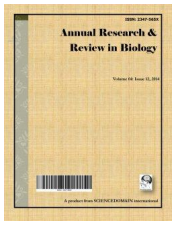

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Comparative Effects of Light and Water Stresses on Antioxidant Enzymes Activity of Three Ecotypes of *Jatropha curcas* Seedlings

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GZ and JS designed the study, wrote the protocol and interpreted the data. Authors CG and RFO anchored the field study, gathered the initial data and performed preliminary data analysis. Authors SDK and RFO managed literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Jatropha curcas is a Euphorbiaceae whose seed lipids attract biofuel industry attention. However, the effects of environmental stressors inducing resistance mechanisms and affecting the seed yield of the plant are very little investigated. In this study, we evaluated the effect of water stress and sunlight luminosity on the antioxidant enzymes activities of *Jatropha curcas*. Seeds of three *Jatropha curcas* ecotypes coming from Burkina Faso were sown in reduced sunlight luminosity

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condition in the greenhouse. A 20 day water stress and/or 20 day direct sunlight stress was applied to 33 weeks old plants. Control plants grew in the greenhouse under a reduced sunlight quantity and received one liter water daily. The activities of leaf antioxidant enzymes, catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were measured using a spectrophotometer. The results showed that plants of Sahelian zone containing the greatest proline content had the highest enzymatic activity thereby indicating a better adaptation of the sahelian ecotype of *J. curcas* to water deficit and direct sunlight luminosity. The effect of water stress on enzymatic activities was more pronounced than the effect of direct sunlight luminosity. Our results suggest that the mechanisms modulating the enzymatic activity of young plants of *J. curcas* controlled by water deficit, such as inhibition of photosynthesis and photorespiration reinforcing, were earlier set up.

Keywords: Jatropha curcas; seedlings; water deficit; sunlight; soluble proteins; antioxidant enzymes; proline.

1. INTRODUCTION

Jatropha curcas is a shrub originated in Latin America also occurred in arid areas of Africa and Asia [1]. This plant belongs to the family of Euphorbiaceae and contains much toxic latex which, besides helping resistance to water shortages, fights pathogens attacks [2]. *J. curcas* seeds are rich in oil and are considered by biofuel industries as a source of high-quality biodiesel [3,4].

In their environment, plants are more often confronted with major factors of stress such excessive sunlight, insufficient water intake, very high temperatures, air and soil pollutions, pathogens attacks. These factors of stress act independently or simultaneously, making more or less difficult for the plant to withstand and adapt to them. In response to stresses, plant is able to induce morphological, physiological, biochemical and molecular changes [6]. Cross-tolerance occurred in plants results of the fact that the resistance acquired in case of a stress can be used to face another type of stress and the antioxidant system is an example [7]. Antioxidant system acts in response to an intra-cellular oxidative stress caused by environmental stresses. Under the effect of biotic or abiotic stress, reactive oxygen species (ROS) pile up in order to compensate the over-reduction of involved molecules [8]. Among ROS, hydrogen peroxides (H_2O_2) is the most stable and plays a central role in the signaling, the development regulation and plants adaptation to biotic and abiotic stresses [9]. However, oxidative burns can occur to stressed plants and must be controlled by the antioxidant system including molecules and antioxidant enzymes for the maintenance of cellular redox homeostasis [10]. Glutathione and ascorbate are antioxidant molecules [11,12]. Catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are antioxidant enzymes [13]. These antioxidant enzymes were and still are the object of many plants studies such as *A. thaliana* because of their synergistic ability to detoxify the cell of H_2O_2 [14]. CAT uses two molecules of H_2O_2 and converts it into two molecules of H_2O and one molecule of O_2 in the cytosol, the chloroplast, the mitochondria and the peroxisome [1]. In cytosol, chloroplast and peroxysome, APX catalyses the reduction of H_2O_2 in H_2O using ascorbate as electrons donor [10]. Glutathione reductase uses NADPH to reduce glutathione in cytosol, chloroplast and mitochondria [15].

In our study, we followed the effect of sunlight stress combined with water stress on antioxidant enzymes activity, mainly CAT, APX and GR, to account for the ability of the plant to overcome such environmental stresses that can affect the seed yield.

2. MATERIALS AND METHODS

2.1 Plant Materials and Treatments

The seeds of *J. curcas* were collected at Mansila, Gampela and Bobo-Dioulasso. These localities of Burkina Faso belong to the three respective climatic zones, which are the Sahelian zone (annual rainfall average between 300 and 600 mm; annual temperature average of 29°C; seasonal temperature range of 11°C; sunshine of 6-10h/day), the Sudano-Sahelian zone (600 to 900 mm for annual rainfall average, 28°C for annual temperature average, 8°C for seasonal temperature range; 6-10h/day for sunshine) and the Sudanian zone (annual rainfall average between 900 to 1200 mm; annual temperature average of 27°C; seasonal temperature range of 5°C; sunshine of 6-10 h/day). Three seeds per ecotype were sown in each plastic pot (20 cm

deep, 22 cm upper diameter, 17 cm of lower diameter, 6 liters capacity) containing six kilograms of substrate made essentially of sand (63.51%), silt (23.42%) and clay (13.07%). Two hundred and eighty eight (288) pots containing seeds were sheltered in the greenhouse, an enclosure with transparent roof and wire-netting walls, receiving a reduced sunlight quantity average of 122.43±39.46 µmol photons $m^{-2}s^{-1}$. Each pot received 0.5 L water daily until the lifting, and then one seedling was kept in each pot. From the lifting to the stressor application, meaning 33 days after sowing, the seedlings received 1Lwater daily. Three abiotic stress factors have been considered: direct sunlight luminosity, water deficit and direct sunlight luminosity associated to water deficit.

2.2 Experimental Device

Ninety six (96) pots of plant were used for each of the three stressors (direct sunlight luminosity, water deficit and direct sunlight luminosity associated to water deficit). For each factor of stress a complete randomization block of four repetitions was taken as the experimental device. Two levels per stressor were applied to the three ecotypes and each repetition involved four sub-repetitions for each ecotype. The experimental device was set up in the Sudano-Sahelian zone, at the University of Ouagadougou with GPS coordinates of 1°49' west longitude and 12°37' north latitude. The greenhouse was used as a control condition for the light factor (sunlight quantity of 122.43±39.46 µmol photons $m^{-2}s^{-1}$and 1 L water daily) as compared with non-greenhouse conditions (572.36±70.67 µmol photon $m^{-2}s^{-1}$ and 1 L water daily). Water stress was applied in the greenhouse by depriving plants of water and comparing them with control plants which receive 1 L water daily. The third stressor application consisted in exposing plant to excessive sunlight luminosity of 572.36±70,67 µmol photon $m^{-2}s^{-1}$ and depriving them of water (outside the greenhouse), and comparing them with their control plants which grew on reduced sunlight quantity (122.43±39.46 µmol photons $m^{-2}s^{-1}$) and received 1 L water daily (inside the greenhouse). The study covered a total period of March to April 2011. Each stressor was applied for 20 days.

2.3 Extraction and Determination of Soluble Proteins

For proteins extraction, a 100 mg of leaf tissue was harvested into liquid nitrogen the 21st day following the stressor application and stored at -80°C. On removal of the -80°C freezer, the samples were immediately placed in a mortar cooled with liquid nitrogen for total soluble proteins extraction [16]. In addition of 20 mg of polyvinyl pyrrolidone, frozen the leaf was ground with a pestle in 1.5 ml of cold extraction buffer (0.1 mM NaH_2PO_4, 1 mM EDTA, pH 7.5, 0.1 M ascorbate). The ground tissue was transferred in a 2 ml Eppendorf tube. Two (2) ml Eppendorf tubes containing 1.2 ml of acetone 80% each received 50 µl of the ground tissue for next chlorophyll assay and the remaining extract then spun at 4°C for 10 min. Five hundred (500) µL of supernatant were deposited on a NAP-5 column for proteins desalting. 1 ml of phosphate buffer (0.1 mM NaH_2PO_4, 1mM EDTA, pH 7.5) was used to elute fixed proteins [16]. The resulting extract was stored on ice for as little time as possible prior to taking absorbance readings.

The protein dosage was carried out with a spectrophotometer at 595 nm using the method of Bradford [17]. Different concentrations (0 à 10 µg) of bovine serum albumin containing 1ml of Coomassie blue were used to establish the calibration range. For each sample, 50 µl of diluted extracts (for 0.7< absorbance <2) were mixed to 1 ml of Coomassie blue and stored for 10 minutes in the dark before the absorbance reading [17]. The spectrophotometer directly gave the protein content then expressed in mg protein.g^{-1} fresh the leaf.

2.4 Determination of Chlorophyll Content

The total chlorophyll were extracted from 1.2 ml of acetone 80% in 50 µl of grinding and based on Queval et al. [16] method. After 3 hours standing away from light, a 10 minute centrifugation at 14,000 rpm was performed. The collected supernatant was used to read the absorbance in a spectrophotometer at the two wave lengths of 645nm and 663 nm against a blank composed of acetone 80% [16]. The total concentration of chlorophyll is determined by the formula: Chlorophyll concentration = $8.02 \times A^{663nm} + 20.2 \times A^{645nm}$ and expressed in mg chlorophyll.g^{-1} fresh leaf).

2.5 Determination of Proline Content

The proline assay adapted to the leaf was performed according to [18] method. At 510 nm the absorbance of the leaf extract and the absorbance of standard proline solutions to 32 µg.ml^{-1} were taken to deduce the proline content

(expressed in µg proline.mg^{-1} protein) in the extract. Proline content (µg.mg^{-1}protein) = (A_e/A_s) x (M_s/M_{fw} x80)/T_p (A_e: absorbance of the leaf extract; A_s: absorbance of proline standard solution; M_s: masse of proline in standard solution (µg); M_{fw}: leaf fresh weight (g); T_p: protein content (mg.g^{-1} fresh leaf); 80: Factor of dilution). One hundred (100) mg of fresh leaf were ground in 4 ml of water with a mortar and pestle. Each solution (standard proline and extract) was shared in 3 tubes for absorbance readings and led to calculate a mean. A 5 ml tube contained 0.5 ml of standard proline or ground the leaf or water for the blank received 1 ml of formic acid 100% and 1 ml ethylene glycol 3%. After a vigorous mixing during 15 minutes at room temperature each tube was placed 15 minutes in a boiling bath. 2.5 ml of 2-propanol 50% were added in each tube then placed for 10 minutes in a water bath of 70°C. When the tube took the room temperature 45 minutes later 1 ml was taken of its content of the absorbance reading at 510 nm with a spectrophotometer.

2.6 Determination of Antioxydant Enzyme Activity

All of the enzyme assays were measured using a spectrophotometer [19]. All samples were corrected for the amount of total protein in the extract. For CAT and GR activities, desalted protein extract resulting in the elution with phosphate buffer (0.1 mM NaH_2PO_4, 1 mM EDTA, pH 7.5) was used for the assay whereas phosphate buffer added to 0.1 M ascorbate (extraction buffer) served for APX assay [19].

2.6.1 Catalase activity

CAT expressed in mmol H_2O_2.mg^{-1}.min^{-1} was measured at 240 nm by following the decrease in absorbance of H_2O_2 due to its consumption by CAT. For each sample, an average has been calculated by taking account of two series of measurements carried out according to the amount of extract (50 µl and 100 µl). Kinetic establishment needed 930 µl (or 880 µl) of phosphate buffer (0.1 mM NaH_2PO_4, 1 mM EDTA), 20 µl of 2 M H_2O_2 and 50 µl (or 100 µl) of desalted protein extract were added at last to initiate the reaction. The blank was constituted of an aliquot containing phosphate buffer.

2.6.2 Ascorbate peroxydase activity

The assay for ascorbate peroxidase (APX) was monitored in the presence of H_2O_2 and ascorbate. The absorbance decrease of ascorbate oxidized in DHA by APX was followed at 290 nm. An aliquot of extraction buffer (0.1 mM NaH_2PO_4, 1 mM EDTA, pH 7.5, and 0.1 M ascorbate) was considered as blank. To establish a mean of absorbance, two series of measurements per sample were performed according to the amount of extract (50 µl and 100 µl). Kinetic required 890 µl (or 840 µl) of phosphate buffer (0.1 mM NaH_2PO_4, 1 mM EDTA), 50 µl of 10 mM ascorbate, 50 µl (or 100 µl) of protein extract and 10 µl of 20 mM H_2O_2 for reaction initiation. APX is expressed in µmol ascorbate.mg^{-1}protéine.min^{-1}.

2.6.3 Glutathione reductase (GR) assay

The decrease in NADPH absorbance by GR, using NADPH and oxidized glutathione (GSSG) as substrates, was followed at 340 nm. Two series of absorbance measurements per sample were performed according to the amount of extracting 50 µl and 100 µl from the calculate absorbance average. For the kinetic establishment, 930 µl (or 880 µl) of phosphate buffer (0.1 mM NaH_2PO_4, 1 mM EDTA), 10 µl of NADPH 10 mM, 50 µl (or 100 µl) of extract and 10 µl of GSSG 50 mM were added at last to initiate the reaction while the blank was of constituting of phosphate buffer. The average of absorbance led to GR unit in µmol NADPH.mg^{-1} protein.min^{-1}. In our study, the percentages of protein content, chlorophyll content, proline content and antioxidant enzymes activity in treated plant comparatively to their control plants have been considered for the results.

2.7 Statistical Analysis

The software GenStat Discovery Edition 4 –VSN International was used to analyze the results of the dosages. An ANOVA test was used to compare the variance between the averages. The test of Student Newman-Keuls was used to compare significant different averages two by two. Any variable whose P-value is less than or equal to 0.05 was considered as significant. Error bars in the figures represent the standard error.

3. RESULTS

In total, 288 plants were used for this study. Fig. 1 represents the percentage of protein, chlorophyll and proline contents in treated plants comparatively to their control plants.

3.1 Protein Content of Plants Exposed to Sunlight and/or Water Deficit

When exposed to direct sunlight luminosity, Bobo-Dioulasso plants produced the most quantity of protein corresponding to 2.7 and 1.3 fold of the protein content in Mansila and Gampela plants respectively (Fig. 1A). Protein content of Mansila and Gampela plants decreased (similarly) under water deficit (Fig. 1B) or under direct sunlight exposure associate to water deficit (more decreased protein content for Mansila) (Fig. 1C).

The association for direct sunlight and water deficit led to an increase in protein content for Bobo- Dioulasso plants (Fig. 1C). For each ecotype, the compared protein contents of the 3 stressors (direct sunlight, water deficit and direct sunlight associate to water deficit) are reported on Table 1.

Table 1 compares protein, chlorophyll and proline content of each ecotype of plant under direct sunlight exposition, water deficit and direct sunlight associated to water deficit. Direct sunlight exposure and water deficit had a synergic effect on the reduction of protein content of Mansila plants (P-value = 0,013; Table 1). Under hydrous deficit coupled or not to direct sunlight exposition, Gampela plants showed a decreased percentage of protein content opposed to the increased percentage noted in direct sunlight condition (P-value < 0,001; Table 1). For the plants of Bobo-Dioulasso, the effect of direct sunlight associated or not to water deficit increased similarly the protein content while water deficit applied to didn't affect the protein content (P-value < 0,001; Table 1).

3.2 Chlorophyll Content of Plants Exposed to Sunlight and/or Water Deficit

Chlorophyll content failed in plants of the 3 ecotypes exposed to direct sunlight luminosity coupled or not to hydrous stress (Fig. 1A and 1C). It slightly increased to Mansila plants deprived of water (Fig. 1B). The comparing effects of the 3 stress factors of the chlorophyll content of each ecotype revealed a considerable reduction caused by direct sunlight (P-value < 0,001 and P-value = 0,002; Table 1). The effect of water deficit was only marked on Mansila plants which increased their protein content of 28% (Table 1).

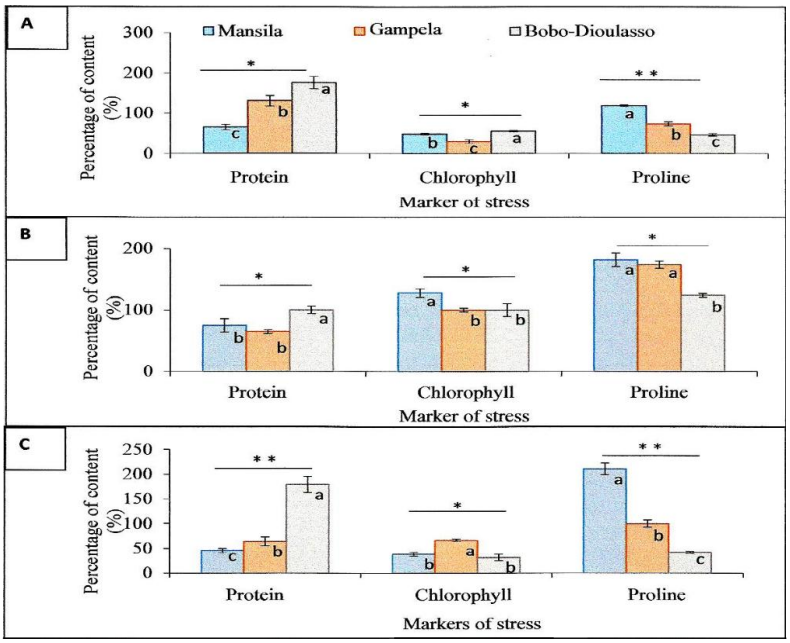

Fig. 1. Percentage of protein, chlorophyll and proline contents in treated plants relative to control plants

*A. Direct sunlight; B. Water deficit; C. Direct sunlight associated to water deficit. Averages (n = 4) in 3 successive columns assigned by the same letter do not differ among themselves on the threshold of 5%; *: P-value ≤ 0,050; **: P-value < 0,001*

Table 1. Protein, chlorophyll or proline content of each ecotype of plant under direct sunlight exposition, water deficit and direct sunlight associated to water deficit. Averages (n = 4) on a line assigned by the same letter do not differ among themselves on the threshold of 5%; %: percentage relative to the control

Parameter	Ecotype	Factor of stress			P-value
		Direct sunlight	Water deficit	Direct sunlight +water deficit	
Protein (%)	Mansila	65±7 [a]	75±11 [a]	46±4 [b]	0,013
	Gampela	131±13 [a]	65±13 [b]	64±9 [b]	<0,001
	Bobo-Dioulasso	176±15 [a]	100±6 [b]	179±16 [a]	<0,001
Chlorophyll (%)	Mansila	48±11 [b]	128±7 [a]	38±4 [b]	0,002
	Gampela	30±5 [c]	100±3 [a]	67±2 [b]	<0,001
	Bobo-Dioulasso	55±2 [b]	100±11 [a]	32±2 [c]	0,002
Proline (%)	Mansila	119±3 [c]	182±11 [b]	211±12 [a]	0,008
	Gampela	73±5 [c]	174±6 [a]	100±7 [b]	<0,001
	Bobo-Dioulasso	46±2 [b]	124±3 [a]	42±2 [b]	0,004

3.3 Proline Content of Plants Exposed to Sunlight and/or Water Deficit

According to Fig. 1A, Mansila plants exposed to direct sunlight raised their proline quantity of 19% while a drop concerned the plants of the two other ecotypes (reduction of 27% and 54% for Gampela and Bobo-Dioulasso plants respectively). When the plants were deprived of water, proline content raised for the 3 ecotypes with a greater quantity in Mansila and Gampela plants (Fig. 1B). The association for direct sunlight and hydrous deficit led to an increase in proline contents of Mansila plants and a decrease in Bobo-Dioulasso ecotype (P-value (Fig. 1C).

For plants of Mansila ecotype, direct sunlight and water deficit acted for the proline increasing (P-value = 0,008; Table 1) whereas they have antagonist actions on proline production of Gampela (P-value < 0,001) and Bobo-Dioulasso (P-value = 0,004) ecotypes. The effect of direct sunlight on the reduction of proline content is more pronounced than the positive effect (increase) of hydrous deficit for Bobo-Dioulasso ecotype.

3.4 Influence of Sunlight, Water Deficit and the Two Associated Factors on Antioxidant Enzymes

The activity of the 3 studied enzymes, catalase, ascorbate peroxydase and glutathione reductase in treated plants have been expressed in percentage relatively to the enzymatic activity in the control plants (Fig. 2). For each ecotype of plants, the comparative effects of the 3 stress factors applied are shown in Table 2.

3.4.1 Catalase (CAT) activity of plants exposed to sunlight and/or water deficit

Plants exposed to direct sunlight luminosity have reduced CAT activity to 73% for Gampela and Bobo-Dioulasso ecotypes while a rise of 49% was observed for Mansila ecotype (Fig. 2A). A decreased CAT activity was also noted for the 3 ecotypes of plants deprived of water (Fig. 2B) and for Gampela and Bobo-Dioulasso plants treated by direct sunlight exposure coupled to hydrous deficit (Fig. 2C). Considering the action of the 3 factors of stress on CAT activity of each ecotype, direct sunlight and hydrous deficit showed antagonist effects for Mansila ecotype which are characterized by an increasing rate of 49% and a reduction rate of 17% respectively (P-value < 0,001; Table 2). However, the 2 factors of stress led to a drop in the CAT activity for Gampela or Bobo-Dioulasso ecotype and had synergic action when applied together on plants (P-value < 0,001; Table 2).

3.4.2 Ascorbate peroxydase (APX) activity of plants exposed to sunlight and/or water deficit

During stressors exposure, APX activity went up in plants except Gampela and Bobo-Dioulasso plants submitted to direct sunlight which exhibited a reduction rate nearly of 27% (Figs. 2A, 2B, 2C). Then, direct sunlight and water deficit acted in synergy to enhance APX activity in Mansila plants (P-value < 0,001; Table 2).

Concerning Gampela and Bobo-Dioulasso ecotypes, plants deprived of water and associated or not to direct sunlight exposure

showed a great APX activity comparatively to the drop of activity in plants only exposed to direct sunlight (P-value < 0,001; Table 2).

3.4.3 Glutathion reductase (GR) activity of plants exposed to sunlight and/or water deficit

A decrease (of 80%) in GR activity has been observed only in Gampela and Bobo-Dioulasso plants exposed to direct sunlight luminosity whereas Mansila plants grew in GR activity (57%) (Fig. 2A).

Under the two others factors, treated plants raised GR activity 1.3 or 2.7 times more than their control plants (Figs. 2B and 2C). For Mansila ecotype, the stressors (direct sunlight, water deficit, direct sunlight associated to water deficit) enhanced considerably GR activity of plants (P-value < 0,001; Table 2) while direct sunlight and hydrous deficit had antagonist effect on GR activity of Gampela and Bobo-Dioulasso plants with a dominant effect of water deficit on GR activity increase (P-value < 0,001; Table 2).

4. DISCUSSION

In our study, the proline content was negatively associated with protein content treated plants of ecotypes. The decreased protein contents opposed to enhanced proline quantity of Mansila plants 20 days exposed to direct sunlight and in the plants of the 3 ecotypes deprived of 20 days water marks a possible degradation of proteins or an alteration in protein biosynthesis, generating proline accumulation [20,21]. It also comes from a possible neo synthesis of the amino acid [22]. According to some authors, the enhanced proline content is an adapted reaction plant to abiotic stress [23,24]. For Gampela and Bobo-Dioulasso plants, the increased protein content contrasting with the reduction of proline amount of direct sunlight quantity supposes a use of proline (and other amino acids) for the neo synthesis of proteins subjected to improve the resistance of plant against stress [25].

Particularly, direct sunlight exposure and hydrous deficit acted in synergy on proteins (decrease) and proline (increase) content in leaves of Mansila plants. However, direct sunlight had a major effect on protein (augmentation) and proline (diminution) contents of Bobo-Dioulasso plants and hydrous deficit effect was predominant on the protein accumulation for Gampela ecotype; these results reflect the synergic or antagonist effects of a stress factor on another according to the biochemical quantitative parameters and the ecotype.

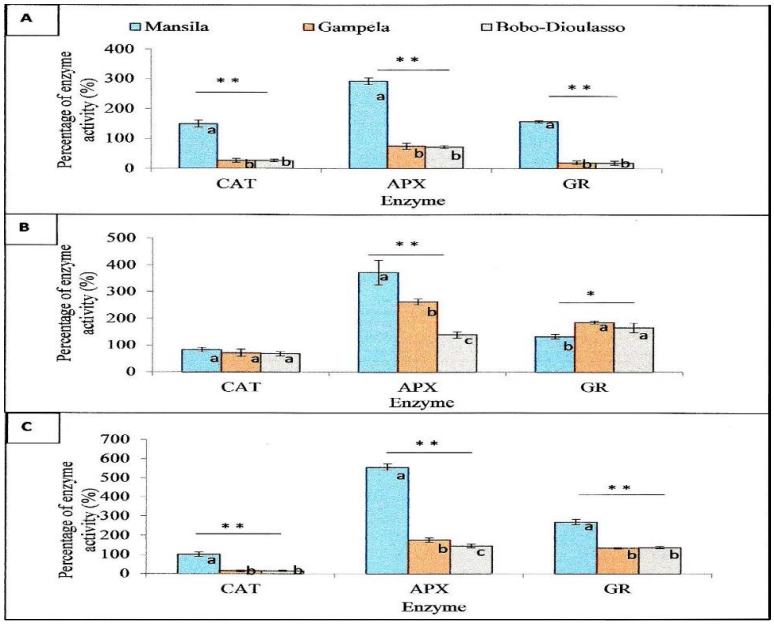

Fig. 1. Percentage of catalase (CAT), ascorbate peroxydase (APX) and glutathion reductase (GR) activity in treated plants relative to control plants

*A. Direct sunlight. B. Water deficit; C. Direct sunlight associated to water deficit Averages (n = 4) in 3 successive columns assigned by the same letter do not differ among themselves on the threshold of 5%; *: P-value ≤ 0,050; **: P-value < 0,001*

Table 2. Compare catalase, ascorbate peroxydase and glutathione reductase content of each ecotype of plant under direct sunlight exposition, water deficit and direct sunlight associated to water deficit. Averages (n = 4) on a line assigned by the same letter do not differ among themselves on the threshold of 5%; %: percentage relative to the control

Parameter	Ecotype	Factor of stress			P-value
		Direct sunlight	Water deficit	Direct sunlight +water deficit	
Catalase (%)	Mansila	149±12 [a]	83±9 [b]	100±12 [b]	<0,001
	Gampela	27±7 [b]	71±13[a]	14±4 [c]	<0,001
	Bobo-Dioulasso	27±5 [b]	68±8 [a]	15±3 [c]	<0,001
Ascorbate	Mansila	292±12 [b]	372±20 [a]	557±12 [c]	<0,001
Peroxydase (%)	Gampela	75±10 [c]	262±10 [a]	176±12 [b]	<0,001
	Bobo-Dioulasso	72±4 [b]	139±12 [a]	145±9 [c]	<0,001
Glutathion	Mansila	157±4 [c]	132±9 [b]	269±15 [a]	<0,001
reductase (%)	Gampela	20±6 [c]	185±6 [a]	133±5 [b]	<0,001
	Bobo-Dioulasso	19±6 [b]	165±18 [a]	137±6 [b]	<0,001

Chlorophyll quantity too depends on the type of stressor and ecotype. An increase (or no change) of chlorophyll amount of Mansila (or Gampela and Bobo-Dioulasso) plants influenced by hydrous deficit can be perceive as a mean to enhance (or maintain) the photosynthetic activity and protect photosystems. The drop of the chlorophyll content linked to direct sunlight associated or not to water deficit has also been observed in soybean plants under chronic water deficit [26]. This can lead to a reduction of photosynthesis. It comes probably from chlorophyll degradation or an imbalanced state between chlorophyll, amino acids and proteins production. Chlorophylls and proline having glutamate like common precursor [27], the rise of the proline content in Mansila plants and the elevated proteins amount in Gampela and Bobo-Dioulasso plants would disadvantage the accumulation of chlorophyll. The fall of the chlorophyll content only caused by direct sunlight coupled or not to water deficit suggests the control of 20 days luminosity exposure of 572 $\mu mol.m^2.s\text{-}^1$ effect on that of 20 days water deficit hydrous.

Changes in antioxidant enzymes activity have been observed according to the stressor and ecotype. Mansila plants raised CAT, APX and GR activity in order to protect the cells against oxidative burns caused by the excessive light quantity [28,29]. Gampela and Bobo-Dioulasso showed a similar decreasing in the enzymatic activity which can result in saturation of ROS on the active site of enzyme leading to its inactivation. Regarding the case of excessive excitation energy (EEE) on inactivation of antioxidant enzyme, we can suppose that Gampela and Bobo-Dioulasso plants perceive a 572 $\mu mol.m^{-2}.s^{-1}$ sunlight luminosity like a factor release of EEE. A subsequent neo synthesis of enzymes concords with the elevated protein content for the two ecotypes. In plants under water deficit, the decrease in CAT activity versus APX and GR increasing activity for the 3 ecotypes confirms the relation between these 3 antioxidant enzymes in the catabolism of excessive hydrogen peroxide (H_2O_2) in cell [30]. Stomacal closure in response to the hydrous deficit for limiting water loss from leaves reduces the dioxide carbon amount on Carboxylation site of RuBisCO and supports its oxygenase activity leading to a production of H_2O_2 in peroxisome [31]. Concerning CAT activity decrease, some studies indicated that it can be due to toxicity of ROS [32]. In our study, the decrease in CAT activity seems to be linked to excessive production of H_2O_2 molecules as catalase is the first enzyme of H_2O_2 detoxification. Then APX and GR take over for H_2O_2 detoxification by enhancing their activities. Independent to ecotype, water deficit effect was more pronounced on antioxidant enzyme activity when combined to direct sunlight suggesting that enzymes activity is better controlled by the water deficit action in *J. curcas*.

5. CONCLUSION

Our results clearly show that the activity of the studied enzymes of antioxidant system varies significantly depending on the stressor and the ecotype. Mansila treated plants showed the highest enzymatic activity and the greatest proline content which characterize a best adaptation to the abiotic stress. For *Jatropha curcas* seedlings ecotypes the effect of 20 days water deficit is predominant on that of 20 days

direct sunlight exposure. The relationship among the actions of catalase, ascorbate peroxidase and glutathione reductase in the detoxification of excessive H_2O_2 is suspected in deprived plants of water. The study of the mutants as performed by some authors contributes to the understanding of the mechanism of resistance via the antioxidant system. For example, the mutant *cat2* in *A. thaliana* which is deficient in catalase accumulates H_2O_2 molecules related to an accumulation of oxidized glutathione which leads to a rise of GR activity. Also, among the overexpressed genes in c*at2* and *cat2gr1* mutants, appear encoding gene of the enzymes APX1. Further studies would be carried out to evaluate the effect of water stress and/or sunlight stress on the expression of genes encoding the synthesis of these antioxidant enzymes in *J. curcas*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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