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Original Research Article

Expression of *APX1*, *JcHSP1* and *JcHSP2* genes in three *Jatropha curcas* L. ecotypes under light and water stress in Burkina Faso

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This study aims to evaluate the expression of genes implicated in three *Jatropha curcas* ecotypes mechanisms of protection against drought in Burkina Faso. Three drought component factors (sunlight intensity, water deficit, and sunlight associated with water deficit) and three genes including *APX1* (cytosolic ascorbate peroxidase), *JcHSP1* and *JcHSP2* (heat shock proteins) were considered. Plants were grown until 33 days after sowing in a mesh shelter with the translucent plastic roof (at 122 µmol photons.m^{-2}.s^{-1}) and receiving 1 liter of tap water every three days underwent three separate treatments for 20 days: direct sunlight at 572 µmol photons.m^{-2}.s^{-1}, watering suspension, and a treatment was combined the two previous ones. Gene expression at the leaf level was sought by reverse transcription and visualized by electrophoresis on the agarose gel. The results showed a conditioned overexpression of genes. *APX1* was associated with water stress and ecotype. *JcHSP1* and *JcHSP2* were related to all environmental factors, while *JcHSP2* was also associated with ecotype. We have also shown a predominance of the water stress effect on gene expression. Our results suggest that *APX1*, *JcHSP1* and *JcHSP2* genes are involved in the adaptive molecular response of *J. curcas* ecotypes to drought and could be "positive" molecular markers for water stress.

Keywords: *Jatropha curcas*, Ecotype, Sunlight, Water deficit, RT-PCR.

INTRODUCTION

Jatropha curcas (*J. curcas*) is a shrub in the Euphorbiaceae family that is native to Central and Southern America and is widely distributed in African and Asian countries. It is also known as desert green gold or Indian pine nut (Heller, 1996). *J. curcas* is used as a pasture hedge, medicinal plant, and biofuel source in rural areas in Burkina Faso (Bazongo

et al., 2015a; Bazongo et al., 2015b; Ouédraogo et al., 2013; Pandey et al., 2012). The plant is known to be drought resistant (Paramathma et al., 2007). However, few molecular studies have been conducted to improve this qualitative trait and, thus, productivity (Sama et al., 2018; Sama et al., 2020; Tiendrebeogo et al., 2019; Tiendrébéogo et al., 2021).

In a previous study, we evaluated the biochemical characteristics and the antioxidant activities of *J. curcas* enzymes (Ouédraogo et al., 2016). In the present work, the objective is to deepen research on the ability of the plant to withstand bad weather, most often combining water deficit and sunlight excess. Considering *J. curcas* ecotypes, the effect of two stress factors applied together or separately was evaluated on the expression of genes encoding the synthesis of *J. curcas* heat shock proteins 1 and 2 (*JcHSP1* and *JcHSP2)*, and its cytosolic peroxidase ascorbate gene *APX1*. Indeed, HSPs are involved in the plant processes in response to thermic stress (Thakur et al., 2020) and other environmental stresses (Maimbo et al., 2007; Scharf et al., 2012; Song et al., 2009). Among HSPs there are small HSPs (sHSPs) characterized by their lower molecular weight (Shaw et al., 2016; Wang et al., 2004). *JcHSP1* and *JcSHP2* are sHSPs involved in seed dehydration during the seed maturation (Omar et al., 2011). Due to the plant's resistance to drought, the expression of these genes was investigated in this study. As for *APX1*, an enzyme involved in the response to oxidative stress linked to environmental hazards (Yoshimura et al., 2000; Li et al., 2005; Li et al., 2005; Yoshimura et al., 2000), its expression was sought in plants affected by drought factors with the view to deepening the findings of the study carried out on its enzymatic activity (Ouédraogo et al., 2016).

MATERIAL AND METHODS

Plant material and study site

Seeds from Mansila (in the Sahelian zone), Gampela (Sudano-Sahelian zone), and Bobo-Dioulasso (in the Sudanese zone) were used. The study on molecular parameters was carried out at the Pietro Annigonni Biomolecular Research Center (CERBA) and at the Molecular Biology and Genetics Laboratory (LABIOGENE) at the UFR / SVT (Training and Research Unit in Life and Earth science) of Joseph Ki-Zerbo University. Plant leaves of each ecotype previously-stored in the cold at -80°C were used as vegetal material.

Seeds sowing

Under a mesh shelter with a translucent plastic roof, the greenhouse, 05 kg of soil composed of sand (63.51%), clay (23.42%), and silt (13.07%), were placed in pots and watered to field capacity (1 liter) the day before sowing. Five seeds per ecotype (Mansila, Gampela, Bobo-Dioulasso) per pot were sown. The plants generated were watered with 1 liter of tap water every 3 days before removing them 14 days after sowing (DAS). Each plant per pot was watered at the same rate up to 33 DAS.

Plant treatment

Three treatments of 20 days each were applied from 33 DAS. The light treatment consisted of placing the plants of each ecotype outside the greenhouse (with 122 µmol photons.m^{-2}.s^{-1}) to 572 µmol photons.m^{-2}.s^{-1} (direct sunlight condition). The treatment with water resulted in suspending the watering of plants in the greenhouse (water deficit condition). The treatment combining the two environmental stress factors considered plants outside the greenhouse with watering suspension (direct sunlight combined with water deficit). The control condition was the condition of plants that are watered with one liter every 03 days in the greenhouse.

Experimental device

A block with total randomization with four repetitions constituted the experimental system. The three stress factors used were each at two levels. The two light levels were exposure to attenuated solar luminosity in the greenhouse (122 µmol photons.m^{-2}.s^{-1}; control condition) and to direct solar luminosity outside the greenhouse (572 µmol photons.m^{-2}.s^{-1}) for 20 days. In the greenhouse, daily watering with 1 liter of water (control) and watering suspension for 20 days were the two levels of treatment with water. The two levels associated with the third stress factor were, on the one hand, the exposure to attenuated sunlight simultaneously with a daily watering with 1 liter of water (control). On the other hand the combination of direct solar illumination with water deficit for 20 days. For each stress factor, each of the four repetitions contained four sub-repetitions of treated plants and 96 pots were used to apply each factor on the plants of the three ecotypes (i.e. 2 levels of treatment × 3 ecotypes × 4 repetitions × 4 sub- repetitions).

Extraction of total ribonucleotide acids (RNA)

RNAs were extracted with Cetyltrimethylammonium bromide (CTAB) associated with purification on a silica column as previously described (Sangha et al., 2010). Adapted to the leaves, it consisted in grinding in liquid nitrogen, 100 mg of fresh leaf material, then transferring the ground material into a tube by adding 1 ml of extraction buffer (2 % CTAB, polyvinylpyrrolidone PVP-40, 100 mM Tris HCl pH 8, 25 mM EDTA, 2 M NaCl and 2% β-mercaptoethanol) preheated at 65°C. The mixture was incubated for 30 minutes in a water bath at 65°C. Then, 1 ml of chloroform: isopropanol (24: 1) was added for nucleic acid precipitation. After centrifugation, the supernatant was precipitated again. The supernatant containing the nucleic acids was mixed with 100µl of absolute ethanol, and the mixture was filtered on a silica micro-column according to

Table 1. Genes and primers used

Gene (Genebank accession numbers)	Forward and reverse primers sequences	Size in base pair
APX1 (FJ619044)	For: 5'-ATGGCTAA GAACTATCCA AAAGTAAGCG AAGAGTA-3' Rev: 5'TTAGGCATCAGCAAATCCCAGCTCTG-3'	753
JcHSP1 (FM894116)	For: 5'-ATGGCGT ACTCGGTTG CTCTTAA-3' Rev: 5'-GT-GTTCT GTTCCACGG AGACCTT-3'	842
JcHSP2 (FM891678)	For: 5'-TTCCCTTC ATCTTCTTCC CTCGTC-3' Rev: 5'-TGAACTTG CCACTGCTAC GCTCC-3'	956
ACTIN2 (MM894455)	For: 5'-TGCAGACC GTATGAGCAA GGAGATC-3' Rev: 5'-CCA-GAGGGA CCATTACAGTTGAGCC-3'	758

APX1: cytosolic ascorbate peroxidase 1 gene; *JcHSP1* and *JcHSP2*: heat shock protein gene 1 and 2; *ACTIN2*: actin2 gene.
PCR programs for gene amplifications are shown in Table 2. PCR products were kept at 4°C or stored at -20°C.

Table 2. PCR programs for genes amplification

Gene	PCR steps	Temperature	Time	Number of cycle
APX1	Initial denaturation	95°C	5 min	1
	Denaturation	95°C	1 min	
	Annealing	60°C	1min	40
	Extension	72°C	1min	
	Final Extension	72°C	10 min	1
JcHSP1	Initial denaturation	95°C	3 min	1
	Denaturation	95°C	30 sec	
	Annealing	54°C	50 sec	40
	Extension	72°C	50 sec	
	Final Extension	72°C	7 min	
JcHSP2	Initial denaturation	95°C	3 min	1
	Denaturation	95°C	30 sec	
	Annealing	59°C	50 sec	40
	Extension	72°C	50 sec	
	Final Extension	72°C	7 min	1

APX1: cytosolic ascorbate peroxidase 1 gene; *JcHSP1* and *JcHSP2*: heat shock protein gene 1 and 2; *ACTIN2*: actin2 gene.

the QIAGEN RNA extraction protocol (QIAGEN, Hilden, Germany).

Reverse transcription and semi-quantitative Polymerase Chain Reaction (semi-quantitative RT-PCR)

For reverse transcription was done with an Invitrogen commercial kit (Thermo Fischer Scientific, Germany). Briefly, for each sample, 1 µl of oligo-dT was added to 1 µg of RNA adjusted with water to obtain a final volume of 10 µl in the 1.5 mL Eppendorf tubes. The tubes were incubated for 5 minutes at 65°C, then placed on ice. Ten microliters (10 µl) of a mix consisting of 2 µl of buffer (qScript 10X buffer), 1 µl of dNTP, 2 µl of dithiothreitol (DTT), 1 µl of reverse transcriptase, and 4 µl of water were added to the Eppendorf tubes. The tubes containing the 20 µl of the reaction mixture of was incubated for 25 minutes at 25°C, then 20 minutes at 55°C, and finally 15 minutes at 75°C. The synthesized cDNAs were stored at 4°C.

For each sample, PCR was done in a final volume of 20 µl containing 2 µl of 1X Taq Reaction Buffer (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl2, pH 8.3), 0.4 µl of dNTP, 2 µl of each primer, 2 µl of PVP, 8.2 µl of water, 0.2 µl of DNA polymerase (Ampli Taq, Invitrogen) and 2 µl of cDNA. The primers used and the expected DNA fragments after PCR amplification for APX*1*, *JcHSP1*, *JcHSP2,* and *ACTIN2* genes are shown in Table 1. The *ACTIN2* gene, an actin synthesis gene, served as the internal control (positive) for each gene amplification by PCR.

PCR programs for gene amplifications are shown in Table 2. PCR products were kept at 4°C or stored at -20°C.

Electrophoresis and RT-PCR products quantification

After RT-PCR, 20 µl of the reaction mixture from each sample were added to 4 µl of loading buffer and then left in wells with 1% agarose gel containing Ethidium bromide (BET) for electrophoresis. The gel photographs were then processed to determine the number of pixels per band of each sample concerning the number of pixels in the band corresponding to *ACTIN2*. This helped to obtain a percentage of gene expression concerning the control gene *ACTIN2* to better reflect their face in the cell:

Percentage of gene expression (%) = (pixels number of the interest band/pixels number of the band corresponding to *ACTIN2)* × 100.

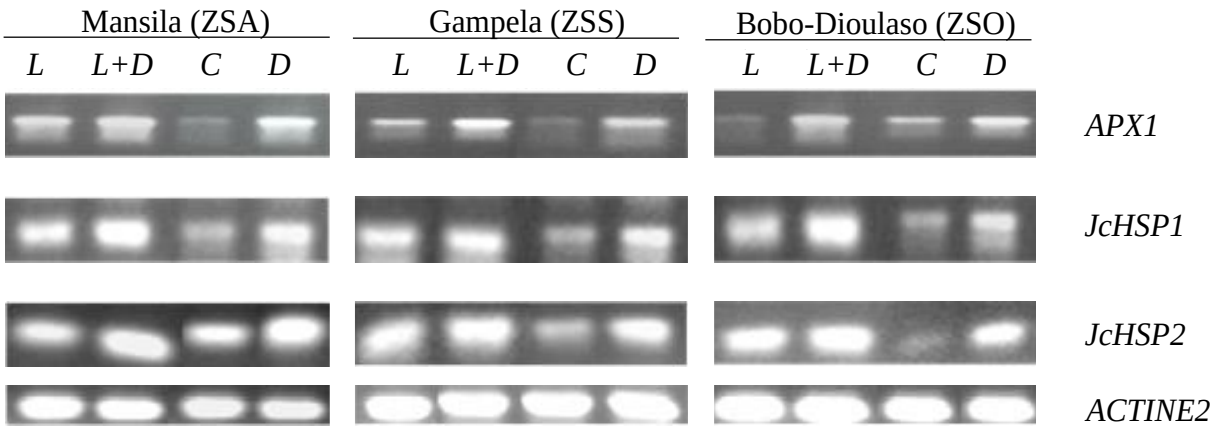

Figure 1: Expression profile of the cDNAs corresponding to *APX1* (753 bp), *JcHSP1* (842 bp) *JcHSP2* (956 bp) genes and to the control gene *ACTIN2* (758 bp). L: direct sunlight; D: Water deficit; L + D: direct sunlight + water deficit; C: control.

Statistical analysis

Data were analyzed using GenStat Discovery software Edition 4 - VSN International software. An ANOVA test was applied to compare the variance between averages. When at least one standard was significantly different from the others, the Student Newman-Keuls test was used to compare them in pairs. Any variable with a P value less than or equal to 0.05 was considered as significant.

RESULTS

The expression profile of the three genes *JcHSP1* and *JcHSP2*, *APX1*, and the positive control gene *ACTIN2* was presented on agarose gel after electrophoresis (Figure 1).

The electrophoretic profile of cDNAs shows bands of varying intensity. The bands corresponding to the *APX1* gene show, in general, a weak signal relative to the control gene *ACTIN2.*

Figures 2A, 2B and 2C show percentages of gene expression reflecting the level of expression of the three genes compared to that of the ACTIN2 control gene to better reflect their expression in the cell. Under the stress factors effect, the 3 genes showed expression percentages in the treated plants higher than in their control ones except for APX1 of the plants from Bobo-Dioulasso (6.46% for the treated plants and 11.87% for the control plants; p <0.001) and of JcHSP2 of the plants from Mansila (same level of expression between control and treated plants; p = 0.873) under direct sunlight (Figure 2A).

Expression of *APX1, JcHSP1* and *JcHSP2* stress factors

Under the direct sunlight, the JcHSP1 and JcHSP2 expression level increased, equivalent to double that observed in the control treatment, while the percentage of *APX1* expression did not change (Table 3). The water deficit, whether or not associated with excess light, increased the level of the *APX1* and *JcHSP1* genes expression three and four times more, respectively (p=0.007 and p<0.001; Table 3) compared to the. As for *JcHSP2*, its expression under water deficit conditions remained close to the value obtained in intense light but also to that noted under the combination of the two factors (p = 0.016; Table 3).

***APX1, JcHSP1, JcHSP2* expression according to the ecotype**

The percentages of gene expression in Table 4 show a significant difference between the ecotypes for *APX1* and *JcHSP2*; in fact, the plants from Bobo-Dioulasso recorded the highest level of expression with APX1 (p<0.001) and the lowest with *JcHSP2* (p=0.003). Those from Gampela and Mansila had low and similar levels for *APX1* but were distinguished by the percentage of JcHSP2 expression, which was the highest in plants from Mansila (p=0.003). No significant difference was observed between ecotypes for the *JcHSP1* gene.

Gene expression according to the gene nature

Of the three genes, *APX1* and *JcHSP1* had similar expression percentages. *JcHSP2* had the highest level of expression, representing twice the other two genes (p = 0.005; Table 5).

DISCUSSION

The band observed after electrophoresis (Figure 1) attests that *APX1*, *JcHSP1* and *JcHSP2* genes were expressed in the considered samples. The increase in the percentage of gene expression of the treated plants compared to their control

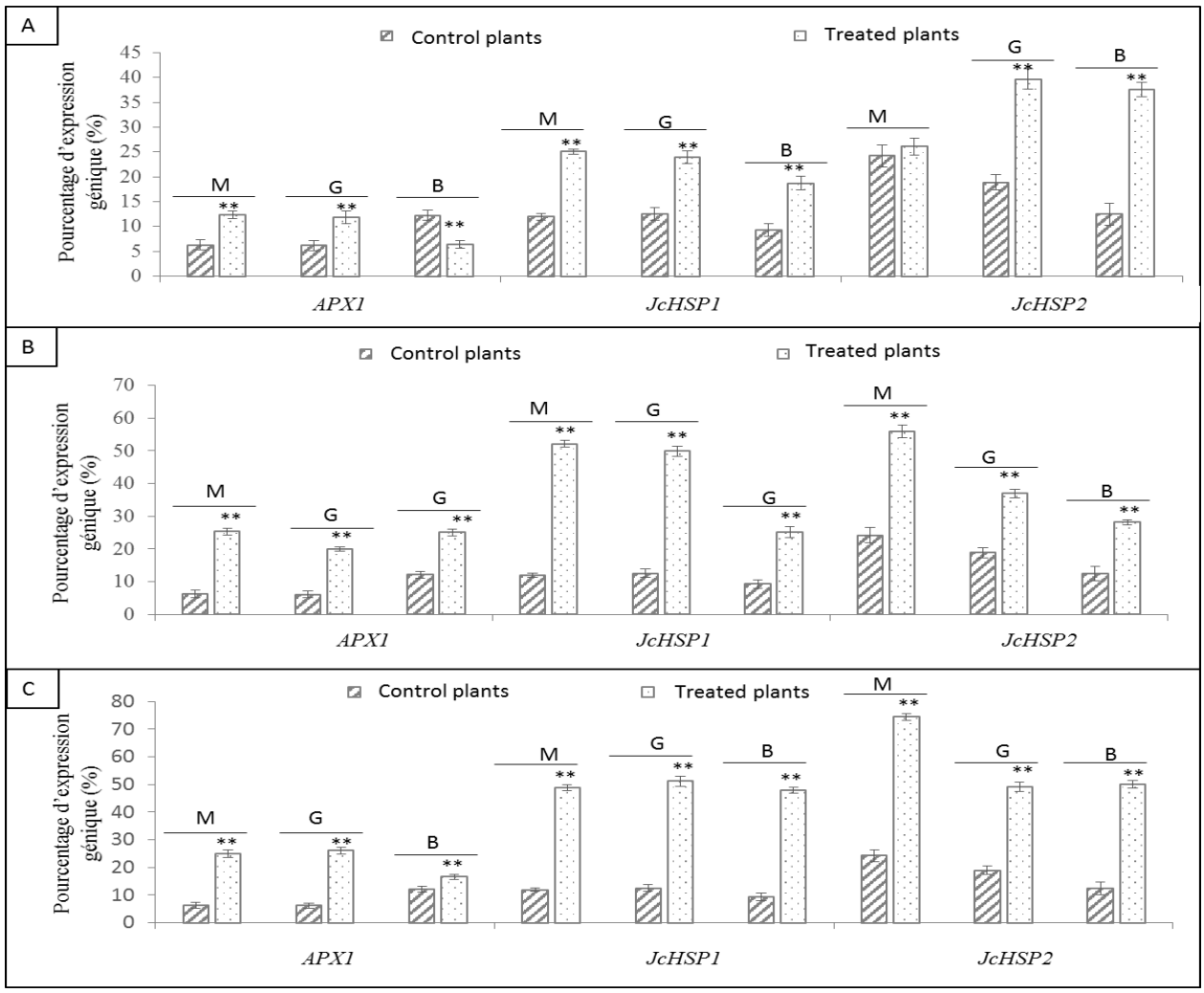

Figure 2: Percentage of gene expression. A. Under the direct sunlight factor (A); under the water deficit factor (B); under the combination of the two factors (C). M: Mansila (Sahelian zone SAZ); G: Gampela (Sudan-Sahelian zone SSZ); B: Bobo-Dioulasso (Sudanese zone SSZ). *APX1*: cytosolic ascorbate peroxidase; *JcHSP1* and *JcHSP2*: heat shock proteins 1 and 2 from *J. curcas.*

**: P-value<0.001; *P-value<0.05.

plants testifies to a gene overexpression resulting from the applied treatment. Likewise, a decrease in percentage reflects underexpression, and similar percentages indicate that the treatment did not affect gene expression. The study of genes following the stress factors that have mostly revealed gene overexpression (Figure 2; Table 3) shows the importance of these genes in responses to environmental conditions. Indeed, the direct sunlight of 572 µmol photons.m^{-2}.s^{-1} intensity stimulated an overexpression of the *JcHSP* genes, a molecular reaction in the plant in response to possible damage to the photosynthetic apparatus. This increase in gene expression level would induce the production of heat shock proteins for the remodeling of proteins denatured under the stress effect (Haq et al., 2019; Timperio et al., 2008). At the level of the photosynthetic apparatus, photosystems, which are protein-pigment complexes, are likely to damage through the production of reactive oxygen species ROS during photorespiration from excess light, like the direct sunlight in our study. The fact that the *APX1* expression did not change under applied light may be justified by the involvement of HSPs in early and efficient responses to environmental stresses. Indeed, the transcription and translation of HSPs is strongly induced at the early stage of the stress perception to promote the folding of neo-synthesized proteins or protect those denatured or aggregated by stress (Ahuja et al., 2010; Haq et al., 2019). Among these proteins are antioxidant enzymes, including APX with high activity when HSP genes are overexpressed (Driedonks et al., 2015; Li et al., 2005). It appears that the sunlight of 572 µmol photons.m^{-2}.s^{-1} applied in this study leading to overexpression of HSP is sufficient to enhance

Table 3. Gene expression under the stress factors

Treatment	Gene expression (%)		
	APX1	**JcHSP1**	**JcHSP2**
Control	8.23±2.82 [b]	11.30±1.39 [c]	18.55±4.82 [c]
Sunlight	10.24±2.68 [b]	22.50±2.74 [b]	34.47±5.97 [b]
Water deficit	23.51±2.45 [a]	41.28±12.21 [a]	40.33±11.56 [ab]
Sunlight + water déficit	22.56±4.23 [a]	49.30±1.39 [a]	57.93±11.68 [a]
P	**0.007**	**<0.001**	**0.016**

APX1: cytosolic ascorbate peroxidase; *JcHSP1* and *JcHSP2*: heat shock proteins 1 and 2 from *J. curcas.* In a column, values followed by letters are significantly different at the threshold of 5%.

Table 4. Gene expression according to the ecotype

Ecotype	Gene expression (%)		
	APX1	***JcHSP1***	***JcHSP2***
Mansila	6.32±1.06% [b]	11.95±0.72% [a]	24.25±2.25% [a]
Gampela	6.17±0.96% [b]	12.05±1.32% [a]	18.93±1.56% [b]
Bobo-Dioulasso	12.22±1.01% [a]	9.38±1.27% [a]	12.47±2.19% [c]
P	**<0.001**	0.066	**0.003**

APX1: cytosolic ascorbate peroxidase; *JcHSP1* and *JcHSP2*: heat shock proteins 1 and 2 from *J. curcas.* In a column, values followed by the same letter are not significantly different on the threshold of 5%.

Table 5. Gene expression according to the gene

	Gene		
	APX1	***JcHSP1***	***JcHSP2***
Expression (%)	8.23±2.82 [b]	11.30±1.39 [b]	18.55±4.82 [a]
P	**0.010**		

APX1: cytosolic ascorbate peroxidase; *JcHSP1* and *JcHSP2*: heat shock proteins 1 and 2 from *J. curcas.* On a line, values followed by different letters are significantly different at the threshold of 5%.

the activity of APX1 enzyme in the adaptive response. The 20-day water deficit, however, resulted in overexpression of *APX1* in addition to that of the *HSP1/2* genes. This finding attests that the three genes are also involved in the plant response to water scarcity (Caverzan et al., 2012; Faize et al., 2011; Reddy et al., 2014) and it also shows that the water deficit led to a more extensive response involving the overexpression of *APX1* for a greater action of the enzyme against the increased production of ROS. In our mentioned subsequent findings, the highest APX activity under water stress compared to light stress (Ouédraogo et al., 2016), which can so be explained here by a liked overexpression of *APX1* gene. The combined action of the two stress factors led to gene expression gene expression levels similar to those obtained under the effect of water deficit alone, thus confirming the greater effect of the lack of water on the expression of the genes studied *Jatropha curcas.*

In addition to the effect of environmental factors on gene expression, the influence of ecotype was shown in this study. Only the word of *APX1* and *JcHSP2* was ecotype-dependent (Table 4). Compared to Mansila plants, plants from Bobo-Dioulasso doubled their level of *APX1* expression while they halved their level of *JcHSP2* expression. The original environment of each ecotype could make our understanding easier. In Mansila (in SAZ), the climate is Sahelian with almost non-existent vegetation and direct sunlight; in Bobo-Dioulasso (in SOZ), the climate is more humid, wooded and shaded; in Gampela (in SSZ), the climate is Sudano-Sahelian and straddles those of Bobo-Dioulasso (SOZ) and Mansila (SAZ). It is then possible that Mansila plants have found a way to adapt to direct solar radiation by further increasing the *JcHSP2* level to overcome photorespiration and photooxidation. As for the plants from Bobo-Dioulasso, a mechanism of resistance to microorganisms favored by the humid climate is believed to be at the origin of APX1 high level, which gene is preferentially involved in the adaptation to biotic stresses (Ampomah-Dwamena et al., 2015; Montero-Barrientos et al., 2010; Pnueli et al., 2003). It should also be noted that gene expression depends on several stress factors including the nature of the stress factor, its duration, the season, the genotype, the tissue (Kozai, 2016). However, under control conditions, the high level of expression of JcHSP2 relative to other genes (Table 5) stipulates gene regulation depending

on the nature of the gene. Considering that *JcHSP1* and *JcSHP2* are involved in dehydration during the maturation process of *Jatropha curcas* seeds (Omar et al., 2011), they are also involved in the growth of *J. curcas* plants with an more marked implication of *JcSHP2*.

Conclusion

The level of *APX1, JcHSP1* and *JcHSP2* genes expression, altered under the influence of stress factors and depending on the ecotype, indicates an adaptive response of *J. curcas* plants at the molecular level. Direct sunlight of 572 µmol photons.$m^{-2}.s^{-1}$ applied to plants for 20 days only affected the *JcHSP* genes, while water deficit involved the three genes. Overexpression of the *JcHSP* genes was made possible by direct sunlight and water deficit, while *APX1* was sensitive to lack of water. Furthermore, the water deficit stimulated gene overexpression with a, more substantial effect than sunlight when the two stress factors were simultaneously applied. The study also shows that, *APX1* and *JcHSP2* genes were dependent- ecotype and that *JcHSP2* appears to be more strongly involved in the growth/development of *J. curcas* plants *JcHSP1* although both are involved in seed maturation.

Conflict of interests

The authors declare that they have no conflicting interests.

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