eureka Research article

Proteomic Analysis of Higher and Lower Altitude Cultivars of *Coffea arabica* Reveals Differences Related to Environmental Adaptations and Coffee Bean Flavour

Received: 31 July 2023
Accepted: 8 October 2023
Published: 6 November 2023

Caitlin R. Fenrich[1]*‡, Philip Lauman[1]‡, and Prabashi M. Wickramasinghe[1]‡
[1] Department of Biological Sciences, University of Alberta
* Corresponding author: cfenrich@ualberta.ca
‡ These authors contributed equally to this work

ABSTRACT

Background Coffee ranks among the most popular beverages in the world and is considered an important global commodity, contributing substantially to the economies of many developing nations. Coffee beans are mainly harvested from two species of coffee plants, *Coffea arabica* and *Coffea canephora*, of which the former species produces beans which are almost universally considered to be superior due to their rich and balanced flavour. *C. arabica* is also known to be less resistant to disease and more sensitive to climatic variables such as temperature, precipitation, and oxygen availability, each of which vary with altitude.

Methods To better understand the impact of altitude on coffee plants, we performed a comprehensive proteomic comparison of two distinct cultivars of *C. arabica*, the high-altitude cultivar Rwanda Shyira (RS) and the lower-altitude cultivar Brazil Flor de Ipe (BFDI), using liquid chromatography MS/MS analysis.

Results We identified a total of 531 proteins, five of which - translation elongation factor 1-γ (EF1γ; AT1G57720), an NB-ARC domain-containing disease resistance protein (AT4G27190), fibrillarin 1 (AT5G52470), a pyruvate kinase family protein (AT3G52990), and a NAD(P)-binding Rossmann-fold superfamily galactose/glucose isomerase (AT2G33590) - exhibited statistically significant differences ($p < 0.05$) in expressional intensity between the two cultivars. Notably, all of these proteins were expressed at a higher intensity in RS except for the NAD(P)-binding Rossmann-fold superfamily protein, which was higher in the BFDI.

Conclusions We propose that heightened expression of EF1γ in the RS cultivar may decrease the concentration of bitter flavonoids, thereby contributing to the improved flavour which is often noted among high-altitude coffee cultivars. Simultaneously, expressional intensity differences identified for the remaining four proteins may correspond to adaptations to cold, hypoxic, and disease stressors at different altitudes and in distinct geographic niches. Overall, substantial proteomic differences were identified between the two elevations, providing greater understanding of the effects of altitude on the *C. arabica* plant and the coffee produced from it, which has implications for the global market.

KEY WORDS: **Coffee, Proteomics, Mass Spectrometry, *Coffea arabica*, Altitude Adaptation, Disease Resistance, Coffee Bean Flavour**

1 | INTRODUCTION

Coffee is considered to be one of the world's most popular beverages, and its production, transport, and consumption is a major contributor to the global economy. In 2022, global production of coffee beans, harvested primarily from the crops of *Coffea arabica* and *Coffea canephora* (also known as *Coffea Robusta)*, is estimated to have reached about 1.04 x 10^{10} kg, amounting to a staggering average of roughly 1.3 kg per capita per annum (Livramento et al., 2017; United States Department of Agriculture, 2022). In the developing world, coffee is the second most important commodity following petroleum, and although coffee is primarily consumed in developed countries, it is harvested and processed almost exclusively in developing countries under a wide array of environmental conditions (Livramento et al., 2017; Talbot, 2004; United States Department of Agriculture, 2022).

Coffee beans harvested from *C. arabica* are almost universally considered to have a stronger, richer, and more balanced taste as compared to the bitter *C. canephora*, consequently accounting for an estimated 55-70% of coffee production worldwide (Livramento et al., 2017; Olechno et al., 2021; United States Department of Agriculture, 2022). Unfortunately, *C. arabica* plants are simultaneously much more sensitive to disease and climatic conditions (Daviron & Ponte, 2005), meaning that the impact of these conditions on *C. arabica* warrants further investigation for the optimization of coffee production.

It is generally agreed that coffee produced from plants grown at higher altitudes has superior taste. This is thought to be the result of climatic conditions that result from higher elevation, primarily lower temperature, as well as lower precipitation and higher humidity, and possibly lower oxygen availability and sunshine hours, rather than as a direct result of higher elevation itself (Avelino et al., 2005; Decazy et al., 2006; Joët et al., 2010; Vaast et al., 2005). Although each of these factors likely plays at least some role, the prevailing hypothesis is that delayed ripening of the beans due to decreased temperatures results in prolonged accumulation of flavour precursors such as sugars, flavonoids, trigonelline, and chlorogenic acids; as a result the beans have a richer and less bitter flavour (Bertrand et al., 2006; Livramento et al., 2017; Vaast et al., 2005).

Although the direct effects of environmental conditions on flavour are undoubtedly substantial, proteomic differences between cultivars resulting from prolonged adaptation to distinct climatic niches are also likely to play a major role in various metabolic processes that underlie certain aspects of coffee plant and thus bean quality. These include disease resistance, homeostatic adjustments to environmental conditions, and possibly synthesis or catabolysis of flavour precursors. Despite the importance of the coffee industry to the global economy, relatively few proteomic studies comparing coffee cultivars or even *Coffea* species have been conducted (Garrett et al., 2013; Livramento et al., 2017; Marques et al., 2022). Although the chemical differences between cultivars growing at different altitudes have been studied extensively (Avelino et al., 2005; Decazy et al., 2006; Pereira et al., 2020; Worku et al., 2018), we are aware of only one study investigating the effects of altitude on *C. arabica* bean proteomics. In this study, the authors compared beans harvested from relatively similar altitudes at the same Brazilian plantation and found virtually no explainable proteomic differences attributable to elevation or elevation-driven environmental parameters (Livramento et al., 2017).

In this study, we take advantage of the substantial improvements in MS/MS technology, computational power, and proteomic database availability that have occurred in recent years to perform a comprehensive comparison of the proteomes of *C. arabica* beans harvested from two highly distinct cultivars. Specifically, the Rwanda Shyira (RS) cultivar grown at high elevation in the Nyabihu district of Western Rwanda, and the Brazil Flor de Ipe (BFDI) cultivar grown at comparatively low elevation in the Minas Gerais province of Southeastern Brazil. Here we report the first instance of a proteomic difference which could be linked to the superior taste of high-altitude *C. arabica*, as well as several major differences which imply adaptation of the RS cultivar of *C. arabica* to abiotic and biotic stressors resulting from high elevation - including hypoxic, cold, and disease stresses.

2 | METHODS

2.1 Protein Extraction

C. arabica beans obtained from Roasti Coffee Co (Sherwood Park, AB) were frozen with liquid nitrogen and ground into a fine powder using a mortar and pestle. Each biological replicate consisted of approximately 10 beans ground into 100 mg fractions (n = 3). Proteins were extracted using 1:3 (w/v) tissue to extraction buffer containing 50mM Tris-Cl (pH 8.0), 4% SDS in HPLC-grade water. DTT was added to each sample to a final concentration of 10 mM. Samples were vortexed and incubated on a heat block for 10 minutes at 95 ˚C. Samples were centrifuged at maximum speed for 5 minutes at room temperature, and the supernatant was then transferred to a fresh 1.5 mL Eppendorf tube. Samples were centrifuged again for 5 minutes at room temperature, and the supernatant was transferred again to a fresh 1.5 mL Eppendorf tube to ensure no particulates. Samples were diluted 1:5 sample to water to ensure SDS was diluted enough to be compatible with the Bradford assay. A Bradford assay was performed on the samples to determine protein concentrations (R^2 = 0.991; data not shown). This allowed us to normalize the sample volumes to 250 uL with extraction buffer (50mM Tris-Cl (pH 8.0), 4% SDS in HPLC-grade water) so that there was a maximum protein concentration of 100 ug. 10 mM DTT was added to each sample, vortexed, and incubated for 10 minutes at room temperature. Samples were alkylated with 30 mM iodoacetamide and incubated for 30 minutes in the dark at room temperature. Samples were digested overnight with 1:100 sequencing grade trypsin (V5113; Promega) to make proteome peptide pools. The peptide pools were then acidified with formic acid to a concentration of 5% (v/v), and were then

Table 1: Environmental and Bean-Specific Parameters pertaining to the two investigated unroasted bean cultivars of *Coffea arabica*.

	Parameters	Brazil Flor de Ipe (BFDI)	Rwanda Shyira (RS)
Environmental Parameters of Growing Region (Kabirigi et al., 2015; Roasti Coffee Co, 2023; WorldData.info, 2023a, 2023b)	**Region of Origin**	Sul de Minas, Minas Gerais, Brazil	Shyira, Nyabihu District, Rwanda
	Altitude (m above sea level)	1100-1150	1850-2300
	Oxygen Availability (% sea level)	88	77-81
	Daytime Temperature Range (°C)	26-30	15-18
	Annual Precipitation (mm)	1500-1800	1100-1600
	Relative Humidity (%)	63-78	68-86
	Sunshine (h/day)	5.5-7.7	4.2-7.8
Bean-specific Parameters (Roasti Coffee Co, 2023)	**Varietal**	Yellow Bourbon, Caturra	Red Bourbon
	Collection Process	Natural	Washed

dried by vacuum concentrator. Peptides were desalted using ZipTip C18 pipette tips (ZTC18S960; Millipore). The peptides were dissolved in 3% acetonitrile (v/v), 0.1 % TFA (v/v) and eluted with 60% acetonitrile (v/v), 0.1 % TFA (v/v). Samples were dried by vacuum concentrator prior to LC-MS/MS analysis.

2.2 Liquid Chromatography MS/MS

C. arabica peptides were analyzed using a Fusion Lumos Orbitrap mass spectrometer (Thermo Scientific) with data-independent acquisition (DIA). Unlike data-dependent acquisition (DDA), DIA allows for fragmentation and subsequent analysis of all peptides, which means there is no bias for proteins with a certain mass to charge (m/z) intensity. For each replicate, 2 µg of the re-suspended peptide was injected using Easy-nLC 1200 system (LC140; Thermo and an Acclaim PepMap 100 C18 trap column (Cat# 164750; Thermo Scientific) followed by a 15 cm Easy-Spray PepMap C18 analytical column (ES906; Thermo Scientific) warmed to 50˚C. Peptides were then eluted at 1.2 µL/min with a segmented solvent B gradient of 0.1 % (v/v) FA in 80 % (v/v) ACN (A998, Fisher) from 4 to 41 % solvent B (0 – 21 min). The FAIMSpro was used with a fixed gas flow of 3.5 L/min with a CV setting of -30, -50, -70. A positive ion spray voltage of 2.3 kV was used with an ion transfer tube temperature of 300°C and an RF lens setting of 40%. All precursor signals were recorded in the Orbitrap using quadrupole transmission in the mass range of 300–1,500 m/z. Spectra were recorded with a resolution of 120,000 at 200 m/z, a target value of 4E5 and the maximum cycle time was set to 3s. Precursor signals were selected for fragmentation with a charge state from +2 to +7 and a signal intensity of at least 1E4. A dynamic exclusion list was used for 30s, and maximum parallelizing ion injections were activated. Data-dependent MS/MS were recorded in the Orbitrap using quadrupole isolation with a window of 2 Da and higher-energy C-trap dissociation (HCD) fragmentation with 30% fragmentation energy.

2.3 Mass Spectrometry Data Analysis

Raw data was processed using MaxQuant software version 1.6.14.0 (15; http://www.maxquant.org/). At the time of writing there has not been a publicly available *C. arabica* proteome sequenced. We instead searched our MaxQuant output against the *C. canephora* proteome (http://www.coffee-genome.org/). Carbamidomethylation of cysteine residues was inputted as a fixed modification (it will be present), while oxidation of methionine was included as a variable modification (it may or may not be present). The fixed modification accounts for cysteine residue interacting with iodoacetamide, while the variable modification allows us to increase our search space of potential sequences for methionine as it can be readily oxidized. We then performed further analysis with Perseus software v2.0.10.0 (16; https://maxquant.net/perseus/). Reverse hits and contaminants were removed, resulting in 531 hits. We performed a log2(x) transformation to determine fold-change between protein expressions. We further filtered based on proteins that had measurements above the detection threshold in at least 3 of the samples across both test groups, resulting in 71 hits. Doing this ensured that threshold values were present in multiple samples and not individual unique events. Data was then median normalized, and imputed with a normal distribution function.

We then performed a one-way ANOVA followed by unpaired Student's T-tests on these 71 hits. 5 of these had *p*-

values <0.05, which we considered as the threshold for statistical significance.

2.4 Bioinformatics

After obtaining significant hits, we queried the fasta headers against the *C. canephora* proteome (http://www.coffee-genome.org/) to obtain their complete protein sequences. We then BLASTed these protein sequences in Phytozome (Goodstein et al., 2012) against *Arabidopsis thaliana* Araport11 to obtain AGI locus codes. It is important to note that sequence similarity does not always translate to exact function, but the number of resources for the *C. canephora* proteome is limited as it has not been sequenced nearly to the extent of *A. thaliana.* The AGI locus codes allowed us to utilize resources such as TAIR (Berardini et al., 2015) to determine gene ontology, molecular functions of the proteins and the developmental stages in which proteins are expressed. Predicted subcellular localization information was obtained using SUBA5 (Hooper et al., 2022) and Ensembl (Cunningham et al., 2022). All figures were made using GraphPad Prism 9.3.1.

3 | RESULTS

We selected coffee beans harvested from two *C. arabica* cultivars, Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI), which were grown in two regions with considerable differences in altitude and, as a result, several environmental variables including temperature, precipitation, humidity, oxygen availability, and sunshine hours (Table 1). Following MS/MS we identified a total of 531 proteins, five of which exhibited statistically significant differences in expressional intensity between the RS and BFDI cultivars (Figure 1). These proteins have various molecular functions related to flavour precursor turnover and responses to both biotic and abiotic stressors such as cold, hypoxia, and disease (Table 2).

The highest difference in expressional intensity was observed for an NB-ARC domain-containing disease resistance protein (AT4G27190), which had significantly higher expression in RS relative to BFDI (p = 0.0027; Figure 1B). This homolog was identified by performing a BLAST search with NB-ARC domain-containing protein (Cc00t22090.1), identified via coffee genome hub (see Table 2), against the *A.thaliana* proteome, and was selected because it had the highest query coverage (74%) and identical molecular function. Localized to the cytosol and plasma membrane, NB-ARC is composed of three key domains: a nucleotide-binding (NB) domain, and two ARC domains, ARC-1 and ARC-2, which are

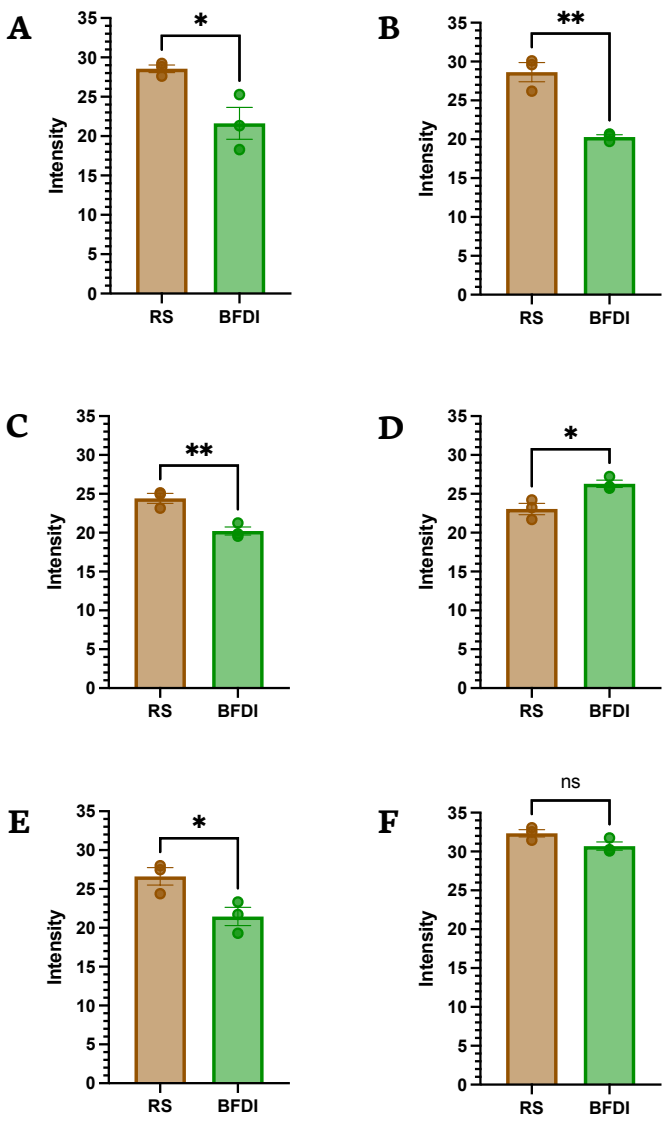

Figure 1: Differences in expressional intensity of proteins with the highest identified expression variability between *Coffea arabica* cultivars Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI). Significant differences in expression between RS (brown) and BFDI (green) were identified for Translation Elongation Factor 1-γ (AT1G57720; **A**), an NB-ARC Domain-Containing Disease Resistance Protein (AT4G27190; **B**), Fibrillarin 1 (AT5G52470; **C**), Pyruvate Kinase Family Protein (AT3G52990; **D**), and an NAD(P)-Binding Rossmann-fold Superfamily Protein (AT2G33590; **E**), while the difference in expression of Galactose-Mutarotase-like Superfamily Protein (AT3G47800; **F**), despite being the next highest of all proteins queried in this study, was not statistically significant. Proteins with statistically significant differences in expression were identified using a one-way ANOVA followed by unpaired Student's T-tests using Perseus v2.0.10.0, with $p < 0.05$ indicating statistical significance. Bars represent the mean values of three biological replicates, shown as points about the mean, while error bars indicate standard error of the mean of these replicates.

well conserved across plant species (Hooper et al., 2022; van der Biezen & Jones, 1998; Van Ooijen et al., 2008). The ARC domains have been found to regulate several important proteins, from which they received their name: apoptotic protease-activating factor-1 (APAF-1), R proteins, and *Caenorhabditis elegans* death-4 protein (CED-4; Takken et al., 2006; Van Ooijen et al., 2008)). NB-ARC is also an ATPase domain (Van Ooijen et al., 2008).

The second highest difference in expressional intensity was observed for the gamma chain of translation elongation factor 1 (EF1-γ; AT1G57720), which had significantly higher expression in RS relative to BFDI (p = 0.0289; Figure 1A). In addition to its canonical roles in translation, EF1-γ is known to be involved in copper ion binding through an as-of-yet unidentified motif (Kung et al., 2006) and, interestingly, contains a glutathione-s-transferase (GST) domain (Koonin et al., 1994). Indeed, EF1-γ functions as a soluble cytosolic GST in several species of plants, and rice EF1-γ was also shown to produce GST activity when expressed ectopically in *Escherichia coli* (Jain et al., 2010; Kobayashi et al., 2001).

A smaller but significant difference in expressional intensity was also observed for fibrillarin 1 (AT5G52990), which had higher expression in RS relative to BFDI (p = 0.007; Figure 1C). Fibrillarin is a highly conserved methyltransferase involved in processing of primary ribosomal transcripts, and is pivotal for ribosome biogenesis (Rodriguez-Corona et al., 2015). Fibrillarin is a core component of the small nucleolar ribonucleoprotein (snoRNP) that methylates rRNA (Tollervey et al., 1993) and is also involved in early steps of ribosomal transcription initiation (Yildirim et al., 2013). It is primarily localized in the fibrillar center and dense fibrillar component of the nucleoli where active rDNA transcription and rRNA processing take place, and is also found in Cajal bodies in the cell nucleus.

Table 2: Proteomic Data Pertaining to Five Proteins with Significant Variation in Expressional Intensity between *Coffea arabica* cultivars Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI)

FASTA header for *C. canephora* [Coffee Genome Hub] MaxQuant (Cox & Mann, 2008) **Perseus** (Tyanova et al., 2016)	**% coverage of peptide sequences**	**NCBI Accession No. (NCBI)**	**Top BLAST hit for *A. thaliana***	**AGI [Phytozome]** (Goodstein et al., 2012)	**Molecular Functions [TAIR]** (Berardini et al., 2015)	**Subcellular Localization [SUBA5]** (Hooper et al., 2022)
Cc00t22090.1 NB-ARC domain-containing protein	2.6	CDP20838	NB-ARC domain-containing disease resistance protein.	AT4G27190	ADP binding, regulation of APAF-1, R proteins and CED-4	Cytosol, plasma membrane
Cc10t09370.1 Elongation factor 1-gamma 2	34.7	XP_027093104	Translation elongation factor EF1, gamma chain	AT1G57720	Copper ion binding glutathione transferase (GST) activity	Cytosol, mitochondrion, vacuole, cell wall, plasma membrane
Cc02t02560.1 Mediator of RNA polymerase II transcription subunit 36a-like	6.1	XP_027103727.1	Fibrillarin 1	AT5G52470	RNA binding, histone H2AQ104 methyltransferase activity, mRNA binding, rRNA methyltransferase activity, snoRNA binding	Nucleus
Cc02t10330.1 Pyruvate kinase 1, cytosolic	29.6	XP_027111400.1	Pyruvate kinase family protein	AT3G52990	mRNA binding, magnesium ion binding, potassium ion binding, pyruvate kinase activity	Plastid, cytosol
Cc00t08940.1 Epimerase domain-containing protein	32.9	CDP19334.1	NAD(P)-binding Rossmann-fold superfamily protein	AT2G33590	oxidoreductase activity (acts on CH-OH group of electron donors, NAD or NADP as acceptor).	Nucleus, cytosol, mitochondrion, plastid, plasma membrane

Another small but significant difference in expressional intensity was observed for pyruvate kinase 1 (AT3G52990), a cytosolic enzyme which was the only protein identified in our dataset to have higher expression in BFDI relative to RS (p = 0.0205; Figure 1D). The cytosolic pyruvate kinase (cPK) is a critical glycolytic enzyme which catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP for the synthesis of ATP during glycolysis (Wulfert et al., 2020). In addition to their roles in cellular energy homeostasis, different isoforms of cPKs are known to be differentially regulated by allosteric effectors, and have different enzyme subgroup associations and expression patterns in *A. thaliana* (Wulfert et al., 2020).

A substantial difference in expression was also identified for an NADP-Binding Rossmann-fold superfamily protein (AT2G33590), which had higher expressional intensity in RS relative to BFDI (p = 0.0334; Figure 1E). Primarily localized to the cytosol, this protein contains domains involved in the catabolic processing of galactose to glucose. Interestingly, another protein in our dataset, which was identified to have only a slight (not statistically significant) difference in expression between RS and BFDI (p = 0.081; Figure 1F), is a galactose mutarotase-like superfamily protein (AT3G47800) which is also involved in the catabolic conversion of galactose to glucose (Berardini et al., 2015; Hooper et al., 2022; Thoden et al., 2001).

4 | DISCUSSION

4.1 Increased expression of elongation factor 1-γ in the high-altitude C. arabica cultivar Rwanda Shyira may suggest a novel explanation for the superior taste quality of high-altitude coffee.

Glutathione-s-transferases (GSTs) such as EF1-γ are responsible for the transfer of glutathione groups to xenobiotic compounds with the intent of decreasing toxicity, promoting further metabolism, or directly facilitating the excretion of the targeted compound (Hayes et al., 2004). Interestingly, GST concentration and activity has been shown to increase in the saliva of individuals fed with diets rich in coffee, which contains flavonoids, and broccoli, which contains isothiocyanates (Sreerama et al., 1995). This implies that GSTs might be responsible for the detoxification of these compounds in human saliva (Sreerama et al., 1995). Several GSTs participate in the detoxification of isothiocyanates in *Drosophila melanogaster*, while in the taste buds of *Rattus norvegicus* GSTs are localized to Type II cells - which are known to be involved in the detection of bitter, umami and sweet molecules, providing further support for a role for GSTs in the detection and detoxification of bitter compounds across several species (Gonzalez et al., 2018; Schwartz et al., 2023). Detoxification of bitter or otherwise unpleasant-tasting compounds in the taste buds is adaptive not only due to the reduction of toxicity, but also because the decreased concentration of the free molecule results in decreased binding to bitter molecule receptors. The lack of a strong bitter sensation indicates to the organism that the consumed amount of the bitter food is tolerable. In contrast, a strong bitter taste may indicate that binding to receptors remains high because detoxification enzymes have been saturated, suggesting that the consumed dose is unacceptable and could be toxic (Dagan-Wiener et al., 2019; Gonzalez et al., 2018; Schwartz et al., 2023). This phenomenon has been described extensively for Proline Rich Proteins (PRPs), which are present in saliva and are able to bind and sequester polyphenols - simultaneously detoxifying them and reducing their free concentration, which has the effect of reducing their binding to bitter taste receptors (Matsuo, 2000).

Recently, Schwartz et al. found that GSTs are able to bind isothiocyanates and flavonoids, as well as caffeine. The authors further demonstrated that GSTs are able to transfer glutathione to isothiocyanates in *ex vivo* human saliva samples, but whether this same mechanism extends to flavonoids remains to be elucidated (Schwartz et al., 2022). While the effect of glutathione transfer to these substrates on perception of bitterness remains unknown, it is likely that this mechanism of detoxification simultaneously prevents the binding of the bitter molecules to their receptors on gustatory cells, as described for PRPs, meaning that increased GST activity in the saliva can reasonably be hypothesized to decrease the sensation of bitterness. Concordantly, the heightened expression of the GST domain-containing EF1-γ in the RS cultivar of *C. arabica* could potentially be associated with modification of endogenously occurring flavonoids and other bitter molecules, possibly reducing the bitterness of coffee brewed with beans of that cultivar.

Coffee quality is typically associated with stronger non-bitter flavours, and a corresponding reduction of the overall bitter taste; *C. canephora* beans, for instance, are more bitter and therefore considered inferior (Olechno et al., 2021). It is generally agreed that coffee beans harvested from plants grown at higher altitudes are superior in quality, and this is thought to be mainly due to the fact that lower temperatures delay the ripening process and thus allow flavour precursors to accumulate for a longer period of time, thereby enhancing the flavours of the beans (Avelino et al., 2005; Livramento et al.,

2017; Vaast et al., 2005). Although a number of studies have investigated the effects of altitude on metabolite composition in coffee, little is known about the effects of this variable on protein composition, and the few studies performed on this subject have failed to identify proteins which are known or even speculated to be associated with coffee flavour (Livramento et al., 2017).

Here we present the tantalizing conjecture that heightened expression of EF1-γ in the RS cultivar of *C. arabica* may decrease the concentration of bitter molecules such as flavonoids and caffeine through the function of its GST domain, thereby contributing to the improvement of taste quality of this high-altitude cultivar by decreasing its overall bitterness. Since GSTs are not known to interact with other, non-bitter flavour precursors, this proposed mechanism might improve the overall taste of the resulting coffee. Although caffeine, flavonoids, and other bitter molecules found in coffee are not known to be cytotoxic in plants, flavonoids are thought to have antifungal properties (Galeotti et al., 2008) , while caffeine is known to protect plants against predation by insects and other herbivores, as well as infection by bacteria and fungi (Baumann & Gabriel, 1984; Frischknecht et al., 1986; Mohammed & Al-Bayati, 2009; Nathanson, 1984) . Given that stresses from herbivory and bacterial and fungal colonization are thought to be reduced at lower temperatures and thus higher altitudes (Desaint et al., 2021; Wu et al., 2021) , bitter antimicrobial and anti-herbivory compounds may not be necessary in high concentrations at such altitudes and could be repurposed for alternative functions, thereby potentially explaining the adaptive value of the postulated selective breakdown of caffeine and flavonoids by high-altitude *C. arabica*. Although this preliminary postulation is interesting, it remains uncertain whether heighted EF1-γ expression is a property of all high-altitude cultivars of *C. arabica* or only the RS cultivar being investigated in this study. Clearly, further research is required to validate and refine this conjecture.

4.2 Increased expression of NB-ARC domain-containing disease resistance protein in the C. arabica cultivar Rwanda Shyira may reflect increased effector-triggered immunity as an adaptive response to lower temperatures at higher altitudes.

In *A. thaliana*, the NB-ARC domain-containing protein functions as a regulator of immunity and disease resistance through the regulation of resistance (R) proteins (Berardini et al., 2015; Cunningham et al., 2022; Van Ooijen et al., 2008; Zhu et al., 2010). Of the two ARC domains found in NB-ARC, the ARC-2 domain contains a highly conserved methionine-histidine-aspartate sequence (Van Ooijen et al., 2008; L. Wu et al., 2014). This has been identified as being a key regulator of R proteins in *Nicotiana benthamiana*, and these R proteins activate effector-triggered immune (ETI) responses in response to the expression of avirulent (Avr) genes by invading pathogens (Van Ooijen et al., 2008; L. Wu et al., 2014).

The role of elevation in the development and expression of plant defenses remains controversial, with certain studies finding positive correlations between elevation and plant defense and others reporting the opposite. Wu et al. speculate that these inconsistent findings are likely due to other elevation-dependent abiotic factors such as temperature, rather than elevation itself (R. Wu et al., 2021). Interestingly, Cheng et al. identified that *A. thaliana* increases ETI signalling at temperatures ranging from 10˚C to 23˚C, but shifts to increasing pattern-triggered immunity (PTI) at temperatures ranging from 23˚C to 32˚C. This phenomenon is likely due to the co-evolution of plants with bacteria, since it has been found that pathogenic bacteria secrete Avr-factors at lower temperatures - forcing plants to increase R proteins and therefore ETI signalling and in order to respond to these Avrs (Cheng et al., 2013; Van Dijk et al., 1999). These findings explain the greatly increased expression of the NB-ARC domain-containing disease resistance protein in beans of the RS cultivar of *C. arabica*, since the Shiyra region of Rwanda from which these beans were harvested has a lower temperature range (15-18˚C; Table 1), at which R protein expression and ETI signalling, which are regulated by the NB-ARC proteins, are prevalent.

Interestingly, three putative R proteins (all of them RGA3) were found in our initial 531 protein hits (prior to filtering, see Methods), but most of these had expression intensities below the detection threshold. One RGA3 protein, Cc00t11180.1, however, was expressed at an intensity above the limit of detection in one replicate of the RS cultivar, suggesting that further MS analysis with a longer gradient might allow for increased detection of at least some of these proteins and permit further analysis.

4.3 Increased expression of fibrillarin in the high-altitude C. arabica cultivar Rwanda Shyira provides insights into the role of this protein in C. arabica immunity and cold stress responses.

In plants, evidence exists for fibrillarin involvement in several epigenetic nucleolar mechanisms. Specifically, fibrillarin is capable of methylating histone H2A while bound to rDNA (Loza-Muller et al., 2015), and in RNA polymerase I-mediated transcription, the protein complex FACT (facilitates chromatin transcription) interacts with methylated histone

H2A to reorganize nucleosomes in the active promoters for rRNA (Tessarz et al., 2014). Moreover, plant fibrillarin is also capable of interacting with histone H2A to carry out rDNA promoter methylation (Loza-Muller et al., 2015). Fibrillarin is also shown to have ribonuclease activity (Rodriguez-Corona et al., 2017) and is part of a protein complex that possesses endonuclease activity required for proper processing of rRNA (Dragon et al., 2002). Intriguingly, fibrillarin has been found to play an indispensable role in the infection process of several plant umbraviruses, which co-opt and utilize the protein as an essential component of translocatable viral ribonucleoproteins (RNPs). In particular, the ORF3 protein of the groundnut rosette virus has been shown to directly interact with fibrillarin to facilitate nuclear localization and assembly of movement-competent infectious RNP particles, which are required for systemic infection of the plant (Sang et al., 2007). Furthermore, fibrillarin knockdown suppresses the long-distance movement of groundnut rosette virus particles and prevents systemic infection of the plant, suggesting that lowered expression of fibrillarin could be a potential host defense mechanism against viruses with nucleolar replication phases, such as the groundnut rosette virus.

Fascinatingly, the prevalence and distribution of infection with groundnut rosette virus is substantially lower in South America relative to the African continent (Naidu et al., 1998; Okello et al., 2014; Thottappilly, 1992) which could be at least partially explained by the decreased expressional intensity of fibrillarin in the BFDI cultivar. Whether decreased expression of fibrillarin is common among South American *C. arabica* cultivars or is unique to the BFDI cultivar is unclear, however, and further proteomic investigations into South American cultivars will therefore be necessary to validate this conjecture.

Plant fibrillarin has also been shown to act as part (subunit 36a) of a multi protein complex - mediator of RNA polymerase II transcription - which provides an interface for communication between transcription regulation proteins and core promoters (Bäckström et al., 2007). Interestingly, the mediator16 subunit of the transcriptional coactivator complex has additionally been shown to regulate cold-responsive gene expression in *A. thaliana* (Hemsley et al., 2014). Due to its high altitude, the Shyira region of the Nyabihu district of Rwanda has a daytime temperature range of 15-18˚C, considerably lower than the Sul de Minas region of Brazil (Table 1). This may explain the heightened expression of fibrillarin in the RS cultivar relative to BFDI (Figure 1C), since the RS cultivar of *C. arabica* may require higher fibrillarin expression to appropriately modulate Cold On-Regulated (COR) genes in response to lower temperatures (Hemsley et al., 2014).

Taken together, these data might place fibrillarin at the center of a fascinating evolutionary trade-off in which the RS cultivar of *C. arabica* increases the expression of fibrillarin in response to temperature stresses, but is consequently more susceptible to systemic infection by umbraviruses such as the groundnut rosette virus.

4.4 Decreased expression of pyruvate kinase 1 in the high-altitude C. arabica cultivar Rwanda Shyira raises questions about the levels and mechanisms of cold adaptation in coffee plants.

Low temperatures can strongly affect plant metabolism and respiration rate, and photosynthesis is known to be strongly suppressed in coffee leaves at temperatures below 13-18°C, which greatly limits the supply of ATP for cellular metabolism (Partell et al., 2010; Ramalho et al., 2003). Plants growing at such temperatures might therefore be expected to increase expression of respiratory enzymes such as pyruvate kinase. As discussed previously, daytime temperatures in the Nyabihu district of Rwanda are considerably lower than in the Sul de Minas region of Brazil (Table 1), and our findings regarding the relative expressional intensities of cPK (Figure 1D) are therefore challenging to understand. Interestingly, Partell et al. found that a relatively cold-resistant genotype of *C. arabica*, Catucai IPR 102, exhibits increased activity by respiratory enzymes such as malate dehydrogenase and pyruvate kinase, in addition to possessing higher concentrations of soluble sugars involved in osmoregulation and membrane stabilization (Partell et al., 2010). Although Partell et al. did not investigate cPK expression levels, it is conceivable that the cPK enzymes of cold-resistant plants are optimized for higher enzymatic performance at lower temperatures, allowing the organism to catalyze reactions with a lower overall amount of the enzyme. This could indicate that the RS cultivar of *C. arabica* is cold adapted, which is reasonable given its environment, and therefore exhibits a lower expression of cPK since its cPK is optimized for higher enzymatic activity at lower temperatures. This is only speculative, however, since the enzymatic activity of RS cPK is not known, and further investigations comparing the cPK enzyme kinetics of cold-adapted and non-adapted strains in cold temperatures will therefore be necessary to shed more light on this unexpected finding.

4.5 Increased expression of a galactose/glucose isomerase in the high-altitude C. arabica cultivar Rwanda Shyira could be an adaptive response to hypoxic stress.

The identified NADP-Binding Rossmann-fold superfamily protein is known to respond to hypoxic and water-deprivation conditions (Berardini et al., 2015; Hooper et al., 2022), possibly explaining the increased expression of this protein in the RS cultivar since the Shyira region of Rwanda is at a substantially increased elevation and consequently has decreased oxygen availability (Table 1). This protein primarily acts as an oxidoreductase which oxidizes CH-OH groups using NAD and NADP as electron-accepting cofactors, but also contains an NAD-dependent epimerase/dehydratase domain which is known to be involved in the conversion of UDP-galactose to UDP-glucose during the process of galactose metabolism (Allard et al., 2001; Berardini et al., 2015; Thoden et al., 2001). Both domains play roles in different forms of substrate isomerization, suggesting that AT2G33590 may be a galactose/glucose isomerase. Curiously, a galactose mutarotase-like superfamily protein (AT3G47800), which is also a cytosolic epimerase involved in the catabolic processing of galactose to glucose via UDP-galactose/glucose conversion (Berardini et al., 2015; Thoden et al., 2001), exhibited relatively similar levels of expressional intensity between the RS and BFDI cultivars (Figure 1F).

Given that the galactose mutarotase-like protein is not known to be associated with hypoxic stress and appears to have similar expression levels in RS and BFDI, while the hypoxia-associated NADP-binding Rossmann-fold protein has increased expression in high altitude-grown RS, it is conceivable that the latter protein is overexpressed under hypoxic conditions in order to generate a larger pool of glucose molecules which can be metabolized through various pathways to offset the effects of hypoxic stress. While intriguing, this preliminary conjecture ought to be validated through measurement of enzyme kinetics and glucose production under these varying conditions of altitude and oxygen availability.

5 | CONCLUSIONS

Overall, these findings serve as a starting point for further studies on the effects of endogenous glutathione-s-transferases (GSTs) on coffee bean flavour and the function of *Coffea arabica* immunoregulatory proteins, as well as research into the modifications of enzyme kinetics in response to low temperatures and hypoxia. In subsequent proteomic work, longer MS gradients will permit the detection of a broader range of protein expressional intensities and thus allow for qualitative identification of other proteins which might be associated with our existing findings (such as the RGA3 or galactose mutarotase-like superfamily proteins). MS/MS analysis of other *C. arabica* cultivars would provide further insight into our results and allow us to determine if the presence of these proteins is exclusive to RS and BFDI or generalizable to all *C. arabica* cultivars from different elevations. These results would allow us to better understand whether the proteomic effects of altitude explain the superior yield, taste, popularity, and market performance of certain cultivars of *C. arabica*. Ultimately, our findings contribute to understanding the role that these various proteins play in the taste quality of *C. arabica* beans, and the responses this organism has to abiotic and biotic stressors caused by high elevation - providing further insights which could improve the production and quality of one of the most popular commodities worldwide.

ACKNOWLEDGEMENTS

We would like to thank Dr. R. Glen Uhrig for performing MS/MS analysis and Maria C. R. Gallo for performing the overnight digestion. We would also like to thank Roasti Coffee Co: Roastery and Coffee Bar in Sherwood Park, Alberta for supplying the raw coffee beans and Emma Trevor for procuring them.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Allard, S. T. M., Giraud, M. F., & Naismith, J. H. (2001). Epimerases: Structure, function and mechanism. *Cellular and Molecular Life Sciences*, *58*(11), 1650–1665. https://doi.org/10.1007/PL00000803

Avelino, J., Barboza, B., Araya, J. C., Fonseca, C., Davrieux, F., Guyot, B., & Cilas, C. (2005). Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa María de Dota. *Journal of the Science of Food and Agriculture*, *85*(11), 1869–1876. https://doi.org/10.1002/jsfa.2188

Bäckström, S., Elfving, N., Nilsson, R., Wingsle, G., & Björklund, S. (2007). Purification of a Plant Mediator from Arabidopsis thaliana Identifies PFT1 as the Med25 Subunit. *Molecular Cell*, *26*(5), 717–729. https://doi.org/10.1016/j.molcel.2007.05.007

Baumann, T. W., & Gabriel, H. (1984). Metabolism and excretion of caffeine during germination of coffea arabica l. Plant and Cell Physiology, 25(8), 1431–1436. https://doi.org/10.1093/oxfordjournals.pcp.a076854

Berardini, T. Z., Reiser, L., Li, D., Mezheritsky, Y., Muller, R., Strait, E., & Huala, E. (2015). The Arabidopsis information resource: making and mining the "gold standard" annotated reference plant genome. *Genesis*, *53*(8), 474–485.

Bertrand, B., Vaast, P., Alpizar, E., Etienne, H., Davrieux, F., & Charmetant, P. (2006). Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. *Tree Physiology*, *26*(9), 1239–1248. https://doi.org/10.1093/treephys/26.9.1239

Cheng, C., Gao, X., Feng, B., Sheen, J., Shan, L., & He, P. (2013). Differential temperature operation of plant immune responses. *Nature Communications*, *4*(2530). https://doi.org/10.1038/ncomms3530

Cox, J., & Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature Biotechnology*, *26*(12), 1367–1372. https://doi.org/10.1038/nbt.1511

Cunningham, F., Allen, J. E., Allen, J., Alvarez-Jarreta, J., Amode, M. R., Armean, I. M., & Flicek, P. (2022). Ensembl 2022. *Nucleic Acids Research*, *50*(D1), 988–995.

Dagan-Wiener, A., Di Pizio, A., Nissim, I., Bahia, M. S., Dubovski, N., Margulis, E., & Niv, M. Y. (2019). Bitterdb: Taste ligands and receptors database in 2019. *Nucleic Acids Research*, *47*(D1), D1179–D1185. https://doi.org/10.1093/nar/gky974

Daviron, B., & Ponte, S. (2005). *The Coffee Paradox: Global Markets, Commodity Trade and the Elusive Promise of Development*. Zed Books.

Decazy, F., Avelino, J., Guyot, B., Perriot, J., Pineda, C., & Cilas, C. (2006). Quality of Different Honduran Coffees in Relation to Several Environments. *Journal of Food Science*, *68*(7), 2356–2361.

Desaint, H., Aoun, N., Deslandes, L., Vailleau, F., Roux, F., & Berthomé, R. (2021). Fight hard or die trying: when plants face pathogens under heat stress. New Phytologist, 229(2), 712–734. https://doi.org/10.1111/nph.16965

Dragon, F., Compagnone-Post, P. A., Mitchell, B. M., Porwancher, K. A., Wehner, K. A., Wormsley, S., Settlage, R. E., Shabanowitz, J., Osheim, Y., Beyer, A. L., Hunt, D. F., & Baserga, S. J. (2002). A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. *Nature*, *417*(6892), 967–970. https://doi.org/10.1038/nature00769

Frischknecht, P. M., Ulmer-Dufek, J., & Baumann, T. W. (1986). Purine alkaloid formation in buds and developing leaflets of Coffea arabica: Expression of an optimal defence strategy? Phytochemistry, 25(3), 613–616. https://doi.org/10.1016/0031-9422(86)88009-8

Galeotti, F., Barile, E., Curir, P., Dolci, M., & Lanzotti, V. (2008). Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity. Phytochemistry Letters, 1(1), 44–48. https://doi.org/10.1016/j.phytol.2007.10.001

Garrett, R., Schmidt, E. M., Pereira, L. F. P., Kitzberger, C. S. G., Scholz, M. B. S., Eberlin, M. N., & Rezende, C. M. (2013). Discrimination of arabica coffee cultivars by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry and chemometrics. *Lwt*, *50*(2), 496–502. https://doi.org/10.1016/j.lwt.2012.08.016

Gonzalez, D., Fraichard, S., Grassein, P., Delarue, P., Senet, P., Nicolaï, A., Chavanne, E., Mucher, E., Artur, Y., Ferveur, J. F., Heydel, J. M., Briand, L., & Neiers, F. (2018). Characterization of a Drosophila glutathione transferase involved in isothiocyanate detoxification. *Insect Biochemistry and Molecular Biology*, *95*(March), 33–43. https://doi.org/10.1016/j.ibmb.2018.03.004

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S. (2012). Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Research*, *40*(D1), 1178–1186. https://doi.org/10.1093/nar/gkr944

Hayes, J. D., Flanagan, J. U., & Jowsey, I. R. (2004). Glutathione Transferases. *Annual Review of Pharmacology and Toxicology*, *45*, 51–88.

Hemsley, P. A., Hurst, C. H., Kaliyadasa, E., Lamb, R., Knight, M. R., De Cothi, E. A., Steele, J. F., & Knight, H. (2014). The Arabidopsis mediator complex subunits MED16, MED14, and MED2 regulate mediator and RNA polymerase II recruitment to CBF-responsive cold-regulated genes. *Plant Cell*, *26*(1), 465–484. https://doi.org/10.1105/tpc.113.117796

Hooper, C., Castleden, I., Tanz, S., Grasso, S., Aryamanesh, N., & Millar, A. (2022). *Subcellular Localisation database for Arabidopsis proteins version 5*. https://doi.org/10.26182/8dht-4017

Jain, M., Ghanashyam, C., & Bhattacharjee, A. (2010). Comprehensive expression analysis suggests overlapping and specific roles of rice glutathione S-transferase genes during development and stress responses. *BMC Genomics*, *11*(1), 1–17. https://doi.org/10.1186/1471-2164-11-73

Joët, T., Laffargue, A., Descroix, F., Doulbeau, S., Bertrand, B., kochko, A. de, & Dussert, S. (2010). Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chemistry*, *118*(3), 693–701. https://doi.org/10.1016/j.foodchem.2009.05.048

Kabirigi, M., Musana, B., Ngetich, F., Mugwe, J., Mukuralinda, A., & Nabahungu, N. L. (2015). Applicability of conservation agriculture for climate change adaptation in Rwanda's situation. *Journal of Soil Science and Environmental Management*, *6*(9), 241–248. https://doi.org/10.5897/JSSEM15.0508

Kobayashi, S., Kidou, S., & Ejiri, S. (2001). Detection and Characterization of Glutathione S-Transferase Activity in Rice EF-1BB'γ and EF-1γ Expressed in Escherichia coli. *Biochemical and Biophysical Research Communications*, *288*(3), 509–514.

Koonin, E. V., Tatusov, R. L., Altschul, S. F., Bryant, S. H., Mushegian, A. R., Bork, P., & Valencia, A. (1994). Eukaryotic translation elongation factor 1γ contains a glutathione transferase domain—Study of a diverse, ancient protein super family using motif search and structural modeling. *Protein Science*, *3*(11), 2045–2055. https://doi.org/10.1002/pro.5560031117

Kung, C. C. S., Huang, W. N., Huang, Y. C., & Yeh, K. C. (2006). Proteomic survey of copper-binding proteins in Arabidopsis roots by immobilized metal affinity chromatography and mass spectrometry. *Proteomics*, *6*(9), 2746–2758. https://doi.org/10.1002/pmic.200500108

Livramento, K., Borém, F., Torres, L., Silva, F., Livramento, D., & Paiva, L. (2017). Proteomic Analysis of Natural and Demucilaged Coffee Beans from Plantations at Different Altitudes in the Mantiqueira Mountains. *Journal of Experimental Agriculture International*, *19*(4), 1–15. https://doi.org/10.9734/jeai/2017/38343

Loza-Muller, L., Rodríguez-Corona, U., Sobol, M., Rodríguez-Zapata, L. C., Hozak, P., & Castano, E. (2015). Fibrillarin methylates H2A in RNA polymerase I trans-active promoters in Brassica oleracea. *Frontiers in Plant Science*, *6*(NOVEMBER), 1–11. https://doi.org/10.3389/fpls.2015.00976

Marques, I., Gouveia, D., Gaillard, J. C., Martins, S., Semedo, M. C., Lidon, F. C., Damatta, F. M., Ribeiro-Barros, A. I., Armengaud, J., & Ramalho, J. C. (2022). Next-Generation Proteomics Reveals a Greater Antioxidative Response to Drought in Coffea arabica Than in Coffea canephora. *Agronomy*, *12*(1). https://doi.org/10.3390/agronomy12010148

Matsuo, R. (2000). Role of Saliva in the Maintenance of Taste Sensitivity. *Critical Reviews in Oral Biology & Medicine*, *11*(2), 216–229.

Mohammed, M. J., & Al-Bayati, F. A. (2009). Isolation, identification and purification of caffeine from Coffea arabica L. and Camellia sinensis L.: A combination antibacterial study. *International Journal of Green Pharmacy*, 3(1), 52–57. https://doi.org/10.4103/0973-8258.49375

Naidu, R. A., Bottenberg, H., Subrahmanyam, P., Kimmins, F. M., Robinson, D. J., & Thresh, J. M. (1998). Epidemiology of groundnut rosette virus disease: Current status and future research needs. *Annals of Applied Biology*, *132*(3), 525–548. https://doi.org/10.1111/j.1744-7348.1998.tb05227.x

Nathanson, J. . (1984). Caffeine and Related Methylxanthines: Possible Naturally Occuring Pesticides. *Science*, 226(4), 184–187.

Okello, D., Akello, L., Tukamuhabwa, P., Odong, T., Ochwo-Ssemakula, M., Adriko, J., & Deom, C. (2014). Groundnut rosette disease symptoms types distribution and management of the disease in Uganda. *African Journal of Plant Science*, *8*(3), 153–163. https://doi.org/10.5897/ajps2014.1164

Olechno, E., Puścion-Jakubik, A., Zujko, M. E., & Socha, K. (2021). Influence of various factors on caffeine content in coffee brews. *Foods*, *10*(6), 1–29. https://doi.org/10.3390/foods10061208

Partell, F. L., Vieira, H. D., Rodrigues, A. P. D., Pais, I., Campostrini, E., Chaves, M. M. C. C., & Ramalho, J. C. (2010). Cold induced changes on sugar contents and respiratory enzyme activities in coffee genotypes. *Ciencia Rural*, *40*(4), 781–786. https://doi.org/10.1590/s0103-84782010005000041

Pereira, P. V, da Silveira, D. L., Schwan, R. F., de Assis Silva, S., Coelho, J. M., & Bernardes, P. C. (2020). Effect of altitude and terrain aspect on the chemical composition of

Coffea canephora cherries and sensory characteristics of the beverage. *Journal of Science of Food and Agriculture*, *101*(6), 2570–2575.

Ramalho, J. C., Quartin, V. L., Leitão, E., Campos, P. S., Carelli, M. L. C., Fahl, J. I., & Nunes, M. A. (2003). Cold Acclimation Ability and Photosynthesis among Species of the Tropical Coffea Genus. *Plant Biology*, *5*(6), 631–641. https://doi.org/10.1055/s-2003-44688

Roasti Coffee Co. (2023). *Roasti Coffee*.

Rodriguez-Corona, U., Pereira-Santana, A., Sobol, M., Rodriguez-Zapata, L. C., Hozak, P., & Castano, E. (2017). Novel ribonuclease activity differs between fibrillarins from arabidopsis thaliana. *Frontiers in Plant Science*, *8*(October), 1–10. https://doi.org/10.3389/fpls.2017.01878

Rodriguez-Corona, U., Sobol, M., Rodriguez-Zapata, L. C., Hozak, P., & Castano, E. (2015). Fibrillarin from Archaea to human. *Biology of the Cell*, *107*(6), 159–174. https://doi.org/10.1111/boc.201400077

Sang, H. K., MacFarlane, S., Kalinina, N. O., Rakitina, D. V., Ryabov, E. V., Gillespie, T., Haupt, S., Brown, J. W. S., & Taliansky, M. (2007). Interaction of a plant virus-encoded protein with the major nucleolar protein fibrillarin is required for systemic virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(26), 11115–11120. https://doi.org/10.1073/pnas.0704632104

Schwartz, M., Boichot, V., Fraichard, S., Muradova, M., Senet, P., Nicolai, A., Lirussi, F., Bas, M., Canon, F., Heydel, J. M., & Neiers, F. (2023). Role of Insect and Mammal Glutathione Transferases in Chemoperception. *Biomolecules*, *13*(2), 1–18. https://doi.org/10.3390/biom13020322

Schwartz, M., Brignot, H., Feron, G., Hummel, T., Zhu, Y., von Koskull, D., Heydel, J. M., Lirussi, F., Canon, F., & Neiers, F. (2022). Role of human salivary enzymes in bitter taste perception. *Food Chemistry*, *386*. https://doi.org/10.1016/j.foodchem.2022.132798

Sreerama, L., Hedge, M. W., & Sladek, N. E. (1995). *Identification Saliva Subjects and Who of a Class Increased and 3 Aldehyde of This Ingest Dehydrogenase Enzyme , in the Large Saliva of in Human Levels Glutathione Continually Quantities of Coffee or Broccoli ' . 1.*

Takken, F. L. W., Albrecht, M., & Tameling, W. I. L. (2006). Resistance proteins: molecular switches of plant defence. *Current Opinion in Plant Biology*, *9*(4), 383–390.

Talbot, J. M. (2004). *Grounds for Agreement: The Political Economy of the Coffee Commodity Chain*. Rowman & Littlefield.

Tessarz, P., Santos-Rosa, H., Robson, S. C., Sylvestersen, K. B., Nelson, C. J., Nielsen, M. L., & Kouzarides, T. (2014). Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature*, *505*(7484), 564–568. https://doi.org/10.1038/nature12819

Thoden, J. B., Wohlers, T. M., Fridovich-Keil, J. L., & Holden, H. M. (2001). Human UDP-galactose 4-epimerase. Accommodation of UDP-N-acetylglucosamine within the active site. *Journal of Biological Chemistry*, *276*(18), 15131–15136. https://doi.org/10.1074/jbc.M100220200

Thottappilly, G. (1992). Plant Virus Diseases of Importance to African Agriculture. *Journal of Phytopathology*, *134*(4), 265–288. https://doi.org/10.1111/j.1439-0434.1992.tb01236.x

Tollervey, D., Lehtonen, H., Jansen, F., Kern, H., & Hurt, E. C. (1993). Temperature-Sensitive Mutations Demonstrate Roles for Yeast Fibrillarin in Pre-rRNA Processing, Pre-rRNA Methylation, and Ribosome Assembly. *Cell*, *72*, 443–457.

Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M., & Cox, J. (2016). The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods*, *13*(9), 731–740. https://doi.org/10.1038/nmeth.3901

United States Department of Agriculture. (2022). *Coffee : World Markets and Trade Ending Stocks to Rise*. *December*, 1–9. https://apps.fas.usda.gov/psdonline/circulars/coffee.pdf

Vaast, P., Bertrand, B., Perriot, J.-J., Guyot, B., & Genard, M. (2005). Fruit thinning and shade improve bean characteristics and beverage quality of coffee (Coffea arabica L.) under optimal conditions. *Journal of the Science of Food and Agriculture*, *86*(2), 197–204.

van der Biezen, E. A., & Jones, J. D. . (1998). The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. *Current Biology*, *8*(7), 226–228.

Van Dijk, K., Fouts, D. E., Rehm, A. H., Hill, A. R., Collmer, A., & Alfano, J. R. (1999). The Avr (effector) proteins HrmA (HopPsyA) and AvrPto are secreted in culture from Pseudomonas syringae pathovars via the Hrp (type III) protein secretion system in a temperature- and pH-

sensitive manner. *Journal of Bacteriology*, *181*(16), 4790–4797. https://doi.org/10.1128/jb.181.16.4790-4797.1999

Van Ooijen, G., Mayr, G., Kasiem, M. M. A., Albrecht, M., Cornelissen, B. J. C., & Takken, F. L. W. (2008). Structure-function analysis of the NB-ARC domain of plant disease resistance proteins. *Journal of Experimental Botany*, *59*(6), 1383–1397. https://doi.org/10.1093/jxb/ern045

Worku, M., de Meulenaer, B., Duchateau, L., & Boeckx, P. (2018). Effect of altitude on biochemical composition and quality of green arabica coffee beans can be affected by shade and postharvest processing method. *Food Research International*, *105*(March 2017), 278–285. https://doi.org/10.1016/j.foodres.2017.11.016

WorldData.info. (2023a). *Climate in Minas Gerais*.

WorldData.info. (2023b). *Climate in Western Province Rwanda*.

Wu, L., Chen, H., Curtis, C., & Fu, Z. Q. (2014). Go in for the kill: How plants deploy effector-triggered immunity to combat pathogens. *Virulence*, *5*(7), 710–721. https://doi.org/10.4161/viru.29755

Wu, R., Lev-Yadun, S., Sun, L., Sun, H., & Song, B. (2021). Higher Elevations Tend to Have Higher Proportion of Plant Species With Glandular Trichomes. *Frontiers in Plant Science*, *12*(April), 1–10. https://doi.org/10.3389/fpls.2021.632464

Wulfert, S., Schilasky, S., & Krueger, S. (2020). Transcriptional and biochemical characterization of cytosolic pyruvate kinases in Arabidopsis thaliana. *Plants*, *9*(3). https://doi.org/10.3390/plants9030353

Yildirim, S., Castano, E., Sobol, M., Philimonenko, V. V., Dzijak, R., Venit, T., & Hozák, P. (2013). Involvement of phosphatidylinositol 4,5-bisphosphate in RNA polymerase I transcription. *Journal of Cell Science*, *126*(12), 2730–2739. https://doi.org/10.1242/jcs.123661

Zhu, Y., Qian, W., & Hua, J. (2010). Temperature modulates plant defense responses through NB-LRR proteins. *PLoS Pathogens*, *6*(4), 1–12. https://doi.org/10.1371/journal.ppat.1000844

How to cite this article:

Fenrich, C.R., Lauman, P., & Wickramasinghe, P.M. (2023). Proteomic Analysis of Higher and Lower Altitude Cultivars of *Coffea arabica* Reveals Differences Related to Environmental Adaptations and Coffee Bean Flavour. *Eureka*. 8 (2). https://doi.org/10.29173/eureka28796