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Antioxidant response to cold stress in two oil plants of the genus *Jatropha*

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ABSTRACT

Spanò C., Bottega S., Ruffini Castiglione M., Pedranzani H.E. (2017): Antioxidant response to cold stress in two oil plants of the genus *Jatropha*. Plant Soil Environ., 63: 271–276.

Jatropha curcas and *J. macrocarpa*, suitable for production of biodiesel oil from their seeds, are able to live in arid and semi-arid regions, where most crops cannot survive. *J. curcas* is characterized by higher oil quality and seed yield, but it is not a good candidate for oil production in arid areas with freezing temperatures, due to its sensitivity to chilling in comparison to *J. macrocarpa*. In this work, for the first time, the effects of cold stress and different mechanisms activated in these conditions have been studied in the two species. Seedlings were treated with low non-freezing temperatures with or without a previous acclimation period. Water status, pigment content, oxidative stress and antioxidant response were studied in acclimated and non-acclimated plants. The key features that differentiate *J. macrocarpa* from *J. curcas* were the ability to accumulate, at low temperatures, high concentrations of pigments and glutathione and significantly higher activities of ascorbate peroxidase. These data could explain the greater resistance to low temperatures of *J. macrocarpa*. A period of acclimation was not able to improve cold tolerance of *J. curcas* and this confirms its limited adaptability to arid areas with freezing temperatures.

Keywords: cold acclimation; damage; enzymes; hydrogen peroxide; liquid bio-fuel; reactive oxygen species

An increasing demand for friendly energy sources is growing worldwide with a consequent interest in plants suitable for the production of biodiesel oil from their seeds. Among these oil crops, plants of the genus *Jatropha* can adapt to marginal conditions for agriculture production. Their ability to live in arid and semi-arid regions, where most crops cannot survive (Francis et al. 2005), makes these plants of a particular interest. *J. curcas* produces seeds with oil of good quality which is easily transformed into liquid bio-fuel (Achten et al. 2008) while *J. macrocarpa* produces oil of low quality (Wassner et al. 2012). Despite the higher oil quality and seed yield, *J. curcas*, due to its sensitivity to chilling (Ao et al. 2013) is not a good candidate for oil production in arid areas with freezing temperatures. The ability of *J. macrocarpa* to survive in such arid lands, which is also due to some specific anatomic adaptive traits (Tavecchio et al. 2016), makes this species an interesting example of plant which has adapted to living in these restrictive conditions.

Low temperatures induce in plants the typical symptoms of chilling injury such as wilting, yellowing of leaves, inhibition of growth and disturbances in water status, mineral nutrition, respiration and photosynthesis, this last due to a decrease in chlorophyll and the disorganization of the photosynthetic apparatus (Jouyban et al. 2013). As with other stress conditions, low temperatures can induce in plants the generation of reactive oxygen species (ROS), such as hydrogen peroxide.

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At high concentrations, these molecules can induce oxidative damage to cellular structure oxidizing proteins, lipids and nucleic acids, playing in this way a key role in transduction of the chilling injury (Lukatkin 2002, Hu et al. 2008). However, at lower concentrations, ROS can exert a positive function as signalling molecules which are able to elicit a plant protective response. Plants have developed a complex antioxidant defense machinery both enzymatic and non-enzymatic. The low molecular weight of antioxidants ascorbate and glutathione can cooperate with the enzymes of the ascorbate-glutathione cycle, such as ascorbate peroxidase (APX) to scavenge ROS. Glutathione peroxidase (GPX), guaiacol peroxidase (POX) and catalase (CAT) are other important enzymes that are able to detoxify hydrogen peroxide (Mittler 2002). The capacity of plants to withstand restrictive environmental conditions mostly depends on their ability to activate an adequate antioxidant response. The exposure of a plant to a chilling temperature, called cold acclimation, can induce molecular, biochemical and physiological changes leading to a higher cold tolerance (Sanghera et al. 2011).

With the aim of having a greater insight into the mechanisms underlying the different cold tolerance in the two species, oxidative stress and antioxidant response were studied in seedlings of *J. curcas* and *J. macrocarpa* treated with low non-freezing temperatures with or without a previous acclimation period. The study focuses on evaluation of different mechanisms activated in cold conditions and whether the period of acclimation could improve the performance of the two species under study.

MATERIAL AND METHODS

Experimental design. Seeds of *Jatropha curcas* L. and *J. macrocarpa* Griseb. (Secretary of Agriculture and Natural Resources from La Rioja, Argentina: 29°26'S, 66°50'W) were surface-sterilized using 5% sodium hypochlorite. The seeds were dehulled to break physical dormancy and germinated in Petri dishes. After five days 30 seedlings were planted in pots filled with agricultural soil and transferred to a growth chamber at 28°C, with 14-h light (400 µmol/m^2/s). Ten plants were left at 28°C (control); the other twenty plants were treated with low temperatures. In detail, 10 plants were transferred after 45 days to a growth chamber at 15°C for five days (acclimated); the other ten plants were kept at 28°C (non-acclimated). Acclimated and non-acclimated plants were transferred to a growth chamber at 4°C for two days.

Water content, relative water content and pigments. The percentage of leaf water content percentage was estimated on the fresh weight basis with the formula:

$$Water\ content = \frac{FW - DW}{FW} \times 100$$

Where: FW – fresh weight; DW – dry weight.

The leaf relative water content (RWC) was determined according to Balestri et al. (2014) and calculated with the formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where: TW – turgid weight.

The fresh weight was obtained by weighing the fresh leaves. Turgid weight was determined after water immersion of leaves overnight. The leaves were then dried in an oven at 60°C to constant weight and reweighed to obtain the dry weight.

Chlorophylls (*a*, *b* and total) and carotenoids were extracted from leaves and determined, according to Hassanzadeh et al. (2009) and to Lichtenthaler (1987), respectively.

Hydrogen peroxide and thiobarbituric acid reactive substances (TBARS). Hydrogen peroxide (H_2O_2) content was determined in leaves according to Jana and Choudhuri (1982). Lipid peroxidation in leaves was estimated by determining the amount of TBARS according to Wang et al. (2013) with minor modifications. The leaves were homogenized with 5% trichloroacetic acid (TCA) and the extraction solution was mixed with TBA reagent (5% w/v TCA + 0.5% w/v thiobarbituric acid). The level of TBARS was measured as specific absorbance at 532 nm by subtracting the non-specific absorbance at 600 nm.

Ascorbate and glutathione. Ascorbate (ASA) was extracted from leaves and determined according to Kampfenkel et al. (1995) with minor modifications. Total ascorbate was determined after the reduction of dehydroascorbate to ASA by dithiothreitol. Glutathione was extracted and determined in leaves according to Gossett et al. (1994) by the 5,5'-dithio-bis-nitrobenzoic acid (DTNB)-glutathione reductase recycling.

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Enzyme extraction and assays. Enzyme leaf extraction was made according to Spanò et al. (2013). Ascorbate peroxidase, glutathione peroxidase, catalase and guaiacol peroxidase activities were measured according to Nakano and Asada (1981), Navari-Izzo et al. (1997), Aebi (1984) and Arezki et al. (2001), respectively.

Protein content was determined according to Bradford (1976), using bovine serum albumin as standard.

Statistical analysis. The reported data were the mean of at least three (until nine) replicates in each experimental group (pools of leaves derived from ten plants). Statistical significance was determined by ANOVA tests followed by post hoc Bonferroni multiple comparison test. Post hoc statistical significance ($P < 0.05$) is indicated in figures and tables by different letters.

RESULTS AND DISCUSSION

The present results show that cold treatment induced different responses in terms of hydric state, pigment content, oxidative damage markers and antioxidant machinery activation in *J. curcas* and *J. macrocarpa*.

The fact that high water content makes plants more susceptible to mechanical damage from freezing has been accepted for a long time and the ability of plants to reduce their water content during acclimation is generally considered necessary to attain freezing tolerance by reducing the rate of ice formation and growth (Gusta et al. 2004). While the cold treatment always induced a decrease in water content, only *J. macrocarpa* reduced it during the acclimation period (Figure 1). Relative water content is probably the most appropriate parameter for determining water status of plants as the physiological consequence of cellular water deficit. *J. macrocarpa* dramatically reduced its relative water content (Figure 1) showing, unlike *J. curcas*, a typical response of hardening plants (Hao et al. 2009). In fact, the decline in RWC could be the result of osmotic adjustment, correlated to the increase in frost tolerance (Burchett et al. 2006).

Following the cold treatment, in accordance with its hardiness, *J. macrocarpa* showed a significant increase in pigment content, which was necessary to maintain the photosynthetic process in restrictive conditions, and the maximum values characterized acclimated plants (Table 1). The higher chlorophyll content always recorded in *J. macrocarpa* is in accordance with its higher numbers of palisade layers (Tavecchio et al. 2016). In comparison with control plants, the increase, of carotenoids/total chl ratio (Table 1) in cold-treated *J. macrocarpa* leaves could ensure greater protection, thanks to the activity of non-photochemical quenching, typical of carotenoids, in restrictive conditions. The cold-induced decrease in chl *a*/*b* ratio (Table 1) in *J. curcas* could be the symptom of a higher investment in antenna enhancing incident light capitation in stress conditions with increased probability of photoinhibition (Bascuñán-Godoy et al. 2012).

Hydrogen peroxide is a relatively stable ROS induced in plants by many stress conditions. An adaptive role has been suggested for H_2O_2 in increasing chilling tolerance in maize (Prasad et al. 1994) and in tomato (Zhou et al. 2012). *J. macrocarpa* had hydrogen peroxide contents always

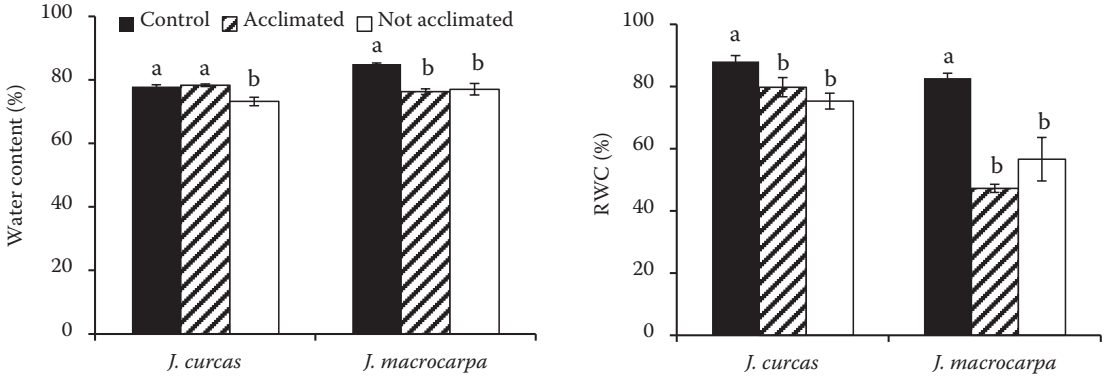

Figure 1. Water content and relative water content (RWC) in control, acclimated and non- acclimated plants of *Jatropha curcas* and *J. macrocarpa*. Values are means of triplicate and vertical bars represent standard errors. Different letters denote significant differences at $P < 0.05$

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Table 1. Pigment contents in control, acclimated and non-acclimated plants of *Jatropha curcas* and *J. macrocarpa*

	J. curcas			*J. macrocarpa*		
	control	acclimated	non-acclimated	control	acclimated	non-acclimated
Total chlorophyll (mg/g FW)	1.61 ± 0.10^{a}	1.74 ± 0.19^{a}	1.83 ± 0.05^{a}	1.92 ± 0.22^{c}	3.66 ± 0.14^{a}	2.81 ± 0.16^{b}
Carotenoids (mg/g FW)	0.29 ± 0.03^{a}	0.34 ± 0.04^{a}	0.33 ± 0.05^{a}	0.18 ± 0.01^{c}	0.49 ± 0.02^{a}	0.41 ± 0.02^{b}
Chl *a*/*b*	3.24 ± 0.06^{a}	3.06 ± 0.04^{ab}	2.95 ± 0.06^{b}	2.97 ± 0.10^{a}	2.92 ± 0.11^{a}	2.80 ± 0.10^{a}
Carotenoids/total Chl	0.18 ± 0.01^{a}	0.19 ± 0.02^{a}	0.18 ± 0.00^{a}	0.10 ± 0.01^{b}	0.14 ± 0.00^{a}	0.15 ± 0.00^{a}

Values are means of at least three replications ± standard errors. Different letters denote significant differences at $P < 0.05$. FW – fresh weight

significantly lower than *J. curcas* but its ability to increase the content of this signalling molecule, in cold conditions able to elicit a plant protective response, can be considered as an adaptive trait (Figure 2).

The maximum values of TBARS, an indirect measure of lipid peroxidation and of membrane damage, were always detected in acclimated plants (Figure 2). It should be noted that the damage was always lower in *J. macrocarpa* than in *J. curcas* under the same treatment. No positive correlation was observed between hydrogen peroxide content and TBARS, suggesting that membrane damage was not, or at least not completely, H_2O_2-dependent.

The pattern of enzymatic and non-enzymatic antioxidants differed in *J. curcas* and *J. macrocarpa* (Table 1). The role of ascorbate and glutathione in plant stress tolerance has been emphasized (Lukatkin and Anjum 2014). While there were not major differences in ascorbate concentration among the treatments, glutathione showed the most dramatic changes with particularly high values in acclimated plants (Table 2). The concentrations of GSH were always higher in *J. macrocarpa* than in *J. curcas* (Table 2). This could be another important adaptive trait, as data in literature envisage the role of glutathione in protecting plants from low temperatures (Kocsy et al. 2001, 2002). The strong increase in glutathione content could largely compensate for the slight decrease in ascorbate concentration recorded in *J. macrocarpa* (Table 2).

The patterns of antioxidant enzymes were different in the two species (Table 2) and on the whole, the major differences were recorded in the APX activity of *J. curcas* where in cold-treated plants the activity of this enzyme decreased by more than six times compared to the control. An important protective action of APX at low temperatures has been hypothesized (Wang et al. 2005) and the increase in APX activity in cold-treated *J. macrocarpa*, together with the relatively higher CAT activities could compensate for the relatively low POX activities.

In conclusion, the key features that differentiate *J. macrocarpa* from *J. curcas* consist in its ability

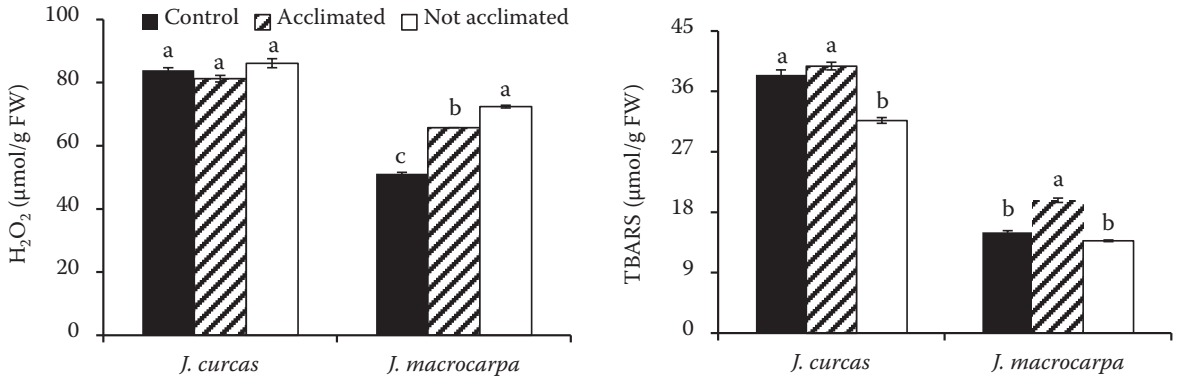

Figure 2. Hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) in control, acclimated and non-acclimated plants of *Jatropha curcas* and *J. macrocarpa*. Values are means of triplicate and vertical bars represent standard errors. Different letters denote significant differences at $P < 0.05$

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Table 2. Biochemical parameters in control, acclimated and non-acclimated plants of *Jatropha curcas* and *J. macrocarpa*

		J. curcas			*J. macrocarpa*		
		control	acclimated	non-acclimated	control	acclimated	non-acclimated
Total ascorbate (mg/g FW)		1.33 ± 0.01^{b}	1.38 ± 0.01^{b}	1.71 ± 0.00^{a}	0.92 ± 0.02^{a}	0.81 ± 0.00^{c}	0.85 ± 0.01^{b}
Total glutathione (nmol/g FW)		6.47 ± 1.10^{b}	28.81 ± 0.80^{a}	9.66 ± 1.18^{b}	44.15 ± 2.76^{b}	166.76 ± 5.59^{a}	44.35 ± 2.78^{b}
APX	(U/mg protein)	1.62 ± 0.01^{b}	0.22 ± 0.00^{a}	0.24 ± 0.01^{a}	0.98 ± 0.04^{c}	1.32 ± 0.02^{b}	1.53 ± 0.01^{a}
GPX		1.11 ± 0.08^{a}	0.97 ± 0.09^{a}	1.15 ± 0.14^{a}	1.25 ± 0.14^{a}	0.88 ± 0.24^{a}	1.27 ± 0.31^{a}
POX		0.12 ± 0.00^{c}	0.28 ± 0.00^{b}	0.32 ± 0.00^{a}	0.084 ± 0.09^{b}	0.084 ± 0.003^{b}	0.11 ± 0.00^{a}
CAT		4.79 ± 0.46^{a}	3.65 ± 0.44^{a}	4.03 ± 0.34^{a}	5.29 ± 0.68^{a}	4.58 ± 0.60^{a}	5.40 ± 0.45^{a}

Values are means of at least three replications ± standard errors. Different letters denote significant differences at $P < 0.05$. FW – fresh weight; APX – ascorbate peroxidase; GPX – glutathione peroxidase; POX – guaiacol peroxidase; CAT – catalase; U – enzymatic units

to accumulate relatively high concentrations of pigments glutathione in cold conditions, and in its significantly higher activities of APX as a response to the increase in hydrogen peroxide content. The ability to accumulate high concentrations of these protective molecules in cold conditions, in particular after acclimation, could explain the greater resistance to low temperatures of *J. macrocarpa*. Acclimation was not able to improve the cold tolerance of *J. curcas* confirming its limited adaptability to arid areas with freezing temperatures.

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