

Full Length Research Paper

Effects of pre-sowing treatments on *Jatropha curcas* seed germination and seedling growth

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This study aimed to investigate the effects of different pre-sowing treatments on *Jatropha curcas* seed germination rate and seedling early growth. Growth and vigour of the seedlings was assessed through the measurements of growth parameters, in order to identify the best pre-sowing treatment, which guarantees both the highest seed germination rate and the best development and growth of the seedlings. *J. curcas* seeds of the 'Indian' cultivar were collected in Tamale region (Ghana) and subjected to five different pre-sowing treatments: i) control; ii) soaking in 30°C water for 24 h; iii) hammer shell cracking; iv) warm stratification at 37°C for 24 h; v) hammer shell cracking plus warm stratification at 37°C for 24 h. Amongst the sixteen indices considered in the experiment (six germination indices and ten growth rate indices), results revealed that the tested pre-sowing treatments influenced much more seed germination than seedling growth. Shell cracking treatment enhanced seed germination and warm stratification promoted emergence rate and seedling growth as compared to the other tested treatments.

Key words: Physic nut, biodiesel, rural development, vegetable oil, land use.

INTRODUCTION

Jatropha curcas L., a drought avoidant perennial small tree, is autochthonous of Mexico and tropical America, and was then largely spread out in India, Africa and South East Asia (Achten et al., 2010a). Nowadays, *J. curcas* grows in tropical and subtropical regions in a wide range of climatic conditions from semiarid to humid (Achten et al., 2010a). In the last decades, *J. curcas* has become popular thanks to its wide capabilities and plethora of uses, including biodiesel production, which are the cause of an increasing of hectares of *J. curcas* yearly planted at global level (Fairless, 2007; Kant and

Wu, 2011). *J. curcas* seeds contain about 25 to 35% or more of oil (Freitas et al., 2011; Verma and Verma, 2014), which can be extracted and used as lighting and cooking fuel, to manufacture soap, medicine or bio-pesticide and, after further chemical treatments, to produce biodiesel, a renewable energy source alternative to conventional petrodiesel (Martínez-Herrera et al., 2006; Pompelli et al., 2010; Contran et al., 2013; Sunil et al., 2013; Sushma, 2014). Besides the economic value derived from *J. curcas* oil and its derived products, *J. curcas* strength as a crop derives from its potential

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adaptability to grow on low-nutrient soils and under arid and semi-arid conditions, avoiding *J. curcas* competition against food crops. Furthermore, the plant itself offers the ecological advantage to mitigate soil degradation and to restore marginal land or abandoned farmland (Reubens et al., 2011).

Nevertheless the positive impacts that could be generated by the use of *J. curcas* in arid and semi-arid areas of developing countries, the high potential of this tree has not been reached so far and *J. curcas* is still a (semi-) wild undomesticated plant. Its basic agronomic needs are only partially understood, the growing and management practices are not enough documented for a lot of areas of new introduction, and the environmental effects should more deeply investigated (Achten et al., 2010a, b, c; Contran et al., 2013; Yamada and Sentelhas, 2014). Studies on vegetative (cutting) or generative (seed) propagation of *J. curcas*, representing a critical stage in the plant-life cycle, have been carried out so far (Ginwal et al., 2005; Achten et al., 2008; Kumar and Sharma, 2008; Islam et al., 2009; Severino et al., 2010; Windauer et al., 2012; Moncaleano-Escandon et al., 2013).

High variability of seed germination has been recorded as influenced by the observed genotype (cultivar, seedling or population), time after harvest and storage conditions, environmental characteristics of plant growing, pre-sowing and after-sowing treatments (temperature and water potential of seed tissues and substrates) (Islam et al., 2009; Pompelli et al., 2010; Windauer et al., 2012; Duong et al., 2013; Moncaleano-Escandon et al., 2013). Some authors report on a loss of seed viability and germinability after medium and long term storage (Duong et al., 2013; Moncaleano-Escandon et al., 2013), while others suppose that the presence of seed coat, may be the responsible of a physical dormancy, and furthermore generates the need to remove this inhibition by pre-sowing treatments (Baskin and Baskin, 1998; Islam et al., 2009; Windauer et al., 2011).

In order to enhance germination percentage, seeds have been subjected to pre-germination treatments before sowing, with the aim to break the seed coat, favour the embryo hydration and consequently increase the germination percentage as compared to untreated seeds. Among the studies on the effects of pre-sowing treatments of *J. curcas* seeds on different germination parameters, Islam et al. (2009) demonstrated that *J. curcas* seeds, kept under stone sand and moistened with water for 72 h before sowing, showed a significantly higher germination percentage than the untreated and directly sown control in all the twenty different genotypes tested in the experiment. Windauer et al. (2012) tested the effects of different temperatures (from 15 to 35°C) on *J. curcas* seed germination percentage. This study revealed that an incubation of seeds at 25°C before sowing caused the highest final germination percentage,

even if at 30°C seeds germinated faster than any other temperature. Furthermore, positive results were reached for seed of various tropical tree species, previously treated with hot water, which is considered one of the cheapest, easiest and replicable techniques to induce seed dormancy-breaking (Wang and Hanson, 2008). Only few studies were found in literature on the effects of pre-sowing treatments on the growth of *J. curcas* seedlings (Islam et al., 2009; Pompelli et al., 2010; Moncaleano-Escandon et al., 2013).

The aim of this study was to investigate the effects of different pre-sowing treatments on germination behaviour of the seeds of 'Indian' cultivar, which in spite of the agronomic value showed some difficulties to obtain a good rate of propagation. Growth rate and vigour of the seedlings through the measurements of growth parameters were also assessed.

MATERIALS AND METHODS

The experiment was performed in a growth chamber of the Department of Agriculture of the University of Sassari (Italy) and carried out on *J. curcas* seeds of the 'Indian' cultivar. This cultivar has been chosen, since it is one of the most common cultivar used in Ghana and India and largely adopted in many countries for small-scale extensive plantations, promoted by cooperation projects, and large-scale intensive plantations, operated by multinational companies (Acheampong and Campion, 2014). *J. curcas* seeds were collected in October 2011 from Ghana Yendi road Farm, Tamale, Northern Region of Ghana. The area of Tamale is classified as a tropical savannah climate zone (Peel et al., 2007), characterized by a pronounced dry season (from October to March), in which precipitation is less than 60 mm. The average annual precipitation is 1179 mm (MOFA, 2011). The average annual temperature is 27.8°C (min 22.3°C - max 33.4°C) (Climatedata, 2014). Seed were stored at 18 (±2)°C and 75% relative humidity for six months until the start of the experiment (April, 2012).

J. curcas seeds were subjected to five different pre-sowing treatments as follows: i) untreated control, in which seeds were directly sown in pot in a depth of 1 cm; ii) seed soaking in 30°C water for 24 h; iii) hammer shell cracking, in which seeds were mechanically scarified by cracking with a hammer to weaken the shell; iv) warm stratification at 37°C for 24 h, in which seeds were mixed with an equal volume of a moist medium (peat) in a close container and maintained at 37°C for 24 h; v) hammer shell cracking plus warm stratification at 37°C for 24 h, in which seeds were mechanically scarified by cracking with a hammer to weaken the shell and then mixed with an equal volume of a moist medium (peat) in a close container and maintained at 37°C for 24 h. Germination test was carried out in a growth chamber at 28°C, under an 8/16 light/dark regime at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A completely randomised design with four replications per treatment was used, according to the Seeds Analysis Rules (ISTA, 2004). Seeds were sown in pot (15 cm diameter, 10 cm height) filled with potting mix medium (dry matter 30%, organic matter 20%, fertilizer NPK 12:14:24 1 kg/m³) and fully irrigated every day with a total of 55 ml per pot of distilled water during the first two weeks of the experiment, 100 ml per pot during the period between 15 and 25 days and with 150 ml per pot between 26 and 35 days.

Number of emerged seeds and first true leaf expansion were recorded by everyday monitoring from the sown for 35 days. The seed emergence criterion was visible protrusion from the surface of

soil (AOSA, 1983). Emerged seed was considered germinated (Ranal and De Santana, 2006). According to Cornelissen et al. (2003), after 35 days from sown, seedlings developed by germinated seeds were separated into cotyledons, leaves, stem, and roots (washed). The following destructive measurements were carried out: i) cotyledons (fresh and dry weight and total cotyledons area), ii) leaf (fresh and dry weight and total leaf area), iii) stem (length, basal diameter, fresh and dry weight), and iv) root (length, diameter, fresh and dry). Dry weight was measured when samples at 100°C reached a constant weight (around 48 h). Total cotyledon area and leaf area were measured by an Area Meter (LI-3100C Area meter, Licor), which provided a scanner of the leaf blade and allowed further biometric determination by means of a specific software. Measured data on several parameters related to germination process and seedling growth were allowed for calculation, as reported in Table 1.

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test). Percent values were transformed by arcsine-square root prior to analysis. Analysis of variance (ANOVA) was applied to test the effect of Treatments. For the ANOVA of seed germination parameters, the statistical unit was the replication (N=4). For the ANOVA of seedling growth parameters, the statistical unit was the single seedling (N=14); since the number of germinated seeds in water soaking treatment was very low, this treatment was not considered for the ANOVA test of seedling growth parameters. A Tukey HSD test was applied to compare the above effects between homogeneous groups. Tests of significance were made at a $p \leq 0.05$ confidence level. Analyses were processed by using STATISTICA 6.0 Package for Windows (StatSoft, Inc. 2013).

RESULTS

The statistical analysis revealed that the tested pre-sowing treatments have various effects on different germination parameters of *J. curcas* seeds. In particular, germination ability, emergence rate, emergence index, and mean emergence time were different between treatments and control, while true leaf expansion and seedling vigour index did not revealed any difference (Table 2).

More in detail, water soaking treated seeds showed the lowest percentage of germinability (7.5%) while shell cracking treated seeds showed the highest percentage of germination (>80%) (Figure 1). Seeds exposed to shell cracking, warm stratification and the combination of these two pre-sowing treatments (shell cracking plus warm stratification) had higher emergence rate and, consequently, lower mean emergence time compared to control untreated seeds (Figure 1). Furthermore, the highest value in emergence rate (24 day⁻¹) was found in warm stratification treated seeds, which showed higher emergence rate also compared to shell cracking treated seeds (Figure 1). Both seeds treated with the sole shell cracking and with the combination of shell cracking plus warm stratification showed statistically higher emergency index than untreated control seeds, while warm stratification treated seeds did not showed a significant difference from control, shell cracking, and shell cracking plus warm stratification treated seeds (Figure 1). True leaf expansion and seedling vigour index do not provide

significant differences among treatments. However, these data have been reported for a complete information on the experiment results, as well to corroborate the good performance of the warm stratification in the case of the true leaf expansion and of the shell cracking and warm stratification in the case of the seedling vigour index.

After 35 days from the sown, amongst the ten growth parameters, only cotyledon size, leaf size, and specific root length showed significant differences among treatments (Table 3). Since the number of germinated seeds in water soaking treatment was very low (7.5% on 40 seeds), this treatment was not considered for the analyses of seedling growth parameters.

Warm stratification treated seedlings showed the highest cotyledon size, which is significantly higher than in shell cracking plus warm stratification treated seedlings (Figure 2). Maximum leaf size was observed in warm stratification treated seedlings, which also revealed significant differences, as compared with control, shell cracking and shell cracking plus warm stratification treated seedlings (Figure 2). Any significant difference among treatments was observed for specific cotyledon area and cotyledon dry matter content. These data, however, were reported as complementary information, as well as specific leaf area and leaf dry matter content that are in accordance with the highest leaf size of the seedlings of the warm stratification treatments.

Shell cracking plus warm stratification treated seedlings showed the highest specific root length values amongst the five tested pre-sowing treatments (Figure 3). Stem specific density, stem dry matter content and root:shoot ratio do not provide significant differences among treatments and were showed for a complete information on the experiment findings.

DISCUSSION

Considering the strong variability and genotype dependence of previous experimental results obtained by many authors, our test focused on the effects of five different seed pre-sowing treatments on germination and growth of *J. curcas* seedlings of the commonly used 'Indian' cultivar. In fact, in spite of the large diffusion, few scientific data were recorded regarding the seed germinability of this cultivar.

Results of the present investigation indicated that among the pre-sowing treatments only shell cracking significantly influenced seed germination. With the exception of water soaking, treatments had a positive effect on the early growth and extension of *J. curcas* tissues, as demonstrated by a significant increasing of the emergence rate and the emergence index, and a significant reduction of the emergence time. We were expecting a much higher performance of soaking seeds in water at 30°C for 24 h, since Islam et al. (2009) demonstrated that *J. curcas* seeds soaked in water had a

Table 1. Estimation of parameters related to germination process and seedling growth.

Variable	Formula	References
Germination process		
Germinability (G) [%]	$G = (N_g / N) * 100$	(ISTA, 2004)
True leaf expansion (TI) [%]	$TI = (NI / G) * 100$	-
Emergence rate (Er) [day^{-1}]	$Er = [\sum Nd / (\sum D * Nd)] * 100$	Kotowski (1926)
Emergence index (EI) [day^{-1}]	$EI = \sum (Nd / D)$	AOSA (1983)
Mean emergence time (MET) [day]	$MET = (\sum D * Nd) / \sum Nd$	Ellis and Roberts (1981)
Seedling vigor index (SVI) [cm %]	$SVI = [(L_s + L_r) * G] / 100$	Abdul-Baki and Anderson (1973)
Seedling functional traits		
Cotyledon size (Cs) [mm^2]	$Cs = Ac$	Cornelissen et al. (2003)
Specific cotyledon area (SCA) [$\text{mm}^2 \text{mg}^{-1}$]	$SCA = Ac / DWc$	Cornelissen et al. (2003)
Cotyledon dry matter content (CDMC) [mg g^{-1}]	$CDMC = DWc / FWc$	Cornelissen et al. (2003)
Leaf size (Ls) [mm^2]	$Ls = Al$	Cornelissen et al. (2003)
Specific leaf area (SLA) [$\text{mm}^2 \text{mg}^{-1}$]	$SLA = Al / DWI$	Cornelissen et al. (2003)
Leaf dry matter content (LDMC) [mg g^{-1}]	$LDCM = DWI / FWI$	Cornelissen et al. (2003)
Stem specific density (SSD) [mg cm^{-3}]	$SSD = DWs / Vs$	Cornelissen et al. (2003)
Stem dry matter content (SDMC) [mg g^{-1}]	$SDCM = DWs / FWs$	Cornelissen et al. (2003)
Specific root length (SRL) [cm mg^{-1}]	$SRL = Lr / DWr$	Cornelissen et al. (2003)
Root:shoot ratio (RSr)	$RSr = DWr / DWc+l+s$	-

Legend: D = number of days counted from the beginning of germination; N = total number of seed; N_g = total number of germinated seeds; N_d = number of seeds germinated on day D after sowing; N_i = number of expanded true leaf when seed is germinated; N_5 = number of seeds germinated on day 5 after sowing; N_{15} = number of seeds germinated on day 15 after sowing; L_s = average stem length (cm); L_r = average root length (cm); Ac = cotyledon area [mm^2]; DWc = cotyledon dry weight [mg]; FWc = cotyledon fresh weight [g]; Al = leaf area [mm^2]; DWI = leaf dry weight [mg]; FWI = leaf fresh weight [g]; DWs = stem dry weight [mg]; FWs = stem fresh weight [g]; DWr = root dry weight [mg]; $DWc+l+s$ = cotyledons plus stem plus leaf dry weight [g].

Table 2. F values of one-way analysis of variance for the effects of treatment on germination process.

Dependent variable	Treatment
d.f.	4
Germinability	6.7**
True leaf expansion	1.2 ^{ns}
d.f.	3
Emergence rate	22.6***
Emergence index	7.7*
Mean emergence time	10.9***
Seedling vigor index	1.9 ^{ns}

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = $p > 0.05$ (not significant). d.f. represents the degrees of freedom.

significantly higher germination than control due to the rupture of seed coat. Anyway, water soaking treatment caused a significant seed germinability reduction as compared to all the other treatments. It is possible to suppose that for the 'Indian' cultivar this practice may be effective only in an earlier or later time of application with respect to the six months of seed storage applied before treatments.

Seedling dry matter, nutrient allocation, and plant structural strength seem to be not affected by treatments (Figure 2). On the contrary, warm stratification at 37°C induced an increase in aboveground seedling growth since, after 35 days from the sown, seedlings showed a significantly higher expansion of both cotyledon and primary leaf size. Furthermore, in shell cracking plus water stratification treated seedlings, an antagonistic

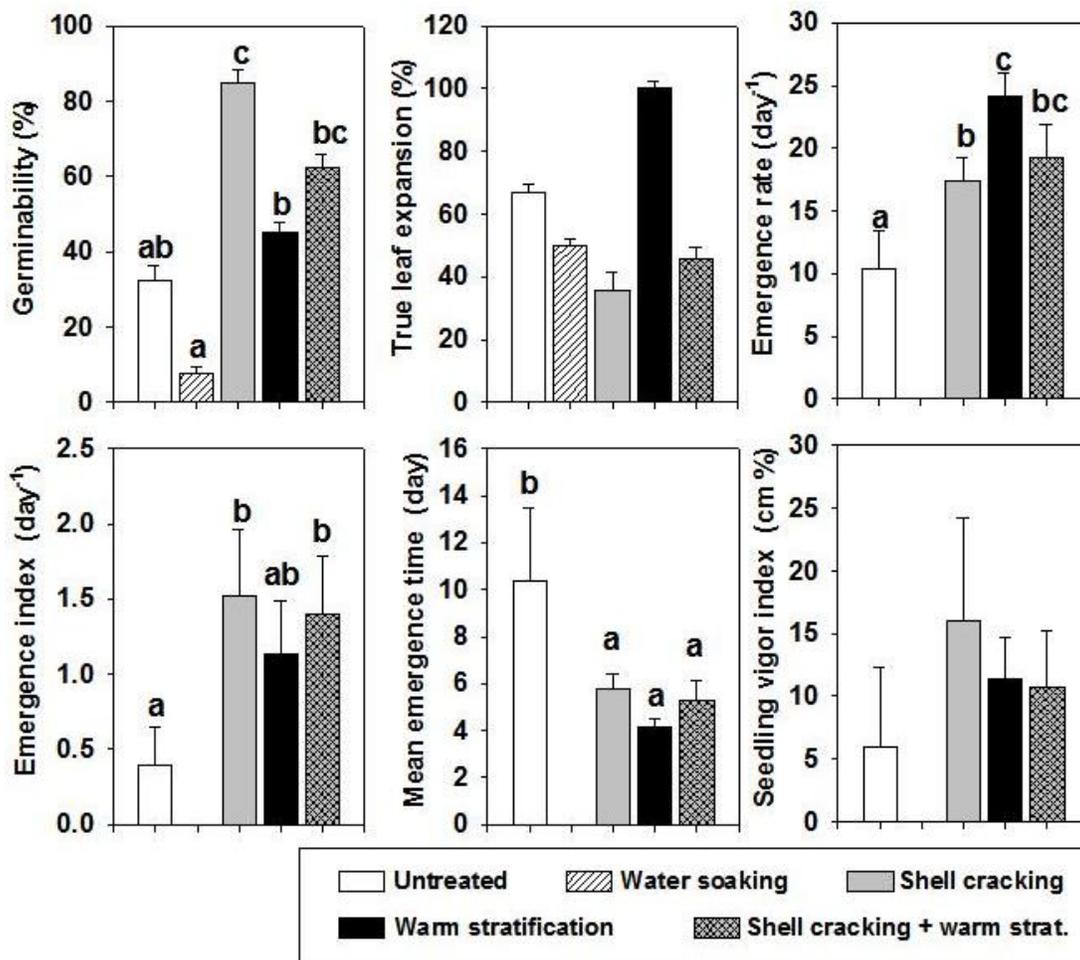


Figure 1. Germinability, true leaf expansion, emergence rate, emergence index, mean emergence time, and seedling vigour index of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means \pm S.D. (N=4). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=4).

Table 3. *F* values of one-way analysis of variance for the effects of treatment on *J. curcas* seedling growth.

Dependent variable	Treatment
d.f.	4
Cotyledon size	6.1**
Specific cotyledon area	0.5 ^{ns}
Cot. dry matter content	1.7 ^{ns}
Leaf size	6.6***
Specific leaf area	1.8 ^{ns}
Leaf dry matter content	0.9 ^{ns}
Stem specific density	0.4 ^{ns}
Stem dry matter content	1.6 ^{ns}
Specific root length	3.6*
Root:shoot ratio	2.1 ^{ns}

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = $p > 0.05$ (not significant). d.f. represents the degrees of freedom.

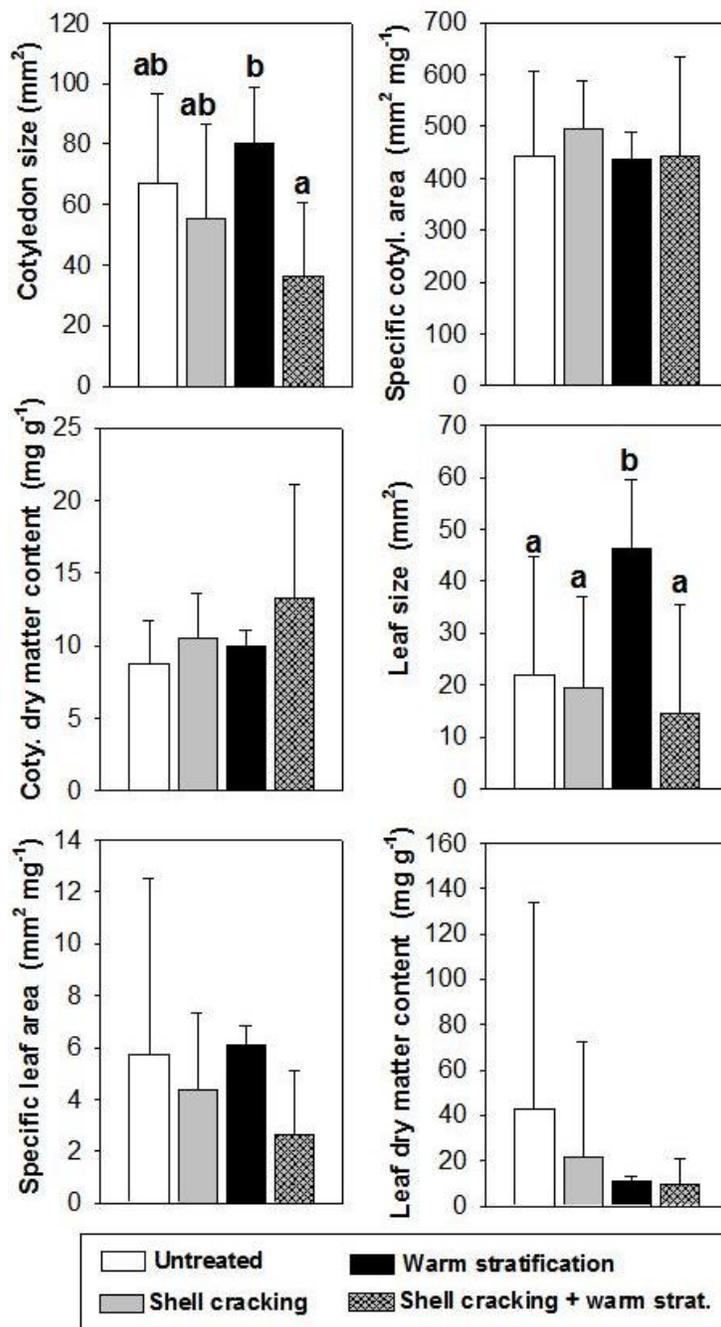


Figure 2. Cotyledon size, specific cotyledon area, cotyledon dry matter content, leaf size, specific leaf area, and leaf dry matter content of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means ±S.D. (N=14). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=14).

effect of the combined treatment on the aboveground part growth of the seedlings was observed, while an additive effect on root system growth was found (Figures 2 and

3). No correlation was found between germination and seedling growth indicators, thus showing some differences with respect to previous observations (Islam

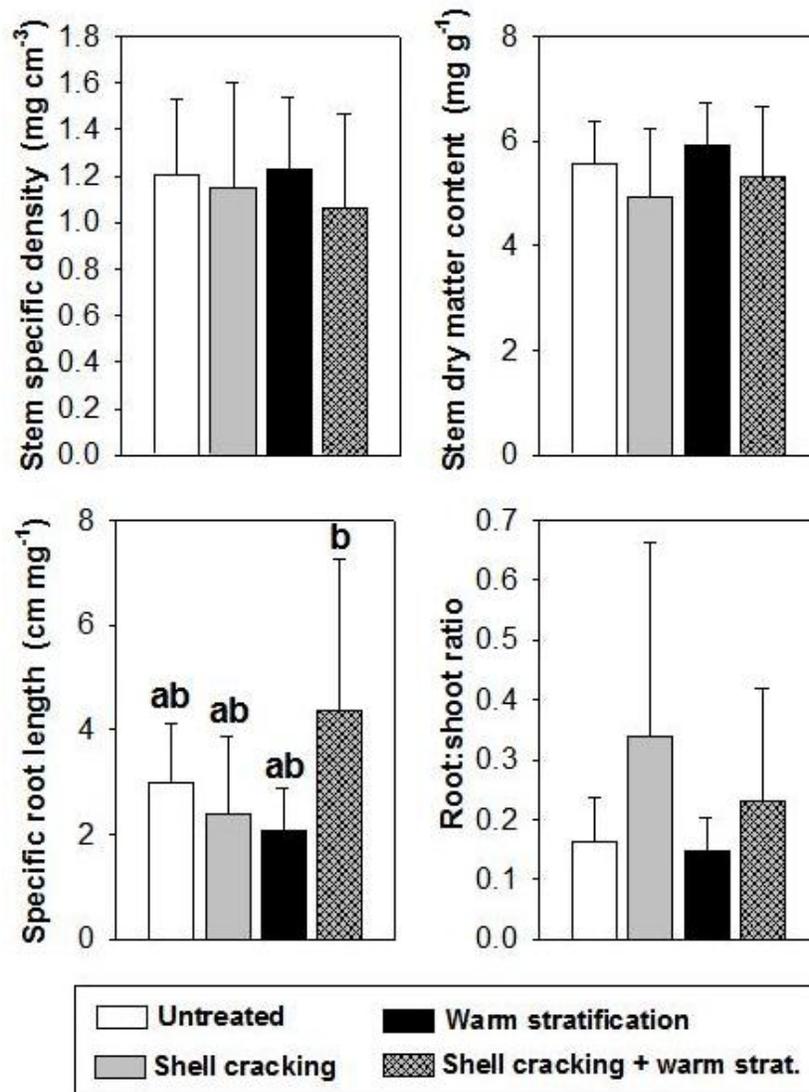


Figure 3. Stem specific density, stem dry matter content, specific root length, and root:shoot ratio of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means \pm S.D. (N=14). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=14).

et al., 2009; Moncaleano-Escandon et al., 2013).

Amongst the tested treatments, warm stratification treatment may be preferred to the other treatments since it expressed a good performance on seed germination and promotes the seedling growth. Pregermination by warm stratification of seeds of tropical plants is common practice of propagation for some important crops like oil palm (*Elaeis guineensis* Jacq.) (Green et al., 2013). The effect of this practice is not only to promote seed hydration but also to enhance the biosynthesis of stress-response substances, like heat-shock proteins, which can play a role in the fast removal of inhibitors of seed

germination (Collada et al., 1997; Bailly, 2004; Berjak and Pammenter, 2008). However, the right combination between treatment temperature, time and optimum temperature for germination, in the case of *J. curcas*, should be further investigated. Probably, the treatment temperature at 37°C in our experiment was too high because of the final germination rate inhibition risk, as observed by Windauer et al. (2011) at 35°C. This treatment requires also a certain degree of investment, due to the fact that needed a growth chamber to maintain a constant temperature for a certain period. For this reason, warm stratification treatment could be difficult to

be applied on *J. curcas* seeds collected and processed by farmers in remote areas of developing countries, in which *J. curcas* is generally cultivated at a small scale. Consequently, scarification treatment, allowing a higher root growth, could be a replicable and economic solution to be promoted at small scale.

Further research is needed to both test other different *J. curcas* low-cost pre-sowing treatments, which can be easily practiced in rural areas of developing countries, and monitor the seedling growth for a longer period (> 35 days), in order to collect more data and better evaluate their development. Another aspect to be investigated could be the interaction between seed storage methods and pre-sowing treatments, with the aim to find the optimal combination of these two factors, which could increase both seed germination and seedling growth parameters. In fact, *J. curcas* seed has a relatively short period of viability and high seed storage temperatures, which are common in tropical areas where *J. curcas* grows, and strongly speed up the loss of seed germination (Moncaleano-Escandon et al., 2013).

Conflict of Interest

The authors have not declared any conflict of interest.

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