Research & Development
2023; 4(4): 150-154
http://www.sciencepublishinggroup.com/j/rd
doi: 10.11648/j.rd.20230404.15
ISSN: 2994-7057 (Online)

Comparison of Hybrid Arabica Coffee *(Coffea arabica)* Plants Regenerated from Seed, Tissue Culture and Cutting for Yield, Organoleptic and Biochemical Quality Characters

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To cite this article:

Berihu Mengs, Desalegn Alemayehu, Abrar Sualeh, Tamene Haile, Bealu Girma. Comparison of Hybrid Arabica Coffee *(Coffea arabica)* Plants Regenerated from Seed, Tissue Culture and Cutting for Yield, Organoleptic and Biochemical Quality Characters. *Research & Development*. Vol. 4, No. 4, 2023, pp. 150-154. doi: 10.11648/j.rd.20230404.15

Received: October 19, 2023; **Accepted**: November 3, 2023; **Published**: November 11, 2023

Abstract: Coffee is consumed not only for its typical flavor but also for its stimulating effect and its health benefits. Arabica coffee is a stimulant beverage, source of foreign currency for Ethiopia and contributes more than 35% of the total export earnings. The Heterosis of these hybrid coffees had were 60% yield advantage as compared to pure-line cultivars. However, the seedlings' sources of hybrid coffee were difficult to address the demands of stakeholders via seed due to segregation. Hybrid coffee seedling sources were raised via hand pollination, cutting and tissue culture methods. Hence, the issue stakeholders assumed that, tissue culture seedlings were similar to genetically modified organisms (GMO) rather than true to type issue. Therefore, to intervene in the perception of stakeholders experimental trials were applied on tissue culture, seed and cutting seedling sources at Jimma and Agaro for yield, organoleptic quality and biochemical contents. The result revealed that, there is no significant difference among mean treatments in all parameters of yield, organoleptic quality and biochemical contents within the range of yield and quality and biochemical Arabica coffee. Therefore, the hypotheses of true-to-type and diseases free planets were raised and maintained via the experimental results of means analysis among means of the treatments.

Keywords: Organoleptic, Biochemical, Heterosis, True-to-Type

1. Introduction

Coffee (Coffeaarabica L.) is a non-alcoholic stimulant beverage crop that belongs to the family Rubiaceae genus of *Coffea*. It is the world's favorite beverage and the second-most traded commodity after oil [10]. Ethiopia is currently producing an estimated of 8.1 million bags that would rank the country as Africa's largest coffee producer, fifth and tenth Arabica coffee producer and export worldwide respectively [14]. Coffee is one of the major sources of export in the Ethiopian economy as it contributes 35% of Ethiopia's total export earnings [3]. However, due to biotic and abiotic stresses the production was also very low. Breeding strategies aiming to develop new F1 hybrid cultivars (from two parents) of Arabica coffee were proposed simultaneously in Ethiopia [6] and in Central America [7]. In Central America, the strategy was based on crossing American cultivars with wild Ethiopian accessions. Like many other hybrid plants, *C. arabica* F1 hybrids possess genetic and agronomic advantages [11], such as higher and more stable yields, more vigor, disease resistance, better cup quality, and adaptability to agroforestry systems [8]. In order to tackle these production constraints, Jimma Agricultural Research Center (JARC) developed three hybrid coffee cultivars Aba buna, MelkoCH_2 and Gawe with an average yield of 23.8, 24 and 26 qt/ha, respectively [5]. In addition, [4] reported higher hybrid performance for yield (18%-60%) among the cross of elite breeding materials in Ethiopia. Fortunately, the demand for hybrid seed and seedling from coffee farmers as well as from coffee plantation owners were high. Whatever the case, the fact that it was still possible to identifying the off-types, topography variations and disease susceptible plants in the F1 hybrid

population is a disadvantages of hybrids propagated from seeds when compared with clonal F1 hybrids [12]. Although off-types exist in F1 hybrid clones propagated by SE and are the result of somaclonal variation (aneuploidy), we have shown that a series of measures in the laboratory and nursery drastically limits their incidence of occurrence in the field <1% [9]. However, these higher yielding coffee varieties were not distributed to coffee growers, due to lack of efficient seedling production techniques. Disease free and true-to-type large-scale micro propagation of *C. arabica* hybrid seedlings were raised via tissue culture, Seed and cutting were planted over locations. Therefore, the objective of this research was to assess yield, cup quality and biochemical component of coffee seedlings raised from tissue culture (TC), seed, and cutting at various locations.

2. Materials and Methods

Three hybrid coffee varieties propagated by seed, cutting and Tissue Culture (TC) seedlings were planted at Jimma and Agaro. The experiments were conducted by Randomized Complete Block Design (RCBD) 2*3*9 = 54 with two locations, three replications and nine treatments for yields. Spacing between Plants and rows has been designed 2m*2m respectively.

2.1. Coffee Sample Preparation

The experimental study was conducted at Jimma and Agaro with an elevation range of 1600 – 1751 (masl) mid-altitudes and 1529 mm average rainfall per annum. Six kilograms of red ripe coffee cherries were harvested from each randomly selected coffee farm during the peak harvesting season. Red fresh cherries were collected and wet processed by pulping using a single disc hand pulpier. Successively, the fresh parchment coffee was fermented, soaked and washed. The washed parchment coffee was uniformly dried to a moisture level of 11.0%. Finally, dried parchment coffee samples were mechanically hulled and cleaned. Samples of 200 gram (g) clean undamaged coffee beans were prepared from each treatment for cup and biochemical quality analysis. The clean pulped coffee bean sample was subdivided into two of 100g green beans for cup quality and 100g roasted beans for biochemical analysis. The sample of 100g green beans were roasted at 200^{o}C heated coffee roaster machines (Probat BRZ6, Germany) to medium level for 8 min. After air-cooling, each roasted coffee sample was ground into medium (<0.5mm) using an electrical coffee grinder (Mahlkoing, Germany). A sample of 100g of green beans per sample were ground to a fine powder size (<0.5mm) using green coffee grinder for analysis of green biochemical contents.

2.2. Cup Quality Analysis

Cup quality evaluation was done at the JARC coffee quality evaluation laboratory. Coffee samples were medium roasted and medium ground. The hot beverage was prepared by brewing 8g roasted and ground coffee in 180 mL of hot water (95^{o}C). At the palatable temperature of about 60^{o}C cup evaluation was done following the Coffee Quality Lab manual procedure of JARC [2]. Eight cup evaluation criteria were used. Aromatic intensity (AI), aromatic quality (AQ), astringency (AS) and bitterness (BI) were evaluated on 0 to 5 scales. Acidity (AC), body, flavor and overall cup quality (OCQ) were assessed on 0 to 10 scales. Cup evaluation was done by a panel of three experienced certified Q-grade cuppers [2].

2.3. Moisture Content Analysis

The moisture content of coffee samples was determined according to the procedure suggested by AOAC (2000). About two grams (W1) of the green coffee powder was weighed on a dish. The samples were dried in an oven set at 136^{o}C for one hour, cooled and reweighed (W2). The loss in weight was calculated as the moisture loss and the value was expressed in percentage. The moisture content levels were used to obtain the dry matter content of the green coffee samples. The percentage of dry matter content was calculated according to Eq. (1) (AOAC, 2000).

$$\% \text{ Dray matter} = \frac{W2}{W1} \text{X } 100 \quad (1)$$

2.4. Biochemical Content Analysis

The green bean coffee samples of caffeine (CAF) chlorogenic acids (CGA) and trigonelline (TRG) extraction were done following the method adapted from [16] with some modifications by [1]. About 0.5 g of finely ground coffee powder was accurately weighed into a 50 mL Erlenmeyer flask. 50 mL of heated (95^{o}C) distilled water was added and stirred for 20 min on the hot plate. Then the extract was filtered using No. 4 What-man filter paper and subsequently filtered through 0.22 µm pore size and the 10µL specimen was injected into the High performance liquid chromatography (HPLC) (Agilent 1260 Infinity, Germany). Simultaneous determination of CAF, CGA and TRG was done using the HPLC system consisting of Discovery C_{18} column with Isocratic flow of 0.7mL/min methodology adapted from [16] with some modification. The column used was a size of 4.6X 250 mm 5µm particles (Waters, Taunton, USA). Elute compounds with gradients containing 5% acetic acid (A) and acetonitrile (B) were as follows:- 0–4 min: 4% B; 4–8 min: 10%B; 8–12 min: 90%B; 12–15 min: 0% B; and 15–17 min: 4%B, at a flow rate of 0.7 mL/min. Three minute post run was used. CAF and TRG were detected at 272 nm, while CGA was detected at 320 nm. Calibration curves of CAF, CGA and TRG standards using triplicate measurements were used to quantify those compounds. CAF and TRG were evaluated from 10, 20, 40, 50, 100 and 200 µg/mL, whereas CGA was evaluated over the range 10, 20, 100, 200 and 500 µg/mL. CAF, CGA and TRG were identified by comparing the retention times of CAF standard (99 %) (Fischer Scientific), TRG standard (Sigma Aldrich) and CGA standard (Acros organics) and their concentrations calculated

from peak areas using calibration equations. Calibration curves were made using the standard concentration and area of the sample subsequently used to calculate the composition of the respective biochemical component using the area generated after a retention time [13].

3. Results and Discussion

The seedling sources raised from TC, seed and cutting were evaluated in two locations for three years for yield, organoleptic tests and biochemical contents. The result revealed that there is no significant difference among means in all parameters of yield, organoleptic and biochemical contents. Therefore, the hypothesis of true-to-type was supported via the means analysis of the experimental results in Tables (1, 2 and 3).

The yield performance of comparison of seed, cutting and tissue culture seedling.

Three hybrid coffee seedlings propagated via TC, seed and Cutting were evaluated for three years at Jimma and Agaro in RCBD with three replications. In the first year yields were higher for stem cutting propagation method at Jimma in all varieties (Table 1). However, there is non- significant difference at Agaro. In addition to that, there is also a significant different among means on the propagation methods in the third year when we compared cutting and TC but there is a non-significant difference mean in seed and tissue culture (TC). Therefore, there is no significant difference among means over years and over locations in all varieties and propagation methods.

Table 1. *Comparison of yield data (Kg/ha) over years and over locations of three hybrid coffee seedlings raised via Tissue Culture (TC), Seed and Cutting.*

Treatments	2012		2013		2014		Over years		Over Loca.
	Jimma	Agaro	Jimma	Agaro	Jimma	Agaro	Jimma	Agaro	Mean
Ab buna TC	706^{ab}	1122	2690	1208	2961^{ab}	2515^{ab}	2110	1615	1867
Ababuna Seed	342^{c}	722	2887	1062	2739^{ab}	2316^{ab}	2098	1367	1678
Ababuna Cutting	846^{a}	1049	3069	1180	2394^{ab}	3231^{a}	2103	1820	1962
Melko CH_2 TC	448^{bc}	913	2579	924	1515^{b}	2024^{b}	1514	1287	1400
Melko CH_2 Seed	670^{abc}	809	2983	1207	2928^{ab}	2608^{ab}	2194	1541	1867
Melko CH_2 Cutting	657^{abc}	1157	2779	1297	3504^{a}	2471^{ab}	2313	1642	1978
Gawe TC	395^{bc}	829	2374	1037	2281^{ab}	1924^{b}	1683	1263	1473
Gawe Seed	446^{bc}	817	3498	1093	2441^{ab}	2834^{ab}	2128	1581	1855
Gawe Cutting	818^{a}	1015	3369	1072	2883^{ab}	2634^{ab}	2357	1574	1965
Mean	592	937	2914.2	1120	2627	2506	2044	1521.1	1783
F-test	*	NS	NS	NS	*	*	NS	NS	NS
LSD @ 5%	162	497	1124.4	373	1632	1016	844	557	710
CV %	27.4	28	18.1	20.4	24.2	18	27	14.4	20

Means followed by the same letters are not significant from each other's; NS = non-significantly different at P<0.05, TC = Tissue culture

3.1. The Effect of Propagation Methods on the Organoleptic Quality of Hybrid Coffee Seedlings

The result indicates that, all samples collected from Jimma and Agaro were assessed for bean size, raw and cup quality shows very good and highly acceptable overall quality standard in improved hybrid coffees and propagation methods in both locations. The ranges of (80 - 100 %) improved *A. coffae* in Q-grade cuppers [2]. In general, there is no significant difference among seedlings propagation methods in overall organoleptic quality standards in both locations. In the world, there is considerable economic importance in producing the highest lion share of large size exportable beans as well as excellent and very good overall quality aspects of Arabica coffee with acceptable to healthy aspects [15].

Table 2. *The Screening of Been size, Raw, cup and overall quality result at Jimma and Agaro.*

Treatments	Jimma				Agaro			
	Been Size	Raw (40%)	Cup (60%)	Overall (100%)	Been Size	Raw (40%)	Cup (60%)	Overall (100%)
Aba buna TC	98.00	35.83	47.00	82.83	98.00	39.00	43.67	82.67
Aba buna Seed	98.00	35.33	46.83	82.16	99.00	38.33	43.17	81.50
Aba buna Cutting	99.00	36.50	49.17	85.67	99.00	38.50	43.67	82.17
Melko CH_2 TC	99.00	35.33	47.17	82.50	99.00	39.33	44.17	83.50
Melko CH_2 Seed	97.00	35.50	45.50	81.00	98.00	38.33	43.33	81.66
Melko CH_2 Cutting	98.00	35.67	47.50	83.17	98.00	38.67	44.33	83.00
Gawe TC	99.00	36.17	46.83	83.00	99.00	39.00	43.83	82.83
Gawe Seed	98.00	36.00	46.17	82.17	98.00	39.00	44.17	83.17
Gawe Cutting	99.00	36.33	46.50	82.83	98.00	38.50	44.50	83.00
Mean	98.33	35.85	46.96	82.81	98.44	38.74	43.87	82.61
F-test		ns	ns	ns		ns	ns	ns
CV %		1.92	4.96	2.84		1.48	2.98	1.84

NS = non-significantly different at P < 0.05, TC = Tissue culture

3.2. Biochemical Composition of Seed, Cutting and TC Seedlings Raised Coffee Trees

The result indicates that all samples collected from Jimma and Agaro were determined for caffeine, chlorogenic acid and trigonelline. The mean showed 1.24%, 5.9% and 0.96% related with the international ranges of caffeine, chlorogenic acid and trigonelline respectively in both locations. In general, the F-test showed that there is no significant difference among means in both locations of biochemical contents of all propagation methods and hybrid coffee varieties.

Table 3. *Biochemical contents of green bean caffeine, chlorogenic acids and trigonelline for different seedling sources at Jimma and Agaro.*

Location	Seedlings Source	Caffeine (%)	Chlorogenic acid (%)	Trigonelline (%)
Melko	Ababuna TC	1.22	5.76	0.84
Melko	Ababuna Seed	1.23	5.73	0.84
Melko	Ababuna Cutting	1.30	6.48	0.94
Melko	MCH_2- TC	1.11	5.76	0.88
Melko	MCH_2-Seed	1.49	6.35	1.17
Melko	MCH_2 Cutting	1.11	5.66	0.96
Melko	Gawe TC	1.12	6.42	1.14
Melko	Gawe Seed	1.25	7.09	1.10
Melko	Gawe Cutting	1.06	5.91	0.99
Agaro	Ababuna TC	1.42	6.09	1.20
Agaro	Ababuna Seed	1.24	5.67	0.92
Agaro	Ababuna Cutting	1.46	6.01	1.10
Agaro	MCH_2 TC	1.06	5.01	0.94
Agaro	MCH_2 Seed	1.15	5.22	0.98
Agaro	MCH_2 Cutting	0.94	5.54	0.83
Agaro	Gawe TC	1.34	5.47	0.77
Agaro	Gawe Seed	1.40	6.16	0.86
Agaro	Gawe Cutting	1.36	5.88	0.83
	Mean	1.24	5.9	0.96
	F- test	ns	ns	ns
	CV %	3.83	2.47	4.09

NS = non-significantly different at P<0.05, TC = Tissue culture

4. Conclusions

The results of the present study showed that the treatments of varieties with propagation methods over years and across locations for yield were not significantly different among means treatment, which is not turn influenced the yield by propagation methods. The green bean size, raw cup and overall quality of hybrid coffee in both locations have also been similar within mean treatments. The inherent chemical constituent of green coffee is among the quality characteristics which influenced by the physical appearance and organoleptic cup quality of the coffee beans. Moreover, these very good and highly acceptable in overall quality standards which enhances the biochemical contents attributes the ranges of Arabica coffee whatever the proportion methods and varietal differences were varied. Obviously, the hypothesis of true-to-type in tissue culture technics for somatic embryogenesis propagation methods maintained by the inheritance of F_1 progeny raised via hand pollination and cutting in the means analysis of the experimental results.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

Acknowledgments

The authors acknowledge the Quality Laboratory of Headquarters in Ethiopian Institute of Agricultural Research (EIAR) for examining and analysis of biochemical contents such as CAF, CGA and TRG via High performance liquid chromatography (HPLC). In addition to that, we would like to say thanks to the Ethiopian Institute of Agricultural Research (EIAR) for financial support. Moreover, the Authors also acknowledged that researchers of Agaro sub-center who have contributed from land preparation to yield data collection, and the team of JARC Coffee cuppers.

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