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Short communication

VARIATION FOR GREEN BEAN CAFFEINE, CHLOROGENIC ACID, SUCROSE AND TRIGOLLINE CONTENTS AMONG ETHIOPIAN ARABICA COFFEE ACCESSIONS

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ABSTRACT: Green bean biochemical composition affects cup quality. However, the green bean biochemical composition of coffee accessions grown in northwestern Ethiopia was unknown. Therefore, this study was conducted to assess the presence of green bean biochemical composition variation among 42 *Coffea arabica* L accessions. Caffeine, chlorogenic acid, sucrose and trigonelline were extracted from dry processed green beans of 42 arabica coffee accessions grown at Finoteselam coffee trial site, northwestern Ethiopia and quantified with HPLC. Accessions were significantly different in green bean caffeine, chlorogenic acid, sucrose and trigonelline contents and values ranged from 0.91–1.32, 2.34–4.67, 5.30–8.98 and 1.04–1.71 percent in dry weight basis, respectively. F35, 7440, 491, 38191, 991 and 1681 had relatively low (<1.00%) caffeine content and seem valuable to develop low caffeine coffee variety. Coffee berry disease resistant accessions, 74112 and 74158, had relatively higher green bean chlorogenic acid content indicating the association. Ageze is known for better cup quality and had the highest green bean sucrose content. Therefore, accessions are diverse in green bean biochemical composition and knowledge of green bean biochemical composition seems helpful to develop varieties with desirable cup quality and disease resistance.

Key words/phrases: ***Coffea arabica*, decaffeinated coffee, Ethiopia, Northwestern Ethiopia**

INTRODUCTION

Coffee is an important commodity in the world economy, generating on average US$ 9.7 billion annually (ITC, 2002). It is grown in over 80 countries, exported to more than 165 nations (Tsegaye Berhane, 2002) and provides livelihood for 25 million coffee farming families around the world (ICO, 2004). About 7.2 billion ton coffee was harvested from 10.4 million ha land in 2003 (FAO, 2004). However, for the last few years the price of coffee was steadily declining due to overproduction coupled with undiversified uses. Hence, quality coffee production and diversification of coffee outputs could be important strategies for coffee producer nations.

Green bean caffeine, chlorogenic acid, sucrose and trigonelline content affect cup quality. Chlorogenic acid and caffeine participate in defence mechanisms against phytopathogens subsequently helps to increase productivity (Tefsetewolde Biratu 1997; Ky *et al.*, 1999). Caffeine is also used as additive in soft drinks and for the preparation of drugs in different pharmaceutical companies. Therefore, assessment of green bean biochemical composition variability is vital to develop varieties with desirable cup quality, resistance to phytopathogens and diversify coffee outputs. However, such information are lacking for coffee accessions currently grown in Ethiopia. Therefore, the objective of this study was to assess green bean caffeine, chlorogenic acid, sucrose and trigonelline contents variability among 42 arabica coffee accessions originally collected from different parts of Ethiopia.

MATERIALS AND METHODS

Accessions were five years old and maintained at Finoteselam coffee trial site, Ethiopia, with recommended agronomic practices and under shade. The trial site is located at 10°67`N and 37°11`E with an altitude of 1850 m above sea level.

On average it receives 950 mm rainfall and 20.8°C temperature annually. Its soil had a pH of 6.5 and contain 3.02% organic carbon, 5.21% organic matter, 0.13% total nitrogen and 10.11 ppm available phosphorous. Healthy and mature berries harvested by hand from five trees of each accession, dried and cleaned-off parchment. For each accession 300 beans frozen in liquid nitrogen and ground to fine flour using coffee grinder. This flour was used to estimate dry weight and alkaloids (caffeine and trigonelline), chlorogenic acid and sucrose contents. Dry weight was determined by overnight oven drying of three samples (250 grams each) per accession at 105°C.

Chlorogenic acid, sucrose and alkaloids were extracted following the methods of Ky *et al.* (1997), Ky *et al.* (2000) and Ky *et al.* (2001), respectively. Extracts were filtered (0.45 μm) and used for alkaloids, chlorogenic acid and sucrose content measurement using High Performance Liquid Chromatography (HPLC). Alkaloids and chlorogenic acid were measured using Hewlett Packard system consisting of Quaternary pump, auto-sampler, Shimadzu SPD 10A UV-vis detector, C18 pre-column and 250 x 4.6 mm Phenomenex Luna 18(2) column with 5μm pore size. Sucrose was measured using Waters Breeze System equipped with Differential Refractive Index Detector and Waters' SUGARPACK1 300 x 7.8 mm column. Alkaloids and chlorogenic acid were analysed according to the elution programme described by Barre *et al.* (1998) and Ky *et al.* (1999), respectively. Sucrose was analysed using de-ionized water as a mobile phase. Samples and sucrose standard (20μl) were automatically injected into the system and the flow rate was 0.5 ml/min. Quantification was done according to a known sucrose standard from Sigma Chemical Corporation.

Statistical analysis

Statistical analyses were performed using means calculated from results of three samples. Statistical significance was measured using critical differences (CD) at 0.05 and 0.01 probability levels. Dendrogram was constructed by Unweighted Pair Group Method with Arithmetic Average (UPGMA) using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

RESULTS

Accessions were significantly different for green bean caffeine, chlorogenic acid, sucrose and trigonelline content (Table 1). Caffeine content ranged from 0.91 to 1.32% with an average of 1.10% in dry weight basis (dwb). Among all accessions, 38191 and 5691 had the lowest and highest caffeine content, respectively. Caffeine was the least variable biochemical compound. Chlorogenic acid was the second highest biochemical compound present in coffee green bean and values varied from 2.34 to 4.67%. The lowest and highest chlorogenic acid content recorded from green beans of 2991 and Ageze, respectively.

Sucrose was the highest biochemical compound present in coffee green beans with an average value of 7.21%. It was also the most variable biochemical compound. Of all accessions, 991 and Ageze had the lowest and highest sucrose content, respectively. Green bean trigonelline content ranged from 1.04 to 1.71% with an average value of 1.33%. The lowest and highest trigonelline content recorded from green beans of 61291 and 20671, respectively.

Correlation analysis revealed the presence of positive association of green bean caffeine content with green bean chlorogenic acid, sucrose and trigonelline content. However, only the association between green bean caffeine content and chlorogenic acid content was statistically significant. The association between green bean chlorogenic acid and sucrose content, chlorogenic acid and trigonelline and sucrose and trigonelline were positive and statistically non-significant.

Cluster analysis performed using data of green bean caffeine, chlorogenic acid, sucrose and trigonelline content allow to classify 42 accessions into four clusters and two singletons (Fig. 1). Accessions assigned in cluster I had below average and average green bean caffeine, chlorogenic acid and trigonelline content. On the other hand,

accessions assigned in cluster II had below average green bean chlorogenic acid and sucrose contents.

Accessions assigned in cluster III were characterised by above average green bean caffeine, trigonelline and sucrose contents and below average chlorogenic acid content. Three accessions originally collected from Metu, southwestern Ethiopia, were assigned in cluster IV. This group of accessions were characterised with above average caffeine, chlorogenic acid and trigonelline content. The two singletons namely 5691 and 20671 were characterized with the highest caffeine and trigonelline content, respectively.

Table 1. Green bean caffeine, chlorogenic acid, sucrose and trigonelline content (%) of 42 *C. arabica* accessions grown at Finote Selam, Ethiopia.

Collection Location	Accession /Genotype	Caffeine	Trigonelline	Chlorogenic acids	Sucrose
SWE	F34	1.09±0.01	1.41±0.15	3.62±0.53	6.78±0.68
SWE	F35	0.93±0.03	1.35±0.04	3.18±0.28	7.66±0.17
GERA	741	1.06±0.01	1.24±0.02	3.58±0.16	6.78±0.63
SWE	754	1.11±0.01	1.38±0.05	3.21±0.57	7.23±0.18
SWE	7440	0.98±0.03	1.39±0.13	3.68±0.22	6.63±0.25
SWE	7454	1.01±0.02	1.17±0.02	3.78±0.29	6.23±0.97
SWE	7487	1.05±0.02	1.23±0.11	3.05±0.09	7.79±0.34
METU	74110	1.25±0.05	1.63±0.23	3.44±0.09	7.13±0.12
METU	74112	1.13±0.02	1.60±0.18	4.09±0.40	7.74±0.13
METU	74158	1.24±0.04	1.28±0.26	4.41±0.27	6.89±0.04
METU	74165	1.23±0.02	1.52±0.27	4.16±0.05	7.77±0.64
WOLEGA	9384	1.18±0.02	1.41±0.02	3.55±0.21	7.24±0.57
MAJI	20071	1.14±0.06	1.27±0.14	3.85±0.06	7.45±0.29
SWE	ABABUNA	1.27±0.09	1.35±0.03	3.90±0.20	7.59±0.55
SWE	7455	1.17±0.16	1.23±0.33	3.49±0.18	8.61±0.92
SWE	1681	0.97±0.13	1.16±0.02	3.10±0.28	8.04±0.51
SWE	GEISHA	1.12±0.23	1.38±0.04	2.68±0.36	8.30±0. 75
PORTUGAL	CATIMOR	1.16±0.10	1.28±0.04	2.77±0.14	5.48±0. 75
MAJI	20671	1.10±0.16	1.71±0.08	3.13±0.28	8.67±0.61
WELLO	1087	1.23±0.02	1.48±0.06	3.28±0.19	7.72±0.31
GONDER	AGEZE	1.23±0.05	1.22±0.10	4.67±1.06	8.98±0.12
ACHEFER	5291	1.10±0.03	1.25±0.16	2.85±0.13	6.96±0.24
MECHA	4891	1.19±0.03	1.38±0.04	3.14±0.50	6.59±0.18
ANKESHA	391	1.23±0.11	1.34±0.03	4.03±0.71	7.40±0.21
ANKESHA	6091	1.08±0.12	1.39±0.08	2.78±0.19	6.28±0.46
BANJA	57291	1.12±0.03	1.41±0.12	2.73±0.08	5.69±0.61
DANGELA	5691	1.32±0.06	1.11±0.16	2.72±0.40	7.32±0.28
F. SELAM	491	0.94±0.01	1.40±0.06	2.99±0.09	7.16±0.26
B. DAR	291	1.01±0.11	1.31±0.08	2.82±0.18	6.75±0.63
F.SELAM	38191	0.91±0.09	1.31±0.09	3.68±0.40	7.22±0.36
MECHA	991	0.96±0.15	1.36±0.14	3.09±0.43	5.30±0.49
ACHEFER	5391	1.13±0.10	1.43±0.03	3.52±0,25	6.73±0.97
DEJEN	61391	1.04±0.13	1.34±0.06	2.70±0.27	7.81±0.41
DEJEN	61291	1.08±0.03	1.04±0.18	3.77±0.22	7.30±0. 71
MECHA	7191	1.02±0.07	1.20±0.06	2.62±0.22	6.63±0.90
MECHA	10191	1.07±0.20	1.28±0.07	3.50±0.49	6.48±0.25
MECHA	1191	1.08±0.04	1.22±0.08	2.36±0.12	8.09±0.28
BURIE	2291	1.02±0.03	1.31±0.12	2.78±0.09	8.36±0.43
B. DAR	2991	1.11±0.03	1.28±0.02	2.34±0.10	6.21±0.60
BURIE	30291	1.06±0.03	1.39±0.05	3.34±0.34	7.68±0.66
MECHA	4591	1.08±0.13	1.29±0.00	3.04±0.08	7.29±0.49
ACHEFER	5091	1.11±0.05	1.20±0.06	3.61±0.53	6.93±0. 70
Mean		1.10	1.33	3.31	7.21
Stdv.		0.098	0.132	0.545	0.824
Minimum		0.91	1.04	2.34	5.30
Maximum		1.32	1.71	4.67	8.98
CD at 0.05		0.20	0.27	1.10	1.66
CD at 0.01		0.26	0.36	1.47	2.23

Stdv.= standard deviation; CD= critical difference

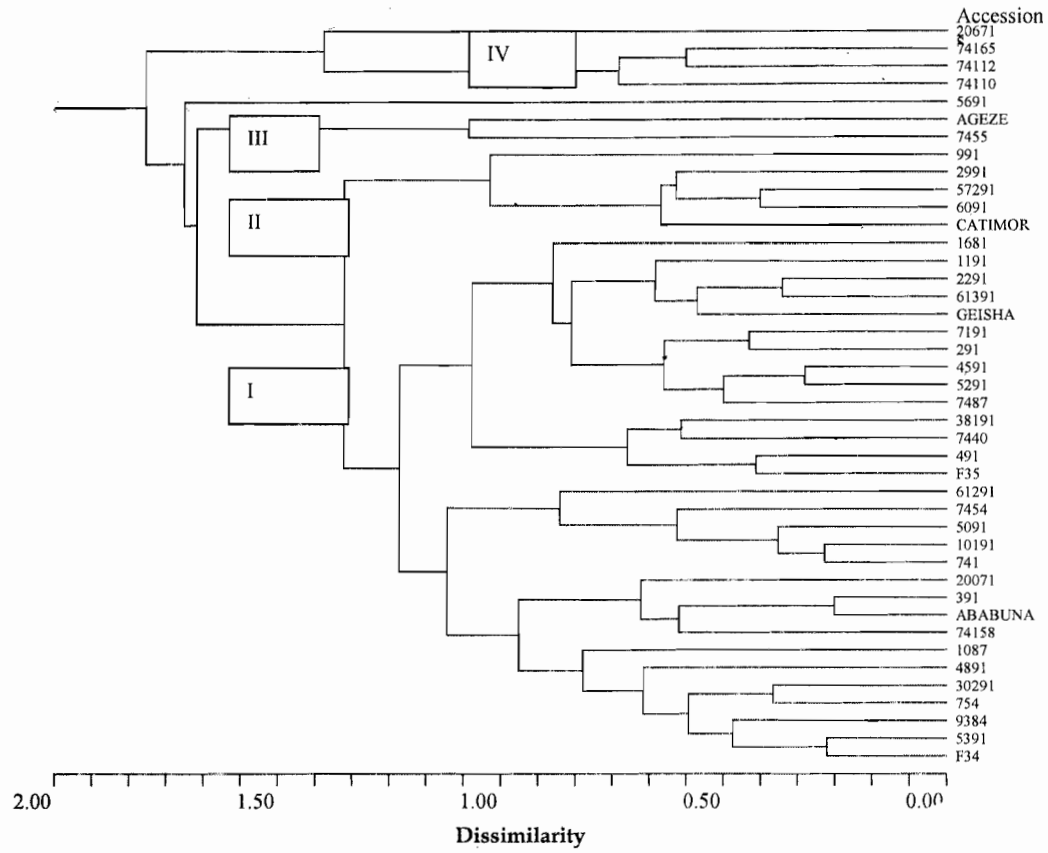

Fig. 1. Dendrogram of 42 *C. arabica* genotypes constructed by cluster analysis using green bean caffeine, chlorogenic acids, sucrose and trigonelline contents.

DISCUSSION

Evaluated 42 arabica coffee accessions were significantly different in caffeine content. Similarly, Silvarolla *et al.* (2000) and Ky *et al.* (2001) reported the presence of significant difference among 99 and 38 *C. arabica* accessions in green bean caffeine content, respectively. In addition, Silvarolla *et al.* (2004) identified three naturally decaffeinated arabica coffee trees among 300 accessions collected from Ethiopia. Therefore, the present as well as previous research results demonstrated the presence of caffeine content variability in arabica coffee.

Coffee discovered and become popular beverage due to the alertness caused by caffeine. However, currently the demand for decaffeinated coffee is increasing and consumption increased to about 10% worldwide (Silvarolla *et al.*, 2004). Organic solvents or supper critical carbon dioxide is used to decaffeinate coffee, with the final product containing less than 0.5% caffeine (Silvarolla *et al.*, 2000). However, during the process of decaffeination flavour precursors such as sugars, oil and phenolic compounds are also extracted consequently reduces cup quality. Considering the problem associated to the process of decaffeination, development of naturally decaffeinated coffee varieties is a desirable solution and several studies were conducted to assess caffeine content variability and mode of inheritance of caffeine. Caffeine content is a quantitative trait governed in polygenic manner, but slightly influenced by exogenous factors (Baumann *et al.*, 1998). Therefore, green bean caffeine content can be improved through crossing and subsequent selections. Accessions such as F35, 7440, 1681, 491, 38191 and 991 had relatively low (less than 1.00% dwb) green bean caffeine content. Therefore, these accessions may serve as sources of desirable genes to develop low caffeine coffee varieties. Yigzaw Dessalegn (2006) reported

statistically significant and negative correlation between desirable cup quality and green bean caffeine content. Therefore, selection for low caffeine content and better cup quality can be made simultaneously.

Chlorogenic acid is a phenolic compound commonly found in coffee green bean and has several functions. Accessions were significantly different in green bean chlorogenic acid content. Similarly, Ky *et al.* (2001) reported significant green bean chlorogenic acid content variability among 38 *C. arabica* accessions. Coffee berry disease resistant accessions had relatively higher chlorogenic acid content than susceptible accessions. Likewise Aerts and Baumann (1994) reported the anti-pathogenic and allelopathic properties of chlorogenic acid. Therefore, association between coffee berry disease resistance and green bean chlorogenic acid content could be future area of investigation. Accessions were also significantly different in green bean trigonelline content and values were in accord with the report of Ky *et al.* (2001). Trigonelline give rise to appreciated flavour products. Its degradation during roasting results in niacin, nicotinamide and a range of aroma volatiles (Ky *et al.*, 2001).

Sucrose is an important precursor for coffee flavour. The higher the sucrose content in green beans, the more intense coffee flavour (Ky *et al.*, 2000). Similarly, Yigzaw Dessalegn (2006) reported significant and positive correlation between green bean sucrose content and coffee flavour. Accessions were significantly different in green bean sucrose content and the result was in accord with the report of Ky *et al.* (2001). On average, accessions from southwestern Ethiopia had higher green bean sucrose content compared to accessions from northwestern Ethiopia. This could be since most accessions from southwestern Ethiopia are improved varieties.

The UPGMA method of cluster analysis independently distinguished all accessions and classified into four clusters and two singletons, indicating the presence of adequate variability for these biochemical compounds. Similarly, Ky *et al.* (2001) reported the presence of high biochemical diversity in arabica coffee. Therefore, these accessions should be independently conserved and could be used as resource to develop varieties with desirable biochemical composition in the future. Other Ethiopian arabica coffee accessions should also be assessed to identify accessions either with lower or higher green bean caffeine, chlorogenic acids, trigonelline and sucrose contents for different purposes.

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