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Genetic profiling of locally registered Philippine coffee using molecular markers linked to resistance against diseases and pests

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Abstract. *Santos NRS, Magat MB, Mondragon MV, Cao EP, Santos DMC. 2023. Genetic profiling of locally registered Philippine coffee using molecular markers linked to resistance against diseases and pests. Biodiversitas 24: 4136-4144.* Coffee is a major commodity in the Philippines, but diseases and pests have hampered local production. Natural resistance could help increase production by eradicating infestation or lessening the symptoms of infection and minimizing the need for chemical control. Hence, this study aims to screen local *Coffea arabica* varieties, whose beans are prized for their superior taste and aroma, using genetic markers. These markers are linked to resistance against Coffee Leaf Rust (CLR, caused by *Hemileia vastatrix*), Coffee Berry Disease (CBD, caused by *Colletotrichum kahawae*), and Root Knot Nematode (RKN, *Meloidogyne* spp.). The Arabica samples were obtained from the Bureau of Plant Industry in Baguio City, Benguet, a main distributor of coffee seedlings to farmers. *C. canephora* and *C. liberica* trees from Cavite State University, Indang, Cavite, were used as control samples. Results reveal that the registered NSIC-2008-Cf-A-05 (Red Bourbon) Arabica tree contains a unique haplotype in a region of chromosome 3. This region has been linked to the *SH3* gene, which confers resistance against CLR, a promising result for infested areas. However, all Arabica samples are inferred to be susceptible to CBD and RKN. Hence, NSIC-2008-Cf-A-05 is a potential source for resistance genes specifically against CLR in future breeding programs.

Keywords: Arabica, *Coffea*, haplotype, SCAR, SSR

INTRODUCTION

Coffee (*Coffea* sp.) is a major market in the Philippines. Thus, the government and private stakeholders have undertaken steps to improve local production, as reported in the Philippine Coffee Industry Roadmap 2021-2025 (Department of Agriculture, RP 2022). According to the same report, coffee production at the local level depends on the following *Coffea* species: *C. canephora* Pierre ex A.Froehner (Robusta: 66%), *C. arabica* L. (Arabica: 25%), *C. liberica* Hiern var. *dewevrei* (Excelsa: 8%), and *C. liberica* Hiern var. *liberica* (Liberica or "Barako": 1%). However, much of the coffee production at the global level depends on two species: *C. arabica* (Arabica: ~60%) and *C. canephora* (Robusta: ~40%) (International Coffee Organization 2021). Special preference is given to Arabica coffee because of its better beverage quality (Department of Agriculture, RP 2022). However, an issue with this species is its general susceptibility to diseases and pests compared to its Robusta counterpart (Van der Vossen et al. 2015). Diseases and pests have been reported as a main problem in the Philippines according to the Coffee Roadmap. Hence, they may be one of the reasons for the large difference in global (~60%) and local (25%) utilization of Arabica coffee.

Despite Arabica's general susceptibility, some of its varieties have been noted to be naturally resistant. By investigating these varieties, previous studies have developed molecular genetic markers which can determine whether a specific tree has the potential to be resistant or susceptible. A list of such markers has already been collated in a previous study (Yu et al. 2021). Thus, this study aims to screen Arabica trees at the Bureau of Plant Industry branch in Baguio City, Benguet (BPI-Baguio) using these resistance-linked markers. Other coffee species from the Cavite State University in Indang, Cavite (CavSU) were used as control samples. This study will accomplish the following objectives: 1) assess the genetic profile of Arabica trees available at BPI-Baguio concerning resistance-linked markers, and 2) determine if any trees exhibit specific bands linked to resistance.

At the time of this study, BPI-Baguio has registered three Arabica varieties at the National Seed Industry Council (NSIC). BPI-Baguio is one of the institutions identified by the Coffee Roadmap as a main source of coffee seedlings for farmers (Department of Agriculture, RP 2022). Three distinct Arabica mother trees are being used to propagate coffee seedlings in BPI-Baguio for wider cultivation. These trees are registered as NSIC-2008-Cf-A-05 (Red Bourbon), NSIC-2008-Cf-A-06 (Caturra), and NSIC-2008-Cf-A-07 (Yellow

Bourbon). At sampling, non-registered Arabica trees (Catimor, San Ramon, and Typica) were maintained within the nursery site. Bourbon and Typica represent some of the oldest coffee cultivars of *C. arabica,* while Caturra and San Ramon are their dwarf mutants, respectively (Alemu and Dufera 2017; Pruvot-Woehl et al. 2020). The Bourbon and Caturra varieties, in turn, can further be subdivided according to the color of their berry fruits (Red, Yellow). Meanwhile, Catimor is a cross between Caturra and the highly resistant Hibrido de Timor variety, a natural hybrid between *C. arabica* and *C. canephora* (Silva et al. 2018).

In addition to the Arabica trees from BPI-Baguio, this study included coffee trees of other species as control specimens. The Robusta, Excelsa, and Liberica control trees in this study are owned by CavSU. The Robusta and Liberica trees are registered as NSIC-2007-Cf-R-07 and NSIC-2007-Cf-L-01, respectively. The Excelsa tree was not yet NSIC-registered at the time of this study. Several studies have reported that certain resistance genes in Arabica trees originated from other species. These genes include those which confer resistance against coffee berry disease (Gichimu et al. 2014), coffee leaf rust (Alkimim et al. 2017), and root-knot nematode (Barrantes et al. 2020).

MATERIALS AND METHODS

Plant material and DNA extraction

Young leaves were collected from selected coffee trees in BPI-Baguio and CavSU. These include three clone trees from each NSIC-registered Arabica tree (NSIC-2008-Cf-A-05: Red Bourbon, NSIC-2008-Cf-A-06: Caturra, and NSIC-2008-Cf-A-07: Yellow Bourbon) at BPI-Baguio. Other non-registered Arabica trees that were present at BPI-Baguio were also sampled, and these include one Catimor, one San Ramon, two Typica trees. Lastly, one tree for each of the following: NSIC-2007-Cf-R-07: Robusta, NSIC-2007-Cf-L-01: Liberica, and Excelsa were collected at CavSU. Pertinent information for the NSIC-registered trees, such as their morphological traits and resistance profiles, can be downloaded from the NSIC website (National Seed Industry Council, RP).

DNA extraction was performed using the cetyltrimethyl-ammonium bromide (CTAB) method described by Healey et al. (2014) but with modifications. The following instructions were adjusted for each sample. Using liquid nitrogen, 100 mg of leaf tissue was frozen and ground into a fine powder using a mortar and pestle. The powder was placed in a 2 mL centrifuge tube and immersed in 1 mL pre-heated CTAB extraction buffer with 0.3% (v/v) β-mercapto-ethanol. The tube was placed in a 65°C water bath, with regular mixing by inversion every 10 min for 1hr. The tube was centrifuged for 5 min at 5,000 ×g using Spectrafuge™ 24D Digital Lab Microcentrifuge. The supernatant was transferred to a new 2mL centrifuge tube.

Therefore, to remove proteins and RNA, one volume (1 volume = same amount as supernatant from the previous step) of chloroform: isoamyl alcohol was added. The tube was mixed by inversion for 5 min and then centrifuged for 10 min at 5,000 ×g. The aqueous phase was pipetted to a new 2 mL centrifuge tube, avoiding the aqueous/organic layer interface. Afterward, 0.5 µL of RNAse A (10 mg/mL) was added. The tube was then incubated at 37°C for 15 min with periodic gentle mixing. After incubation, one volume of chloroform: isoamyl alcohol was added. The tube was mixed by inversion for 5 min and then centrifuged for 10 min at 5,000 ×g. The aqueous phase was pipetted to a new PCR tube, again taking care to avoid the organic layer.

Furthermore, 0.5 volume of 5M NaCl was added to precipitate the DNA. The tube was then mixed gently by inversion. Up to 3 volumes of cold 95% ethanol were added until the entire tube was filled. The tube was again mixed gently by inversion, placed into a -20°C freezer, and then incubated for 1hr. After incubation, the tube was centrifuged for 10 min at 5,000 ×g. The supernatant was carefully decanted away, and the DNA pellets were washed with 300 µL of 70% ethanol. The tubes were gently swirled and then centrifuged for 10 min at 5,000 ×g. The supernatant was again carefully decanted, and the DNA pellet was air-dried for 15 min at room temperature. DNA was finally suspended in 100 µL of TE buffer.

DNA concentration and purity were checked using the Take3™ and Take3 Trio™ Micro-Volume Plate reader. The concentration of DNA samples with A_{260}/A_{280} ratio of 1.7-2.0 was adjusted to 25 µg/mL for Marker-PCR amplification.

Molecular markers and PCR amplification

In a previous study (Yu et al. 2021), 39 markers (13 for SCAR and 26 for SSR) linked to resistance against diseases and pests were collected from published literature (Supplementary Data 1). Marker-Polymerase Chain Reaction (PCR) amplification was performed in a volume of 12.5 µL. The melting temperatures provided by the manufacturer (Macrogen Inc., South Korea) were used as a reference for the computation of annealing temperatures, according to Maddocks and Jenkins (2017) (Supplementary Data 1). The PCR mix contained the following: 2.5 µL 5x MyTaq Reaction Buffer (with dNTPs) (Bioline), 0.05 µL (100 pmoles/µL) of each Primer, 0.5 µL 5 U/µL Taq Polymerase (Bioline), 1.0 µL 25 µg/mL DNA, and 8.4 µL double distilled water. PCR was run using the following conditions: initial denaturation at 94°C for 10 min; 35 cycles of denaturation at 94°C for 30 sec., annealing temperature for 30 sec., extension at 72°C for 1min; and a final extension at 72°C for 7 min. PCR reactions were performed using the T-100 Thermal Cycler (Bio-Rad, Singapore).

Gel electrophoresis and analysis

For visual confirmation, PCR products were initially run through 1% agarose gel electrophoresis (AGE) using the Midi plus-2 Horizontal Electrophoresis System, ME15-7-10-

15 (Major Science). The gels were prepared by dissolving 0.50 g of molecular grade agarose powder (Vivantis) in 50 mL of 1.0X Tris/Borate/EDTA (TBE) buffer (Vivantis). The gel mix was microwaved at 1 min intervals until it became clear. Next, 0.5 µL of 10 mg/mL ethidium bromide (Invitrogen®) was added once between intervals. The gel mix was allowed to cool off before being poured into the casting stand (ME15-UV7, 15x7 cm UV tray) containing the 20-well comb (ME15-20-1). After solidification, the gel was placed into the tank containing 1.0X TBE buffer (Vivantis). Gel electrophoresis was run for 30 min at 100 V and 400 mA.

After visual confirmation using AGE, the PCR products were run through 10% polyacrylamide gel electrophoresis (PAGE) using the Mini-PROTEAN® System (Bio-Rad). The gel for each casting stand contained the following: 5 mL of 30% Acrylamide/Bis Solution 29:1, 8.5 mL of distilled water, 1.5 mL of 10X TBE buffer (Vivantis), 150 µL 10% Ammonium Persulfate (Vivantis), and 15 µL of TEMED (Vivantis). After solidification, the gel was placed into the tank containing 1.0X TBE buffer (Vivantis). Gel electrophoresis was run for 85 min at 85 V and 400 mA.

In both gel electrophoreses, 1 µL of each PCR solution was mixed with 1 µL of 6X loading dye (Vivantis) before loading into a well. Hyperladder 25 bp (Bioline®) and Hyperladder 100 bp (Bioline®) were used as molecular weight marker guides. Gels were viewed under UV light at 302 nm wavelength using AlphaImager Mini (ProteinSimple).

The gel images were analyzed using Gel Analyzer 19.1 (Lazar Jr and Lazar Sr). Molecular weight calibration was set to exponential fit, and the bands from the ladders were used as references. Sizes of the bands from the samples were then estimated. Data such as expected band sizes, band size linked to resistance, or band size linked to susceptibility (Supplementary Data 1) were obtained from previous literature including but not limited to the studies listed in Appendix I of Yu et al. (2021). These are then compared with data obtained from this study.

PrimerBlast

The markers were screened through PrimerBlast using the *C. arabica* (Red Caturra) genome (GCA_003713225) and the *C. canephora* genome (GCA_900059795) at GenBank. This was done to determine the markers' location and estimate the bands' expected sizes.

RESULTS AND DISCUSSION

Moreover, 39 resistance-associated SCAR (13) and SSR (26) markers were screened against local coffee trees. Marker-PCR amplification was successful except for one SCAR marker (CaRHvII 1). Results regarding banding patterns are listed in Table 1.

Resistance against Coffee Leaf Rust (CLR)

The SH3 gene region

The *SH3* gene is among multiple *SH* genes (*SH1-SH9*) that confer resistance against CLR caused by the fungal rust, *Hemileia vastatrix*. It is reported to have been introgressed from *C. liberica* into *C. arabica* (Alkimim et al. 2017). Mahé et al. (2008) identified ten markers linked to this particular gene: BA-42-21B-r, BA-48-21O-f, BA-124-12K-f, Sat160, Sat244, Sat281, SP-M5-SH3, SP-M8-SH3, SP-M16-SH3, and SP-M18-SH3. The NSIC-2008-Cf-A-05 (Red Bourbon) samples exhibited banding patterns different from the other Arabica samples for eight markers.

Recent studies have shown that the binding sites of these markers are spread throughout a large region of chromosome 3 of the coffee genome (Cui et al. 2020; Nagaño et al. 2022). These findings suggest that the resistance once associated with a single "*SH3 gene*" is conferred through the combined effects of multiple genes. This study shows that NSIC-2008-Cf-A-05 (Red Bourbon) contains a unique haplotype within said region. However, most of these markers lack data regarding the specific band sizes linked to resistance or susceptibility. Studies such as Prakash et al. (2011) and Gutiérrez-Calle et al. (2021) report band sizes for some *SH3*-linked markers, although they have conflicting data.

All Arabica samples for the BA-48-21O-f marker in this study showed a ~348bp band, but the NSIC-2008-Cf-A-05 (Red Bourbon) samples showed additional ~396bp and ~420bp bands. The Robusta sample showed a ~357bp band, while the Liberica and Excelsa samples showed a ~338bp band. Gutiérrez-Calle et al. (2021) report that their Arabica samples showed a 332bp band. Meanwhile, their resistant EA67 (*C. liberica*) and S.288 (*C. arabica* x *C. liberica*) control samples did not show any band; they attribute this to failure in Marker-PCR amplification. According to Mahé et al. (2008), the presence of a unique band is linked to resistance for this marker. There is the possibility that either the ~396bp or the ~420bp band from this study represents the resistance-linked band for BA-48-21O-f.

For the BA-124-12K-f marker in this study, all Arabica samples showed a ~370bp band, but the NSIC-2008-Cf-A-05 (Red Bourbon) samples showed an additional ~318bp band. The Robusta sample showed ~309bp and ~324bp bands, while the Liberica and Excelsa samples also showed the ~318bp band. Prakash et al. (2011) report that a 320bp band indicates resistance. Meanwhile, Gutiérrez-Calle et al. (2021) report that their Arabica samples showed a 403bp band. Their EA67 control sample showed a 349bp band, while their S.288 control sample showed both 349bp and 403bp bands. According to Mahé et al. (2008), the presence of a unique band is also linked to resistance for this marker. There is the possibility that the ~318bp band from this study, the 320bp from Prakash et al. (2011), and the 349bp band from Gutiérrez-Calle et al. (2021) represent the same resistance-linked band for BA-124-12K-f.

Table 1. Band profiles for *SH3*-linked markers. The bands observed in the gel for each marker are shown alongside the results of PrimerBlast

Marker	PrimerBlast (A)						*Coffea arabica trees at BPI-Baguio (B)*										*Coffea trees at CavSU*		
	C. arabica				*C. canephora*		Caturra		Red Bourbon		Yellow Bourbon		Catimor		San Ramon		Typica 1		Typica 2		*C. canephora* Robusta	*C. liberica* Liberica	*C. liberica* Excelsa
	Caturra				Robusta																		
BA-42-21B-r (SCAR)	**Chr3c**	**378**	Chr3e	404	**Chr3**	**378**	562	**436**	**460**		562	**436**	562	**436**	562	**436**	562	**436**	562	**436**	573	607	562
	Chr3c	374	Chr3e	392			**460**				**460**		**460**		**460**		**460**		**460**		**436**	**460**	**460**
BA-48-21O-f (SCAR)	**Chr3c**	**315**	Chr3e	301	Chr3	315	**348**		420	**348**	**348**		**348**		**348**		**348**		**348**		**357**	**338**	**338**
									396														
BA-124-12K-f (SCAR)	X	X	X	X	Chr3	427	**370**		**370**		**370**		**370**		**370**		**370**		**370**		324	**318**	**318**
					Chr3	403			**318**												309		
					Chr3	401																	
CaRHvII 1 (SCAR)	Chr11c	533	Chr5e	533	Chr11	533	X		X		X		X		X		X		X		X	X	X
CaRHvII 2 (SCAR)	**Chr2c**	**298**	**Chr2e**	**298**	**Chr2**	**298**	**302**		**302**		**302**		**302**		**302**		**302**		**302**		**302**	**302**	**302**
CaRHvII 3 (SCAR)	Chr1c	434	X	X	Chr1	441	**423**		**423**		**423**		**460**		**423**		**423**		**423**		433	449	460
													428									423	
CaRHvII 4 (SCAR)	X	X	Chr8e	207	Chr8	210	**213**		**213**		**213**		**213**		**213**		**213**		**213**		**213**	**213**	**213**
					Chr8	207																**204**	
CaRHvII 5 (SCAR)	Chr9c	259	X	X	Chr9	259	**274**		**274**		**274**		**274**		**274**		**274**		**274**		**274**	**274**	**274**
CaRHvII 6 (SCAR)	X	X	Chr8e	457	**Chr8**	**457**	**456**		**456**		**456**		**456**		**456**		**456**		**456**		**456**	**456**	**456**
SP-M5-SH3 (SCAR)	X	X	X	X	Chr3	160			**161**													**168**	**168**
SP-M8-SH3 (SCAR)	X	X	X	X	**Chr3**	**242**	**255**		**255**		**255**		**255**		**255**		**255**		**255**		**255**	**255**	**255**
SP-M16-SH3 (SCAR)	X	X	X	X	X	X	**109**		**121**		**109**		**109**		**109**		**109**		**109**		**109**	**121**	**122**
SP-M18-SH3 (SCAR)	?	?	?	?	?	?	?		?		?		?		?		?		?		?	?	?
EST-SSR009 (SSR)	Chr1c	160	Chr1e	146	Chr11	152	**178**	**145**	**178**	**145**	**178**	**145**	**178**	**145**	**178**	**145**	**178**	**145**	**178**	**145**	**169**	**180**	**183**	**139**
							156		**156**		**156**		**156**		**156**		**156**		**156**		**151**	**159**	**161**
EST-SSR032 (SSR)	X	X	**Chr2e**	**200**	**Chr2**	**202**	**241**		**241**		**241**		**241**		**241**		**241**		**241**		**241**	**241**	**241**
							211		**211**		**211**		**211**		**211**		**211**		**211**		**211**	**211**	**211**

Marker	PrimerBlast (A)						*Coffea arabica trees at BPI-Baguio (B)*														*Coffea trees at CavSU*					
	C. arabica				*C. canephora*		Caturra		Red		Yellow		Catimor		San		Typica				*C. canephora*		*C. liberica*			
	Caturra				Robusta				Bourbon		Bourbon				Ramon		1		2		Robusta		Liberica		Excelsa	
EST-SSR050	**Chr2c**	**165**	**Chr2e**	**162**	**Chr2**	**165**	**226**		**226**		**226**		**226**		**226**		**226**		**226**		226	174	226	176	**226**	198
(SSR)							**163**		**163**		**163**		**163**		**163**		**163**		**163**		208	**163**	213	**163**	213	**163**
EST-SSR107	**Chr11c**	**159**	Chr11e	169	**Chr11**	**167**	**171**		**171**		**171**		**171**		**171**		**171**		**171**		**171**	147	153		**166**	
(SSR)							**162**		**162**		**162**		**162**		**162**		**162**		**162**		**160**				151	
M11/Sat11	X	X	Chr7e	138	Chr7	144	157		157		157		157		157		157		157		157					
(SSR)							**146**		**146**		**146**		**146**		**146**		**146**		**146**		**146**					
M24	**Chr6c**	**166**	X	X	Chr6	148	**196**		**196**		**196**	*146*	**196**		**196**		**196**		**196**		183	**150**	193	**164**	196	**164**
(SSR)							**169**		**169**		**169**		**169**		**169**		**169**		**169**		**157**		176		187	
M27/Sat27	Chr11c	137	**Chr11e**	**141**	Chr11	135	308	216	273	**147**	273	**147**	308	216	273	**147**	273	**147**	273	**147**	244		252		**152**	
(SSR)							287	165	216		216		287	165	216		216		216		170		174		**145**	
							244	**147**	165		165		244	**147**	165		165		165		**148**		**153**			
M47/Sat47	**Chr11c**	**120**	**Chr11e**	**152**	**Chr11**	**131**	**179**	140	**179**	140	**179**	140	**179**	140	**179**	140	**179**	140	**179**	140	172	140	169	127	150	125
(SSR)							161	130	161	130	161	130	161	130	161	130	161	130	161	130	161		151		140	
							155	**125**	**155**	**125**	**155**	**125**	**155**	**125**	**155**	**125**	**155**	**125**	**155**	**125**	146		145		132	
Sat160	**Chr3c**	**133**	**Chr3e**	**172**	**Chr3**	**133**	**179**	117	**179**		**179**	117	**179**	117	**179**	117	**179**	117	**179**	117	179		**131**		**131**	
(SSR)			Chr3e	103			155	**106**	117		155	**106**	155	**106**	155	**106**	155	**106**	155	**106**	**155**		117		117	
							136		**106**		**136**		**136**		**136**		**136**		**136**		**136**		**106**		**106**	
Sat207	**Chr1c**	**94**	**Chr1e**	**84**	**Chr1**	**90**	106	**86**	106	**86**	106	**86**	106	**86**	106	**86**	106	**86**	106	**86**	**103**		**82**		**93**	
(SSR)							**96**		**96**		**96**		**96**		**96**		**96**		**96**		**93**				**82**	
Sat225	**Chr11c**	**264**	**Chr11e**	**297**	**Chr11**	**277**	354	**301**	354	**301**	354	**301**	354	**301**	354	**301**	354	290	354	**301**	318		334	**281**	329	
(SSR)			**Chr11e**	**264**			324	290	324	290	324	290	332	**284**	324	290	324	**267**	324	290	295		318		**287**	
							309	**267**	309	**267**	309	**267**	324		309	**267**	**301**		309	**267**	**277**		**295**			
Sat227	**Chr11c**	**180**	X	X	Chr11	298	315	208	315	208	315	208	315	208	315	208	315	208	315	208	310	218	223		245	**196**
(SSR)							234	**196**	234	**196**	234	**196**	234	**196**	234	**196**	234	**196**	234	**196**	300	**202**	**193**		220	
																					223	**185**			**213**	
Sat229	?	?	?	?	?	?	**167**		**167**		**167**		**167**		**167**		**167**		**167**		**167**		**167**		**167**	
(SSR)							**121**		**121**		**121**		**121**		**121**		**121**		**121**							
Sat235	**Chr1c**	**225**	X	X	**Chr1**	**222**	295	**253**	295	**253**	295	**253**	295	**253**	295	**253**	295	**253**	295	**253**	332	244				
(SSR)							269	**186**	269	**186**	269	**186**	269	**186**	269	**186**	269	**186**	269	**186**	287	**220**				
							262		262		262		262		262		262		262		256					
Sat244	X	X	X	X	**Chr3**	**302**	**356**	**280**	**367**	292	**356**	**280**	**356**	**280**	**356**	**280**	**356**	**280**	**356**	**280**	**356**		**362**		**372**	
(SSR)							327		337	**277**	327		327		327		327		327		**315**		335		**327**	
							309		**315**		**309**		**309**		**309**		**309**		**309**				**321**			
Sat281	**Chr3c**	**95**	X	X	Chr3	73	**95**		**90**		**95**		**95**		**95**		**95**		**95**		**106**		**166**		**72**	
(SSR)	**Chr3c**	**81**					**83**		**83**		**83**		**83**		**83**		**83**		**83**		**87**					

Marker	PrimerBlast (A)						*Coffea arabica trees at BPI-Baguio (B)*														*Coffea trees at CavSU*					
	C. arabica				*C. canephora*		**Caturra**		**Red**		**Yellow**		**Catimor**		**San**		**Typica**				*C. canephora*		*C. liberica*			
	Caturra				**Robusta**				**Bourbon**		**Bourbon**				**Ramon**		**1**		**2**		**Robusta**		**Liberica**		**Excelsa**	
SSR071	Chr1c	220	Chr1e	220	X	X	247		247		247		247		247		247		247		**245**		**234**		**234**	
(SSR)							**224**		**224**		**224**		**224**		**224**		**224**		**224**		**224**		**224**		**224**	
SSR100	Chr11c	185	Chr11e	185	X	X	212	**195**	212	**195**	212	**195**	212	**195**	212	**195**	212	**195**	212	**195**					**171**	
(SSR)							204	**171**	204	**171**	204	**171**	204	**171**	204	**171**	204	**171**	204	**171**						
SSRCa034	?	?	?	?	?	?	361	331	361	331	361	331	361	331	361	331	361	331	361	331	**304**		**315**		**308**	**276**
(SSR)							352	315	352	315	352	315	352	315	352	315	352	315	352	315					293	
SSRCafé 13	**Chr1c**	**182**	Chr1	179	**Chr1**	**396**	298	193	298	193	298	193	298	193	298	193	298	193	298	193	298	193	207		306	190
(SSR)					**Chr1**	**181**	257	186	257	186	257	186	257	186	257	186	257	186	257	186	257	186	190		291	**184**
							210		210		210		210		210		210		210		210		**184**		207	
SSRCafé 19	**Chr11c**	**168**	Chr11e	170	**Chr11**	**150**	198		198	163	198		198		198		198	163	198		210	**151**	196	**153**	196	**153**
(SSR)	Chr11c	146	Chr11e	146	Chr11	146	**170**		190	**153**	**170**		**170**		**170**		190	**153**	**170**		185		**170**		**170**	
							153		170		**153**		**153**		**153**		170		**153**		173		163		163	
SSRCafé 15	X	X	X	X	X	X	211		211		211		211		211		211		211		194		198	**168**	220	182
(SSR)							196		196		196		196		196		196		196		**180**		185		211	**172**
							187		**187**		**187**		**187**		**187**		**187**		**187**		**172**		**176**		192	**165**
SSRCafé 32	**Chr2c**	**98**	Chr2e	75	**Chr2**	**87**	**113**	87	**113**	87	**113**	87	**113**	87	**113**	87	**113**	87	**113**	87	**83**		**89**		**89**	
(SSR)							**102**	**81**	**102**	**81**	**102**	**81**	**102**	**81**	**102**	**81**	**102**	**81**	**102**	**81**	**77**		**81**		**81**	
SSRCafé 39	Chr1c	172	**Chr1e**	**181**	Chr1	187	224	**187**	224	**187**	224	**187**	224	**187**	224	**187**	224	**187**	224	**187**	224		247	**186**	247	**186**
(SSR)							206	**177**	206	**177**	206	**177**	206	**177**	206	**177**	206	**177**	206	**177**	197		218		218	
							199		199		199		199		199		199		199		**188**		206		206	
SSRCafé 40	**Chr1c**	**290**	X	X	**Chr1**	**577**	**363**	261	**363**	261	**363**	261	391	281	**363**	261	**363**		**363**	261	363	**275**	363		363	
(SSR)					**Chr8**	**554**	341	*240*	341		341		**363**	261	341		**325**		341		345		345		**309**	
							325		**325**		**325**		341	246	**325**		**302**		**325**		335		325			
							302		**302**		**302**		**325**	234	**302**				**302**		**317**		**302**			
							281		281		281		**302**		281				281		296					
SSRCafé 41	Chr2	126	Chr2	112	Chr2	122	148	**125**	148	**125**	148	**125**	148	**125**	148	**125**	148	**125**	148	**125**	142	**121**	**131**	**111**	**119**	
(SSR)							132	**111**	132	**111**	132	**111**	132	**111**	132	**111**	132	**111**	132	**111**	129	111	**121**		**113**	

Note: *C. arabica* is an allotetraploid hybrid between *C. canephora* and *C. eugenioides*. For the *Coffea arabica* (Red Caturra) genome (GCA_003713225) at GenBank, the chromosomes derived from the *C. canephora* subgenome (Chr1c–11c) are distinguished from those derived from the *C. eugenioides* subgenome (Chr1e–11e). Products that have no primer mismatches are in **bold**. "X" indicates the lack of detectable binding sites. "?" indicates the presence of too many binding sites. B. The three registered Arabica trees (Caturra, Red Bourbon, and Yellow Bourbon) are represented by 3 clones each. Inconsistent bands among clones are in *italics* and can be attributed to unintended primer targets. The bands in **bold** represent those with the highest intensity. "?" indicates the presence of too many bands indiscernible from one another. "X" indicates failure of Marker-PCR amplification

For the Sat244 marker in this study, the NSIC-2008-Cf-A-05 (Red Bourbon) samples showed ~277bp, ~292bp, ~315bp, ~337bp, and ~367bp bands, while the rest of the Arabica samples showed ~280bp, ~309bp, ~327bp, and ~356bp bands. Meanwhile, the Robusta sample showed ~315bp and ~356bp bands; the Liberica sample showed ~321bp, ~335bp, and ~362bp bands; and the Excelsa sample showed ~327bp and ~372bp bands. Prakash et al. (2011) report that 300-305bp bands are linked to resistance, while bands less than 300bp are linked to susceptibility. Gutiérrez-Calle et al. (2021) report that their Arabica samples showed 295bp, 319bp, and 321bp bands. Their EA67 control sample showed 329bp and 331bp bands, while their S.288 control sample showed 295bp and 333bp bands. In addition, Georget et al. (2019) report that their resistant coffee sample showed a 306bp band. The conflicting data between these studies could be due to marker nonspecificity, as suggested by data from this study. Nonetheless, Mahé et al. (2008) reported that the presence of a unique band is also linked to resistance for this marker, and the numerous bands present in the NSIC-2008-Cf-A-05 (Red Bourbon) sample are different from those of the rest of the Arabica samples.

For the SP-M8-SH3 marker in this study, a ~255bp band was seen for all the samples. Prakash et al. (2011) also tried to screen their samples with SP-M8-SH3, but their Marker-PCR amplification failed. Gutiérrez-Calle et al. (2021) report that their Arabica samples showed 253bp and 259bp bands, while their EA67 and S.288 control samples showed a 257bp band. Unfortunately, this study's methodology seems unable to resolve the 253bp, 257bp, and 259bp bands. Gutiérrez-Calle et al. (2021) utilized capillary electrophoresis, which has better resolving power than the slab-gel electrophoresis in this study.

The main point of reference for the remaining *SH3*-linked markers is the original study of Mahé et al. (2008). Markers where a particular band is linked to resistance, include SP-M5-SH3, SP-M16-SH3, and SP-M18-SH3. For the SP-M5-SH3 and SP-M16-SH3 markers, there are bands unique to the NSIC-2008-Cf-A-05 (Red Bourbon) samples among the Arabica samples. These bands highly probably represent the resistant alleles for their respective markers. Meanwhile, the SP-M18-SH3 marker is revealed to be highly nonspecific, having multiple binding sites across different chromosomes according to the PrimerBlast results. This explains the difficulty in distinguishing bands in the gel profile. On the other hand, markers where a particular band is linked to susceptibility, include BA-42-21B-r, Sat160, and Sat281. For these markers, bands were detected among the Arabica samples, which are not present in the NSIC-2008-Cf-A-05 (Red Bourbon) samples. These bands probably represent the susceptible alleles for their respective markers.

Altogether, data from this study suggests that the unique haplotype in chromosome 3 of NSIC-2008-Cf-A-05 (Red Bourbon) is similar to the haplotype described by Mahé et al. (2008). For some markers, the samples exhibited bands of almost similar sizes to those linked to resistance, as reported in previous literature. Furthermore, some bands have the same or similar counterparts in the *C. liberica* control samples. As was previously mentioned, the *SH3* haplotype in *C. arabica* was supposedly introgressed from *C. liberica*. Therefore, when comparing the resistance profile of the three registered Arabica species from BPI-Baguio, NSIC-2008-Cf-A-05 (Red Bourbon) appears to be the most generally resistant. The identified haplotype probably contributed to this reported resistance.

Other SH genes

For the other *SH* genes, one group represents natural resistance genes in *C. arabica* (*SH1*, *SH2*, *SH4*, and *SH5*), while another group represents genes introgressed from *C. canephora* (*SH6*, *SH7*, *SH8*, and *SH9*) (Alkimim et al. 2017). A possible candidate of these genes has been characterized as a resistance gene analog (RGA) belonging to the CC-NBS-LRR family (Barka et al. 2020). Unfortunately, the remaining markers linked to resistance against CLR were not specifically linked to these *SH* genes. Nonetheless, PrimerBlast results show that their binding sites are in chromosomes other than chromosome 3, where the *SH3* gene is located.

The CaRHvII 1-6 markers were used by Diola et al. (2011) to screen for resistance against CLR conferred by a resistance gene other than *SH3*. They reported that all six were linked to the same hypothetical resistance gene and constructed a linkage map showing the relative locations of each. PrimerBlast results, however, show that the marker binding sites are actually spread across different chromosomes. Among these, the sample from the non-registered Catimor tree showed a unique banding pattern for CaRHvII 3. The Catimor sample showed a ~460bp, while the other Arabica samples did not. Interestingly, the band is also present in the Excelsa control sample. This band is closest to the reported 459bp band, representing the resistant allele. PrimerBlast results place the CaRHvII 3 marker in chromosome 1. Hence, the Catimor tree may have a unique resistance gene in chromosome 1 not present in the other Arabica samples.

Van der Vossen et al. (2015) report an *SH* gene linked to Sat27 and Sat47, with Sat27 being closer to the gene according to their linkage map. According to PrimerBlast results (Table 1), the binding sites for these markers are located in chromosome 11. Based on this study, there were no detected variations among the Arabica samples for Sat47. For Sat27, on the other hand, the banding pattern of NSIC-2008-Cf-A-06 (Caturra) and the Catimor samples were similar. However, the unique bands shared between the two are far from the expected product size suggesting nonspecific targets. As mentioned, Catimor is a cross between the Caturra and the Hibrido de Timor varieties (Silva et al. 2018). Hence, the data suggest that the Catimor tree from BPI-Baguio inherited alleles from its Caturra parent.

Among the remaining markers linked to resistance against CLR, the Catimor sample once again showed a unique banding pattern for Sat225. For this marker, all Arabica samples showed a ~301bp band. In addition, the Catimor samples showed a ~284bp band, while the rest of the Arabica samples showed a ~267bp band. While data from previous literature is lacking, it is important to note

that the ~284bp band of the Catimor sample is closer to the lowest bands for the Robusta, Liberica, and Excelsa control samples, which are ~277bp, ~281bp, and ~287bp, respectively. PrimerBlast results also place the binding site of Sat225 in chromosome 11 (Table 1).

Resistance against Coffee Berry Disease (CBD)

CBD, caused by the fungal *Colletotrichum kahawae*, is among the diseases in the NSIC registered coffee list. However, there is no data yet regarding the reactions of the registered Arabica varieties from BPI-Baguio to said disease. Three genes have been identified as conferring resistance against CBD: the *R*, *K*, and *T* genes named after Rume Sudan, K7, and Hibrido de Timor varieties, respectively (Gichimu et al. 2014). The Sat207 and Sat235 markers were reported to be linked to the *T* gene, which has been renamed as the *Ck-1* gene (Gichimu et al. 2014; Alkimim et al. 2017). The *Ck-1* gene was also reportedly introgressed from *C. canephora* into their Catimor samples via the Hibrido de Timor parent. In this study, the Robusta sample displayed a ~93bp band for Sat207 and a ~220bp band for Sat235, which could represent the resistance-linked 89bp and 227bp bands, respectively, according to previous literature (Georget et al. 2019). None of the Arabica samples, including the non-registered Catimor, showed resistance-linked bands.

Meanwhile, Kiguongo et al. (2014) reported the M24 and Sat227 markers as independently linked to resistance against CBD while studying Rume Sudan, an Arabica variety with the *R* and *K* genes. It was not determined, however, which marker is linked to which gene. In this study, none of the Arabica samples showed any bands which could represent the resistance-linked 210bp band for M24 nor the 200bp band for Sat227. Overall, data suggest that the Arabica samples from BPI-Baguio are susceptible to CBD. However, a more recent study utilizing quantitative trait loci (QTL) mapping in Rume Sudan has linked its CBD resistance to regions in chromosomes 1 and 2 (Gimase et al. 2020b). Based on Primerblast (Table 3), the binding sites for the M24 and Sat227 markers are located in chromosomes 6 and 11, respectively. The presence of multiple sites linked to CBD resistance suggests that there could be more than just the *R* and *K* genes. SNP markers linked to the regions in chromosomes 1 and 2 have already been identified and dubbed as *Ck-2* and *Ck-3* (Gimase et al. 2020a). These SNP markers are beyond the scope of this study, however.

Resistance against Root Knot Nematode (RKN)

RKN caused by *Meloidogyne* spp. is not among the pests in the NSIC list of registered coffee but they are starting to cause problems in plantations (Balagot L 2022, pers. com.; Baltazar MD 2023, pers. com.). A *Mex-1* gene, which confers resistance against RKN, has been identified and reportedly introgressed into *C. arabica* from *C. canephora* via Hibrido de Timor (Barrantes et al. 2020). Van der Vossen et al. (2015) place this *Mex-1* gene alongside the *SH* gene linked to Sat27 and Sat47. That gives additional importance to the genetic variation observed for Sat27, discussed in the previous section, as it may not only be linked to an *SH* gene but also to the *Mex-1* gene.

Lastly, the SSRCafé markers were used by Pereira et al. (2016) to screen for resistance against RKN in the hybrid progeny of Hibrido de Timor and Catuaí Amarelo, a susceptible Arabica variety. The ones included in this study are markers that displayed bands linked to resistance or susceptibility. The Arabica samples did not show the resistance-linked bands reported from the previous study for most of these markers. For SSRCafé 40, the Catimor sample did show a ~247bp band. This is close to the 250bp band Pereira et al. (2016) reported as negatively correlated with gall index. However, data suggests that the Arabica samples from BPI-Baguio are also likely susceptible to RKN.

In conclusion, this study screened 39 resistance-associated SCAR (13) and SSR (26) markers on registered and non-registered Arabica samples from BPI-Baguio. Robusta, Liberica, and Excelsa samples from CavSU were used as controls. Among the Arabica samples, a unique haplotype located in chromosome 3 has been identified in the registered NSIC-2008-Cf-A-05 (Red Bourbon) trees. This region of chromosome 3 has been linked to the *SH3* gene, which confers resistance against the fungal CLR. Analyses of data from this study and previous literature suggest that this haplotype contains the resistant alleles for most *SH3*-linked markers. Currently, the NSIC database reports NSIC-2008-Cf-A-05 (Red Bourbon) and NSIC-2008-Cf-A-07 (Yellow Bourbon) as moderately resistant to CLR as opposed to NSIC-2008-Cf-A-06 (Caturra), which is susceptible. A more careful study should re-assess the performance of seedlings derived from NSIC-2008-Cf-A-05 (Red Bourbon) and NSIC-2008-Cf-A-07 (Yellow Bourbon) against CLR in the field to determine if the former shows greater resistance than the latter. If proven as such, seedlings derived from the Red Bourbon tree can be considered for planting in CLR-infected areas. In addition, this tree could serve as a source tree for resistance genes, from chromosome 3, in future marker-assisted breeding programs (Nasiro and Teferi 2019; Saavedra et al. 2023) within the Philippines.

Among the non-registered Arabica trees from BPI-Baguio, the Catimor tree contained unique genetic variations concerning the resistance-linked markers. Some variations are inferred to represent the resistant allele, while others are inferred to represent the susceptible allele. Lastly, the Arabica trees in BPI-Baguio are inferred to be susceptible to CBD and RKN, given the current set of markers. Future studies on resistance in Philippine coffee can focus on other diseases and/or pests such as leaf scorch, berry borers, and leaf miners (Van der Vossen et al. 2015) or focus on other types of genetic markers such as SNP markers (Gimase et al. 2020a; Nonato et al. 2021; De Faria Silva et al. 2022).

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