditional way over a fire. Characteristics scored were: acidity: 1 (fair), 2 (medium), 3 (medium pointed); body: 1 (fair), 2 (medium), 3 (medium to full); flavor: 1 (fair), 2 (average), 3 (fairly good); overall standard: 1 (fair), 2 (average), 3 (fairly good). Green bean physical characteristics were scored as: bean shape: 1 (round), 2 (long); bean size (screen size): small or 1 (<14 mm), medium or 2 (14–16 mm), bold or 3 (>17 mm); bean uniformity: 1 (mixed), 2 (uniform); 100 bean weight (g).

Caffeine content determination

About 100 dry processed green beans per genotype were crushed with a coffee bean grinder to a fine powder. This was divided into six samples: three were

used to estimate the dry matter content and three for extraction and analysis of caffeine.[9]

Caffeine was extracted and purified.[9] Each sample was extracted in a 50 mL capped tube. In each tube 50 mg green bean powder, 500 mg magnesium oxide, and 25 mL double-distilled water were added. Tubes were heated for 20 min at 121 °C in an autoclave; extracts were filtered through 0.45 μm filters and analyzed by high-performance liquid chromatography (HPLC) on a Hewlett-Packard (Palo Alto, CA, USA) system consisting of a quaternary pump, autosampler, a UV-visible variable wavelength detector (Spp 10A, Shimadzu, Kyoto, Japan), C_{18} pre-column, and a 250 × 4.6 mm Phenomenex Luna (Torrance, CA, USA) 18(2) column with a 5 μm particle size. Two degassed solvents were used for the elution of caffeine:[14] solvent A was 2 mmol L^{-1} phosphoric acid, pH 2.7, containing 5% methanol; solvent B was methanol containing 5% 2 mmol L^{-1} phosphoric acid, pH 3.9. Samples and standards (10 μL) were analyzed at room temperature at 1 mL min^{-1} flow rate and a linear gradient of 35–100% solvent B. Ultraviolet detection was carried out at 273 nm wavelength and quantification was made by peak area measurement and comparison with a caffeine standard (Sigma Chemical Co., St Louis, Missouri, MO, USA).

Statistical analysis

Relationships among genotypes and correlation coefficients among caffeine content, cup quality, and green bean physical characteristics were estimated using the unweighted pair group arithmetic average (UPGMA) method of cluster analysis and correlation matrix analysis packages of NCSS 2000, respectively.[15]

RESULTS AND DISCUSSION

The green bean caffeine content of the 42 arabica coffee genotypes ranged from 9.1 to 13.2 g kg^{-1} with an overall average of 11.0 g kg^{-1} d.m.b. (Table 1). The lowest and highest green bean caffeine contents were recorded from genotypes AD2691 and AD2291, respectively. Previous studies also showed caffeine content variability ranging from 0.62% to 1.21%, 0.76% to 1.82%, and 0.96% to 1.62% among nine, 21 and 38 *Coffea arabica* genotypes, respectively.[8–10] Therefore the results of the present study were in agreement with previous findings.

Six genotypes – AD0291, AD0591, AD2491, AD2691, AD2791, and AD2891 – had caffeine contents of less than 10.0 g kg^{-1}. AD0291, AD0591, and AD2891 were collected from southwest Ethiopia, while the remaining three were obtained from the northwest. Therefore, genotypes with low caffeine content are available in both regions and may serve as sources of desirable genes for the development of coffee varieties with low caffeine content for each adaptation zone or region. Moreover, some of these genotypes (AD0291 and AD0591) had coffee berry disease (CBD) resistance genes and produce reasonable yields under optimum management conditions. Most of the genotypes (66.7%) had caffeine content in the range of 10.1–12.0g kg^{-1}. Eight genotypes – AD0891, AD1091, AD1191, AD1291, AD1591, AD1691, AD1991, and AD2291 – had greater than 12.0g kg^{-1} caffeine content (Table 1). Genotypes such as AD0891, AD1091, AD1191, and AD1591 were collected from southwest Ethiopia, while the remaining four genotypes were obtained from the northwest. Therefore, variation for caffeine content was observed among genotypes collected in each region, in the same range as reported in other studies.[9]

The caffeine content of coffee beans is genotypically defined in a quantitative, polygenic manner, and is influenced by exogenous factors.[16] In contrast to this, high (0.80) narrow-sense heritability for caffeine content was reported in coffee.[17] The result of the present study, together with previous research findings, showed the presence of caffeine content variability among arabica coffee genotypes of Ethiopia and the potential for caffeine content improvement in arabica coffee.

There were no significant differences in caffeine content between entries collected from the two regions. Genotypes collected from northwest Ethiopia had caffeine content ranging from 9.1 to 13.2 g kg^{-1} with an overall average of 10.9 g kg^{-1}, while those from the southwest ranged from 9.3 to 12.7g kg^{-1}, with an overall average of 11.2g kg^{-1} (data not shown).

Caffeine content had negative and significant associations with all cup quality attributes of coffee such as acidity, body, flavor, and overall standard of the liquor (Table 2). However, the magnitude of their association was relatively low and ranged from −0.30 to −0.41. Among cup quality attributes, caffeine content showed the highest level of association with acidity but the lowest level of association with overall standard of the liquor. Genotypes with medium pointed acidity, medium to full body, fairly good flavor, and fairly good overall standard of liquor had relatively low caffeine content. On the other hand, genotypes with high caffeine content were found to be poor in cup quality. Therefore, simultaneous selection both for low caffeine content and good cup quality is possible. Moreover, indirect selection for a low caffeine content or better cup quality seems feasible in arabica coffee by using either of the traits as a selection parameter. In agreement with this, a number of researchers attribute the cup quality difference between arabica and robusta coffee to their differences in green bean biochemical composition (caffeine, chlorogenic acids, sucrose, trigonelline, etc.).[9,17] The better cup quality of arabica coffee mainly originated from its high sucrose and trigonelline, but low caffeine and chlorogenic acids content, compared to robusta coffee (*Coffea canephora* Pierre).

Correlations between caffeine content and green bean physical characteristics were negative, but not

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Table 2. Spearman's coefficients of rank correlation among cup quality, caffeine content, and green bean physical characteristics

	Body	Flavor	OVAS	Bean size	Bean uniformity	Bean shape	Bean weight	Caffeine content
Acidity	0.86**	0.88**	0.71**	0.22	0.19	0.18	0.22	−0.41**
Body		0.93**	0.69**	0.36*	0.21	0.19	0.29	−0.34*
Flavor			0.78**	0.28	0.27	0.16	0.25	−0.36*
OVAS				0.22	0.36*	0.02	0.29	−0.30*
Bean size					0.01	−0.12	0.79**	−0.17
Bean shape						0.22	0.07	−0.06
Bean uniformity							−0.12	−0.04
Bean weight								−0.08

* $p \leq 0.05$; ** $p \leq 0.01$; OVAS, overall liquor quality standard.

significant (Table 2). Beans with a low 100-bean weight had relatively more caffeine than heavy beans. Similarly, mixed beans had more caffeine than uniform beans. In addition, large-sized beans had relatively low caffeine content compared to small-sized beans. Therefore, simultaneous selection both for low caffeine content and desirable green bean physical characteristics is possible. Usually coffee genotypes bear light, mixed, and small-size beans under stress conditions. Therefore, caffeine biosynthesis and accumulation in beans may be more pronounced during stress than favorable conditions.

The relationship of genotypes based on cup quality, caffeine content, and green bean physical characteristics was analyzed using the UPGMA method of cluster analysis and the result is presented in Fig. 1. Cluster analysis grouped coffee genotypes into two major groups. The first group comprised 11 coffee genotypes, which are characterized by poor cup quality, high caffeine content, and undesirable green bean physical characteristics. The second cluster comprised the remaining 31 coffee genotypes and further bifurcated into two sub-clusters. The first sub-cluster comprised 26 coffee genotypes mostly with average cup quality and low to medium caffeine content. The second sub-cluster comprised five coffee genotypes, namely AD0191, AD0691, AD0791, AD1691, and AD1891. These genotypes were

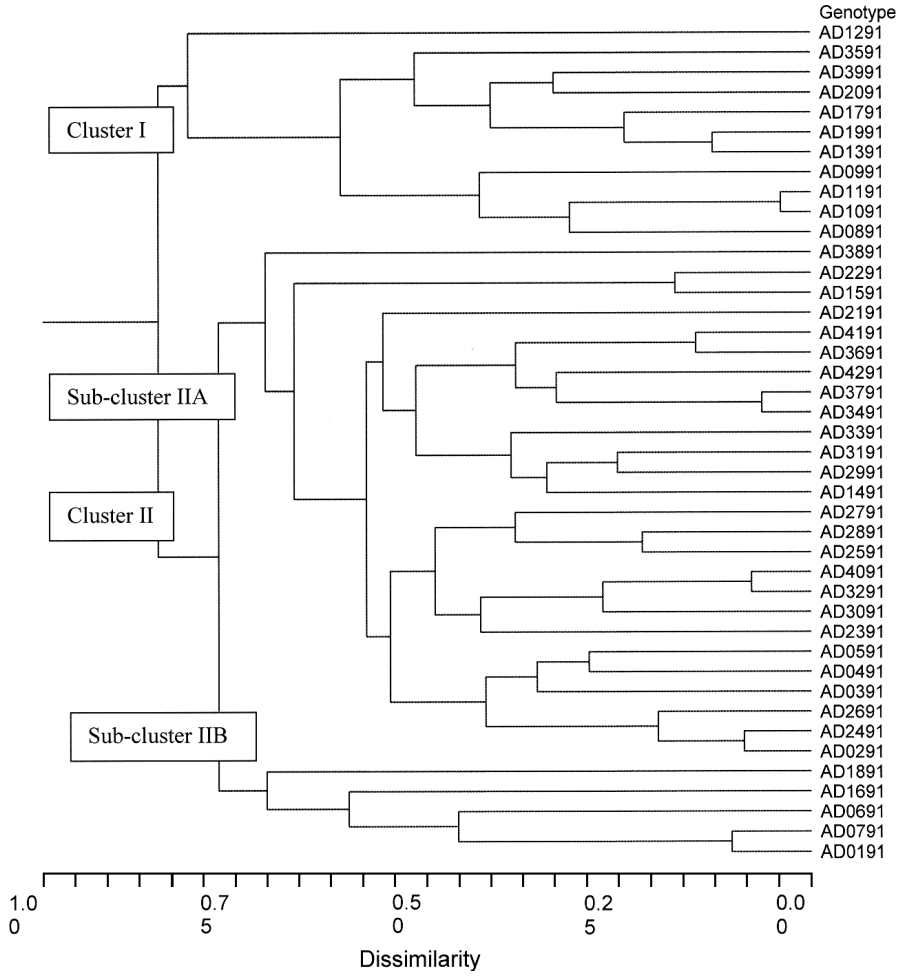

Figure 1. Dendrogram of 42 coffee genotypes constructed using cup quality, green bean physical characteristics and caffeine content.

characterized by good cup quality, desirable green bean physical characteristics, and medium caffeine content. Therefore, these genotypes are an asset for the cup quality and green bean physical characteristics improvement program in arabica coffee in the region. Genotypes were not clustered according to either their collection region or similarity of vegetative characteristics. This indicates the presence of coffee genetic resource diversity in each region, especially in cup quality, green bean physical characteristics, and green bean caffeine content. It also indicates the lack of association between cup quality, green bean physical characteristics, and caffeine content with other agro-morphological characteristics of coffee.

CONCLUSIONS

Variation in caffeine content was observed among evaluated coffee genotypes. These genetic resources could be exploited for caffeine content selection in arabica coffee in the future. Caffeine content showed negative and significant associations with all cup quality parameters. Therefore, simultaneous genetic improvement for better cup quality and low caffeine content is possible. The associations between caffeine content and green bean physical characteristics were also negative but not significant. Therefore, green bean physical characteristics are not useful for the indirect selection of low caffeine content in arabica coffee.

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