Journal of Agricultural Science; Vol. 8, No. 9; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Regulated Lignin Structural Units and Soil Organic Carbon Content by Cowpea Peroxidase

Godson O. Osuji[1], Wenceslaus C. Madu[2], Eustace Duffus[1], Paul Johnson[1], Aruna Weerasooriya[1], Peter A. Y. Ampim[1], Laura Carson[1], Sanique South[1], Dwiesha Johnson[1], Ali Fares[1] & Alton Johnson[1]

[1] College of Agriculture and Human Sciences, Prairie View A&M University, Prairie View, Texas, USA

[2] Imo State Polytechnic, Umuagwo, Owerri, Nigeria

Correspondence: Godson O. Osuji, Cooperative Agricultural Research Center, Prairie View A&M University, P.O. Box 519-2008, Prairie View, Texas 77446, USA. E-mail: goosuji@pvamu.edu

Received: April 6, 2016 Accepted: July 7, 2016 Online Published: August 15, 2016

doi:10.5539/jas.v8n9p12 URL: http://dx.doi.org/10.5539/jas.v8n9p12

Abstract

Peroxidases participate in lignin biosynthesis, but there is no biochemical resolution between the structural units of lignin and soil organic carbon (SOC) contents. Black-eyed beans are staple high-protein foods for millions of at-risk populations in every continent. Its cultivation in semiarid zones could be leveraged to maximize SOC sequestration. Cowpea was treated with stoichiometric mixes of mineral nutrients. Peroxidase was electrophoretically purified from leaves, and assayed for o-dianisidine (guaiacyl units) and pyrogallol (p-hydroxyphenyl units) substrate specificities. Lignin, and SOC compositions were determined by gravimetry. Sulfate-treated cowpea produced the highest lignin (318.88 $kg{\cdot}ha^{-1}$) because the o-dianisidine maximum velocity (V_{max}) value (0.36 $\mu M{\cdot}min^{-1}{\cdot}mg^{-1}$) was higher than that for the pyrogallol (0.08 $\mu M{\cdot}min^{-1}{\cdot}mg^{-1}$), but the SOC (64.75 $kg{\cdot}ha^{-1}$) was low due to the guaiacyl being higher than p-hydroxyphenyl units. Peroxidase V_{max} value was low (0.12 $\mu M{\cdot}min^{-1}{\cdot}mg^{-1}$) for both substrates in the control cowpea, and accordingly lignin (268.44 $kg{\cdot}ha^{-1}$) and SOC (42.33 $kg{\cdot}ha^{-1}$) compositions were very low. The pyrogallol V_{max} value (0.5 $\mu M{\cdot}min^{-1}{\cdot}mg^{-1}$) was lower than the o-dianisidine value (1.0 $\mu M{\cdot}min^{-1}{\cdot}mg^{-1}$) for KK-treated cowpea, and accordingly the lignin contents (227.4 $kg{\cdot}ha^{-1}$) possessed variable compositions of guaiacyl and p-hydroxyphenyl units, leading to very high SOC composition (214.56 $kg{\cdot}ha^{-1}$). The high SOC sequestration technology involving fertilization with stoichiometric mixes of mineral nutrients could enable limited resource farmers who cultivate cowpeas as cover crop in the Sahel to improve SOM while producing their staple crop.

Keywords: carbon sequestration, syringyl, guaiacyl, stoichiometric mineral ratios, Sahel soil

1. Introduction

The interpretation of crop rotation, the dynamic fulcrum of plant-soil relationship, in terms of crop's peroxidase biology (Wight et al., 2012, 2014) has opened the gateway for more extensive understanding of the agronomic mechanisms of soil organic carbon (SOC) sequestration. Soil organic matter (SOM) is rich in lignin. Plant peroxidases participate in the biosynthesis of lignin, a complex biomaterial whose carbon content is associated with the improvement of the aggregation, chemical, and agricultural properties of the soil (Bauer & Black, 1994; Li-xia & Jian-jun, 2003; Eynard et al., 2004; Qiu et al., 2012; Chintala et al., 2015a). Lignin is a product of the polymerization of one or more of sinapyl, cinnamyl, and coniferyl alcohols precursors to give rise to the syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) units respectively in the lignin structure (Higuachi, 1985; Fukushima, 2001; Kalsoom et al., 2015). Agronomic practices regulate peroxidase activities (Wight et al., 2012, 2014), but the control of the chemical compositions of lignin by plant-soil relationship has not been studied. The aim was to explain the structural compositions of lignin and soil organic carbon contents in terms of the biology of plant peroxidases so as to freshen-up the biochemical understanding of plant-soil interactions.

The major source of stable organic carbon content of the soil is lignin because lignin is inherently recalcitrant to microbial degradation (Tian et al., 1992). Accordingly, lignin is very important in *in vivo* water transport-mechanical support functions in plant tissues; enhancement of soil chemical, physical, biological, and agricultural properties; and as a stable sink of global carbon reserve cycle in the soil (Qualls et al., 2003; Campbell & Sederoff, 1996; Sommer & Bossio, 2014). Whereas the carbon to nitrogen ratios of soil organic materials control the rate of mineralized labile fraction of crop residues, the metabolic control of the stable

organic carbon of the soil has not been studied (Brady & Weil, 2002; Chintala et al., 2015b). Lignins that are composed of methoxylated units are more resistant to white-rot fungal degradation (Skyba et al., 2013). Therefore, there is differential microbial degradation rate that is dependent on the chemical composition of the lignin. Methoxylated syringyl units make hardwood lignin more readily hydrolyzed during pulping (Chiang & Funaoka, 1990; Nunes et al., 2010). On the other hand, p-hydroxyphenyl units without methoxy groups tend to be hydrophobic, which property increases the chemical inertness of lignin to ligninase (Kirk & Farrell, 1987). This resistance of lignin to microbial degradation enhances lignin persistence in soils. Lignin is therefore a significant component of global carbon cycle (Campbell & Sederoff, 1996; Li-xia & Jian-jun, 2003; Bose et al., 2009; Fageria, 2012; Bot & Benites, 2005).

Many soils world-wide possess very low organic matter reserves despite the regular return of crop residues to the soil, and the prevalence of high water activity in the soil (Batjes, 2001; IITA, 1984). Biology is yet to provide some explanation for the low levels of SOM contents. Cowpea (*Vigna unguiculata*) was selected for this project because in the Sahel zone of West Africa anthropological and diversification center of the crop (Kiple & Kriemhild, 2000), it is used as a cover crop; but the SOM is very low being about 0.5%; and the dry grain yield is low, being about 1000 kg/ha (IITA, 1984). The Sahel is home to some at-risk population where cowpea is the folklore (lucky new year food) staple crop that provides medicinal remedies for common health maladies, and the bulk of the dietary protein and vitamins (IITA, 1985). Cowpea (black-eyed beans) provides favorite dishes in every continent (Yummy Recipes, 2015). Being cultivated in the tropics, mild temperate, semiarid, and subtropics, increasing the p-hydroxyphenyl units of cowpea lignin might enhance the persistence of organic carbon content of the soil and minimize the emission of carbon dioxide. Ability to alter the biology of peroxidase could enable the determination of the optimal agronomic practices for improving the p-hydroxyphenyl units of the lignin synthesized by cowpea thereby might prolong the life of the organic matter, and increase the agricultural productivity of the soil.

2. Materials and Methods

2.1 Treatment of Cowpea with Mineral Salt Solutions

Cowpea (*Vigna unguiculata* purple hull) seeds were planted in 120 × 120 × 30 cm (width × length × depth) boxes, each filled with 3 bags of professional growing mix (Sungro Horticulture, Bellevue, Washington, USA) mixed with 2 bags of organic matter-rich top soil (Landscapers Pride, New Waverly, Texas, USA). Each box was set up on level ground in the field on a weed-blocking plastic mat. About 25 seeds were planted per box. There was replanting to make up for ungerminated seeds. The applied mineral salt compositions were based on the model molar combinations (Osuji et al., 2011, 2003/4) that activate the plant's metabolism. The first box was left as the untreated control; the second box (NPKS) was treated with 1L of combined NH_4Cl (25 mM), Na_3PO_4 (20 mM), Na_2SO_4 (50 mM), and KCl (4 mM) solution; the third box (KKPP) was treated with 1L of combined KCl (8 mM), and Na_3PO_4 (40 mM) solution; the fourth box (KS) was treated with 1 L of combined and KCl (4 mM), and Na_2SO_4 (50 mM) solution; the fifth box (Sulfate) was treated with 1 L of Na_2SO_4 (50 mM) solution; the sixth box (KK) was treated with 1 L of KCl (8 mM) solution; the seventh box (PPN) was treated with 1 L combined Na_3PO_4 (40 mM) and NH_4Cl (25 mM) solution; the eighth box (KKK) was treated with 1 L KCl (12 mM) solution; the ninth box (KKS) was treated with 1 L combined KCl (8 mM) and Na_2SO_4 (50 mM) solution; the tenth box (KKN) was treated with 1 L combined KCl (8 mM) and NH_4Cl (25 mM) solution; and the eleventh box (KN) was treated with 1 L combined KCl (4 mM) and NH_4Cl (25 mM) solution. Each mineral treatment was triplicated giving a total of 33 boxes in the experiment. The boxes were watered every other week. Mineral nutrient solutions were applied sequentially, first at pre-flowering stage (2 weeks after seed germination), second at flowering, and third at post-flowering. While the pods were still green, about 250 g of green shoot was harvested from the three boxes of each treatment, immediately immersed in liquid nitrogen, and transferred to -80 ^{o}C freezer for storage. When the leaves turned yellow (cowpea maturity), pods were harvested per box, shelled, weighed separately, and the seeds were stored at room temperature. When the cowpea shoots had dried, they were harvested per treatment, dried at 60 ^{o}C to constant weight, and stored in paper bags at room temperature. Soil samples were collected from 2 to 20 cm depth in each box on the same day the dry shoots were harvested, and stored at room temperature.

2.2 Analyses for Cowpea Yield

Dry and milled (composited) shoots (100 g) per experimental treatment, sent to Universal Testing, Quincy, Illinois, USA were custom analyzed for lignin, and neutral detergent fiber (NDF) using proprietary modifications of Van Soest and Wine (1968) standard gravimetric methods. Soil samples (3 kg per experimental treatment)

were custom analyzed for pH and organic carbon contents by Soil, Water and Forage Testing Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, USA.

2.3 Cowpea Peroxidase Enzymology

Peroxidase was extracted from cowpea leaves (50 g) by high speed homogenization with 100 mL of ice-cold 50 mM Na_2HPO_4 buffer pH 6.0 containing 25% polyvinylpyrrolidone (w/v) and 0.1% β-mercaptoethanol as described before (Wight et al., 2012). The homogenate was centrifuged at 4000 g for 30 min at 4 ^oC to pellet cell debris. The supernatant was frozen at -80 ^oC, thawed at 5 ^oC, and centrifuged at 9000 g for 30 min at 4 ^oC. The supernatant was made 50% saturated with solid $(NH_4)_2SO_4$, and the protein precipitated was pelleted by centrifugation at 9000 g for 30 min at 4 ^oC. The pellet was dissolved in minimum volume of extraction buffer, and dialyzed against 3 changes of 10 mM Tris-HCl buffer (pH 8.0) at 5 ^oC, over 36 h, each change being 4 L. Protein precipitate at the end of dialysis was removed by centrifugation (9000 g 30 min 4 ^oC).

Partially purified cowpea peroxidase containing ~1 g protein was made 4 M with deionized urea and 2% with Bio-Lyte ampholyte (pH 3-10, 40% w/v). This solution was applied to Rotofor cell (Bio-Rad Laboratories, Hercules CA, USA), and focused for 3.5 h at 15W constant power and at 4 ^oC. Rotofor fractions were harvested, their pH values measured. Ampholyte and urea were removed from the fractions by dialyzing at 4 ^oC against 3 changes of 10 mM Tris-HCl buffer (pH 8.0) over 36 h, each change being 4 L. Dialyzed Rotofor fractions were stored in the fridge at 4 ^oC, and peroxidase remained active for at least 3 weeks.

Dialyzed Rotofor fractions of peroxidase (0.2 mL) were prepared with bromophenol blue-glycerol protein loading buffer (Davis et al., 1986) and loaded into the wells of a slab of 7.5% native polyacrylamide gel (PAG), and electrophoresed (Bio-Rad protean ii cell, constant 100 V at 4 ^oC) until the bromophenol blue dye was at the lower edge of the gel. Peroxidase activity was detected by staining (Quesada et al., 1990) the electrophoresed gel in a solution of 100 mL of 50 mM Na_2HPO_4 containing 6.0 mM o-dianisidine, or 12.0 mM pyrogallol and 8.8 mM hydrogen peroxide at room temperature in the dark until the peroxidase isoenzyme bands became visible (30-45 min). The stained gel was rinsed with distilled water, photographed, and the peroxidase bands digitalized using UN-SCAN-IT software (Silk Scientific, Utah, and USA).

Peroxidase activity was determined (Wight et al., 2012) at fixed 0.6 mM o-dianisidine, or pyrogallol concentration, and varied (0.3-1.8 mM) hydrogen peroxide concentrations, in 3 mL of 50 mM Na_2HPO_4 solution pH 6.0. The activity was measured at 27 ^oC using Nanodrop spectrophotometer and calculated for o-dianisidine at A_{460} ($\epsilon_{460\,nm}$: 11.3 $mM^{-1}cm^{-1}$) concentration; or for pyrogallol at A_{420} ($\epsilon_{420\,nm}$: 12.0 $mM^{-1}cm^{-1}$) concentration for double reciprocal plots. Peroxidase kinetic assays per experimental cowpea were repeated three times, and the kinetic constants derived were identical. Protein concentrations were determined by the Folin-Ciocalteau reagent and lysozyme as protein standard.

3. Results

3.1 Carbon Sequestration

The mixture of 3 bags of professional growing mix (Sungro Horticulture, Bellevue, Washington, USA) and 2 bags of organic matter-rich top soil (Landscapers Pride, New Waverly, Texas, USA) was the experimental soil used in this research project. It had organic carbon content of about 8.83 ppm. After supporting a growth cycle of cowpea, the organic carbon content was unchanged. This meant that the control cowpea without mineral nutrient treatment utilized the organic carbon at the same rate as it sequestered new organic matter into the soil. The NPKS-treated cowpea similarly did not alter the organic carbon content (Table 1). Mineral salt fertilizations that induced large sequestration of organic carbon included PPN treatment (45.61 ppm), KK treatment (44.70 ppm), KS treatment (29.65 ppm), KKN treatment (27.76 ppm).

Table 1. Control of soil organic carbon contents through stoichiometric mineral salts fertilization of cowpea

Mineral Treatments	Soil organic carbon (ppm)	pH
KKS	4.63	6.7
KKK	4.69	6.7
NPKS	9.17	6.8
Control	8.82	6.9
KN	16.15	7.0
PPN	45.61	7.1
KKN	27.76	7.1
KKPP	20.99	7.1
KS	29.65	7.2
S	13.49	7.2
KK	44.70	7.3

KKPP treatment (20.99 ppm), and sulfate treatment (13.49 ppm). Therefore, mineral salt treatment of cowpea enhanced and maximized the soil organic carbon contents by ten folds. Two treatments (KKK and KKS) induced the loss of organic carbon from the soil (Table 1). Therefore, when inappropriate mixes of mineral nutrients are applied, they decrease the metabolic ability of cowpea to sequester carbon into the soil. This may account for the repeatedly reported low organic matter contents of West African soils, the diversification center of cowpea (Kolawole et al., 2002; Kiple & Kriemhild, 2000; Bationo et al., 2007).

3.2 Peroxidase Isoenzymes of Cowpea

Free solution isoelectric focusing (Rotofor) purification and scanning quantitation of bands on the polyacrylamide gel showed that the o-dianisidine-active isoenzymes were different from the pyrogallol-active isoenzymes (Figures 1 and 2) in terms of their pI values and isoform distribution patterns on the gel landscape suggesting that the lignin polymerized by the pyrogallol peroxidase could be different in chemical units from that polymerized by the o-dianisidine peroxidase. The o-dianisidine-active peroxidase isoenzymes were acidic whereas the pyrogallol-active peroxidase isoenzymes were neutral and more alkaline per treated cowpea. Therefore, the Rotofor fractionation step is important for visual illumination of peroxidase biology. Also, both the o-dianisidine and pyrogallol substrates showed that peroxidases responded to the different mineral salt treatments, each peroxidase fingerprint being characteristic for the specific mineral treatment (Figures 1 and 2). Similar specific responses of sorghum peroxidase to agronomic treatments of biomass sorghum have been reported (Wight et al., 2012, 2014).

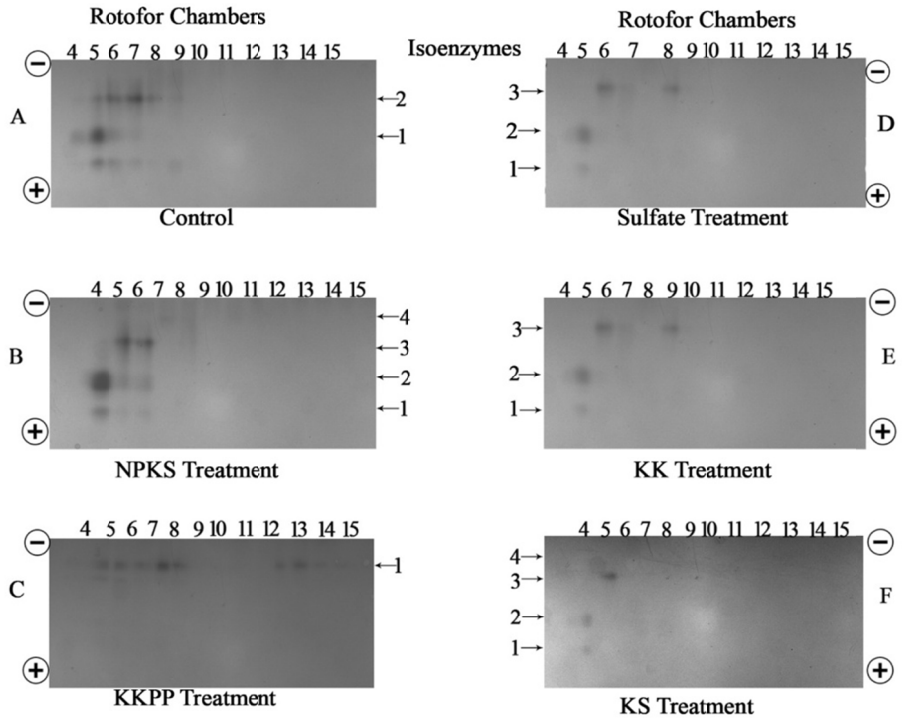

Figure 1. o-dianisidine-active peroxidase of cowpea

Note. Distribution patterns of the o-dianisidine-active peroxidase isoenzymes from mineral nutrient treated, and control untreated cowpea. Partially purified peroxidase extract from the cowpea leaves was subjected to free solution isoelectric focusing followed by native PAGE. The electrophoresed gel was stained with o-dianisidine solution. The control was the untreated cowpea; NPKS was the cowpea treated with 1L of combined NH_4Cl (25 mM), Na_3PO_4 (20 mM), Na_2SO_4 (50 mM), and KCl (4 mM) solution; KKPP was the cowpea treated with 1L of combined KCl (8 mM), and Na_3PO_4 (40 mM) solution; Sulfate was the cowpea treated with 1L of Na_2SO_4 (50 mM) solution; KK was the cowpea treated with 1L of KCl (8 mM) solution; KS was the cowpea treated with 1L of combined and KCl (4 mM), and Na_2SO_4 (50 mM) solution. The pI values of peroxidase isoenzymes in Rotofor chambers 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 were 4.55, 4.90, 5.0, 5.25, 5.76, 6.35, 6.35, 7.18, 8.08, 8.08, 8.52, 8.86, and 8.86 respectively.

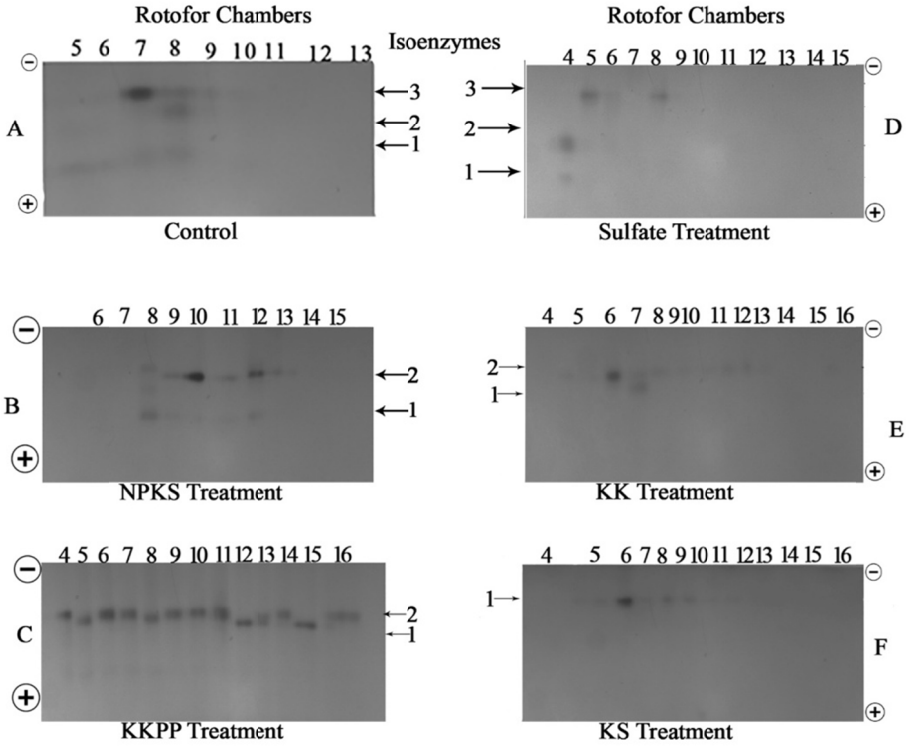

Figure 2. Pyrogallol-active peroxidase of cowpea

Note. Distribution pattern of the pyrogallol-active peroxidase isoenzymes from mineral nutrient treated, and control untreated cowpea. Partially purified peroxidase extract from the cowpea leaves was subjected to free solution isoelectric focusing followed by native PAGE. The electrophoresed gel was stained with pyrogallol solution. The control was the untreated cowpea; NPKS was the cowpea treated with 1 L of combined NH_4Cl (25 mM), Na_3PO_4 (20 mM), Na_2SO_4 (50 mM), and KCl (4 mM) solution; KKPP was the cowpea treated with 1 L of combined KCl (8 mM), and Na_3PO_4 (40 mM) solution; Sulfate was the cowpea treated with 1 L of Na_2SO_4 (50 mM) solution; KK was the cowpea treated with 1 L of KCl (8 mM) solution; KS was the cowpea treated with 1 L of combined and KCl (4 mM), and Na_2SO_4 (50 mM) solution. The pI values of peroxidase isoenzymes in Rotofor chambers 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 were 4.55, 4.90, 5.0, 5.25, 5.76, 6.35, 6.35, 7.18, 8.08, 8.08, 8.52, 8.86, and 8.86 respectively.

Table 2. Control of cowpea peroxidase activity and lignin contents by stoichiometric mineral salts

Mineral treatments	Total lignin ($Kg \cdot ha^{-1}$)	Pyrogallol		o-dianisidine	
		V_{max} [a]	K_m [b]	V_{max} [a]	K_m [b]
NPKS	269.8	0.12	05	0.26	1.0
Control	268.4	0.12	1.7	0.12	0.45
S	318.9	0.08	0.15	0.36	1.53
KK	227.4	0.5	4.0	1.0	4.0
KS	284.6	0.06	0.24	0.25	1.0
KKPP	121.8	0.33	5.0	0.33	0.83

Note. a: V_{max} is $\mu M \cdot min^{-1} \cdot mg^{-1}$ protein; b: K_m is μM.

3.3 Metabolic Regulation of Cowpea Lignin and Soil Organic Carbon by Mineral Nutrients

The hydrogen peroxide Michaelis constant (K_m) values for cowpea peroxidase were low (Table 2) similar to those for biomass sorghum (Wight et al., 2014), and were also within the ranges characteristic of plant tissue/cellular concentrations of hydrogen peroxide (Cheeseman, 2006; Pradedova et al., 2013). Therefore, the K_m values were the common denominator for comparing the peroxidase biology and for estimating the lignin chemical compositions. In each experimental cowpea, the maximum velocity (V_{max}) value for the o-dianisidine

reaction was either the same or higher than that for the pyrogallol reaction (Table 2). o-dianisidine is a derivative of the guaiacyl whilst pyrogallol is a derivative of p-hydroxyphenyl units of lignin. Therefore, the peroxidase V_{max} values mean that cowpea lignins have equal or are richer in guaiacyl (G) than in p-hydroxyphenyl (H) units. This is in agreement with the general observation which is based on lignin chromatographic analyses (Lupoi et al., 2014; Nunes et al., 2010; Spark, 2006) that syringyl (S) units are as abundant as G units in angiosperm lignins, with the H units being in smaller amounts (Kirk & Farrell, 1987). Peroxidase activity is suitable for determination of the chemical units of lignin. Cowpea experimental treatments (control, and NPKS) where the V_{max} values (Table 2) for the o-dianisidine peroxidase reactions were same or higher than that for the pyrogallol gave similar lignin yields per unit area of land (268.44-269.77 kg·ha^{-1}), the G units being higher than the H units. Their soil organic carbon contents were approximately equal (Table 1).

The V_{max} value for the o-dianisidine reaction of the peroxidase for sulfate-treated cowpea was much higher than that for the pyrogallol reaction (Table 2), and at the same time the K_m value (0.15 μM) for the pyrogallol reaction was ten times lower than that (1.53 μM) for the o-dianisidine reaction. The low K_m value for the pyrogallol peroxidase activity of the sulfate-treated cowpea suggested that the lignin composition was richer in H units compared with that of the control cowpea, and accordingly the lignin yield (318.88 kg·ha^{-1}) exceeded that of the control cowpea. Enrichment of the H units relative to the G units remarkably increased the lignin yield and the soil organic carbon content (Table 1).

Similarly, when the peroxidase V_{max} values for the o-dianisidine substrate were higher than that of the pyrogallol substrate as in the KK- and KS-treated cowpeas (Table 2), but the K_m values were either same or slightly lower in the pyrogallol reaction than in the o-dianisidine reaction, the lignin yields were low and they varied widely (227.4-284.59 kg·ha^{-1}) reflecting the wide variability in the compositions of H and G units, and resulting to wide variations in the soil organic carbon contents (Table 1).

When the peroxidase V_{max} values for pyrogallol and o-dianisidine substrates were the same, and the K_m value was very much lower in the o-dianisidine reaction as in the KKPP-treated cowpea (Table 2), the lignin yield was low (121.77 kg·ha^{-1}) similar to the soil organic carbon contents (20.99 ppm) because the lignin was deficient in H units compared with the other cowpeas. These considerations illuminate the biological mechanism that control the H, G, and S units in the structure and biosynthetic yield of lignins. All those cowpeas (control, NPKS-, KKPP-, S-treated) in which the peroxidase V_{max} values were the same or much higher in the o-dianisidine than in the pyrogallol reactions sequestered low organic carbon contents (< 21.0 ppm) into the soil (Table 1) irrespective of the yield of lignin because the H and G units of their lignin compositions were not variable. The KK-, and KS-treated cowpeas where the K_m values were either same or slightly lower in the pyrogallol than in the o-dianisidine reaction (Table 1) introduced much higher recalcitrant organic carbon contents (44.7 ppm, 29.65 ppm respectively) into the soil due to variability in the compositions of G and H units of their lignins.

The mineral treatments (KKS, KKK) that induced the lowest SOC contents also induced soil acidity (Table 1). This is in agreement with the pH buffering function of soil organic matter. An advantage of stoichiometric mixes of mineral nutrients is that in addition to being mineral nutrients, they also act as electromagnets as they reprogram the metabolic pathways according to the prevailing pH gradient (Osuji et al., 2011) thereby coordinating biological mechanisms for increased crop performance and yield, peroxidase being a target site of the action of the stoichiometric mineral nutrients. Because stoichiometric mixes of mineral nutrients are soluble, they exert their combined signal integration-discrimination effects on plant metabolism synchronously. In contrast, the mineral nutrient compositions of commercial fertilizers are differentially soluble, most of them having delayed release to the crop, they exert their effects on plant metabolism non-synchronously and ineffectively coordinated.

4. Discussion

The internal repeats in the stoichiometric compositions of the mineral nutrient solutions minimized the extent of stochastic experimental variations, and accordingly the numbers of field plot replications. Each stoichiometric mineral nutrient composition was related to at least two others thereby promoting the practical interplay of nutrient antagonism or synergism. The responses of cowpea lignin and peroxidases reflected some aspects of mineral nutrient antagonism. Sulfate treatment of cowpea did not induce any inhibition on the o-dianisidine and pyrogallol activities of the peroxidase (Table 2); KS-treatment induced uncompetitive inhibition leading to the decrease of the lignin content. Similarly, KK-treatment induced non-competitive inhibition on the peroxidase activities (Table 2), but the KKPP treatment induced competitive inhibition kinetics on the enzyme. These push-pull reactions in the cell wall metabolism were the basic biological factors that regulated soil-cowpea relationship by differentially increasing the lignin yields and organic carbon sequestration into the soil. Carbon to

nitrogen ratio (USDA NRCS, 1977) may not be the commanding factor responsible for the amount of stable organic carbon sequestered by crop plants into the soil. As Chintala et al. (2015a, 2015b) reported, hydrophobicity especially of the lignin content may also influence the mineralization of soil organic matter.

The structural units of lignin, a complex biomaterial, is analyzed via oxidative degradation followed by spectroscopic characterization of the resultant syringyl and guaiacyl units (Lupoi et al., 2014; Nunes et al., 2010; Spark, 2006). But chemical degradation of lignin does not reveal the biological control of the structural composition. Therefore, the biochemical (Tables 1 and 2) alternative by which the activity of peroxidase was applied for deducing the structural units could supplement the spectroscopic approach to provide a complete understanding of the mechanistic function of the complex biomaterial in sequestering carbon in the soil.

In cowpea's native ecosystems in Africa Sahel and humid tropics, the soils have low nutrient and organic carbon contents (Bates, 2001; IITA, 1984, 1985; Bationo et al., 2007) and are inadequate to support high yields of crops (Bot & Benites, 2005). However, under stoichiometric mixes of mineral nutrients (KK, PPN, and KS), the cowpea was able to increase the organic carbon contents of the soil by as much as four folds (Table 1). Therefore, the repeatedly observed low organic matter content of some West African soils (Agboola, 1978; Kolawole et al., 2002) could be remedied by application of the appropriate molar mixtures of mineral nutrients to the cowpea crops.

Cowpea (black-eyed beans, Southern peas) is cultivated in Africa, USA, Asia, South America, Southern Europe, Australia) by mainly limited resource farmers. Africa produces about 68% while Brazil/USA/Asia account for 17% of the world crop (Gomez, 2004). Cowpea meals (lobia, waakye, akara, moi moi, chili beans etc.) are rich in protein, vitamins, minerals, and so they have anthropological ties with millions of cultures who associate them with good-luck. Therefore, cowpea cultivation could be leveraged to achieve increased sequestration of stable organic carbon to the soil. Compared with the KKK-, and KKS-fertilized plots, the PPN-fertilized cowpea maximized the SOC about ten folds (Table 1). It is excellent to focus attention on the minimization of greenhouse gas emission as the significant approach for the control of some of the adverse effects of climate change. It is also excellent on the flip side to focus attention on the mitigation of greenhouse gas emission by maximizing stable organic carbon sequestration into the soil. The global popularity of cowpea meals could be a strong advantage for adopting cowpea cultivation in the strategy to mitigate some adverse effects of climate change. Mineral salt concentrations mixed according to their reactive molar ratios are based on the responses of biomass biology to the varied concentrations of the mineral salts (Osuji et al., 2003/4). The weathered 'one-mineral nutrient-fits-all' agronomic formulae could be the root cause for the declining productivity of organic matter-deficient West African soils (Kolawole et al., 2002; Agboola, 1978). Agricultural extension could spearhead the transfer of the stable organic carbon technology to limited resource farmers.

5. Conclusion

The agricultural importance of lignin is related to its syringyl (S), p-hydroxyphenyl (H), and guaiacyl (G) compositions. Soil organic matter is rich in lignin because lignin is difficult to degrade by microbes. The responses of the structural units of lignin to environmental and agronomic conditions have not been studied. Lignin compositions of cowpea shoots, and SOC compositions were determined by gravimetry. The higher the V_{max} values were for o-dianisidine peroxidase substrate as in the control, NPKS-, KKPP-, and SO_4^{-2}-treated cowpeas, the higher were the compositions of G units in the lignin, and the lower were the SOC contents. Conversely, the higher the V_{max} values were for pyrogallol peroxidase substrate as in the KK-, and KS-treated cowpeas, the higher were the H units in the lignin structural composition, and the higher were the sequestered SOC contents. Therefore, some mineral nutrient ion combinations induced higher H structural unit compositions on cowpea lignin and higher percentage sequestration of organic carbon into the soil. Cultivation of cowpea under appropriate mineral fertilization in sub-Sahara Africa and similar arid zones of the world could help to increase the SOC composition, improve soil fertility, and crop yield.

Acknowledgements

This project was funded by NIFA-USDA-Allen funds made to Prairie View A&M University. The Institutional Review Board of the University approved and monitored the project.

References

Agboola, A. A. (1978). Influence of soil organic matter on cowpea's response to N fertilizer. *Agronomy Journal, 70*, 25-28. http://dx.doi.org/10.2134/agronj1978.00021962007000010007x

Bationo, A., Kihara, J., Varlauwe, B., Waswa, B., & Kimetu, J. (2007). Soil organic carbon dynamics functions and management in West African agro-ecosystems. *Agricultural Systems, 94*, 13-25. http://dx.doi.org/10.1016/j.agsy.2005.08.011

Batjes, N. H. (2001). Options for increasing carbon sequestration in West African soils: An exploratory study with special focus on Senegal. *Land Degradation & Development, 12*, 131-142. http://dx.doi.org/10.1002/ldr.v12:2/issuetoc

Bauer, A., & Black, A. L. (1994). Quantification of the effects of soil organic matter content on soil productivity. *Soil Science Society of America Journal, 58*, 15-193. http://dx.doi.org/10.2136/sssaj1994.03615995005800010027x

Bose, S. K., Francis, R. C., Govender, M., Bush, T., & Spark, A. (2009). Lignin content versus syringyl to guaiacyl ratio amongst poplars. *Bioresource Technology, 100*, 1628-1633. http://dx.doi.org/101016/j.biortech.2008.08.046

Bot, A., & Benites, J. (2005). The importance of soil organic matter. Retrieved April 3, 2014, from http://www.fao/docrep/009/a100e/a100e4.htm

Brady, N. C., & Weil, R. R. (2002). *The nature and properties of soils* (13th ed.). Prentice Hall.

Campbell, M. M., & Sederoff, R. R. (1996). Variation in lignin content and composition. *Plant Physiology, 110*, 3-13.

Cheeseman, J. M. (2006). Hydrogen peroxide concentrations in leaves under natural conditions. *J. Experimental Botany, 57*, 2435-2444. http://dx.doi.org/10.1093/jxb/er1004

Chiang, V. L., & Funaoka, M. (1990). The difference between guaiacyl and guaiacyl-syringyl lignins in their responses to kraft delignification. *Holzforchung, 44*, 309-313. http://dx.doi.org/10.1515/hfsg.1990.44.4.309

Chintala, R., Owen, R. K., Schumacher, T. E., Spokas, K. A., McDonald, L. M., Malo, D. D., … Bleakley, B. (2015b). Denitrification kinetics in biomass and biochar amended soils of different landscape positions. *Environmental Science and Pollution Research, 22*(7), 5152-5163. http://dx.doi.org/10.1007/s11356-014-3762-2

Chintala, R., Schumacher, T. E., Kumar, S., Malo, D. D., Rice, J., Bleakley, B., … Gu, Z. R. (2015a). Molecular characterization of biochar materials and their influence on microbiological properties of soil. *Journal of Hazardous Materials, 279*, 244-256. http://dx.doi.org/10.1016/j.jhazmat.2014.06.074

Davis, L. G., Dibner, M. D., & Battey, J. F. (1986). *Basic Methods in Molecular Biology*. Elsevier, New York.

Eynard, A., Schumacher, T. E., Lindstrom, M. J., & Malo, D. D. (2004). Porosity and poresoze distribution in cultivated Ustolls and Usterts. *Soil Science Society of America Journal, 68*, 1927-1934. http://dx.doi.org/10.2136/sssaj2004.1927

Fageria, N. K. (2012). Role of soil organic matter in maintaining sustainability of cropping systems. *Communications Soil Science Plant Analysis, 43*, 2063-2113. http://dx.doi.org/10.1080/00103624.2012.697234

Fukushima, K. (2001). Regulation of syringyl to guaiacyl ratio in lignin biosynthesis. *J. Plant Research, 114*, 499-508. http://dx.doi.org/10.1007/PL00014017

Gomez, C. (2004). *Cowpea: Post-harvest operations*. FAO, Rome. Retrieved January 4, 2016, from http://www.fao.org/.../inpho/docs/Post_Harvest_Compendium_-_Cowpeas.pdf

Higuachi, T. (1985). Biosynthesis and biodegradation of wood components. In T. Higuachi (Ed.), *Biosynthesis of lignin* (pp. 141-160). Academic Press New York.

IITA. (1984). *Nigeria Annual Report* (pp. 67-117). International Institute of Tropical Agriculture, Ibadan.

Kalsoom, U., Bhatti, H. N., & Asgher, M. (2015). Characterization of plant peroxidases and their potential for degradation of dyes: A review. *Appl. Biochem. Biotechnol., 176*, 1529-1550. http://dx.doi.org/10.1007/s12010-015-1674-3

Kiple, K. F., & Kriemhild, C. O. (2000). *The Cambridge World History of Food*. Cambridge, UK: Cambridge University Press, England.

Kirk, T. K., & Farrell, R. L. (1987). Enzymatic combustion: The microbial degradation of lignin. *Annual Review of Microbiology, 41*, 465-505. http://dx.doi.org/10.1146/annurev.mi.41.100187.002341

Kolawole, G. O., Tian, G., & Sing, B. B. (2002). Differential response of cowpea lines to application of P fertilizer. In C. A. Fatokun, S. A. Tarawali, B. B. Singh, P. M. Kormawa, & M. I. Tamo (Eds.), *Challenges and opportunities for enhancing sustainable cowpea production* (pp. 319-328). Ibadan, Nigeria Publisher.

Kouyate, Z., Krasova-Wade, T., Yattara, I., & Neyra, M. (2012). Effects of cropping systems and cowpea variety on symbiotic potential and yields of cowpea and pearl millet in Sudano-Sahelian zone of Mali. *International J. Agronomy, 2012*. http://dx.doi.org/10.1155/2012/761391

Li-xia, Y., & Jian, P. (2003). Dynamics models of soil organic carbon. *Journal of Forestry Research, 14*, 323-330. http://dx.doi.org/10.1007/BF02857862

Lupoi, J. S., Singh, S., & Davis, M. (2014). High-throughput prediction of eucalypt lignin syringyl/guaiacyl content using multivariate analysis: A comparison between mid-infrared, near-infrared, and Raman spectroscopies for model development. *Biotechnology for Biofuels, 7*, 93-105. http://dx.doi.org/10.1186/1754-6834-7-93

Nishanth, B., Rajkumar, J. S. I., & Sundaram, M. (2013). Sequestration of atmospheric carbon through forage crops cultivated in Ramayanpatti, Tirunelveli district, Tamilnadu, India. *Research J. Agriculture and Forestry Sciences, 1*(3), 11-14.

Nunes, C. A., Lima, C. F., & Barbosa, L. C. A. (2010). Determination of *Eucalyptus* spp. lignin S/G ratio: A comparison between methods. *Bioresource Technology, 101*, 4056-4061. http://dx.doi.org/10.1016/j.biortech.2010.01.012

Osuji, G. O., Brown, T. K., South, S. M., Duncan, J. C., & Johnson, D. (2011). Doubling of crop yield through permutation of metabolic pathways. *Advances Bioscience Biotechnology, 2*, 364-379. http://dx.doi.org/10.4236/abb.2011.25054

Osuji, G. O., Mangaroo, A. S., Reyes, J., Bulgin, A., & Wright, V. (2003/4). Biomass enhancement in maize and soybean in response to glutamate dehydrogenase isomerization. *Biologia Plantarum, 47*, 45-52. http://dx.doi.org/10.1023/A:1027324713682

Parthasarathi. T. (2011). *Carbon sequestration in agriculture*. Lambert Academic Publishing, India.

Pradedova, E. V., Trukhan, I. S., Nimaeva, O. D., & Salyaev, R. R. K. (2013). Hydrogen peroxide generation in the vacuoles of red beet root cells. *Doklady Biological Sciences, 442*, 1-4. http://dx.doi.org/10.1134/s0012496613020129

Qiu, L., Wei, X., Zhang, X., & Cheng, J. (2012). Soil organic carbon losses due to land use change in a semiarid grassland. *Plant and Soil, 355*, 299-309. http://dx.doi.org/10.1007/s11104-011-1099-x

Qualls, R. G., Takiyama, A., & Wershaw, R. L. (2003). Formation and losses of humic substances during decomposition in a pine forest floor. *Soil Science Society of America Journal, 67*, 899-909. http://dx.doi.org/2136/sssaj2003.8990

Quesada, M. A., Tigier, H. A., Bukovac, M. J., & Valpuesta, V. (1990). Purification of an anionic isoperoxidase from peach seeds and its immunochemical comparison with other anionic isoperoxidases. *Physiologia Plantarum, 79*, 623-628. http://dx.doi.org/1111/j.1399-3054.1990.tb00035.x

Skyba, O., Douglas, C. J., & Mansfield, S. D. (2013). Syringyl-rich lignin renders poplars more resistant to degradation by wood decay fungi. *Applied and Environmental Microbiology, 79*, 2560-2571. http://dx.doi.org/10.1128/AEM.03182-12

Sommer, R., & Bossio, D. (2014). Dynamics and climate change mitigation potential of soil organic carbon sequestration. *Journal of Environmental Management, 144*, 63-87. http://dx.doi.org/10.1016/j.jenvman.2014.05.017

Spark, A. (2006). *Analysis of syringyl and guaiacyl (S/G) ratio in lignin*. Retrieved October 16, 2015, from http://researchspace.csir.co.za/dspace/bitstream/10204/3300/1/Spark_2007.pdf

Tian, G., Kang, B. T., & Broussard, L. (1992). Biological effects of plant residues with contrasting chemical composition under humid tropical conditions: Decomposition and nutrient release. *Soil Biology and Biochemistry, 24*, 1051-1060. http://dx.doi.org/1016/0038-0717(92)90035-V

UC Davis. (2015). *University of California's Sustainable Agriculture Research and Education Programs cover crop resource page*. Retrieved October 16, 2015, from http://ucanr.org/sites/asi/db/covercrops.cfm?crop_id=15

USDA NRCS. (1977). Relationship of carbon to nitrogen in crop residues. *Conservation Agronomy Technical Notes* (No. 30). Retrieved January 19, 2016, from http://www.nm.nrcs.usda.gov/Technical/tech-notes /agro/AG30.pdf

Van Soest, P. J., & Wine, R. H. (1968). Determination of lignin and cellulose in acid detergent fiber with permanganate. *Journal of the Association of Official Analytical Chemists, 51*, 780-785.

Wight, J. P., Hons, F. M., & Osuji, G. O. (2014). *R*esponses of bioenergy sorghum cell wall metabolism to agronomic practices. *Advances in Biological Chemistry, 4*, 67-78. http://dx.doi.org/10.4236/abc.2014.41010

Wight, J. P., Hons, F. M., South, S. M., & Osuji, G. O. (2012). Purification of active peroxidase isoenzymes and their responses to nitrogen fertilization and rotation of biomass sorghum. *American Journal Plant Sciences, 3*, 1422-1429. http://dx.doi.org/10.4236/ajps.2012.310172

Yummy Recipes. (2015). *Spicy black-eyed peas and tomato soup*. Retrieved December 29, 2015, from http://www.yummy.com/recipies/black-eyed-peas-side-dish