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Characterization of Storage Protein Profile of Robusta Coffee (*Coffea canephora*) Seedlings by Sodium Dodecyl Sulfate–Polyacrylamide gel Electrophoresis (SDS – PAGE)

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ABSTRACT

Coffee storage proteins are connected with beverage quality and proteins contribute to the formation of the aromas and flavours of coffee beverages. Previously it has been reported that coffee seeds contain a legumin-like protein as the main reserve protein, constituted of two subunits i.e., α and β of approximately 35 and 20 kDa. The present research is conducted to characterize the principal storage protein profile of *Coffea canephora* (var robusta coffee - Sl 274) endosperm cultivated under organic and integrated nutrition modes at Western Ghats of India by two-dimensional SDS-PAGE technique for the first-time. The most abundant polypeptide spots observed on mature coffee grain 2DE profiles were found to be subunits of the same protein and existed as multiple isoforms. Resilient sequence similarity was found to the 11S family of plant storage proteins. The structure is typical of the 11S type which occurs as a precursor of 55 kDa and is observed under denaturing and reducing conditions on SDS-PAGE storage protein profiles.

Keywords Coffee, Storage proteins, Two-dimensional SDS polyacrylamide gel electrophoresis, Organic, Integrated nutrition modes.

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INTRODUCTION

Coffee is one of the most widely produced and traded agricultural commodities around the world. Coffee is cultivated in 80 countries and exported by over 50 in Central and South America, Africa and Asia. More than a 100 million people are involved in producing and processing coffee. Therefore, coffee production has a significant impact on the economic development of the coffee producing areas and their environment. In India, Coffee occupies a pride among the plantations crops grown. As an agro-based rural enterprise primarily, this industry is a source of employment for over one million people in cultivation, processing and trading sectors. India accounts for about 4.5% of world coffee production and the industry provides employment to 6 lakh workforce. During FY 2020 – 2021, India has produced 342,000 MT of Coffee

and Exported 16410 MT to 50 different Countries and gained foreign exchange of Rs 278905 lakhs during FY 2020 – 2021 exchequer, which include Italy (33435 MT - 20.37%), Germany (33435 MT - 11.72%), Belgium (13901 MT - 8.47%), Russian Federation (7823 MT - 4.77%) and USA (3813 MT - 2.32%) (Coffee Statistics 2021).

Coffee is a perennial plant and evergreen in nature. The coffee plant belongs to the *Rubiaceae* family, *Coffea* genus and comprises of more than 70 different species. However, commercially only two species are cultivated i.e., *Coffea arabica* and *Coffea canephora* var *robusta*. In India, the consequences of leaf rust and white stem borer (WSB) on *Coffea arabica* fortified Indian planters to introduce *Coffea canephora* (Robusta coffee) during 1903-1906. For achieving sustainable eco-friendly coffee production through rust tolerance, high productivity, wide adaptability and improved quality, Central Coffee Research Institute (CCRI) developed superior and improved Robusta cultivar (Sl.274) and distributed for commercial cultivation (Coffee Guide 2014). Previously remarkable research work has been done on the species. Though, knowledge at the biochemical and molecular biological levels is still limited (Carneiro 1997). Such information is necessary in order to introduce or modify traits of technical quality or disease resistance in the species or to assistance in breeding programs. The storage proteins are the most copious in the endosperm and therefore considered as prime contenders for biochemical and molecular biology studies (John Rogersa 1999). Proteins are important precursors of aromas and flavors of the coffee beverage due to reactions with sugars during roasting. On average, coffee beans contains 10% of protein content (Clifford 1985) and serve as significant component for beverage quality (Amorim *et al.* 1975, Arnold and Ludwig 1996, Melo and Amorim 1975). Among the green coffee storage proteins, precisely the 11S storage protein account for 45% of the total protein content of green coffee beans and represent an important reservoir for free amino acids and peptides (Montavón *et al.* 2003, Rodrigues *et al.* 2010). Proteins composition in coffee beans are influenced by growing conditions and negative correlations with accumulation have been observed between them (Joet *et al.* 2010). In general, geographical areas of cultivation, agronomic factors (genetic origin, soil fertility and nutrient management), environmental conditions (altitude, temperature, hydric-demand), harvesting and post harvesting circumstances and processing methods (roasting and storage) can impact the composition of coffees (Silva *et al.* 2005, Leroy *et al.* 2006, Mullen *et al.* 2013).

On average, coffee beans contains 10% of protein content (Clifford 1985) and serve as significant components for beverage quality (Amorim *et al.* 1975, Arnold and Ludwig 1996, Melo and Amorim, 1975). However, despite their obvious role in many chemical reactions during maturation, storage and roasting, very diminutive information is known about coffee seed proteins (Sandra *et al.* 2001, Montavon *et al.* 2003). Earlier a few studies have been conducted on *Coffee arabica*, with the aim to establish a correlation between water soluble proteins and their importance in the coffee beverage quality coffee quality (Centi-Grossi *et al.* 1969, Amorim and Josephson 1975, Amorim and Amorim 1977, Bade and Stegemann 1982). Bade and Stegemann (1982) differentiated proteins from seeds of different coffee species according to the profiles obtained with several eletrophoretic systems. Preliminary electrophoretic evidence is provided for a secondary family of 11S proteins in certain robusta coffee varieties (John Rogersa *et al.* 1999). Studies conducted by Acuña *et al.* (1999) also confirmed 11S proteins as the main storage proteins in coffee seeds. Legumin (11S) storage proteins begin to accumulate when the endosperm is growing up and they account for approximately 45% of the total proteins of the mature arabica coffee bean (Rogers *et al.* 1999a). Marraccini *et al.* (2001) reported similar observations for robusta coffee (*C. canephora*). Preliminary electrophoretic results also indicated a secondary family of 11S proteins in Robusta coffee (*Coffea canephora*) (Fig. 1). The close sequence similarity with other 11S-type plant storage proteins supports the assumption of a storage function within the coffee grain. Low sulfur content may be a characteristic of the majority of 11S proteins and it has been suggested that this ensures the capacity of the seed to continue to synthesize storage proteins in environments deficient in this element (Shewry 1995). Rose *et al.* (1970) studied the SDS gel patterns of Soft and Rio coffee proteins, compared to standard

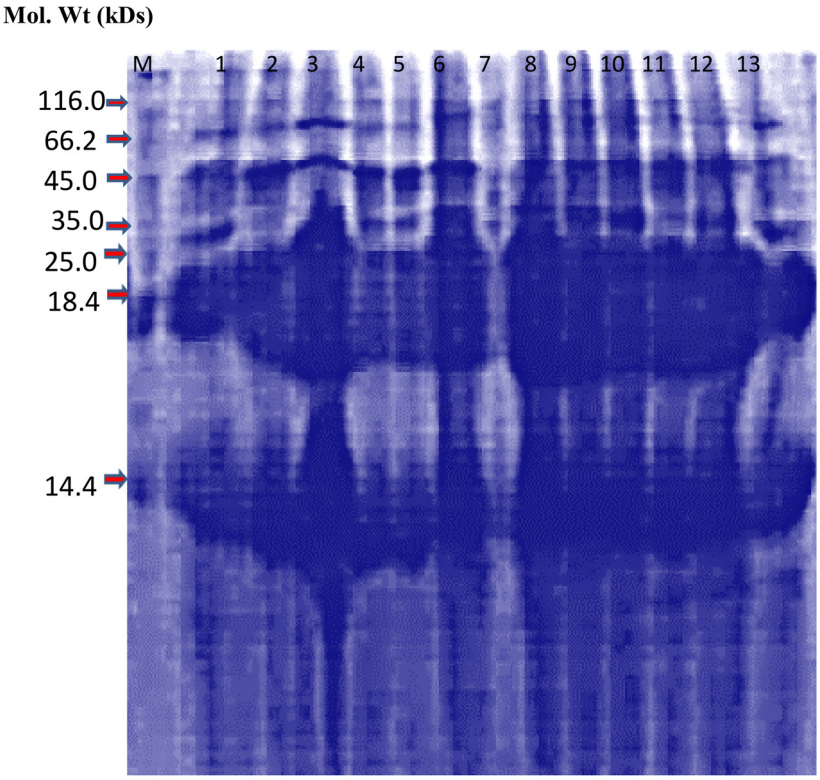

Fig. 1. Storage protein profile of robusta coffee seedlings.

bovine whey protein species of known molecular weights which given the approximate molecular weight distribution.

Luthe (1992) analyzed the protein profiles of several dicotyledoneous species, including arabica coffee (*Coffea arabica*). Two main bands were observed in denaturing polyacrylamide gel electrophoresis (SDS-PAGE) and because of their resemblance to the molecular weight of the acidic and basic subunits of legumins (author classified them as α and β subunits of a legumin-like protein). Supporting Luthe's investigation, Milton Massao Shimizu and Paulo Mazzafera (2000) using SDS-PAGE and gel filtration to determine the molecular weight of the native proteins, reported that each band is probably composed of six subunits. The typical structure of an 11S storage protein consists of 3−6 monomers, which migrate into storage vacuoles (protein bodies) and generate by hydrophobic interactions the tri- and hexameric quaternary forms, with molecular weights of 150− 400 kDa (Shutov and Vaintraub 1987). The rupture of the disulfide bonds in 11S monomers releases under reducing conditions the α (acidic) and β (basic) subunits (Shutov and Vaintraub 1987). The 11S globulin monomers were identified in *Coffea arabica* with a molecular weight of 55 kDa and consisted of 33 kDa (α) and 24 kDa (β) subunits (Acuña *et al.* 1999, Rogers *et al.* 1999). Similarly, Montavon *et al.* (2003), Nunes and Coimbra (2002) concluded that, *Coffea canephora* had an abundant protein monomer at 58 kDa, producing the corresponding two subunit fractions with 32−38 kDa (α) and 20−22 kDa (β). Supplementary comprehensive studies of the coffee seed legumin conducted by different scientists, (Montavon *et al.* 2003a, Acuña *et al.* 1999, Rogers *et al.* 1999) proposed the presence of different isoforms. In this current investigation, the SDS PAGE was performed to check these main storage protein compositions in robusta coffee endosperm.

MATERIALS AND METHODS

Study location: This field experiment was carried out at nine selected robusta coffee estates located at Western Ghats of India, i.e., Koppa region of Chikkamagaluru District, Chikkamagaluru district is situated in the south western part of Karnataka State, between 12º 54′ and 13º 53′ north latitude and between 75º 04′ and 76º 21′ east longitudes. 2,509 m above sea level, with an average mean annual Rainfall of 2908 mm. The tropical climate prevails in the study location, the relative humidity ranges from 27 to 80% and the average wind speed ranges from between 4 to 7 km/hr. The climate in study location is having three distinct seasons; 1) Summer season - March to early June, 2) Monsoon season – early June to September, however very less rainfall occurs during October to November due to impact North East Monsoon, 3) winter season initiates in mid-November and ends in mid-February. Among the selected 9 coffee estates, four estates practice organic mode of nutrition, while four estates follow integrated nutrition management practice and one estate where no nutrition management is practiced (absolute control). Varying shade pattern (open and thick) and irrigation (blossom, backing and winter) are the differentiation factors in the selected estates practicing exclusive organic cultivation and integrated nutrient management. The experiment was laid out in Randomized Block Design (RBD) with 25 plants per treatment (plot size- 112 m^2) with four replications. The selected estates under organic cultivation were practicing organic farming since preceding four years. The other cultural practices were carried out as per the package of practices (Anonymous, 2003). The treatment details are as follows

T^1- Control
T^2 - Organic nutrition*, thick shade (TS - 50 to 60% canopy) + Irrigation - I (winter)
T^3 - Organic nutrition*, thick shade (TS - 50 to 60% canopy) + Irrigation -II (Blossom & Backing)
T^4 - Organic nutrition*, optimum shade (OS - 25 to 30% canopy) + Irrigation - II (Blossom & Backing)
T^5 - Organic nutrition*, optimum shade (OS - 25 to 30% canopy) + Irrigation - I (winter)
T^6 - INM#, thick shade (TS - 50 to 60% canopy) + Irrigation - I (winter)
T^7 - INM#, thick shade (TS - 50 to 60% canopy) + Irrigation (Blossom & Backing) - II
T^8 - INM#, optimum shade (OS - 25 to 30% canopy) + Irrigation – II (Blossom & Backing)
T^9 - INM#, optimum shade (OS - 25 to 30% canopy) + Irrigation - I (winter)

*** Organic nutrition** -100% organics [Farm Yard Manure and Compost -2.5 tones ha^{-1}, Rock phosphate 0.2 tones ha^{-1}],

 # Integrated nutrition [50% recommended dose of fertilizer (Anonymous 2003) + 50% organic manures]

Winter-irrigation (I): At least four irrigations at winter, blossoming, backing and summer (interval of twenty days), extended if dry spell continuous

Blossom backing irrigation (II): Irrigations at blossoming and backing

Experimental design and sample collection: The experiment was laid out in Randomized Block Design (RBD) with 25 plants per treatment (plot size- 112 m^2) with four replications. The organics estates were selected where organic farming practices were practiced in the preceding four years. The other cultural practices were carried out as per the package of practices (Anonymous 2003). Representative coffee fruits from all nine robusta growing coffee estates were collected during harvesting period (February-March). After harvesting of the fruits they were wet processed to remove pulp and mucilage from the fruits. Further, they were sun dried up to 10% moisture level and stored using standard methods followed in parchment coffee (Anonymous 2003).

Protein analysis in coffee bean by SDS-PAGE: The finely milled coffee bean samples were defatted in n-hexane to about 24 hrs to remove fat content. Further, in the defatted samples proteins were extracted using pestle and mortar at 4ºC in sodium borate buffer as described by (Shimizu and Mazzafera 2000). Electrophoretic protein profiles were obtained by subjecting reduced proteins to discontinuous SDS-PAGE (Laemmli 1970), with 17% of acrylamide in the main gel. Separating Gel Buffer: 1.5M Tris-HCl of pH 8.8 was prepared and stored at 40C. Stacking Gel Buffer: 1M Tris-HCl of pH 6.8 was prepared and stored at

40C. Sodium Dodecyl Sulfate solution: 2% aqueous solution of SDS was prepared. Electrophoresis buffer: 3.0 g of Tris base and 14.4 g of glycine was dissolved in water and the final volume was made up to 1liter. The final pH was adjusted to 8.3 with glycine solution. Proteins were stained with Coomassie Brilliant Blue R and visualized using gel document system (Syngene Gene snap).

RESULTS AND DISCUSSION

The coffee bean samples collected during the experimental period were analyzed using electrophoresis (SDS- PAGE) for determining the storage proteins. The protein banding pattern of robusta coffee (*Coffea canephora* Sl. 274) from different treatments were compared and presented in the image. The SDS electrophoresis characterization profile show that legumin like proteins are the main storage protein found in endosperm of coffee beans, similar investigations were also reported earlier in several coffee species. Mainly two types of bands i.e., medium and high intensity were noted in the storage protein profile. Similarly, in arabica coffee beans two main bands were observed in denaturing polyacrylamide gel electrophoresis (SDS-PAGE) (Luthe 1992). The low molecular weight proteins (14.4 and 18.4 kDs) were observed as thick bands, while medium to high molecular weights (25 to 116 kDs) bands were observed as thin bands. The results are in line with (27 and 29 kDa subunits) in vegetative tissues of soybean (Staswick 1988), in coffee bean (55 kDa) Rogers *et al.* (1999), Paulo Mazzafera (2000). The monomorphic banding pattern among the treatments was dominated in the gel (71) scored, among which seven bands were polymorphic. However, significant differences between banding pattern in the different treatments were not observed. Since proteins were isolated from the coffee beans of single variety (Sl. 274) it is expected to have constitutional in nature with predominantly structural properties. These results allow us to understand the influence of the nutrient management practices on coffee storage proteins. Since the coffee plant productivity and final beverage quality are influenced by the nutrient management, geographical areas of cultivation, agronomic factors, environmental conditions, harvesting and post harvesting circumstances and processing methods are also affect the coffee beverage quality. Further, this data may be correlated with other biochemical and bean physical attributes to draw a valid conclusion on the coffee quality attributes.

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