

Discrimination of *Coffea arabica* Hybrids of the Composite Cultivar Ruiru 11 by Sensorial Evaluation and Biochemical Characterization

¹C.W. Kathurima, ²G.M. Kenji, ²S.N. Muhoho, ³R. Boulanger and ³F. Davrieux

¹Coffee Research Foundation, P.O. Box 4 (00232) Ruiru-Kenya

²Jomo Kenyatta University of Agriculture and technology P.O. Box 60000, Nairobi, Kenya

³CIRAD - Performance of Tropical Production and Processing Systems Department, UMR Qualisud, TA B-95/16 73, avenue J.F. Breton 34398 Montpellier Cedex 5

Abstract: The objective of this study was to characterise some Ruiru 11 (R11) hybrids using sensory attributes and biochemical components combined with Principal Component (PC) analysis to discriminate the genotypes according to geographical locations where they were grown. Ten Ruiru 11 hybrids were evaluated in three locations in Kenya (Kitale, Koru and Ruiru) where trials were laid out in a randomized complete block design with each hybrid replicated three times. Seven sensory descriptors were assessed by a panel of seven judges and rated on a 10-point scale. Caffeine, chlorogenic acid, trigonelline, fat, and sucrose were evaluated by predictive models based on Near Infrared (NIR) spectroscopy and by chemometric analysis of the global NIR spectrum. Significant differences ($p < 0.05$) in fragrance, flavor, aftertaste, acidity, body, balance and preference were observed among the hybrids. On average the hybrids CRF-41, CRF-11 and CRF-91 consistently scored highly in the sensory scale while CRF-3 and CRF-5 scored slightly lower. PC analysis on the sensory variables was unable to separate the samples according to geographical locations. Biochemical determinations revealed that Hybrids from Kitale had caffeine levels ranging between 1.52-1.61%, those from Ruiru 1.34-1.59% and in Koru 1.22-1.36%. The levels of sucrose in the hybrids in Koru ranged between 8.99-10.4%, which was less than the levels in the hybrids in Kitale (10.12-11.15%) and in Ruiru (9.91-10.91%). The levels of trigonelline and fat did not differ significantly in the hybrids grown in the three regions. PC analysis on the biochemical variables was able to discriminate the samples according to geographical locations.

Key words: Biochemical, *Coffea arabica*, F1 hybrids, near infra red spectroscopy, Ruiru 11, sensory

INTRODUCTION

Many commercial cultivars of Arabica coffee (*Coffea arabica*) are susceptible to diseases and therefore, the development of coffee varieties resistant to coffee diseases has been a breeding objective of the highest priority in many countries. French Missionaries introduced coffee to Kenya around 1900 A.D. (Mwangi, 1983) and is grown in three altitude zones; the high altitude (over 1700 m above sea level), the medium altitude (between 1580 and 1760 m) and the low altitude (1520-1580 m) above sea level (Jaetzold and Schmidt, 1983). The recommended traditional cultivars in Kenya are K 7 for low altitude areas, SL 28 and SL 34 for low to medium altitude areas with good rainfall (Mwangi, 1983). The cultivated traditional genotypes are susceptible to various pests and diseases of coffee, which are expensive to control. In order to alleviate this problem an extensive breeding program, at the Coffee Research Foundation (CRF), Kenya, saw the release of an Arabica coffee cultivar (cv Ruiru 11) in 1985 (Nyoro and Sprey, 1986).

This cultivar combines resistance to Coffee Berry Disease and Coffee Leaf Rust with high yields, fine cup quality and compact growth and is suitable for all coffee growing areas (Opile and Agwanda, 1993). There are about 60 composite hybrids, each derived from a cross between a specific female and male population. Ruiru 11 progenies comprise of crosses made from pollinating several mother plants (catimors) with bulked pollen from genetically similar males (Omondi *et al.*, 2001).

Agronomic studies have been conducted to evaluate the influence of tree training and plant density on yields of the Ruiru 11 progenies (Njoroge and Kimemia, 1994). Due to its compact growth habit R11 can be planted more densely than the traditional tall varieties. The relative flow of benefits and cost of both the traditional and R11 varieties between the time of establishment and the end of second cycle (10 years) has been compared by Roe and Nyoro (1986). Sensory quality evaluations of related hybrids of Arabica in multilocational trials have been conducted to identify environments that best reveal differences in genetic potentials amongst varieties and

hence useful as selection and/or test sites (Agwanda *et al.*, 2003; Omondi, 2008). A lot of work has already been done in attempting to understand the biochemical composition of green and roasted coffee beans and to associate such chemicals with the cup quality. However the link between such studies and the genetic improvement of quality is however lacking in Kenya. More progress would however be expected if biochemical techniques and sensory approaches are integrated at early stages to detect finer differences between the breeding lines and the existing commercial cultivars. Green coffee beans contain a wide range of different chemical compounds which react and interact at all stages of coffee processing to produce a final product with an even greater diversity and complexity of structure (Clifford, 1985). The characterization of coffee cultivars requires a rapid method of preparation and analysis in order to roughly identify the genotypes. Near Infrared Spectroscopy (NIRS) has already been used to predict the contents of trigonelline and sucrose (which are aroma precursors of appreciated flavors), chlorogenic acids, caffeine and fat in green coffee (Bertrand *et al.*, 2003).

CIRAD has been developing from 1991 NIRS databases (Davrieux *et al.*, 2001) for green coffee (more than 5000 references) and roasted coffee (more than 4000 references). It has been proved efficient to discriminate Robusta and Arabica (Pizarro *et al.*, 2007, Davrieux *et al.*, 2001), to determine the origin of green coffees and the ratio of Robusta/Arabica in coffee blends.

In this study (NIRS) biochemical predictions and sensory attributes coupled with multivariate analysis, were used to discriminate between related hybrids of Arabica coffee grown in three different geographical regions in Kenya.

MATERIALS AND METHODS

Description of study sites: The study was conducted during the 2008/09 main coffee season in three different agro-ecological zones in Kenya. The sites included Ruiru in Central Kenya, Kitale in Western Kenya and Koru in Nyanza province of Kenya. Ruiru is located at 1° 06'S and 36° 45'E at an altitude of 1603M above sea level. The soils are humic nitosols, friable clays, moderate in organic matter and moderately supplied in bases. Kitale is found at 0° 59'S and 35° 01'E at an altitude of 1982M above sea level. The soils are fairly deep sandy clays/loamy clays and full of weatherable minerals. Koru is located at 0° 07'S, 35° 16'E and has an elevation of 1700M above sea level. The soils are eutric nitosols, friable clays, and weakly acidic to neutral, rich in bases, available phosphorous and moderate inorganic matter.

Test materials: Ten Ruiru 11 F1 hybrids were evaluated in this study. The male parents of Ruiru 11 are

outstanding selections from a multiple cross programme involving CBD resistant donors Rume Sudan (*R* gene) and Hibrido De Timor (*T* gene) and high yielding, good quality but susceptible cultivars such as SL28, SL34 and Blue Mountain. The female parents are advanced generations (F3, F4 and F5) of the cultivar Catimor, ex Colombia, which is a hybrid of Hibrido de Timor and Caturra (Omondi *et al.*, 2000). The hybrids evaluated in this study were coded CRF-3, CRF-5, CRF-11, CRF-23, CRF-41, CRF-91, CRF-111, CRF-123, CRF-50, and CRF-131. CRF is the abbreviation for Coffee Research Foundation and the number that follows represent a specific hybrid. From each site thirty (30) samples were analyzed making a total of ninety (90) samples from the three localities.

Experimental layout: The study was on mature coffee laid out in a Randomized Complete Block Design with three replications in each of the sites.

Processing of the samples: Ripe cherries were harvested from a sample size of five trees per replicate during the 2008/09 main coffee seasons. The cherries were bulked and wet processed using standard procedures. The cherry samples were pulped, fermented, washed and the wet parchment dried to final moisture content of 10.5 to 11%. The parchment was then hulled and graded to seven grades based on size, shape and density. Grade AB was used for the study.

Roasting and sensory evaluation: Roasting of the green coffee was done to attain a medium roast using a Probat laboratory roaster within 24 h of evaluation and allowed to rest for at least eight hours. The samples were weighed before and after roasting to determine the uniformity of roasting. The samples were ground immediately prior to cupping, no more than 15 min before infusion with water. Samples were weighed out to the predetermined ratio of 8.25 g per 150 ml of water. Each sample was ground after running a rinsing quantity of the sample through a laboratory grinder (Probat- Type 55 LM 1500) and then grinding each cup's batch individually into the cupping glasses, ensuring that the whole and consistent quantity of sample gets deposited into each cup (five cups per sample). Sensory evaluation procedure described by Lingle (2001) was followed. Seven sensory variables were assessed by a trained panel of seven and rated on a 10-point scale. For the attributes fragrance/aroma, flavor, aftertaste and balance, 1= very poor and 10 = outstanding while for acidity 1= very flat and 10 = outstanding and body 1= very thin and 10 = very bright.

Determination of biochemical components: Portions of coded green coffee samples were placed in small plastic bottles and stored under -80°C. After 24 h of freezing, the

Table 1: Mean of seven descriptive sensory attributes evaluated in the R11 hybrids from each region and the mean of the sensory attributes of each of the hybrids in the three regions combined

Source	Hybrid code	Fragrance	Flavour	Aftertaste	Acidity	Body	Balance	Preference
KITALE	CRF-03	7.45	7.45	7.50	7.52	7.43	7.48	7.50
KORU	CRF-03	7.10	6.81	6.76	6.81	7.05	6.90	6.81
RUIRU	CRF-03	7.38	7.36	7.36	7.48	7.40	7.43	7.48
	Mean	7.31c	7.21d	7.21d	7.27d	7.29d	7.27c	7.26c
KITALE	CRF-05	7.43	7.24	7.38	7.40	7.40	7.33	7.33
KORU	CRF-05	7.10	6.90	7.00	7.02	7.14	7.10	6.98
RUIRU	CRF-05	7.60	7.52	7.55	7.74	7.55	7.69	7.67
	Mean	7.37bc	7.22d	7.31bcd	7.39cd	7.37bcd	7.37bc	7.33c
KITALE	CRF-11	7.48	7.33	7.38	7.48	7.52	7.52	7.48
KORU	CRF-11	7.43	7.33	7.38	7.40	7.45	7.36	7.38
RUIRU	CRF-11	7.76	7.57	7.57	7.74	7.57	7.62	7.69
	Mean	7.56a	7.41abc	7.44abc	7.54bc	7.52ab	7.50ab	7.52ab
KITALE	CRF-23	7.50	7.31	7.31	7.45	7.43	7.40	7.40
KORU	CRF-23	7.29	7.19	7.17	7.31	7.29	7.29	7.26
RUIRU	CRF-23	7.33	7.40	7.48	7.64	7.50	7.50	7.50
	Mean	7.37bc	7.30bcd	7.32bcd	7.47bc	7.40abcd	7.40bc	7.39bc
KITALE	CRF-41	7.55	7.52	7.48	7.62	7.57	7.57	7.60
KORU	CRF-41	7.36	7.21	7.31	7.45	7.48	7.43	7.43
RUIRU	CRF-41	7.71	7.67	7.64	7.90	7.69	7.81	7.79
	Mean	7.54a	7.47ab	7.48abc	7.66a	7.58a	7.60a	7.60a
KITALE	CRF-50	7.29	7.12	7.12	7.31	7.43	7.31	7.29
KORU	CRF-50	7.25	7.00	7.11	7.11	7.18	7.14	7.04
RUIRU	CRF-50	7.55	7.69	7.62	7.90	7.76	7.64	7.71
	Mean	7.38bc	7.30bcd	7.30bcd	7.48abc	7.49abc	7.39bc	7.38bc
KITALE	CRF-91	7.48	7.45	7.48	7.55	7.43	7.50	7.48
KORU	CRF-91	7.43	7.26	7.36	7.38	7.45	7.33	7.38
RUIRU	CRF-91	7.57	7.76	7.69	7.88	7.76	7.79	7.81
	Mean	7.49ab	7.49cd	7.51a	7.60ab	7.55a	7.54ab	7.56ab
KITALE	CRF-111	7.43	7.29	7.33	7.38	7.36	7.29	7.33
KORU	CRF-111	7.29	7.12	7.05	7.12	7.24	7.17	7.17
RUIRU	CRF-111	7.36	7.38	7.33	7.62	7.48	7.48	7.43
	Mean	7.36bc	7.26cd	7.24d	7.37cd	7.36bcd	7.31c	7.31c
KITALE	CRF-123	7.29	7.21	7.31	7.33	7.38	7.26	7.26
KORU	CRF-123	7.31	7.14	7.17	7.17	7.19	7.24	7.21
RUIRU	CRF-123	7.50	7.64	7.57	7.79	7.64	7.64	7.71
	Mean	7.33c	7.24d	7.29bcd	7.33d	7.34cd	7.31c	7.31c
KITALE	CRF-131	7.36	7.24	7.38	7.38	7.29	7.26	7.33
KORU	CRF-131	7.31	7.24	7.21	7.45	7.36	7.40	7.31
RUIRU	CRF-131	7.57	7.68	7.61	7.82	7.64	7.79	7.86
	Mean	7.39bc	7.35abcd	7.38abcd	7.52abc	7.40abcd	7.45bc	7.46abc

Means within a column not sharing a letter are significantly different at $p \leq 0.05$

samples were ground (<0.5 mm) in liquid nitrogen using Retsch ZM 200 Mill. A NIRS 6500 monochromator (Foss NIRS systems, Silver Spring, MD) was used to scan reflectance from 400 to 2500 nm at 2 nm intervals, using ring cups (50 mm in diameter) with about 3 g of fine green coffee powder. Data were saved as the average of 32 scans and stored as $\log(1/R)$, where R was the reflectance at each wavelength and 1 the reflectance of a standard ceramic reference. Spectra were acquired randomly, each sample was measured twice, and the average spectrum was stored. Statistical analyses were performed using Win-ISI II software (Infrasoft International, Port Matilda, PA, and USA). Caffeine, trigonelline, fat and sucrose contents were determined using specific green Arabica coffee calibrations (Davrioux *et al.*, 2003).

Data analysis: Biochemical and sensory data were subjected to Analysis of Variance (ANOVA) using COSTAT statistical software and effects declared

significant at 5% level. Student-Newman-Keuls (SNK5%) test was used to separate the means. The procedure PRINCOMP was then used to perform a principal component analysis using sensory quantitative variables and biochemical characteristics plotted on two dimensions using the first two principal components PC1 and PC2 (SAS, 2005).

RESULTS

Table 1 shows the mean sensory ratings of the R11 hybrids evaluated by the panel. Significant differences ($p < 0.05$) in fragrance, flavor, aftertaste, acidity, body, balance and preference were observed. The sensory traits of the hybrids did not show any discernable trend but generally the hybrids in Koru were rated lower on the sensory scale compared to the other sites. On average the hybrids CRF-41, CRF-11 and CRF-91 consistently scored highly while CRF-3 and CRF-5 scored slightly lower. Similarly (PC) analysis of the seven sensory variables

Table 2: Mean biochemical composition (caffeine, trigonelline, fat, sucrose and chlorogenic acid [CQA] % DWB) of the R11 hybrids in the three regions and mean of each biochemical constituent per hybrid in the three regions combined

Source	Genotype	Caffeine	Sucrose	CGA	Trigonelline	Fat
KITALE	CRF-03	1.53	11.15	8.26	1.18	11.88
KORU	CRF-03	1.35	9.14	8.99	1.22	15.08
RUIRU	CRF-03	1.51	9.91	8.90	1.27	13.17
	Mean	1.46±0.1a	10.07±0.9ab	8.72±0.4a	1.22±0.05a	13.38±1.5a
KITALE	CRF-05	1.55	10.85	8.41	1.18	12.70
KORU	CRF-05	1.26	9.21	8.41	1.23	15.34
RUIRU	CRF-05	1.45	10.07	8.52	1.25	13.46
	Mean	1.42±0.1abc	10.04±0.7ab	8.45±0.2bc	1.22±0.05a	13.83±1.3a
KITALE	CRF-11	1.58	10.93	8.35	1.19	12.17
KORU	CRF-11	1.22	10.40	8.48	1.22	13.38
RUIRU	CRF-11	1.39	10.87	8.71	1.30	12.94
	Mean	1.40±0.2bc	10.73±0.8a	8.51±0.2abc	1.24±0.05a	12.83±0.9a
KITALE	CRF-23	1.52	10.44	7.97	1.16	12.33
KORU	CRF-23	1.36	9.27	8.61	1.13	14.47
RUIRU	CRF-23	1.59	9.96	8.49	1.22	13.19
	Mean	1.49±0.1a	9.89±1.0b	8.36±0.4c	1.17±0.05a	13.33±1.1a
KITALE	CRF-41	1.61	10.61	8.07	1.17	12.01
KORU	CRF-41	1.27	9.88	8.48	1.18	13.51
RUIRU	CRF-41	1.37	10.83	8.54	1.26	12.70
	Mean	1.42±0.2abc	10.44±0.5ab	8.36±0.3c	1.20±0.05a	12.74±1.0a
KITALE	CRF-50	1.59	10.41	8.35	1.17	12.97
KORU	CRF-50	1.26	8.99	8.87	1.12	14.70
RUIRU	CRF-50	1.44	10.28	8.86	1.26	13.31
	Mean	1.43±0.2abc	9.89±0.7b	8.69±0.3ab	1.18±0.7a	13.66±0.9a
KITALE	CRF-91	1.59	10.12	8.29	1.15	12.94
KORU	CRF-91	1.25	9.71	8.60	1.22	14.22
RUIRU	CRF-91	1.34	10.91	8.40	1.19	12.25
	Mean	1.39±0.2bc	10.25±0.8ab	8.43±0.2c	1.19±0.9a	13.14±1.1a
KITALE	CRF-111	1.51	10.81	8.03	1.20	11.91
KORU	CRF-111	1.32	9.40	8.91	1.19	14.25
RUIRU	CRF-111	1.56	9.84	8.84	1.25	13.09
	Mean	1.47±0.1ab	10.02±0.8ab	8.59±0.5abc	1.21±0.4a	13.08±1.2a
KITALE	CRF-123	1.59	10.28	8.28	1.18	12.70
KORU	CRF-123	1.28	9.50	8.87	1.23	14.50
RUIRU	CRF-123	1.44	9.96	8.55	1.29	14.21
	Mean	1.43±0.1abc	9.91±0.6b	8.57±0.3abc	1.23±0.05a	13.81±1.1a
KITALE	CRF-131	1.51	10.36	8.16	1.19	12.55
KORU	CRF-131	1.26	9.25	8.52	1.21	14.73
RUIRU	CRF-131	1.31	10.84	8.57	1.28	12.72
	Mean	1.37±0.1 c	10.06±0.8ab	8.40±0.2c	1.22±0.05a	13.41±1.2a

Means within a column not sharing a letter are significantly different at $p \leq 0.05$

detected significant variations between R11 hybrids in the three localities as illustrated in Fig. 1. Results of the principal component analysis indicated that the first two PCs explained 93.00 and 3.55% (a total of 96.71%) of the total variation respectively. The hybrids coded Ruir-41, Ruir-91, Ruir-50, Ruir-131, Ruir-11 and Ruir-5, from the site Ruiru were placed on the upper part of the PC graph. The hybrids coded, Koru-3, Koru-5, Koru-50, Koru-111 and Koru-123 from the site Koru were placed on the lower part of the PC graph. All the rest of the hybrids from the three localities clustered together with no discernable pattern.

Analysis of variance showed genotype and site as significant factors on the levels of total chlorogenic acids (CQA) and caffeine (at $p < 0.05$). No significant differences ($p < 0.05$) were observed in the levels of trigonelline, and fat among the hybrids. However, locality

was an important factor on the levels of sucrose. Table 2 shows the diversity among R11 hybrids as determined by biochemical levels of caffeine, trigonelline, fat, sucrose and CQA. Hybrids from Kitale had more caffeine levels ranging between 1.52-1.61%, in Ruiru 1.34-1.59% and in Koru 1.22-1.36%. The levels of sucrose in the hybrids in Koru ranged between 8.99-10.4%, which was less than the levels in the hybrids in Kitale 10.12-11.15% and those in Ruiru 9.91-10.91%. The levels of trigonelline and fat did not differ significantly in the hybrids grown in the three regions.

PC analysis showing the diversity of the hybrids from the three geographical locations due to the biochemical variables is presented in Fig. 2. The first three PCs explained 58.04, 23.98 and 11.18% (a total of 93.20%) of the total variation respectively. The Ruiru 11 hybrids from Koru were placed on the upper part of the PCA graph,

hybrids from Ruiru in the middle and those from Kitale in the lower part of the PCA graph.

DISCUSSION

Most of the work done has compared R11 cultivar with the traditional varieties and the cup quality has been found to be similar to the popular commercial cultivars in Kenya (Owuor, 1988; Njoroge *et al.*, 1990; Omondi, 2008). The results of this study agrees with the study of Ojijo (1993) who presented Ruiru 11 as showing great variability in terms of beverage quality although certain progenies presented beverage quality comparable to the standard SL 28 cultivar. Similarly variability in the cup quality of *Coffea canephora* gene introgressed hybrids has been reported by Bertrand *et al.* (2003). On the coffee sensory attribute scale all the hybrids attained scores above 6.5 meaning they were all rated as good by the panel of assessors. However PC analysis was not able to discriminate the hybrids from the three regions by their sensory attributes. Agwanda *et al.* (2003) observed that discrimination on the basis of liquor traits were best observed in the site where moderate moisture stress occurred during bean filling stage. The biochemical constituents of R11 hybrids were within the levels reported in the literature for Arabica coffee (Bertrand *et al.*, 2003). NIRS combined with multivariate calibration methods, has been used to quantify the robusta variety content of roasted coffee samples, (Pizarro *et al.*, 2007). In this study PC analysis based on biochemical constituents derived through NIR, placed Koru coffees far from the coffees from the other two regions (Kitale and Ruiru) as shown in Fig. 2. All the hybrids from the three sites were positioned on their own without overlapping. This shows that NIRS could be applied in discriminating the hybrids from the three regions. Similarly, Bertrand *et al.* (2006) showed that Arabica hybrids could be discriminated according to elevation based on NIR spectra.

CONCLUSION

In this study Principal Component analysis of the biochemical fingerprints of R11 hybrids discriminated the genotypes according to geographical locations where they were grown. However this kind of discrimination was not possible using the sensory data. The work done in this study therefore showed that apart from organoleptic procedures in evaluating coffee characteristics, incorporating quantitative methodologies for assessing coffee would be an added advantage. This study was the first report on the NIR biochemical analysis of Ruiru 11 hybrids grown in Kenya.

ACKNOWLEDGMENT

The authors extend sincere appreciation to Coffee Research Foundation (CRF), CIRAD-FRANCE and the European Union, which supported this study through Quality Coffee Production and Commercialization Support Programme (QCPCP). Assistance from technical and field staff of CRF and CIRAD -UMR QUALISUD is highly appreciated. This work is published with the permission of the Director of Research, CRF, Kenya.

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