Journal of Experimental Agriculture International

Volume 45, Issue 12, Page 64-75, 2023; Article no.JEAI.110553
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Shading and Planting Material Type Effects on Some Physiological and Biochemical Compounds Synthesis in *Desmodium adscendens* Plants Leaves in the Domestication Phase, Daloa, Côte d'Ivoire

Tahia Jean Yves Koffi [a*], Yah Gwladys Gnamien [a], Ahou Nadia Kanga [b], Oussou Kouadio Mathieu Koffi [a], Jean-renaud Allouko [c], Kouame Mathurin Koffi [d], Koutoua Ayolie [a] and Yatty Justin Kouadio [a]

[a] UFR (Faculty) of Agroforestry, Laboratory for Improving Agricultural Production, University Jean Lorougnon Guédé, BP 150 Daloa, Côte d'Ivoire.
[b] University Jean Lorougnon Guédé, IUIPB, University Institute of Landscape and Wood Engineering, Côte d'Ivoire.
[c] UFR (Faculty) of Environment, University Jean Lorougnon Guédé, Côte d'Ivoire.
[d] Water and Forestry Engineer, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2023/v45i122266

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/110553

Original Research Article

Received: 08/10/2023
Accepted: 14/12/2023
Published: 20/12/2023

**Corresponding author: E-mail: koffitahiajean.y@gmail.com;*

ABSTRACT

Desmodium adscendens (Sw.) is a plant of the Fabaceae Family, a wild medicinal plant frequently found in industrial plantations, where it is exposed to pesticide residues. This makes species unsuitable for use as a raw material in pharmaceutical industry. To this end, domestication is one of best ways of cleaning up the species. During this process, the evaluation of a few physiological and biochemical compounds would be an indicator for the choice of planting material and the ecology species. Two types of plant material (seeds and stem) grown under three types of shade (accentuated, moderate, no shade) were tested. The leaves of various 90-day-old plants were used to determine physiological and biochemical parameters various. Results showed that chlorophyll a, carotenoids, polyphenols, total sugars, protein, proline and the activities of tyrosine ammonia-lyase, polyphenol oxidase and catalase were higher in leaves with moderate and no shading. However, under heavy shading, chlorophyll b content, phenylalanine ammonia lyase (PAL) and peroxidase enzyme activities were higher. Whatever shading type, the chl (a) content remained lower than that of chl (b). Also, total sugars, proline, ascorbate peroxidase and catalase were more abundant in the leaves of plants grown from stem. In contrast, phenolics, proteins and PPO are more abundant in leaves of seedlings.

Keywords: Shading; pigment; physiological; biochemical; enzymatic; desmodium adscendens.

1. INTRODUCTION

In Africa, many plant species have so far remained on the bangs of scientific investigation due to a lack of awareness of their interests for mankind [1]. On the other hand, a large proportion of these species contribute strongly, indirectly and/or directly, to the well-being of mankind, and more particularly to many world's populations health [2,3]. According to the World Health Organization (WHO), around 65-80 % of world's population in developing countries, and particularly in Africa, depend essentially on traditional medicinal plants for their primary care due to poverty and lack of access to modern medicine [4,5]. Indeed, today, the plants therapeutic virtues are attracting renewed interest thanks to improvements in modern extractions techniques and advances in structural analysis methods [6]. These different techniques and methods have led to the discovery of many and new active ingredients of interest [7]. It is estimated that two-thirds of active ingredients in today's drugs are obtained either directly by hemi-synthesis or by natural active ingredient modification [8]. Among medicinal plants of interest, *Desmodium adscendens* (Sw.), a wild herbaceous biennial plant of Fabaceae family, native to equatorial zones of Africa and Latin America, remains among the little-known species. And yet, *Desmodium adscendens* (Sw.) enjoys undisputed therapeutic efficacy in health. According to this researcher, this species has become an essential reference in Europe for its involvement in liver pathologies, and in treatments for convulsions, epilepsy, diarrhea and genito-urinary infections [9]. In Côte d'Ivoire, the country of supply, *Desmodium adscendens* (Sw.) grows naturally in industrial (oil palm, rubber, cocoa, etc.) and village plantations, but also in certain lowlands where pesticides (herbicides, fungicides, insecticides, chemical fertilizers) are abused to improve agricultural yields. In addition to chemical threats, the species is also facing increased deforestation [10,11]. So, to produce healthily and safeguard this species to meet the medical industry growing demand, mastering cultivation through domestication has become a major challenge for mankind for any plant species [12]. This approach makes it possible to control all stages in the species production, in line with international standards [13]. Among these, the technique of cultivation under shade seems to be an ideal solution [14]. However, several studies have shown that alternating light on crops strongly influences plant evolution [15]. Thus, with a view to proposing a suitable technical itinerary for this species domestication, the present study aims to determine the impact of shade variation on the evolution of some biochemical and photosynthetic parameters of *Desmodium adscendens* (Sw.) in the domestication phase in Daloa (Côte d'Ivoire).

2. MATERIALS AND METHODS

2.1 Study Site

The experimental plots were set up at the experimental farm of Université Jean Lorougnon

Guédé in Daloa. Daloa, a town in west-central Côte d'Ivoire [16]. The Daloa region soils are predominantly ferralitic, suitable for agriculture and lend themselves to all crops types [17].

2.2 Plant Material

Plant material consisted of *Desmodium adscendens* (Sw.) seed and stem (Fig. 1) harvested from palm, cocoa and rubber plantations in the Assouba locality (Côte d'Ivoire). This area is one of the major ecological zones where *Desmodium adscendens* (Sw.) grows naturally and abundantly.

2.3 Methods

2.3.1 Setting up and trial running

Setting up the trial involved first of all delimiting and cleaning the experimental plot, then designing the shade different types (accentuated shade, moderate shade and no shade). The shading system used is a kind of shed covered either entirely (roof and ¾ of the contour) with palm leaves and perforated black plastic (accentuated shading) or partially covered (only the roof) with palm leaves without plastic (moderate shading). Then, 5 L pots with perforated bottoms and filled with substrate (site soil) were used for the trial. Sowing was carried out at a rate of two seeds or two stem per pot. The stem used had an average length of 20 cm. The crop was watered regularly to keep the substrate slightly moist.

2.3.2. Experimental design

The experiment was carried out in a completely randomized block design with three replicates. Within each replication there are three blocks. Each block represents a treatment (accentuated shade, moderate shade and no shade). Blocks are spaced 10 m apart, with pots 0.5 m x 0.5 m apart. The same substrate (cultivation soil) was used in different blocks.

2.3.3. Physiological and biochemical parameters determination

Physiological and biochemical parameters were determined using fresh leaves from 90-day-old *Desmodium adscendens* (Sw.) plants. Leaves were harvested according to shade type and plant material, then stored at -80 °C for two days prior to manipulation.

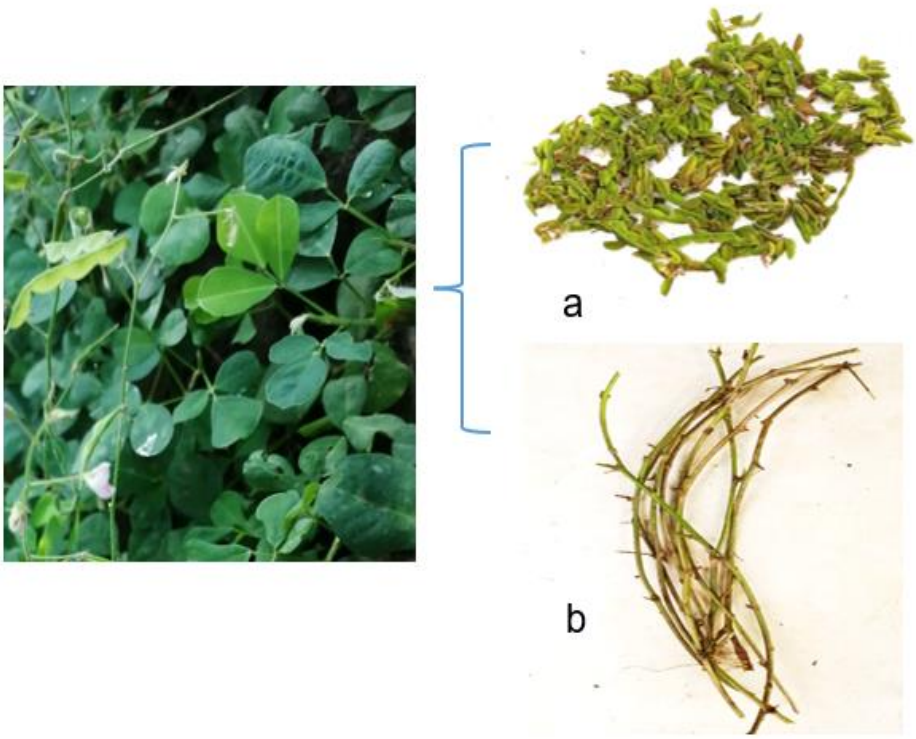

Fig. 1. Plant material, a : seeds ; b : stems

2.3.4 Extraction and assay of physiological and biochemical parameters

Leaf pigments: Chlorophyll pigments and carotenoids extraction and assay were carried out according to method described by Lichtenthaler et al. [18]. A quantity of 0.1 g fresh leaves from each sample was carefully ground in the presence of 95 % acetone. A final volume of 10 mL filtrate from each grind, stored in the dark at 4°C for 48 hours, was then obtained for assay. A 3 mL volume of filtrate was then taken for Spectrophotometer optical density (OD) readings at 663 nm and 647 nm for chlorophyll and 470 nm for carotenoid. Leaf pigment content was determined using Mc KINNEY's formula :

Chlorophyll a (µg/g FM) = 12,25*DO663 - 2,79*DO647 *(V/1000*m)
Chlorophyll b (µg/g FM) = 21,5*DO647 - 5,10*DO663 *(V/1000*m)
Total Chlorophyll (µg/g FM) = 7,15*DO663 + 18,71*DO647*(V/1000*m)
Carotenoïd (µg/g FM) = (1000*DO470 - 1,82*chl a – 8,02*chl b) /198 *(V/1000*m)

- **Phenolic compounds** : Phenolic compounds were extracted using Kouakou [19] method. Leaves

(500 mg) were ground in 10 mL methanol (96 %). The mixture was then incubated in dark for 10 h at 4 °C. After centrifugation at 5,000 rpm for 10 min, the supernatant from each grind obtained was filtered through a 0.45 µm millipore membrane and constituted the crude phenolic extract. Phenolic compounds were determined according to Singh [20] method. To this end, 0.5 mL of 5 M folin-ciocalteu reagent was added to distilled water (0.9 mL). To this was added phenolic extract (0.1 mL). Using a magnetic stirrer, the mixture was stirred at room temperature, then 1.5 mL of 17 % sodium carbonate and distilled water (6 mL) were added to the solution. The resulting solutions were incubated for 30 minutes in a water bath. The coloration intensity (proportional to polyphenol concentration) was read with a spectrophotometer at 765 nm. Total phenol content was determined using a calibration curve with different concentrations of a gallic acid stock solution (200 µg/mL).

- **Total sugars** : Total sugars were extracted in the same way as phenolic compounds.

Total sugars were determined using Dubois et al. [21] method. This method was modified and adapted to plant material. A solution of concentrated sulfuric acid (H2SO4) was used to break the osidic bonds between D-glucose and D-65 fructose, bringing into solution all the sugars present, which were then revealed by phenol. The reaction medium (0.2 mL 5 % phenol and 0.2 mL phenolic extract), made up to 1 mL with distilled water, was then made up to 1 mL with concentrated sulfuric acid (97 %). The final solution was incubated for 5 min in a water bath, then cooled in dark for 30 min. The coloration intensity produced by the reaction was measured with a spectrophotometer at a wavelength of 490 nm against a control containing no sugars. Optical density was converted to total sugars and then expressed in $\mu g.g^{-1}$ of fresh matter using a calibration line (0.01 to 0.1 $mg.mL^{-1}$) constructed from a glucose solution ($1mg.mL^{-1}$).

- **Proteins** : Proteins were extracted and assayed according to Bradford et al. [22] method.

Protein extraction was carried out cold (4 °C). To this end, 500 mg fresh leaves were ground in 0.05 g polivinylpirrolidone (PVP) and 5 mL 0.1 M sodium phosphate buffer (pH 7.9). The resulting grindings were centrifuged at 10,000 rpm for 30 min, and the supernatant obtained constituted the crude extract. Thus, 5.1 mL reaction medium (containing 0.1 mL crude extract and 5 mL Coomassie blue solution) was used. Coomassie blue solution was prepared as follows: 0.2 g Coomassie blue dissolved in 10 mL 95 % ethanol to which 20 mL orthophosphoric acid was added, then the final volume was adjusted to 200 mL with distilled water. The reaction mixture was incubated in an ice bath in the dark for 30 min. Protein content was read by spectrophotometer at 595 nm. In the control tube, the extract was replaced by phosphate buffer. Bovine Serum Albumin (BSA) solution ($250\ \mu g.mL^{-1}$) was used as the reference protein solution. The protein level was determined using the calibration curve, and expressed in micrograms per gram of fresh matter ($\mu g.g^{-1}$ of fresh matter).

- **Proline** : Extraction and determination of proline were carried out according to Dreir and Goring [23] method.

Fresh leaves (100 mg) were ground in 3 mL of methanol 40 %, then placed in a water bath at 85 °C for 30 min. After cooling in melting ice and centrifugation at 4,000 rpm for 10 min, 1 mL of supernatant was removed and 1 mL glacial acetic acid, 25 mg ninhydrin and 1 mL of mixture

(120 mL distilled water, 300 mL acetic acid (CH3COOH) and 80 mL phosphoric acid (H3PO4)) added. The mixture was homogenized by vortexing and boiled at 100 °C for 45 min, until the red color changed. After cooling, 5 mL toluene was added to solution, which was then stirred and left to stand for 30 min. A 3 mL volume of supernatant was collected for spectrophotometric reading of the optical density (OD) at 528 nm. Proline concentration (C) was calculated using the following formula : C (mM/g FM) = DO/Ɛ∗L

Ɛ = 0,9986 $mM.L^{-1}.cm^{-1}$; L : Spectrophotometer tube length (cm).

2.3.5 Enzymatic proteins extraction, purification and assay

A total of six enzyme proteins, grouped into three types according to function, were assayed. These were : Phenylalanine ammonia-lyase PAL and tyrosine ammonia-lyase TAL (phenolic biosynthesis enzymes) ; polyphenoloxidase PPO and peroxidase POD (phenolic degradation enzymes) ; catalase CAT and ascorbate peroxidase APX (antioxidant enzymes).

Extraction and purification : The six enzymes were extracted in the same way as [24]. To this end, fresh leaves (500 mg) were ground in 5 mL of 0.1 M phosphate buffer, 0.05 g of PVP and 0.1 mL of a solution composed of 5 % polyethylene glycol 6000, 0.25 % sodium thiosulphate, 15 % glycerol, 1 mM EDTA and 15 mM mercaptoethanol. After centrifugation at 5000 rpm for 20 min, the supernatant obtained constituted the crude enzyme extract. Enzyme extracts were purified using a DOWEX column. DOWEX was homogenized in enzyme extract, followed by 30 min incubation in the cold. The mixture was then centrifuged at 5000 rpm for 10 min. The supernatant obtained was the purified enzyme extract.

- **Polyphenoloxidase :** Polyphenoloxidase (PPO) assay was performed according to Zhou et al. [25] method. A 3 mL reaction volume consisting 0.2 mL enzyme extract, 1 mL pyrocatechol and 1.8 mL 0.1 M phosphate citrate buffer pH 6.5 was prepared for the assay. Pyrocatechol oxidation was read by spectrophotometer at a wavelength of 500 nm. PPO activity was expressed as enzymatic activity per gram of fresh material (mmol/min/g fresh material), assuming that the molar extinction coefficient of product formed is equal to 1400 M^{-1} cm^{-1}. The assay was carried out for each leaf sample from each shade and material.

- **Peroxidase :** Peroxidase activity (POD) was determined using Santimone [26] method, modified and adapted to plant material. Sodium phosphate (0.1 M) at pH 7.5 was used as phosphate buffer. The reaction mixture consisted of 0.1 mL enzyme extract, 2.9 mL substrate (10^{-2} M guaiacol solution and 10^{-2} M (v/v) hydrogen peroxide (H2O2)). The reaction mixture was stirred and incubated in the dark for 10 min. When adding the enzyme extract to substrate, a one-minute interval was left between each tube. The guaiacol oxidation was read with a spectrophotometer at 470 nm and expressed as enzyme activity per gram fresh material (mmol/min/g fresh material). A control was performed in which the substrate was replaced by 0.1 M sodium phosphate buffer pH 7.5.

- **Determination of phenylalanine ammonia-lyase and tyrosine ammonia-lyase :** Phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) was carried out using Regnier [27] method, modified and adapted to plant material. The base buffer used was 0.2 M sodium borate at pH 8.8. The assay was performed with 0.1 mL enzyme extract, 1 mL 0.1 M phenylalanine for PAL or 0.1 M tyrosine for TAL and 1.9 mL 0.2 M sodium borate buffer at pH 8.8. The reaction mixture was incubated at room temperature for 30 min, and the activities of PAL and TAL were read spectrophotometrically at 290 nm. PAL and TAL activities were expressed as enzyme activity per gram of fresh material (mmol/min/g fresh leaves), assuming that the molar extinction coefficient of the cinnamic acid formed is 19600 M^{-1} cm^{-1} and that of p-coumaric acid is 17600 M^{-1} cm^{-1}.

Ascorbate peroxidase and catalase : Catalase and ascorbate peroxidase activities were determined using Zhou et al. [25] method. This was modified and adapted to plant material. Ascorbate peroxidase assay was carried out using 3 mL reaction volume (0.1 mL enzyme extract and 2.9 mL ascorbic solution). Ascorbate peroxidase activity was read

spectrophotometrically at 290 nm against a blank made with tris-HCL buffer. Activity was expressed in nkat/min/g fresh material. Catalase assay was performed with 3 mL of reaction medium (0.1 mL enzyme extract, 1 mL H2O2, and 1.9 mL Tris-HCl buffer). A control assay was carried out in which hydrogen peroxide (H2O2) was replaced by Tris-HCl buffer. Catalase activity was read spectrophotometrically at 240 nm and expressed in mmol/min/g fresh material. The product molar extinction coefficient formed at the 240 nm wavelength is 36.10^{-6} $M^{-1}.cm^{-1}$.

2.3.6 Data statistical analysis

The chlorophyll pigments, carotenoids, proteins, total sugars and proline contents obtained at leaf level for each factor considered were processed and subjected to analyses of variance using IBM SPSS software. In the event of a significant difference at 5 % threshold, a post hoc test (Newman - Keuls) was performed to classify the different means obtained.

3. RESULTS

3.1 Shading and Plant Material Effect on Leaf Pigment Synthesis

Results analysis revealed that shading had a highly significant effect ($p< 0.01$) on chlorophyll pigments and carotenoids (Caro) content evaluated in *Desmodium adscendens* (Sw.) leaves (Table 1). The different types of shading all stimulated leaf pigment synthesis. Chlorophyll a (Chl a) and carotenoid content increased with decreasing shading intensity. Chlorophyll b (Chl b), however, increases proportionally with shade intensity. Moreover, the highest values of Chl a, Chl b and Carotenoids were observed with seedlings grown from stems.

3.2 Shading and Plant Material Effects on Physiological and Biochemical Parameters

Shade variation had a highly significant effect ($P<0.05$) on the content of phenolic compounds, total sugars, protein and proline (Table 2). Moderate shading favoured the synthesis of phenolic compounds and total sugars. However, protein and proline synthesis increased in the absence of shading. In short, the plants grown from the stem had high levels of phenolic compounds, total sugars, protein and proline.

3.3 Shading and Plant Material Effect on Enzyme Protein Activity

The results of the analysis showed that shading has had a highly significant effect ($p < 0.05$) on the enzymes activity studied (Table 3). The enzymes phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) were more active in shade than in open air. Peroxidase (POD) activity increased with increasing shade. Catalase activity increased with decreasing shade. Overall, the highest enzyme activities were recorded in plants grown from seeds.

Table 1. Leaf pigment content as a function of shading and *Desmodium adscendens* (Sw.) plant material

		Pigment content (ng.g^{-1} fresh leaves)			
Shade type	**Plant material**	**Chl a**	**Chl b**	**Chl t**	**Caro**
Accented	Stems	2.80 ± 2.09 ^{a}	8.50 ± 1.49^{c}	11.31 ± 2.94^{ab}	1.12±0.65^{a}
	Seeds	2.74 ± 1.55^{a}	8.21 ± 3.10^{bc}	10.96 ± 3.96^{ab}	1.01±0.41^{a}
Moderate	Stems	4.99 ± 2.17^{c}	7.94 ± 3.03^{bc}	12.93 ± 4.30^{b}	1.80±0.59^{b}
	Seeds	3.36 ± 1.64^{b}	4.77 ± 1.02^{a}	8.14 ± 2.27^{a}	1.08±0.41^{a}
Unshaded	Stems	5.01 ± 1.27^{c}	5.83 ± 0.91^{ab}	10.84 ± 2.09^{ab}	1.24±0.31^{a}
	Seeds	4.48 ± 1.21^{bc}	6.16 ± 0.98^{b}	10.62 ± 1.98^{ab}	1.02±0.32^{a}
F		3.497	5.261	2.464	3.7
P		0.001	0.001	0.046	0.001

In a column, means followed by the same letter are not significantly different at 5 % probability according to the Newman-Keuls test. P : P-value of the tests ; F : Fischer constancy ; ng : nanogram.

Table 2. Physiological and biochemical compound content of *Desmodium adscendens* (Sw.) leaves as a function of shade and plant material

Shade type	Plant material	Physiological and biochemical content (ng.g-1 fresh leaves)			Proline (mmol/g fresh leaves)
		phenolic compound	sugars	protein	
accented	Stems	4.02 ± 3.96[a]	23.1 ± 23.10[a]	110.41 ± 22.67[a]	0.30 ± 0.14[a]
	Seeds	9.86 ± 2.93[ab]	29.86 ± 10.25[b]	141.5 ± 23.55[b]	0.22 ± 0.04a
moderate	Stem	18.6 ± 4.06[c]	40.81 ± 3.25[c]	130.55 ± 16.83[ab]	0.55 ± 0.18[a]
	Seeds	19.18 ± 16.95[c]	30.15 ± 14.15[b]	130.75 ± 14.90[ab]	0.32 ± 0.07[a]
unshaded	Stems	14.2 ± 5.23[b]	35.51 ± 12.65[bc]	163.28 ± 39.79[c]	5.36 ± 6.04[c]
	Seeds	15.54 ± 4.55[b]	27.94 ± 4.33[ab]	161.16 ± 34.12[c]	2.81 ± 3.12[b]
F		4.524	3.574	5.111	5.112
P		0.002	0.008	0.001	0.001

In a column, means marked with the same letter are not significantly different at 5 % probability according to the Newman-Keuls test. P : P-value of the tests ; F : Fischer constancy.

Table 3. Enzyme activities in *Desmodium adscendens* leaves as a function of shade and plant material

		Enzymatic activity (mmol/min/g fresh leaves)				Activité enzymatique (mol/min/g fresh leaves)	
Shade type	Plant material	PAL	TAL	PPO	POD	APX	CAT
accented	Cuttings	0.52 ± 0.10[bc]	2.18 ± 0.15[a]	7.05 ± 1.51[ab]	696.64 ± 125.95[c]	1064.7 ± 382.05[c]	6332.98 ± 1353.13[a]
	Seeds	0.61 ± 0.10[c]	2.28 ± 0.26[a]	4.61 ± 1.23[a]	850.02 ± 42.06[d]	851.57 ± 98.89[ab]	5391.58 ± 854.88[a]
moderate	Cuttings	0.45 ± 0.007[b]	2.4 ± 0.16[a]	6.04 ± 1.14[ab]	508.27 ± 183.72[b]	1010.7 ± 361.01[c]	7384.4 ± 1328.86[ab]
	Seeds	0.54± 0.006[bc]	2.26 ± 0.10[a]	7.73 ± 3.74[b]	379.65 ± 261.74[b]	784.42 ± 114.19[a]	10376.56 ±4068.33[c]
unshaded	Cuttings	0.48 ± 0.8[b]	2.67 ± 0.37[b]	6.16 ± 1.66[ab]	68.17 ± 25.73[a]	917.35 ± 136.23[b]	9042.44 ± 1673.84[b]
	Seeds	0.36 ± 0.07[a]	2.41 ± 0.12[a]	6.5 ± 1.33[ab]	47.72 ± 21.91[a]	783.13 ± 185.22[a]	9782.48±24440.95[b c]
F		9.122	5.656	2.553	47.562	2.118	7.236
P		0.001	0.001	0.04	0.001	0.04	0.001

In a column, means marked with the same letter are not significantly different at 5 % probability according to the Newman-Keuls test. P : P-value of the tests ; F : Fischer constancy.

4. DISCUSSION

4.1 Shading and Plant Material Effect on Leaf Pigment Synthesis

Appropriate light intensity is a prerequisite for normal plant growth and development [28,29]. In fact, the results obtained revealed that chlorophyll a (chl a) and carotenoid contents are higher when shading decreases, unlike chlorophyll b (chl b). These results could be explained by the fact that the accentuated shade prevents the plant from carrying out photosynthesis normally. As chl b is an accessory pigment, it is synthesised massively to compensate for the chl a content in the accented shade. Similar results were obtained by Kambale et al. [30] on cocoa trees. They emphasised that moderate shading had a positive influence on the physiological parameters of cocoa plants. In addition, [31] found that when cocoa leaves are under heavy shade or full sun, photosynthesis is directly affected. Thus, in low light conditions, plants express developmental changes while increasing the specific leaf area to maximise the amount of light received [32]. With regard to the type of plant material, the highest Chl a, Chl b and carotenoid values were observed in

seedlings taken from stems. Stems are thought to contain sufficient quantities of plant hormones to encourage rapid emergence. This would lead to a significant synthesis of leaf pigments in the plants from the stems [33].

4.2 Shading and Plant Material Effect on Physiological and Biochemical Parameters

The results showed moderate shading favoured the synthesis of phenolic compounds and total sugars. However, protein and proline synthesis increased in the absence of shading. Phenolic compounds are thought to exert specific actions on cell growth while combating environmental stress. Suzuki et al. [34] works showed shading led to hight synthesis of phenolic compounds in *Gynura aurantiaca* leaves than in leaves obtained in full sun. In addition, [35] work on *Arabidopsis thaliana* showed that increasing shading induced the synthesis of leaf polyphenols. On another note, the levels sugar increas would be due to the hydrolysis of starch by α-amylase during photosynthesis. Thus, sugars would have an important role in plant growth. Gao et al. [36] works on *Aralia elata* (miq) and of Greer etn al. [37] on tomato has shown that the soluble sugar content is higher in plants grown in the shade compared with those grown in full sun. These results are at odds with those of Wang et al. [38] on *Ziziphus jujuba* Mill and [39] on grapevine (*Vitis vinifera*). According to them, sugar content reduction is obtained in plants grown in the shade compared with those grown in full sun. As far as protein content is concerned, the increase can be explained by the fact that proteins are involved in regulating plant metabolism. Proteins are primary metabolites that play a vital role in cell function [40,41] revealed an increase in protein content in soybean leaves in absence shading. According to them, active protein synthesis during plant development could be important for cell differentiation. In contrast, in *Aralia elata* (miq) a high protein content was recorded in leaves from the moderate shading treatment [36]. Proline is one of the most effective osmotic regulators in plants. The results of the work carried out revealed that proline content is higher in the absence of shade. This can be explained by the fact that exposure of the leaves to sunlight causes stress, leading to increased accumulation of proline. It has been shown that proline acts as an osmolyte and its increased production confirms osmotic tolerance in plants [42]. However, contrary results were recorded in *Aralia elata* (miq) [36]. They report that with a higher degree of shading there is an increase in proline content. In addition, plants from stem recorded high levels of phenolic compounds, total sugars, protein and proline. This would be justified by the fact that stem, having inherited the genotypic and phenotypic traits of the mother plant, would be more stressed by variations in sunlight intensity due to the levels of shade applied. Indeed, *Desmodium adscendens* grows naturally in humid areas and under shade.

4.3 Shading and Plant Material Effect on Enzyme Protein Activity

The results showed that the enzymes phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), ascorbate peroxidase (APX) and polyphenol oxidases (PPO) were more active in the shade than in the open air. Peroxidase (POD) activity was high with increasing shade. Catalase, on the other hand, increases with decreasing shading. This could be due to the fact that phenylalanine is an enzyme involved in the biosynthesis of phenolic compounds by converting L-phenylalanine into trans-cinnamic acid, that is the precursor for the synthesis of phenolic compounds [43]. However, [44] revealed low activity PAL in leaves from accentuated shading.

The increase in tyrosine ammonia lyase (TAL) activity is thought to be due to the high total phenol content observed. However, [45] results showed that high activity TAL is proportional to the total phenol content. The increased presence of polyphenoloxidase (PPO) in leaves under moderate and no shade is thought to be linked to the increased synthesis of the phenolic compounds. Indeed, this enzyme is responsible for degrading these compounds. Similar results reported by Benjawan et al. [46] in lettuce showed that plants exposed in full sun and those grown under 60 % shade had higher activity of the PPO than those grown under 80 % shade. In addition, [47] mentioned that PPO is more active in sunlight. With regard to peroxidase (POD), a high level of activity was recorded when the shading was accentuated. This increase would explain the strong degradation of phenolic compounds under this type of shading. Similar results reported by Wang et al. [48] revealed low peroxidase activity in soybean (*Glycine max*) leaves grown in full sun. Tests carried out on the antioxidant enzyme ascorbate peroxidase (APx) showed that its activity was higher under strong and moderate shade. These results are

contrary to those of Adriano et al. [49] who reported that ascorbate peroxidase activity increased in prunus grown in full sun. With regard to catalase, the study results showed high catalase activity with moderate shading and without shading.

Similar results were reported by Gao et al. [36] in *Aralia elata* (miq), who pointed out that 50 % shading increased catalase activity. In addition, [49] reported that catalase activity increased in prunus treated in full sun. The presence of catalase in plant cells would therefore be important in increasing resistance to oxidative stress. Also, [50] in *Anoectochilus roxburghii* reported low catalase activity when *A. roxburghii* was subjected to 60 % shading effect.

5. CONCLUSION

The aim of this study was to determine the impact of shade variation on changes in photosynthetic, biochemical and enzymatic parameters of *Desmodium adscendens* (Sw.) in the domestication phase in Daloa (Centre-West, Côte d'Ivoire). The results show that chlorophyll a (chl a) and carotenoid content decreases with shading intensity. On the other hand, chlorophyll b (chl b) synthesis is hignt under accented shading. In addition, the results obtained with biochemical compounds show that polyphenol and total sugar levels are higher under moderate shade. However, protein and proline content increased when there is no shading. With regard to the enzymes are concerned, the results show that phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) are more active in the shade than in the open air.

ACKNOWLEDGEMENTS

We would like to thank Teachers and students of the Laboratory for the Improvement of Agricultural Production at Jean Lorougnon GUEDE University in Daloa, UFR Agroforesterie for the efforts made in carrying out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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