

Journal of Experimental Agriculture International

16(3): 1-13, 2017; Article no.JEAI.33639
Previously known as American Journal of Experimental Agriculture
ISSN: 2231-0606

Physiological Responses to Drought Stress in *Jatropha curcas* Seedlings

N. Contran^{1*}, L. Ledda^{1,2}, M. Mulas^{1,3}, R. Cerana⁴ and M. Lubino¹

¹Desertification Research Center, University of Sassari (NRD-UNISS), Viale Italia 39, 07100 Sassari, Italy.

²Department of Agriculture, University of Sassari, Via Enrico de Nicola 1, 07100 Sassari, Italy.

³Department of Nature and Land Sciences, University of Sassari, Via De Nicola 9, 07100 Sassari, Italy.

⁴Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza Della Scienza 1, 20126 Milano, Italy.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NC, LL, MM and ML designed the study and wrote the experimental protocol. Authors NC and ML conducted the field trial and carried out the majority of the data analyses. Author NC performed the statistical analysis and wrote the first draft of the manuscript. All authors critically reviewed the results, contributed to the discussions and improved the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2017/33639

Editor(s):

(1) Mariusz Cycon, Department and Institute of Microbiology and Virology, School of Pharmacy, Division of Laboratory Medicine, Medical University of Silesia, Poland.

Reviewers:

(1) Raul Antonio Sperotto, Centro Universitário UNIVATES, Brazil.

(2) Abd Allah, Rice Research and Raining Center, Field Crops Research Institute, Egypt.

Complete Peer review History: <http://www.sciencedomain.org/review-history/19183>

Original Research Article

Received 24th April 2017

Accepted 19th May 2017

Published 24th May 2017

ABSTRACT

Aims: The aim of the study was to investigate the physiological mechanisms of *Jatropha curcas* seedlings exposed to drought and the possible influence of seedling age.

Study Design: A pot experiment was carried out using a completely randomized design with two seedling ages (2- and 3-month-old seedlings), two treatments per age (Watered: fully irrigated, and Unwatered: Not irrigated), six replicates (24 pots).

Place and Duration of Study: The experiment was performed in a greenhouse facility located at the Experimental Station "Mauro Deidda" (Department of Agriculture of University of Sassari) at Ottava (Sassari, Italy) between June and September 2011.

*Corresponding author: E-mail: NiclaContran@hotmail.com;

Methodology: To investigate the responses of 2- and 3-month-old *J. curcas* seedlings exposed to drought stress on 4th, 8th, 12th, 19th, and 26th day from treatment's beginning, leaf and soil water content, biometric, gas exchange, and chlorophyll a fluorescence measurements were performed; on 26th day from treatment's beginning, biometric destructive measurements were carried out.

Results: Results support the hypothesis that *J. curcas* is appropriate to be cultivated in areas with limited water availability or prolonged periods of drought and highlight that mechanisms of drought response are highly influenced by seedling age. *J. curcas* seedlings maintained a good leaf water status by means of an effective stomatal closure, associated with a reduced aboveground growth and an increased root:shoot ratio. Under drought stress, 2-month seedlings showed a higher allocation of resources to roots compared to 3-month seedlings. Drought resulted in more detrimental effects on the photosynthetic response of 3-month seedlings, inducing the reduction of stomata conductance and the loss of photosystem II integrity. 2-month seedlings were instead able to activate mechanisms of drought tolerance through the activation of excess energy dissipation mechanisms.

Conclusion: In the early stage of crop establishment, the transplanting of *J. curcas* 2-month seedlings proved to be more effective in order to avoid water stress related consequences.

Keywords: Chlorophyll a fluorescence; gas exchange; Physic nut; water relations; water stress.

1. INTRODUCTION

The biofuel production from *Jatropha curcas* L. plantation has been considered by industries, policy makers and researchers as a possible way to implement the development, particularly in arid and semi-arid regions of developing countries [1-3]. *J. curcas* is a non-edible oil crop and its seeds contain about 25-35% of oil, which can be extracted and converted into biodiesel [4]. However, there are some concerns regarding the cultivation of *J. curcas* for biofuel production. The most critical issue is that the climatic conditions of *J. curcas* plantation spreading are often different from those of its natural distribution, and the major cultivation areas are characterized by high evaporative demand and low water availability. Consequently, *J. curcas* plants often face drought stress and plant yield is generally lower than expected [4,5].

Despite the extraordinary increase of *J. curcas* cultivation areas in the last twenty years, the scientific community has begun only recently to investigate *J. curcas* responses to limited water availability conditions in terms of biomass production and partitioning, plant-water relationships, leaf gas exchange, and osmotic adjustment [6-18]. Drought resistance of *J. curcas* is associated with its ability to maintain water in leaf and root tissues, effectively combining osmotic adjustment with stomatal control mechanisms, in order to allow a continuous growth [10,16]. Stem water is reserved for the fresh leaf flushing as well as for keeping leaves active for several weeks after the beginning of the dry season [6,7]. Similarly to

other stem succulent species with green fleshy stems, under drought conditions *J. curcas* exhibits both CAM and C4 photosynthesis in the succulent stem and CAM metabolism serves primarily to conserve carbon rather than water [19]. Drought stress probably triggers a coordinate down-regulation of photosynthesis (photochemistry and carboxylation phases), which could be modulated by accumulation of sugar and osmotically active solutes [16-18]. The energy excess at PSII level is dissipated by non-photochemical mechanisms associated with enhancement in photorespiration, restricting photo-damages [10,16]. In a recent study Tominaga et al. [20] found that *J. curcas* is able to preserve the integrity of photosystem II (PSII), when stomata are closed under drought conditions, and to regulate thermal dissipation, adjusting PSII quantum efficiency to capacity of CO₂ fixation. In parallel, the antioxidant enzymatic protection was beneficial for oxidative damage protection [8,16]. *J. curcas* root architecture facilitates the exploration of deeper soil horizons, allowing a better water access [21].

Since *J. curcas* seed germination percentage is very low after direct sowing, the best generative propagation technique is the transplanting of pre-germinated seedlings [4]. In arid and semi-arid areas, the transplanting of seedlings is generally scheduled at the end of rainy seasons, making the seedling phase a critical moment to face drought stress. Therefore, it is clear that a deeper understanding of seedling responses to drought is essential to increase *J. curcas* survival and to adopt competitive strategies for *J. curcas* production [18,22]. Additionally, previous field

activities and the management of experimental plantations in arid or semi-arid areas of Ghana and Burkina Faso have allowed us to observe a possible relationship between seedling age at transplantation time and *J. curcas* ability to overcome drought periods. Consequently, experiments with young plants are relevant for assessing the plant performance at field planting age [5,23]. For these reasons, in this study we aimed to investigate both the mechanisms of *J. curcas* seedling resistance to drought and the possible influence of seedling age on it. The responses of 2- and 3-month-old *J. curcas* seedlings, in terms of growth, water relations, leaf gas exchange, and chlorophyll fluorescence exposed to drought stress have been investigated under semi-controlled conditions also with the aim to know the best age for transplant. In addition, studies on drought responses performed on other crops have emphasized how physiological responses to drought could change according to accession. Until now studies have been focused on *J. curcas* accessions of Brazil [9], Ethiopia, India, Thailand [5,7], Indonesia, and Capo Verde Islands [18]. In order to extend our knowledge on the impact of accession on *J. curcas* responses to drought, we have used an Indian cultivar, widely (and really) used in Sub-Saharan regions of Africa, both by industrial companies and rural development programs. Finally, detailed studies on the behavior of *J. curcas* physiological responses to drought stress are gaining more attention because one of the negative effects of the climate change is the expected water shortage in the coming years [24]. Many expectations in the world are focused on the possible role of this species as a tool for aridity adaptation of plant resources.

2. MATERIALS AND METHODS

2.1 Experimental Design

The experiment was performed in the greenhouse facility located at the Experimental Station "Mauro Deidda" (Department of Agriculture of University of Sassari) at Ottava (Sassari, Italy) (40°46'47"N; 8°28'34"E, elevation 221 m asl). Air temperature and humidity inside the greenhouse were automatically recorded with a thermo-hygrograph (Model Siap, Bologna, Italy) every 2 hours from June to October 2011. Air temperature and humidity data are shown in Fig. 1.

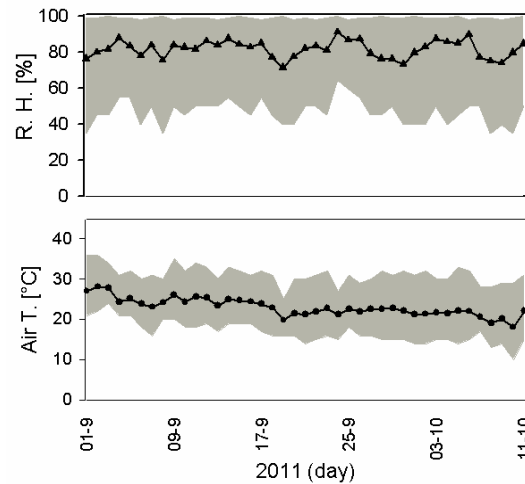


Fig. 1. Daily mean relative humidity (R.H.) and daily mean air temperature (Air T.) in 2011 during the experimental period in the greenhouse facility. Grey areas indicate daily minimum and maximum values of R.H. and Air T

The experiment was carried out on *J. curcas* seedlings of an Indian cultivar. Seeds were collected in November 2010 in Tamale (Ghana Yendi road Farm, Northern Region of Ghana), previously selected on the basis of seed size and weight, and stored at 18(±2) °C until the beginning of the experiment. *J. curcas* seeds were sown in an alveolar plastic board (5 cm diameter and 6 cm height), filled with peat amendment substrate. Sowing was carried out on 16th June (OL – older seedlings) and on 25th July (YO – younger seedlings). After 20 days from the germination, when the first leaf of seedlings completely expanded, a total of 24 seedlings (12 OL seedlings and 12 YO seedlings) were transplanted in pots (40 cm diameter and 60 cm height), filled with 25 kg of soil (2/4 of sieved soil, 1/4 of potting compost and 1/4 of agriperlite). Sieved soil was a sandy-clay-loam overlaid on limestone (Xerochrepts), with an average N content of 0.76‰, and a C/N ratio of 12. Until the beginning of the treatment, seedlings were regularly watered with 1000-1500 ml of water every 2 days. On 12th September 2011, after 2 (OL) and 1 month (YO) from transplanting respectively, seedlings were exposed to treatments: Watered (W, control treatment; 6 OL and 6 YO seedlings) or unwatered (D, drought treatment; 6 OL and 6 YO seedlings). In control treatment, seedlings have been irrigated with 1000-1500 ml of water per pot every 2 days. On the contrary, drought treatment

was imposed by withholding water. Pots were arranged in a completely randomized design. On 4th, 8th, 12th, 19th, and 26th day from treatment's beginning, leaf and soil water content, biometric, gas exchange, and fluorescence measurements were performed on 6 seedlings per treatment and per age. On 26th day from treatment's beginning, biometric destructive measurements were carried out on 5 seedlings per treatment per age.

2.2 Soil and Leaf Water Content

At each measurement day, a cylinder of 238.5 cm³ of soil was collected from each pot between 11:00 and 12:00 a.m. The sampling was always performed between seedling and pot edge to avoid edge effect, and shifting the area of sampling. The soil was immediately replaced, adding in each pot an equal cylinder of 238.5 cm³ of soil collected from a pot subjected to the same water treatment. Soil samples were weighted (soil fresh weight, sFW) and then, after oven drying at 100°C, when samples reached a constant weight (ca. 72 h), weighted again (soil dry weight, sDW). Soil water content (SWC) was calculated as: $SWC = (sFW - sDW) / sDW * 100$. Soil water potential (Ψ_s) was calculated through an empirical relationship between soil water content (SWC) vs soil water potential (Ψ_s). The relation was determined by a water-content vs water-potential curve, constructed by using a pressure plate device (Richard's pressure plate apparatus). Water-content and water-potential measurements were performed on six randomly selected soil samples in a pressure range from 0.02 to 1.5 MPa, founding the relation $\Psi_s = 81.607 * e^{(-0.3118 * SWC)}$ ($R^2 = 0.88$, for $\Psi_s < 1.5$ MPa).

Two leaf discs of 14.5 cm² were collected from one mature leaf between 11:00 and 12:00 a.m. from each plant and at each measurement day. Discs were immediately weighted (fresh weight, FW), immersed in distilled water for 4 h at room temperature, blotted dry, and then weighted (water saturated weight, TW). After oven drying at 80°C, when samples reached a constant weight (ca. 48 h), discs were weighted again (dry weight, DW). Leaf RWC was calculated as: $RWC = (FW - DW) / (TW - DW) * 100$.

2.3 Biometric Measurements

Seedling height (H), stem basal diameter at 1 cm from ground level (Ds), number of leaves (NI), number of fallen leaves (Nfl), and number of secondary branches (Sb) were measured at each measurement day. Total above dry

biomass (AB) was calculated through the allometric relationship, determined by Achten et al. [7], between AB and basal stem diameter (Ds): $AB = 0.029 * Ds^{2.328}$. Collected data were used to evaluate the accuracy of allometric relationship by a linear regression analysis between estimated total above dry biomass and total above dry biomass measured by destructive method, as described below ($R^2 = 0.87$; $p < 0.01$; $N = 20$).

After 26 days from treatment's beginning, seedlings were separated into leaves, stem, and (softly washed) roots, and the following destructive measurements were carried out: Leaf fresh weight (FWl), leaf dry weight (DWl), total leaf area (Al), stem fresh weight (FWs), stem dry weight (DWs), stem volume (Vs), root fresh weight (FWr), root dry weight (DWr), and root length (Lr). Dry weights were measured when samples at 80°C reached a constant weight (ca. 48 h). Total leaf area was measured by an Area Meter (LI-3100C Area meter, LI-Cor, Lincoln, NE, USA). Stem volume was measured by cutting the stem into small sections and by immersing it in a graduated cylinder (500 ml). The amount of displaced water was assumed equal to the volume of the stem section.

Based on destructive measurements, several indexes of seedling growth were calculated: specific leaf area ($SAI = Al / DWl$) [$mm^2 mg^{-1}$], leaf dry matter content ($DMCl = DWl / FWl$) [$mg g^{-1}$], stem specific density ($SDs = DWs / Vs$) [$mg cm^{-3}$], stem dry matter content ($DMCs = DWs / FWs$) [$mg g^{-1}$], specific root length ($SLr = Lr / DWr$) [$mm mg^{-1}$], and root:shoot ratio ($R:S = DWr / (DWl + DWs)$).

2.4 Gas Exchange Measurements

Light-saturated net photosynthesis (A_{max}) and stomatal conductance to water vapour (G_w) were measured at three experimental times: in the morning (7:00-9:00), at midday (12:00-14:00), and in the afternoon (16:00-18:00). Measurements were performed with an infra-red gas-analyser (CIRAS-1 PP-Systems, Herts, UK), equipped with a 2.5-cm² Parkinson leaf cuvette, which controlled leaf temperature (ambient ± 1 °C), leaf-to-air vapour pressure deficit (ambient ± 0.2 kPa), saturating light ($1500 \pm 20 \mu mol photon m^{-2} s^{-1}$) and carbon dioxide (CO_2) concentration ($380 \pm 10 \mu mol mol^{-1}$). Gas exchanges were measured on the central part of the 5th-7th leaf from the tip of all plants. Preliminary light curves (AQ curves) showed that light at $1500 \mu mol photon m^{-2} s^{-1}$ was saturating.

2.5 Chlorophyll a Fluorescence Measurements

Chlorophyll a fluorescence transient was measured *in vivo* in the morning (7:00-9:00), at midday (12:00-14:00), and in the afternoon (16:00-19:00) with a direct fluorometer (Handy PEA, Hansatech Instr., Kings Lynn, UK). Before measurement, leaves were dark-adapted for 40 min with leaf clips. The fluorescence rising transient was induced by saturating red-actinic light ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, peak at 650 nm, duration 1 s). Data were recorded for 1 s, starting from 10 μs after the onset of illumination. Chlorophyll a fluorescence was measured on the central part of the 5th-7th leaf from the tip of all plants. The values of F_0 , ground fluorescence yield in the dark-adapted state (when all reaction centres of PSII are considered open) and F_m , maximal fluorescence yield in the dark (when all reaction centres of PSII are considered closed), were collected. Maximum quantum yield for primary photochemistry (F_v/F_m) was calculated as $(F_m - F_0)/F_m$ [25]. Additionally, biophysical and phenomenological expressions, which quantify the stepwise flow of energy through the photosystem two (PSII), have been calculated by the quantitative analysis of the polyphasic fast fluorescence rise transient, called JIP-test [26]: performance index per absorption flux (PI_{abx}), electron transport probability (ψ_0), quantum yield for electron transport (ϕE_0), quantum yield for energy dissipation (ϕD_0), effective antenna size of an active reaction centre (ABS/RC), maximal trapping rate of PSII (TR_0/RC), electron transport in an active reaction centre (ET_0/RC), and effective dissipation of an active reaction centre (DI_0/RC) (see [27] for parameter definitions). Analysis of the transient was performed with *BioLyzer 3.06* software (by Ronald Maldonado-Rodriguez, Bioenergetics Laboratory, Geneva, Switzerland).

2.6 Statistical Analyses

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's Test). On data collected at treatment's beginning (time 0), a *t*-student test was applied to compare the effect of seedling Age (OL vs YO). On data collected 26 days after treatment's beginning, one-way ANOVA was performed considering four groups: OL-W, OL-D, YO-W, and YO-D. A Tukey HSD test was applied to discriminate the significance of mean differences between homogeneous groups.

Tests of significance were made at a 95% confidence level. Percents were arcsine-square root transformed prior to analysis. The statistical unit was the seedling. Analyses were processed using STATISTICA 6.0 Package for Windows (Stat Soft 2001, Tulsa, OK, USA).

3. RESULTS

3.1 Soil and Leaf Water Content

From June to October, daily mean air temperature inside the greenhouse facility was $24.7 (\pm 2.2) ^\circ\text{C}$, with minimum value of $17.8 (\pm 2.2) ^\circ\text{C}$ and maximum value of $33.1 (\pm 3.2) ^\circ\text{C}$, and daily mean relative humidity was $77.8 (\pm 6.2)\%$, with minimum value of $42.0 (\pm 9.0)\%$ and maximum value of $99.1 (\pm 0.6)\%$ (Fig. 2).

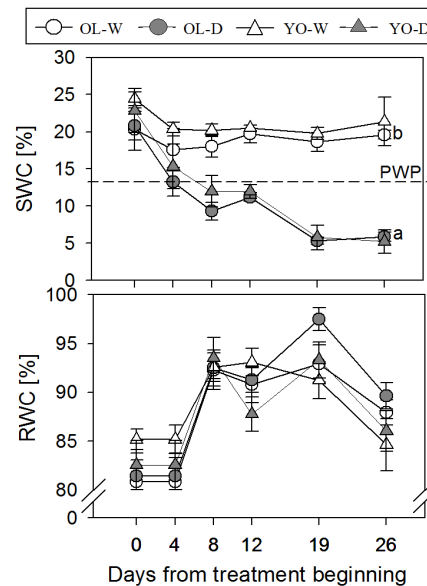


Fig. 2. Soil water content (SWC) and leaf relative water content (RWC) of 3-month-old *J. curcas* seedlings (OL - Circles) or 2-month-old *J. curcas* seedlings (YO - Triangles), exposed to watered (W - White symbols) or Drought (D - Gray symbols) treatments. Values represent means \pm S.E (N=6). The dashed line indicates the value of the soil permanent wilting point (PWP, $\Psi_s = -1.5$ MPa). At treatment's beginning (day zero), significant differences between OL and YO seedlings are marked by asterisks (Student's *t*-test; $P < .05$). At 26th day, the lowercase letters indicate significant differences among groups OL-W, OL-D, YO-W, and YO-D (Tukey HSD; $P < .05$)

Soil water content vs soil water potential curve (data not shown) pointed out the following findings: i) soil water potential of -0.015 MPa, corresponding to the conventional threshold value for field capacity, was observed at a soil water content of 25%; and ii) soil water potential of -1.5 MPa, considered as conventional permanent wilting point (PWP) for agricultural crop plants was observed at a soil water content of 13%. In the watered treatment soil water content was maintained near 20% (Fig. 2), while in drought treatment, after 8 days from treatment's beginning, the soil water content was lower than 13%, corresponding to the conventional permanent wilting point, and then it decreased until the value of 5% (Fig. 2). Despite the drastic reduction of available water for the whole period of drought stress, leaf RWC of unwatered seedlings was comparable with leaf RWC of watered seedlings ($P > .05$, Fig. 2). Additionally, no differences were observed between leaf RWC of older seedlings (OL, 3-month-old) and younger seedlings (YO, 2-month-old) ($P > .05$, Fig. 2).

3.2 Biometric Parameters

At treatment's beginning (day zero), all the biometric parameters (seedling height, stem basal diameter, number of leaves, number of fallen leaves, number of secondary branches, and total above dry biomass) were higher in OL seedlings than in YO seedlings ($P < .001$, Fig. 3).

In the course of the experiment, both in YO and OL seedlings, stem diameter and total dry biomass grew steadily in watered seedlings, while in unwatered seedlings stem diameter and total dry biomass showed little and not significant growth (Fig. 3). After 26 days of drought stress seedling height, number of leaves, and number of lateral branches per plant were not significantly influenced by drought stress (Fig. 3). Additionally, drought stress did not influence the number of fallen leaves of YO seedlings, while unwatered OL seedlings lost more leaves than watered OL seedlings (Fig. 3). Drought stress reduced total leaf area and increased specific leaf area and root:shoot ratio, while stem specific density, root length, stem dry matter content, and specific root length were not significantly influenced (Fig. 4). Unwatered OL seedlings showed lower leaf dry matter content than watered OL seedlings (Fig. 4).

3.3 Photosynthetic Activity

Gas exchange parameters (A_{max} and G_w) measured at the beginning of the treatment were higher in YO seedlings than in OL seedlings ($P < .05$, Fig. 5), with the exception of midday A_{max} . In the course of the experiment, morning and midday A_{max} and G_w of YO seedlings, both watered and unwatered, decreased and reached the values of morning and midday A_{max} and G_w of watered OL seedlings (Fig. 5). These latter values, in fact, remained constant throughout the

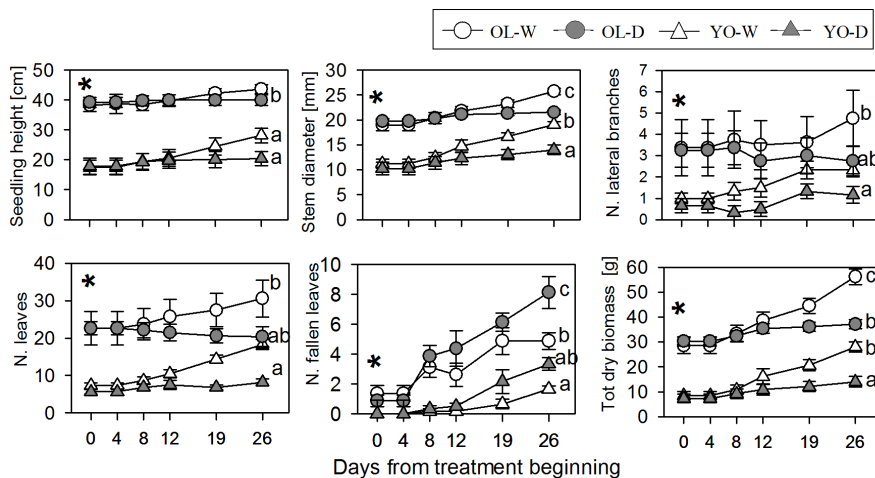


Fig. 3. Biometric parameters of 3-month-old *J. curcas* seedlings (OL - Circles) or 2-month-old *J. curcas* seedlings (YO - Triangles), exposed to Watered (W - White symbols) or Drought (D - Gray symbols) treatments. Values represent means \pm S.E (N=6). At treatment's beginning (day zero), significant differences between OL and YO seedlings are marked by asterisks (Student's t-test; $P < .05$). At 26th day, the lowercase letters indicate significant differences among groups OL-W, OL-D, YO-W, and YO-D (Tukey HSD; $P < .05$)

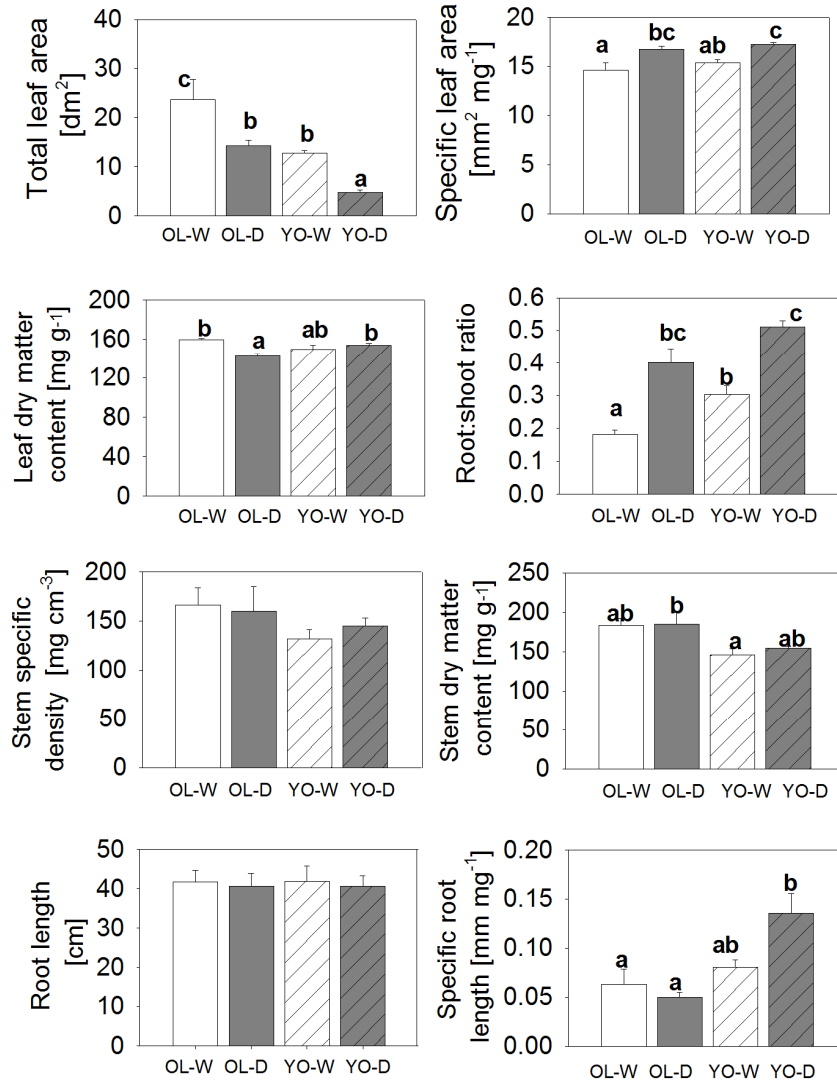


Fig. 4. Growth indexes of 3-month-old *J. curcas* seedlings (OL – no-line patterned columns) or 2-month-old *J. curcas* seedlings (YO – line patterned columns), exposed to Watered (W – White columns) or Drought (D – Gray columns) treatments. Values represent means \pm S.E. (N=5). The lowercase letters indicate significant differences among groups OL-W, OL-D, YO-W, and YO-D (Tukey HSD; $P < .05$)

whole experiment (ANOVA results for time factor of watered OL seedlings: $P > .05$, Fig. 5). Morning A_{max} and G_w of unwatered OL seedling were significantly reduced by drought stress and A_{max} reached negative values after 2 weeks of treatment, indicating that the uptake of CO_2 was lower than the output of CO_2 (Fig. 5). In the afternoon, watered YO and watered OL seedlings showed similar A_{max} and G_w values, while, both in OL and in YO, A_{max} and G_w were significantly reduced by the stress (Fig. 5).

3.4 Photochemical Activity

At the beginning of the treatment, midday F_v/F_m , morning PI_{abx} , ψ_0 and ϕE_0 and afternoon ψ_0 and ET_0/RC were higher in OL seedlings than in YO seedlings ($P < .05$, Fig. 5 and Fig. 6), while morning ABS/RC , TR_0/RC and DI_0/RC and midday ϕD_0 , ABS/RC and DI_0/RC were lower in OL seedlings than in YO seedlings ($P < .05$, Fig. 6).

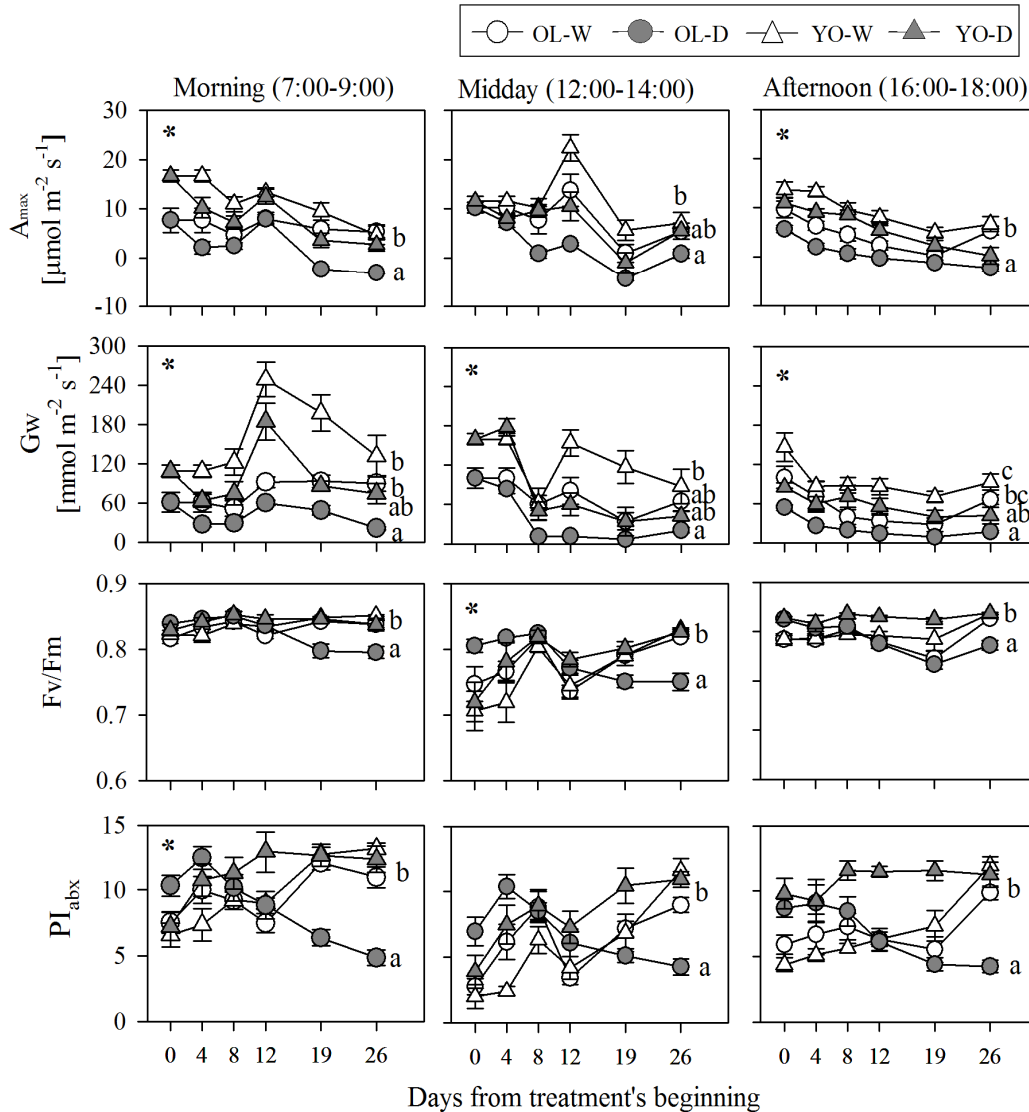


Fig. 5. Light-saturated net photosynthesis (A_{max}) and stomatal conductance to water vapour (G_w), maximum quantum yield for primary photochemistry (F_v/F_m), and performance index per absorption flux (PI_{abs}) measured in the morning, at midday, and in the afternoon, of 3-month-old *J. curcas* seedlings (OL - Circles) or 2-month-old *J. curcas* seedlings (YO – Triangles), exposed to Watered (W – White symbols) or Drought (D – Black symbols) treatments. Values represent means \pm S.E. (N=6). At treatment’s beginning (day zero), significant differences between OL and YO seedlings are marked by asterisks (Student’s t-test; $P < .05$). At 26th day, the lowercase letters indicate significant differences among groups OL-W, OL-D, YO-W, and YO-D (Tukey HSD; $P < .05$)

After 26 days of drought stress, with the exception of ET_0/RC , all the chlorophyll a fluorescence parameters were significantly influenced by drought only in OL seedlings, while in YO seedlings there were no differences between control and treated seedlings (Figs. 5

and 6). At all hours of the day, F_v/F_m , PI_{abs} , ψ_0 , and ϕE_0 were lower in unwatered OL seedlings than in watered OL seedlings, while ϕD_0 , ABS/RC , TR_0/RC and DI_0/RC were higher in unwatered OL seedlings than in watered OL seedlings ($P < .05$, Figs. 5 and 6).

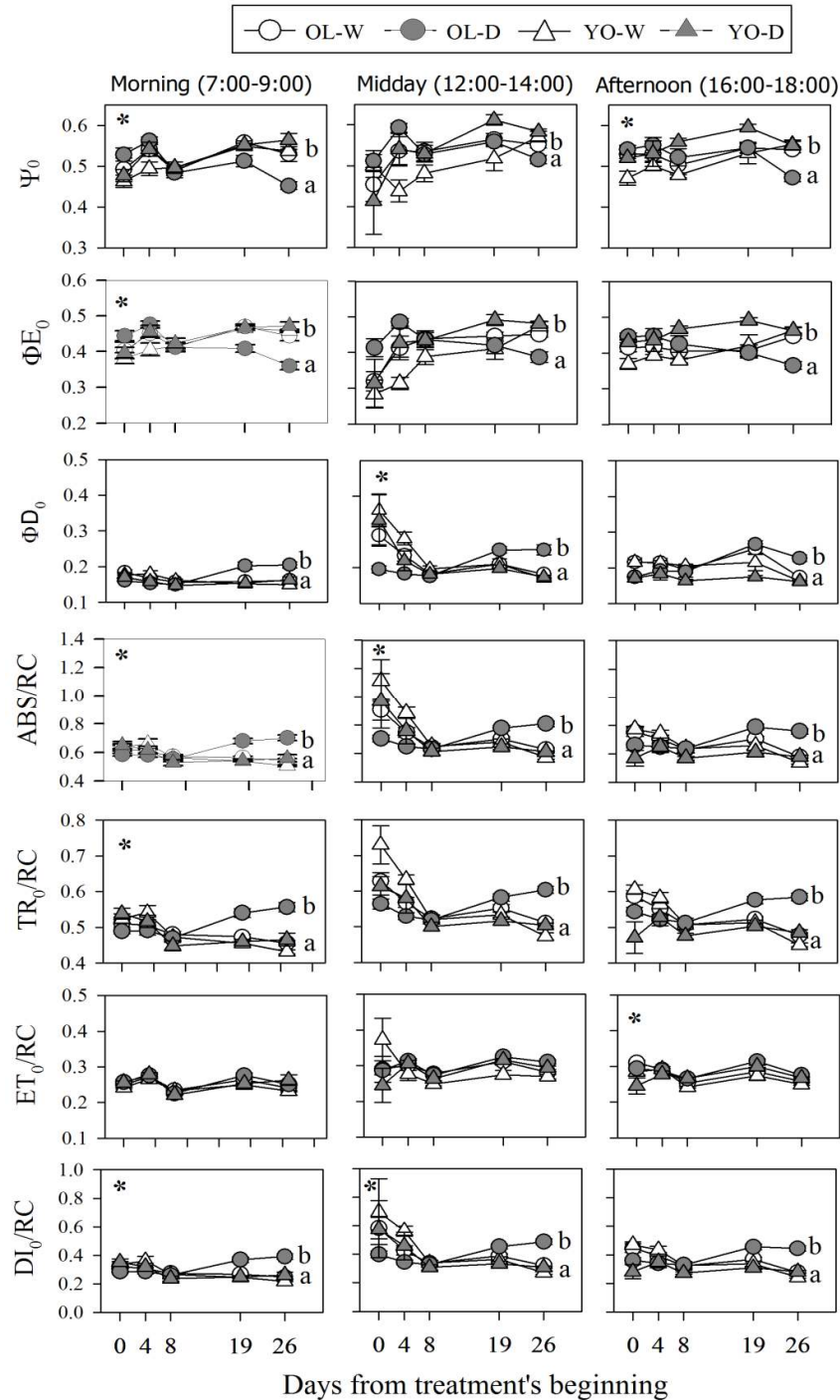


Fig. 6. Chlorophyll a fluorescence parameters (see section 2.5 for acronyms) measured in the morning, at midday, and in the afternoon, of 3-month-old *J. curcas* seedlings (OL - Circles) or 2-month-old *J. curcas* seedlings (YO - Triangles), exposed to Watered (W - White symbols) or Drought (D - Black symbols) treatments. Values represent means \pm S.E. (N=6). At treatment's beginning (day zero), significant differences between OL and YO seedlings are marked by asterisks (Student's t-test; $P < .05$). At 26th day, the lowercase letters indicate significant differences among groups OL-W, OL-D, YO-W, and YO-D (Tukey HSD; $P < .05$)

4. DISCUSSION

Drought affects agricultural productivity around the world, influencing plant growth and yield, especially in arid and semi-arid regions, where plants are often subjected to long periods of water stress [28]. Moreover, worse conditions are expected in the next years as a consequence of climatic changes [24]. Morphological and physiological responses to drought stress may vary considerably among plant species, and the mechanisms, which allow a species to tolerate prolonged periods of water deficit, can involve numerous attributes. Wood plants may employ two different water use strategies: strategies of drought avoidance or drought tolerance [29]. Both strategies involve diverse physiological and biochemical mechanisms that enable a plant to grow and survive even under drought conditions. Nevertheless, these strategies are not mutually exclusive and, in practise, plants may combine a range of response type [30]. There is no clear agreement on the physiological mechanisms specifically involved in the responses of *J. curcas* to drought. Maes et al. [6], Achten et al. [7], and Sapeta et al. [18] found that *J. curcas* is a species with a clear drought avoidance strategy in its leaves and also that this crop has several plant-water relations in common with deciduous stem succulent trees. Generally, drought avoidance is obtained by maintenance of high tissue water potential despite a soil water deficit. On the contrary, the mechanisms triggered in response to drought, as described by Silva et al. [10,16], Pompelli et al. [8], and dos Santos et al. [17], suggest that *J. curcas* tree activates drought tolerance strategy. Generally, drought tolerance is the ability to withstand water deficit with low tissue water potential.

Our results show that the seedlings of an Indian cultivar of *J. curcas*, widely cultivated in Africa, survived prolonged periods of drought stress, characterized by soil water potential constantly lower than permanent wilting point. Despite the stress, leaf relative water content of unwatered *J. curcas* seedlings was maintained at level of watered seedlings, even under extended drought conditions. Possibly, leaves have maintained a good leaf water status by means of an effective stomatal closure, associated with a reduced aboveground growth and an increased root: Shoot ratio. However, as described below, the mechanisms of drought response depend on seedling age.

The growth of unwatered seedlings was prevented by drought, with no difference

between OL and YO seedlings. Under drought conditions, seedlings reduced the total area of the leaves, with important consequences for the leaf energy and water balance. According to Cornelissen et al. [31], the increase of specific leaf area of unwatered seedlings, caused by a reduction in leaf dry weight, suggests that *J. curcas* seedlings under drought reduced their investment in leaf structures. Both in YO and OL seedlings, drought stress increased the ratio between root and shoot. In OL seedlings the increase of root:shoot ratio was due more to a reduction of leaf dry matter content and to a higher leaf abscission than to a greater allocation of resources in the roots. On the contrary, in YO seedlings, the increase of root: Shoot ratio was due both to a decrease in leaf dry weight and to a higher root dry weight. The highest specific root length of unwatered *J. curcas* seedlings suggests that YO seedlings were able to build more roots for a given dry mass investment, and this was achieved by constructing roots of thin diameter or low tissue density [31]. This strategy allows a faster root elongation rate, which results in higher nutrient and water uptake and a higher rate of initial survival [31], but, in the course of time, the production of thinner root could reduce the penetration force on soil and influence the ability of the plant to absorb water from the deeper layers of the soil [32].

Drought induced an afternoon down-regulation of photosynthesis by an increase in diffusive resistances to CO₂ at stomatal level in both YO and OL *J. curcas* seedlings. YO and OL seedlings differed in the photosynthetic response to drought, as prolonged drought stress resulted more detrimental in OL seedlings. Probably this difference depends on the initial condition of photosynthetic activity. In watered condition, the photosynthetic apparatus of OL seedlings is more efficient than that one of YO seedlings, even though OL seedlings had lower net photosynthesis (with the exception of midday), due to a higher stomatal closure. OL seedlings were able to better balance water loss and photosynthetic activity by reducing stomata conductance and maximizing the yield for primary photochemistry, especially in the early hours of the day. This is due to a more efficient electron transport (high ψ_0 , ϕE_0), which leads to a reduced need to dissipate excess of energy (low ϕD_0 , TR0/RC and DI0/RC) [30]. In order to promote their development, YO seedlings probably tried to maximize net photosynthesis, keeping the stomata open, at the expense of a less efficient photochemistry system,

and favouring problems related to the dissipation of excess energy and production of ROS [33]. In this situation, OL seedlings, with already balanced values of gas exchange and probably more acclimated to excess energy, did not present an efficient mechanism for protection against drought-induced oxidative stress, as suggested by the reduction of photosystem II integrity [26]. On the contrary, YO seedlings implemented mechanisms of tolerance, through the fast activation of excess energy dissipation mechanisms (high ϕD_0 , TR0/RC and DI0/RC). In OL seedlings, the further reduction of stomatal conductance caused by drought, even in the early hours of the day, and the consequently considerable reduction of net photosynthesis, led to a down-regulated photosynthetic electron transport through the down-regulation of photosystem II activity. Furthermore, the down-regulation of photosystem II activity also led to a reduction of the maximum quantum yield for primary photochemistry at all hours of the day [34,35].

5. CONCLUSION

The present work supports the hypothesis that *J. curcas* could survive and grow in areas with limited water availability or prolonged periods of drought and results suggest that seedling transplanting should be performed in the earlier phase of the seedling growth. Anyway, before promoting *J. curcas* cultivation in arid and semi-arid regions, a deeper understanding of the response mechanisms of *J. curcas* to drought should be investigated in combination with other co-occurring constraints, such as heat stress and salinity.

ACKNOWLEDGEMENTS

The authors wish to thank Giovanna Seddaiu, Francesco Fava, Simone Mereu, and Roberto Lai for the useful advices and suggestions, Maurizio Pinna, Angelo Ara, Sebastiano Piras, Agostino Piredda, Benedetta Scalas, and Laura Chessa for their help and support, Gabriele Sini e Domenico Carta for the tensiometric curve measurements, and Prof. Pier Paolo Roggero, Prof. Giuseppe Enne and Dr. Davide Bellavite for the given opportunity to carry out this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fairless D. Biofuel: The little shrub that could-maybe. *Nature*. 2007;449:652–655.
2. Behera SK, Srivastava P, Tripathi R, Singh JP, Singh N. Evaluation of plant performance of *Jatropha curcas* L. under different agro-practices for optimizing biomass - A case study. *Biomass Bioenerg*. 2010;34:30-41.
3. Yong JWH, Ng YF, Tan SN, Chew AYL. Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. *Photosynthetica*. 2010;48:208-218.
4. Contran N, Chessa L, Lubino M, Bellavite D, Roggero PP, Enne G. State-of-the art of the *Jatropha curcas* productive chain: From sowing to biodiesel and by-products. *Ind. Crop. Prod*. 2013;42:202-215.
5. Maes WH, Trabucco A, Achten WMJ, Muys B. Climatic growing conditions of *Jatropha curcas* L. *Biomass Bioenerg*. 2009;33:1481-1485.
6. Maes WH, Achten WMJ, Reubens B, Raes D, Samson R, Muys B. Plant-water relationships and growth strategies of *Jatropha curcas* L. seedlings under different levels of drought stress. *J. Arid Environ*. 2009;73:877-884.
7. Achten WMJ, Maes WH, Reubens B, Mathijs E, Singh VP, Verchot L, et al. Biomass production and allocation in *Jatropha curcas* L. seedlings under different levels of drought stress. *Biomass Bioenerg*. 2010;34:667-676.
8. Pompelli MF, Barata-Luís R, Vitorino HS, Goncalves ER, Rolim EV, Santos MG, et al. Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. *Biomass Bioenerg*. 2010;34:1207-1215.
9. Silva EN, Ferreira-Silva SL, Fontenele AV, Ribeiro RV, Viégas RA, Silveira JAG. Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. *J. Plant Phys*. 2010; 167:1157-1164.
10. Silva EN, Ferreira-Silva SL, Viégas RA, Silveira JAG. The role of organic and inorganic solutes in the osmotic adjustment of drought stressed *Jatropha curcas* plants. *Environ. Exp. Bot*. 2010;69: 279-285.

11. Díaz-López L, Gimeno V, Simón I, Martínez V, Rodríguez-Ortega WM, García-Sánchez F. *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. *Agr. Water Manage.* 2012; 105:48-56.
12. Kesava Rao AVR, Wani SP, Singh P, Srinivas K, Srinivasa Rao C. Water requirement and use by *Jatropha curcas* in a semi-arid tropical location. *Biomass Bioenerg.* 2012;39:175-181.
13. Krishnamurthy L, Zaman-Allah M, Marimuthu S, Wani SP, Kesava Rao AVR. Root growth in *Jatropha* and its implication for drought adaptation. *Biomass Bioenerg.* 2012;39:247-252.
14. Matos FS, de Oliveria LR, de Freitas RG, Evaristo AB, Missio RF, Cano MAO, et al. Physiological characterization of leaf senescence of *Jatropha curcas* L. populations. *Biomass Bioenerg.* 2012; 45:57-64.
15. Niu G, Rodriguez D, Mendoza M, Jifon J, Ganjegunte G. Responses of *Jatropha curcas* to salt and drought stresses. *Inter. J. Agron* ; 2012.
16. Silva EN, Ribeiro RV, Ferreira-Silva SL, Vieira SA, Ponte LFA, Silveira JAG. Coordinate changes in photosynthesis, sugar accumulation and antioxidative enzymes improve the performance of *Jatropha curcas* plants under drought stress. *Biomass Bioenerg.* 2012;45: 270-279.
17. dos Santos CM, Verissimo V, de Lins Wanderley Filho HC, Ferreira VM, da Silva Cavalcante PG, et al. Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. *Ind. Crop. Prod.* 2013;41:203-213.
18. Sapeta H, Costa JM, Lourenço T, Maroco J, Van Der Linde P, Oliveira MM. Drought stress response in *Jatropha curcas*: Growth and physiology. *Environ. Exp. Bot.* 2013;85:76-84.
19. Winter K, Holtum J. Cryptic crassulacean acid metabolism (CAM) in *Jatropha curcas* L. *Funct. Plant Biol.* 2015;42:711-717.
20. Tominaga J, Inafukua S, Coetzee T, Kawamitsua Y. Diurnal regulation of photosynthesis in *Jatropha curcas* under drought during summer in a semi-arid region. *Biomass Bioenerg.* 2014;67: 279-287.
21. Reubens B, Achten WMJ, Maes WH, Danjon F, Aerts R, Poesen J, et al. More than biofuel? *Jatropha curcas* root system symmetry and potential for soil erosion control. *J. Arid Environ.* 2011; 75:201-205.
22. Kheira AAA, Atta NMM. Response of *Jatropha curcas* L. to water deficits: Yield, water use efficiency and oilseed characteristics. *Biomass Bioenerg.* 2009; 33:1343-1350.
23. Duarte DM, Guimarães GR, Torres Junior HD, Pereira F, Neves TG, Matos FS. Growth of *J. curcas* seedlings under water deficit condition. *Biosci. J.* 2015;31:1618-1623.
24. Dore MHI. Climate change and changes in global precipitation patterns: What do we know? *Environ. Int.* 2005;31:1167-1181.
25. Maxwell K, Johnson GN. Chlorophyll fluorescence – A practical guide. *J. Exp. Bot.* 2000;51:659-668.
26. Strasser BJ, Strasser RJ. Measuring fast fluorescence transient to address environmental questions: The JIP test. In: Mathis P, editor. *Photosynthesis: From light to biosphere.* Dordrecht: Kluwer Academic; 1995.
27. Contran N, Paoletti E, Manning WJ, Tagliaferro F. Ozone sensitivity and ethylenediurea protection in ash trees assessed by JIP chlorophyll a fluorescence transient analysis. *Photosynthetica.* 2009;47:68-78.
28. Hessine K, Martínez JP, Gandour M, Albouchi A, Soltani A, Abdelly C. Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. *Environ. Exp. Bot.* 2009;67:312-319.
29. assioura JB. Water in the soil-plant-atmosphere continuum. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, editors. *Physiological Plant Ecology II.* Berlin: Springer; 1982.
30. Levitt J. Responses of plants to environmental stresses. Water, radiation, salt and other stresses. New York: Academic Press. 1980;2.
31. Cornelissen HC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, et al. A handbook of protocols for standardised and easy measurement of plant functional

- traits worldwide. Aust. J. Bot. 2003;51: 335-380.
32. Franco JA, Banon S, Vicente MJ, Miralles J, Matinez-Sanchez JJ. Root development in horticultural plants grown under abiotic stress conditions – A review. J. Hortic. Sci. Biotechnol. 2011;86:543-556.
33. Mittler R. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 2006;11:15-19.
34. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 2004;55:373-399.
35. Rossini M, Fava F, Cogliati S, Meroni M, Marchesi A, Panigada C, et al. Assessing canopy PRI from airborne imagery to map water stress in maize. ISPRS J Photogramm Remote Sens. 2013;86: 168-177.

© 2017 Contran et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/19183>