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JATROPHA CURCAS FOR RURAL DEVELOPMENT IN SUB-SAHARAN AFRICA: AGRONOMIC AND SOCIO-ECONOMIC SUSTAINABILITY

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CONTENTS

Summary

In the forthcoming years, 1-2 million hectares of *Jatropha curcas* L. are expected to be annually planted, reaching 12.8 million hectares worldwide by 2015. This considerable expansion is due to its products and by-products multiple uses and its amazing adaptability. *J. curcas* oil extracted by seeds is a promising renewable feedstock for biodiesel production and, together with the oil extraction by-products, it can be used in as cooking/lighting fuel, bio-pesticide, organic fertilizer, combustible fuel, and for soap making, contributing to mitigate environmental problems in developing countries. Nevertheless, *J. curcas* is not a "miracle tree". Indeed, the full potential of *J. curcas* is far from being achieved and its talents are still to be supported by scientific evidences.

The present Ph.D. thesis aims to: (i) detail each phase of *J. curcas* productive chain from sowing to biodiesel and by-products, in order to logically organize the knowledge around *J. curcas* system, and to compare potentialities and criticalities of *J. curcas*; (ii) assess the socio-economic and environmental sustainability of smallholder local and decentralized *J. curcas* plantations, promoted by cooperation rural development cooperation projects in Sub-Saharan Africa; (iii) explore the effects of different pre-sowing treatments on germination behaviour of *J. curcas* seeds and to assess the growth of the seedlings; and (iv) investigate physiological responses, in term of growth and photosynthesis, of *J. curcas* seedlings exposed to a severe soil drought stress. The conducted studies confirmed that community-based initiatives on *J. curcas* plantation could positively contribute to the rural livelihoods in developing countries. However, it is still necessary to fill some knowledge gaps and much more research is required for guaranteeing a full socio-economic and environmental sustainability of *J. curcas* used as a trigger of rural development in Sub-Saharan Africa.

INTRODUCTION

Petroleum demand has risen rapidly due to the world industrialization and modernization. This economic development model has led to an enormous demand for energy, the major part of which derives from fossil sources such as petroleum, coal and natural gas. However, fossil fuels are limited and for this reason alternative fuels derived from renewable energy sources need to be found (Koh and Ghazi, 2011). Biodiesel has become in the recent years more attractive because of its possible environmental benefits (i.e. reduction of greenhouse gases emission) and it is obtained from renewable resources (Divakara et al., 2010; IPCC, 2012). The bio-diesel production from vegetable oils is foreseen to reach about 24 billion litres by 2017 worldwide (OECD-FAO, 2008). In the recent years, many potentialities are being associated to *Jatropha curcas*, a multipurpose tree belonging to the *Euphorbiaceae* family native of tropical America and used to produce a not edible oil (extracted from its seeds), which can be employed for biodiesel production (Achten et al., 2010). The not-edible characteristic is due to the presence of toxic components, such as phorbol esters. Additionally, the capability to grow on poor quality soils allows *J. curcas* to not directly compete against food crops. These reasons make *J. curcas* attractive to be cultivated in areas, such as developing countries, where edible oil are mostly cultivated for human consumption and not for biodiesel production (Gübitz et al., 1999; Devappa et al., 2010).

Furthermore, *J. curcas* seed is very rich in oil (about 25–35%) and the derived biodiesel has similar properties to that one produced from petroleum (Jongschaap et al., 2007; Koh and Ghazi, 2011). In addition to the bio-diesel production, *J. curcas* oil can be used as cooking/lighting fuel, medicine, bio-pesticide, and for soap making (Contran et al., 2013). Moreover, the seed cake, an oil extraction by-product, and fruit husks can be used as organic fertilizer, combustible fuel, or for biogas production (IFAD-FAO, 2010), while other products (i. e. root, bark, latex, leaves juice) have a wide variety of applications in the traditional medicine of developing countries rural communities (Brittaine and Lutaladio, 2010; Grevé et al., 2011). Along with these multiple uses, the expectations around *J. curcas* come from the amazing adaptability of this stem succulent, perennial, and drought avoidant tree even on low-nutrient soils and under arid and semi-arid conditions (Maes et al., 2009; Ye et al., 2009; Achten et al., 2010). Furthermore, the plant itself offers the ecological advantage to mitigate soil degradation and to reclaim marginal land or abandoned farmland, and it can also be used as live-fencing, acting like a livestock and fire barrier to protect fields (Kumar and Sharma, 2008).

For these reasons, some of the countries with highest economic growth rates, such as India and China, have strongly embedded the production of *J. curcas* biodiesel within their Energy policies. In 2003, a two-phase governmental project was launched in India for wide-spread cultivation of *J. curcas* on wasteland. The project aims at planting 12.5 million hectares on government land across the country, and then privatizing the production of *J. curcas* biodiesel. In 2006, China government decided to meet 15% of transportation energy needs with biofuel, leaning on the ambitious plan to raise 11 million hectares of *J. curcas* plantation on marginal lands (Fairless, 2007). In this context, a massive planting program of unprecedented scale encouraged millions of marginal farmers and landless people to plant *J. curcas* (Fairless, 2007; Kant and Wu, 2011). By 2008, *J. curcas* had already been planted over an estimated 900,000 ha globally, of which an overwhelming 85% was in Asia, 13% in Africa and the rest in Latin America, and by 2015 *J. curcas* is expected to be planted on 12.8 million ha worldwide (Kant and Wu, 2011).

However, *J. curcas* is not a “miracle tree”. *J. curcas* capabilities are not easily exploitable and applicable simultaneously. For example, since the lack of moisture and nutrients strictly influence plant yield, trade-offs between marginal land reclamation and profitable oil production have to be taken into consideration (Kant and Wu, 2011). For several reasons, both technical and economical, the full potential of *J. curcas* is far from being achieved, and its talents are still to be supported by scientific evidences (Divakara et al., 2010). *J. curcas* is still an un-domesticated tree and its seed and oil productivity is hugely variable and unknown (Parawira, 2010). Almost every step of cultivation is uncertain. The best agronomic management practices, the selection of suitable plant material, and the potential environmental risks and benefits have to be still investigated to lay out coherent and realistic cultivation plan (Moncaleano-Escandon et al., 2013). Furthermore, the establishment, management and productivity of *J. curcas* under various climatic conditions are not fully documented.

Hence, if the full potential of this species is to be realized, much more research is required into the whole *J. curcas* chain and more information is needed on the actual and potential markets for all its products (Openshaw, 2000). Due to this poor knowledge on several aspects of *J. curcas*, the results achieved so far are not encouraging and all the expectations on this crop are not being confirmed. In India, for example, seed production does not reach the promises. In China, until today, the production of biodiesel from *J. curcas* oil is quite low (Fairless, 2007; Kant and Wu, 2011).

The present doctoral dissertation has been performed in the context of the international cooperation project "GHAJA - Use of *Jatropha* plant to improve sustainable renewable energy development and create income-generating activities: an integrated approach to ensure sustainable livelihood conditions and mitigate land degradation effects in rural areas of Ghana", implemented for six years (2009-2015), within the "Environment and sustainable management of Natural Resources, including energy Thematic Programme (ENRTP)" financed by the European Commission (EuropeAid). The main goals of the present thesis are: (i) to systematise the scientific knowledge on *J. curcas* available so far, taking into consideration all the steps of the whole *J. curcas* chain, (ii) to assess the socio-economic and environmental sustainability of smallholder local and decentralized *J. curcas* plantations and (iii) to investigate some aspects which are still not deeply explored in the literature, through the carrying out of some experimental trials.

The thesis is structured in four chapters, each one structured as an article.

In the first chapter, the summary of a review on *J. curcas* titled "State-of-the-art of the whole *J. curcas* chain, from sowing to biodiesel and by-products" is reported. The main aim of this review was to provide a comprehensive summary of the *J. curcas* system and to compare potentialities and criticalities of *J. curcas* plantation and productive system, highlighting, for each productive step, the agronomical, management, and environmental issues which should be still investigated. In order to achieve this goal, all the available information has been collected from peer-reviewed literature, conference proceedings, books, and project reports and reported. This review has been published in the Journal "Industrial Crops and Products" (2013, vol. 42, 202-215). It was not possible to annex this review to this Ph.D. thesis due to Elsevier copyright restrictions. The review is available on the Elsevier web-site (http://dx.doi.org/10.1016/j.indcrop.2012.05.037).

In the second chapter, a study on the assessment of the socio-economic and environmental sustainability of smallholder local and decentralized *J. curcas* plantations, carried out in the framework of the GHAJA project, is illustrated. Indeed, GHAJA project involved seven rural communities of the West Mamprusi District (Northern Region, Ghana), providing the know-how and allocating financial resources for the realization of smallholder *J. curcas* plantations on abandoned farmland and for the provision of the equipment required for *J. curcas* oilseed and by-products production. Indeed, this diversification of smallholder plantations and the introduction of new sources of income for local populations could lead to greater economic and ecological resilience and strength sustainability actions (Achten et al., 2010; Settle and Garba, 2011; Bond et al., 2012; Dyer et al., 2012). The three sustainability dimensions, social, economic and environmental, described in the Plan of Implementation of the World Summit on Sustainable Development held in Johannesburg in 2002, have been considered in the study (UN, 2002). A Participatory Rural Appraisal (PRA) was conducted in the selected communities, for a total of 402 families provided 428 acres (1-2 acres per family) of abandoned farmland for the establishment of *J. curcas* plantations (Cornwall and Pratt, 2010). Participatory methods (e.g. individual interviews, focus group discussions, questionnaires, resource mapping, and rankings) were used to elicit data on socio-demographic and socio-economic characteristics, energy services, local land uses and cropping patterns, indigenous knowledge and skills on *J. curcas* cultivation and transformation processes. Environmental direct net gains have been calculated and described, while socio-economic consequences have been analysed and pros and cons have been presented.

In the third and fourth chapter, two research studies carried out during the Ph.D. program, are reported. These experiments aimed to investigate some *J. curcas* agronomic and eco-physiological aspects which are not still deeply explored. Out of about 680 articles on *J. curcas* published between 1974 and 2013, only 80 deal with agronomic and eco-physiological aspects. In particular, the two researches described in these chapters, focused on two important issues of *J. curcas*, such as the seed dormancy breaking and the drought resistance, which can be considered as main factors for the success/failure of this species, being used in community-based extensive cultivations promoted by cooperation projects and established in regions characterised by arid and semi-arid climate, such as those of Sub-Saharan Africa.

The first study, illustrated in Chapter 3, focused on two key factors in *J. curcas* production chain which are essential for *J. curcas* cultivation establishment: seed germination and seedling early growth. *J. curcas* poor germination rate relies on a hard and water impermeable seed coat which causes a physical dormancy and only few studies on propagation of *J. curcas* have been carried out so far (Baskin and Baskin, 1998; Islam et al., 2009; Windauer et al., 2012). Indeed, this study aimed to investigate the effects of different pre-sowing treatments on germination behaviour, and to assess the growth and vigour of the seedlings through the measurements of growth parameters (Cornelissen et al., 2003), in order to identify the best treatment which guarantees both the highest seed germination rate and the best development and growth of the seedlings. Five different pre-sowing treatments were tested: (i) control; (ii) soaking in 30 °C water for 24 hours; (iii) hammer shell cracking; iv) warm stratification at 37 °C for 24 hours; (v) hammer shell cracking plus warm stratification at 37 °C for 24 hours and obtained results have been described and discussed.

The second study, described in Chapter 4, aimed to investigate physiological responses, in term of growth and photosynthesis, of *J. curcas* seedlings exposed to a severe soil drought stress. Indeed, *J. curcas* is believed to be drought resistant (Levitt, 1980; Maes et al., 2009a; Achten et al., 2010). Anyway, the physiological mechanisms behind the high drought resistance of *J. curcas* are scarcely described and recent research has shed a new light on the water relations and water requirements of *J. curcas* (Achten et al., 2010; Kesava Rao et al., 2012; dos Santos et al., 2013; Sapeta et al., 2013). The effects of 26 days of water deficit on growth, water relations, leaf gas exchange, and chlorophyll fluorescence of *J. curcas* seedlings of 2 and 3 months have been investigated. The study has been conducted because an improved understanding is essential in order to adopt competitive strategies for improving *J. curcas* production. Additionally, the peculiar characteristics of *J. curcas* and its resistance mechanisms to drought stress make this plant an important object of study in the investigation of plant responses to abiotic factors.

During the Ph.D. program, other experimental trials on *J. curcas* yield are being carried out both in Sassari (Italy) and Tamale (Northern Region, Ghana) on three years old *J. curcas* plantations. In particular, one experiment, carried out in the experimental field of the Savanna Agricultural Research Institute (Nyankpala, Northern Region of Ghana), has the objective both to evaluate the production of some traditional cereals (*Zea mays* and *Sorghum vulgare*) and legumes (*Glycine max* and *Vigna unguiculata*) practised in the area and cultivated in intercropping systems with *J. curcas* and to determine the yield of *J. curcas* when consociated with these traditional food crops. Another experiment is being carried out in the greenhouse facility of the experimental field of the Dipartimento di Agraria of Università degli Studi di Sassari, located in Ottava (Sassari, Italy) and aims to assess the performance, in term of growth, photosynthesis and yield, of *J. curcas* trees exposed to a different soil drought stress. The description of these experiments are not included in the Ph.D. thesis, since they are still on-going, due to the fact that fruits formation in *J. curcas* usually occurs at least three years after transplanting/sowing and it has not occurred yet. Consequently, *J. curcas* yield was not possible to be determined so far. For this reason, these experiments will be accomplished in the next two years and their results will be shown in future publications.

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Chapter 1

State-of-the-art of the *Jatropha curcas* productive chain: from sowing to biodiesel and by-products[I]

Abstract

In the forthcoming years, 1-2 million hectares of *Jatropha curcas* L. are expected to be annually planted, reaching 12.8 million hectares worldwide by 2015. This considerable expansion is due to its products and by-products multiple uses and its amazing adaptability. *J. curcas* oil extracted by seeds is a promising renewable feedstock for biodiesel production and, together with the oil extraction by-products, it can be used as cooking/lighting fuel, bio-pesticide, organic fertilizer, combustible fuel, and for soap making. The capability to grow on poor quality soils not suitable for food crop makes *J. curcas* a possible solution of all the controversies related to biodiesel production. Furthermore, *J. curcas* contributes to mitigate environmental problems, such as marginal land or abandoned farmland reclamation. Nevertheless, *J. curcas* is not a "miracle tree": (i) the full potential of *J. curcas* is far from being achieved and its talents are still to be supported by scientific evidences; (ii) *J. curcas* capabilities are not easily exploitable and applicable simultaneously; (iii) its use is controversial and potentially unsustainable due to the current knowledge gaps about the impacts and potentials of *J. curcas* plantations. The aims of this review are to detail each phase of *J. curcas* productive chain from sowing to biodiesel and by-products, in order to logically organize the knowledge around *J. curcas* system, and to compare potentialities and criticalities of *J. curcas*, highlighting the agronomical, management, and environmental issues which should be still investigated.

Key words: physic nut, biodiesel, vegetable oil, land use, biomass

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[I] This review article has been published in the Journal "Industrial Crops and Products" (2013, vol. 42, 202-215). The review is available on the Elsevier web-site (http://dx.doi.org/10.1016/j.indcrop.2012.05.037).

Chapter 2

Potential and perspectives of *Jatropha curcas* smallholder plantations in Northern Ghana

Abstract

This study analyses and assesses the socio-economic and environmental sustainability of smallholder local and decentralized *Jatropha curcas* plantations in rural communities of Northern Ghana. Plantations were established on abandoned farmland. The processing of *J. crucas* fruits allowed the production of fertilizer, soap, and *J. curcas* energy products (oil, husk, and seed cake) as fuel for cooking or lighting, hence considered as alternative energy source of the traditional fuels. The sustainability of this initiative has been evaluated. Results suggest that community-based *J. curcas* initiatives for local use can be seen as an opportunity for positively contributing to rural livelihoods in Ghana.

Key words: Physic nut, biodiesel, rural development, vegetable oil, land use, Participatory Rural Appraisal

1. Introduction

Ghana is one of the most developed countries of the sub-Saharan area. The economic growth of the country has been estimated close to 8% in 2012 (IMF, 2013) and poverty reduction rates are the best in the Region, as reported by the United Nations (UN, 2011). In 2006, Ghana achieved target A of the first Millennium Development Goal, halving the number of people living below the poverty threshold by 2015, and target B, halving the number of people suffering from hunger (UN, 2011). Despite these successes, Ghana still faces several challenges: Ghana ranks 135 out of 187 countries on the Human Development Index (UNDP, 2011) and 53.6% of its population lives under the poverty threshold, estimated in 2 USD/day (IFAD, 2011). Due to the exponential economic growth during the last decade, the energy demand is high and one of the most difficult challenges which Ghana has to face is the energy supply. About 64% of the total energy supply in Ghana comes from wood-fuel (firewood and charcoal), 9% from electricity, and 27% from petroleum, which is becoming increasingly expensive (Duku et al., 2011). The Ghana government is conducting several efforts to modernise the energy supply sector, but assessments indicate that about 50% of the Ghanaian population has no access to grid-electricity and about 90% has not access to liquefied petroleum gas. Traditional energy sources are therefore considered as the dominant source of energy supply (Kemausuor et al., 2011). Wood-fuel consumption in Ghana is double than other energy sources (Energy Commission Ghana, 2010).

Jatropha curcas L., a valuable multipurpose crop, has recently gained lot of importance especially for the biodiesel production by oilseed. *J. curcas* could allow the production of energy products, without competing with food production and, at the same time, enhancing the socio-economic development, producing environmental benefits, and allowing the production of several economically valuable by-products (Contran et al., 2013). *J. curcas* is a drought-avoidant perennial large shrub or small tree, with a life expectancy of up 50 years. It grows in tropical and subtropical regions, with annual precipitation between 600-1,500 mm (Trabucco et al., 2010). Its high ecological adaptability allows its growth in an ample range of conditions from semiarid to humid (annual rainfall varying from 300 to 3,000 mm) (Maes et al., 2009) and in wide varieties of soil types, including poor quality soils (Ye et al., 2009). *J. curcas* seeds contain about 30-35% of oil per seed dry weight, which can be rather easily expelled or extracted (Jongschaap et al., 2007). The production of oil from *J. curcas* seeds requires two steps: (i) dehusking process (with a decorticator), to separate seeds from fruit husk, and (ii) oil extraction process, to produce oil and seed cake by-product (Figure 1).

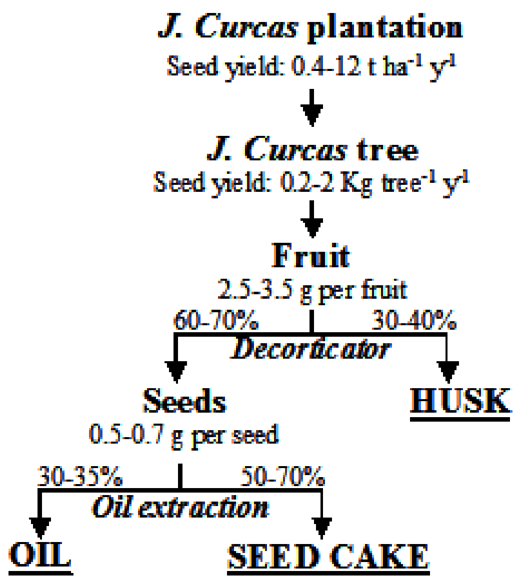

Figure 1. *J. curcas* system.

J. curcas oil can be used as cooking and lighting fuel, adopting special design equipment, and can replace the traditional biomass sources, such as firewood, charcoal, kerosene or petrol. In addition, the oil is utilized for soap making and as medicines and bio-pesticides. Despite all its attributes and multiple uses, the economic interest of large-scale investments on *J. curcas* cultivations depends on the possibility of turning it into biodiesel (Achten et al., 2008). The extraction of oil from *J. curcas* seed generates also important by-products: fruit husks are the by-products of dehusking process, while about 50-70% of the original seed weight remains as de-oiled seed cake (Figure 1) (Brittaine and Lutaladio, 2010; Devappa et al., 2010). Fruits husk and seed cake, having high nutrient content and calorific values, have a wide variety of applications as fuel or organic fertilizer (Ye et al., 2009). However, *J. curcas* is still a (semi-)wild undomesticated plant and its basic agronomic properties are not thoroughly understood, the growing and management practices are poorly documented, and the environmental effects have not been investigated yet (Contran et al., 2013). *J. curcas* yield is still unknown, and a wide yield range is reported in literature: annual dry seed production can range from about 0.4 t to 12 t per ha (Achten et al., 2008; Parawira, 2010).

The properties of this energy crop and its oil have persuaded investors, policy makers and clean development mechanism project developers to consider *J. curcas* as a substitute for fossil fuels, and large-scale investments are trigging all over the world (Achten et al., 2008). Anyway, the current knowledge gaps about the impacts and potentials of *J. curcas* plantation makes large-scale *J. curcas* cultivation for the oil and biodiesel production an hazardous business, with predictable negative repercussions on local populations and environment, such as the plantation of *J. curcas* on productive agricultural lands rather than on marginal lands (Kant and Wu, 2011; Dyer et al., 2012). Due to these controversial aspects, the debate about *J. curcas* plantation is heated (Fairless, 2007). Some of the most important emerging countries, such as India and China, have strongly invested in the large scale production of *J. curcas* biodiesel, in order to meet the energy demand of their dynamic economies. In particular, India government launched a two-phase project for spreading the large scale cultivation of *J. curcas* on wasteland in 2003, aiming at planting 12.5 million hectares. On the other side, China government decided to raise 11 million hectares of *J. curcas* plantation on marginal lands in 2006, aiming in this way at meeting 15% of transportation energy needs (Fairless, 2007; Kant and Wu, 2011). Nevertheless, both in India and in China, the results achieved so far are not reaching the expectations (Fairless, 2007; Kant and Wu, 2011). Segerstedt and Bobert (2013) found that, under current biofuel prices, the yield scenarios (2,000–5,400 kg per ha) make the large-scale *J. curcas* cultivation in Tanzania profitable neither for domestic consumption nor trade. Anyway, the analysis of biofuel production is complex. As shown by Arndt and co-workers (2011), biofuel production could lead to a stronger trade-off between biofuels and food availability when female labour is used intensively. Additionally skills-shortage among female workers also limits the relative poverty reduction. However, the authors conclude their analysis suggesting that only modest improvements in women's education and food crop yields are needed to address food security concerns and ensure broader-based benefits from biofuels investments (Arndt et al., 2011).

Contrary to these large scale industrial *J.curcas* programmes, community-based *J. curcas* initiatives for local use, such as extensive *J. curcas* plantations on poor quality soils, agro-forestry systems in which *J. curcas* is intercropped, and agro-silvo-pastoral practices, can be seen as efficient opportunities to promote rural development in developing countries. The easily integration of *J. curcas* into the rural economy at a village level is able to facilitate access to sustainable and affordable energy, such as oil for cooking or lighting, increase rural income, and create employment opportunities (Achten et al., 2010; Dyer et al., 2012). The diversification of smallholder plantations and the introduction of new sources of income for local populations could lead to greater economic and ecological resilience and strength sustainability actions (Settle and Garba, 2011).

The aim of this paper is to analyse and to asses the socio-economic and environmental sustainability of smallholder local and decentralized *J. curcas* plantations carried out in rural villages of Ghana. The concept of sustainability cannot be defined univocally (Bond et al., 2012). In this paper, the three sustainability dimensions, social, economic and environmental, described in the Plan of Implementation of the World Summit on Sustainable Development held in Johannesburg in 2002, have been considered (UN, 2002). In particular, sustainability has been assumed as the capacity of *J. curcas* smallholder plantations to produce durable net benefits both at environmental level, such as reducing deforestation and soil degradation, and at socio-economical level, such as creating new income generating activities (Bond et al., 2012). Environmental direct net gains have been calculated and described, while socio-economic consequences have been analysed only in their immediate impacts and pros and cons have been presented.

2. SURVEY METHODS

2.1. Study area

The study area was located in the West Mamprusi District (5,013 km^2), in the Northern Region of Ghana, within longitudes 0°35'W and 1°45'W and latitudes 9°55'N and 10°35'N and with Walewale as capital (http://westmamprusi.ghanadistricts.gov.gh). The district 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) (www.climatedata.eu). Seven rural communities were selected in the East part of the West Mamprusi District: Bimbini (10°19´46" N - 1°3´44" W; 258 inhabitants), Janga (10°0´42" N - 0°58´41" W; 2,978 inhabitants), Kparigu (10°17´54" N - 0°38´49" W; 4,000 inhabitants), Loagri (10°15´7" N - 0°49´0" W; 1,906 inhabitants), Nasia (10°9´39" N - 0°49´0" W; 3,000 inhabitants), Wungu (10°19´15" N - 0°50´25" W; 7,601 inhabitants), Yama (10°19´16" N - 1°1´11" W; 2,750 inhabitants).

2.2. Participatory rural appraisal method

A Participatory Rural Appraisal (PRA) was conducted in the selected communities (Cornwall and Pratt, 2010). Data were collected at the beginning of 2010. Participatory methods (e.g. individual interviews, focus group discussions, questionnaires, resource mapping, and rankings) were used to elicit data on socio-demographic and socio-economic characteristics, energy services, local land uses and cropping patterns, indigenous knowledge and skills on *J. curcas* cultivation and transformation processes. PRA was carried out on small groups (max 15 interviewees per group). Groups were selected within the same community, for a total of 402 interviewees. Descriptive statistics, percentage data, and weighted averages of categorical data (±S.D.) have been used to present the results. The percentages of missing data or not answered questions are not reported.

2.3. Agricultural practices and oil extraction activities

In 2010, 30-90 farmers were selected in each community for a total of 402 farmers. Each farmer made 1 or 2 acres of his land available for *J. curcas* plantations, for a total of 428 acres. Plantations were established strictly on abandoned farmland. Abandoned farmland were considered lands unsuitable for crop cultivation or lands unused for at least 2 years, due to the unproductive food production. In Table 1, all the required activities and, for each activity, the year of implementation and the working days per year, estimated for 1 acre plantation over a period of 10 years, are reported. *J. curcas* density plantation was 3m x 2m (666 plants per acre). Direct seed propagation method was used, consisting in sowing 2 seeds at 4-6 cm deep at the beginning of August. Plants were not irrigated and their cultivation was under rainy conditions.

Table 1. *J. curcas* agronomic practices and oil extraction activities. For each activity, the years of implementation and the working days per year are reported, considering a plantation of 1 acre over a period of 10 years.

Activities	Plantation year	Working days per year*
Land cleaning	1° year	1
Ploughing	1° year	1
Sowing	1° year	1
Refilling	1° year	1
Thinning	1° year	1
Weeding	1-10° years	4
Pruning	1-10° years	1
Harvesting	3-10° years	6
Fertilizing	3-10° years	2
Fruit transport	3-10° years	2
Decorticator	3-10° years	1
Seeds dried	3-10° years	1
Expeller	3-10° years	1
Oil and seed cake transport	3-10° years	2
TOT (10 years)		**175**

*Estimated for 1 acre plantation and an average distance of 6 km for transport

2.4. Assessment of environmental and socio-economic sustainability

The socio-economic and environmental sustainability assessment of *J. curcas* smallholder plantations has been performed considering a standard *J. curcas* plantation of 1 acre per household over a period of 10 years, as described in Table 2.

Characteristics of standard *J. curcas* plantation are defined considering a normal water supply (rainfall 700-1,220) and low-medium fertility (Achten et al., 2008; FACT, 2010). Considering that *J. curcas* yield is not still well-kown and annual dry seed production can range from 0.4 to 12 t ha^{-1} (Achten et al., 2008; FACT, 2010), on the basis on direct experience and literature data, a realistic yield of 1000 kg of dry seeds per ha (400 kg per acre) has been considered. The scenario analysed in this evaluation includes three activities: (i) the production of soap from *J. curcas* oilseeds, (ii) the fertilization of the plantations with high nutrient content *J. curcas* by-products, such as seed-cake, in order to promote abandoned farmland reclamation, and (iii) the use of *J. curcas* energy products (oil, husk, and seed cake) as fuel for cooking or lighting, hence considered as alternative energy sources compared to those currently daily used by the interviewees (firewood, charcoal, or kerosene).

Table 2. *J. curcas* standard plantation characteristics and estimated production per acre.

Plant and plantation characteristics	
Fruit weight[a]	3 g per fruit
Husk weight[a]	1 g per fruit
Seed weight[a]	0.65 g per seed
Seed oil[a]	35 % of seed dry weight
Number of plants[a]	666 plants per acre
Yield[a]	405 kg dry seed per acre

***J. curcas* products**	**Estimated production kg $acre^{-1}$**	**Estimated production kg $acre^{-1}$**
	$year^{-1}$	**10 $years^{-1}$**
Fruits[b]	623	4,984
Husk	218	1,744
Oil[c]	85	680
Seed cake	200	1,600

[a] Characteristics are defined considering a normal water supply (rainfall 700-1220) and low-medium fertility (Achten et al., 2008; FACT, 2010).
[b] The total production over a period of 10 years was estimated multiplying 1 year data by factor 8, since *J. curcas* trees are productive starting from the third year of the plantation.
[c] Equivalent to 90 l (Oil density 920 kg m^{-3})

The total energy (quantity per calorific value) of oil, husk, and seed cake produced by the standard *J. curcas* plantation was compared to the total energy of firewood, charcoal, and kerosene consumed for cooking and lighting respectively. Calorific values of the energy sources are reported in Table 3.

The time spent in both *J. curcas* plantation management practices and oil, husk, and seed cake production (Table 1) was compared with the time required to harvest or purchase the proportionate quantity of firewood or charcoal. The quantity (kg) of energy sources and the time spent in collecting the traditional energy sources used by the interviewees, were estimated according to the PRA results.

Table 3. Calorific values of energy sources used by the interviewees and *J. curcas* energy products. In order to avoid overestimated evaluation, the calorific values of *J. curcas* energy products were the lowest values reported in literature, while the calorific values of the energy sources were the highest values reported in literature.

Energy source	Calorific value (MJ kg^{-1})	Reference
Firewood	20	Lamera et al. (1994)
Charcoal	32	Rosillo-Calle et al. (2007)
Kerosene	46	Kalyan and Ishwar (2007)
Husk	11	Jongschaap et al. (2007), Acthen et al. (2008)
Oil	37	
Seed cake	18	

The total cost of oil, husk, and seed cake production from the standard *J. curcas* plantation was compared to the total cost of the proportionate quantity of firewood or charcoal used in 10 years. The data related to the costs had been collected during a survey carried out in Ghana in 2010. Prices and costs were expressed in Ghanaian New Cedi (GHS). In 2010, the official exchange rate GHS/Euro was 0.52 (http://ec.europa.eu). According to the hourly working wage agreed by the local stakeholders, the unit cost per person was equals to 0.6 GHS per hour. With reference to firewood and charcoal, the total cost was calculated considering both the cost of the combustible (replaced by *J. curcas* energy products) and the cost of one person in charge of the energy source collection, estimated according to the PRA results.

3. SOCIO-ECONOMIC PROFILE OF FARMERS

The majority of interviewees involved in the study were men and illiterate and more than half of them were under 50 years old (Figure 2). Households were mainly composed by 6-15 people and about half of interviewees were land owners (Figure 2). Their main activity was farming, from which derived the majority of income. Farm equipment, food, energy, education, and clothing were the main expenses. Details of the socio-economic profile are showed in the Figure 2.

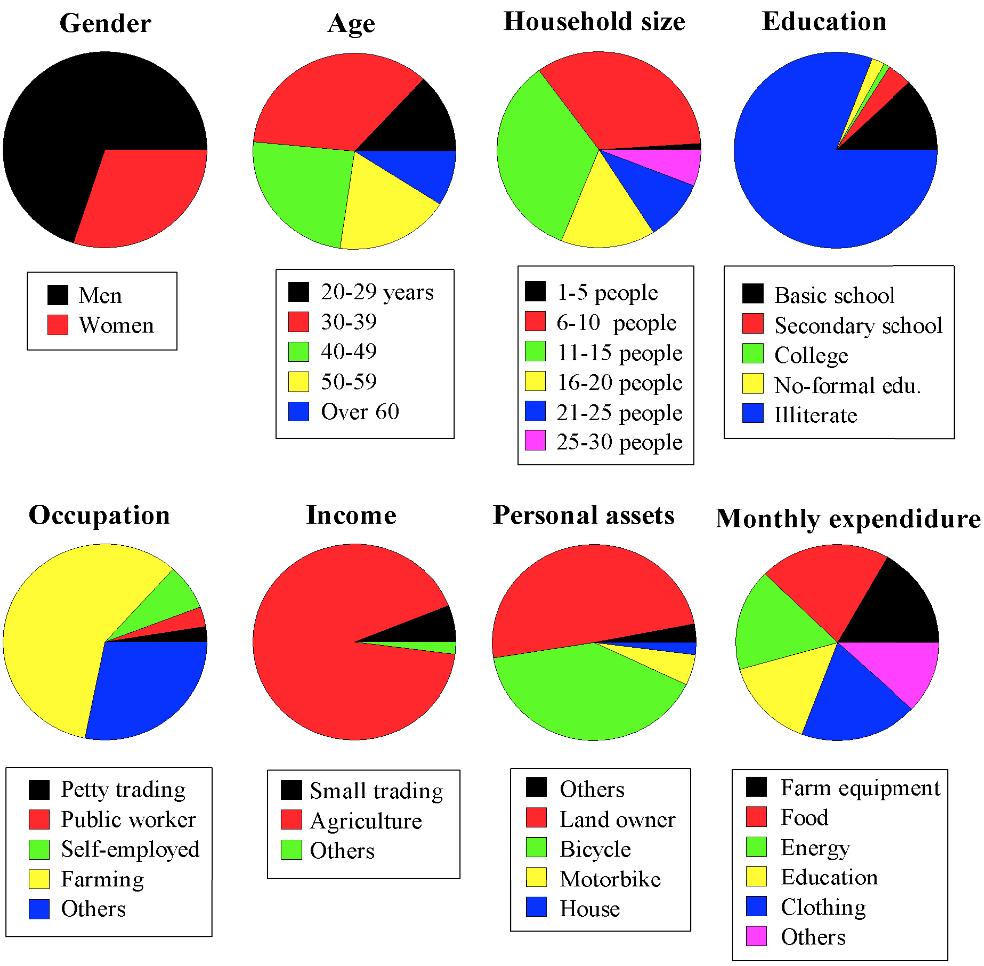

Figure 2. Socio-economic profile of the interviewees involved in the study (N=402). Data are expressed as percentage.

The community chief is the custodian of the lands, but each family could retain the usufruct of lands for personal agricultural practices. More than half of the interviewees owns between 11-30 acres of lands and about 26% of total lands were considered poor quality soils or agriculture unproductive soils (Figure 3).

On fertile soils, the main cultivated crops were cereal and leguminous crops. The yield was quite variable due to the dependence on agronomic inputs, management practices, and climatic annual conditions. The total yield for cereal and leguminous crops was around 4,118 kg (±2,660) per year. The household consumption was about 65% (±30) of the own production.

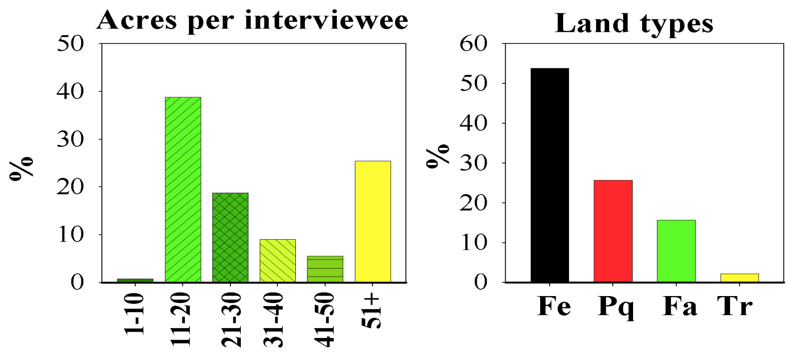

Figure 3. Percentages of acres per each interviewees (left) and percentages of land types (right) in the study area (Fe = fertile soils, Pq = poor quality soils, Fa = fallow soils, Tr = tree planted areas).

Firewood, charcoal, fuel and electricity were the energy sources commonly used in the communities. The uses of various energy sources changed among the communities, due to the differences in availability and accessibility. Most of the interviewees would choose firewood as first energy source, because it is accessible, appreciably cheap, and it is one of the best energy source for cooking purpose (Figure 4a). Each interviewee family used about 104 kg (±55) of firewood per month with an average price around 15-25 GHS. Firewood is generally purchased or harvested by women through the pruning of their own trees (Figure 4b and Figure 5). The main problems in accessing firewood were lack of transport and accidents. Charcoal was generally the second source of energy, utilised to cook and heat water, because it is smokeless than firewood and it is easily accessible (Figure 4a). Each interviewee family used about 56 kg (±39) of charcoal per month, with an average price of around 15-20 GHS. Charcoal was generally purchased by women from their own village or from the nearby market (Figure 4b and Figure 5). The main problems in accessing charcoal were its high price and lack of transport. More than one third of the interviewees would have choose electricity as second source of energy for its efficiency. Electricity was utilized to provide energy for household use or to power grinding mills (Figure 4a).

The interviewees spent around 15-25 GHS per month. Interviewees accessed electricity from their own village and the responsibility to buy electricity or pay the electricity bills was mainly in charge of men (Figure 4b and Figure 5). The main problem in accessing electricity was its high price. Kerosene was the main oil fuel source, because it is easy to use and it is the best energy source for lighting purpose (Figure 4a). Each interviewee family used about 5.2 l (±2.5) of kerosene per month, equivalent to 4.1 kg per month (±2), with an average price of around 15-24 GHS. Fuel was generally purchased by men from their own village or from the nearby market (Figure 4b and Figure 5). The main problems in accessing kerosene were its high price and lack of transport. On average, interviewees spent approximately 2-3 hours per week for collecting or purchasing energy sources, except for firewood harvest, which took about 12 hours a week (Figure 4c).

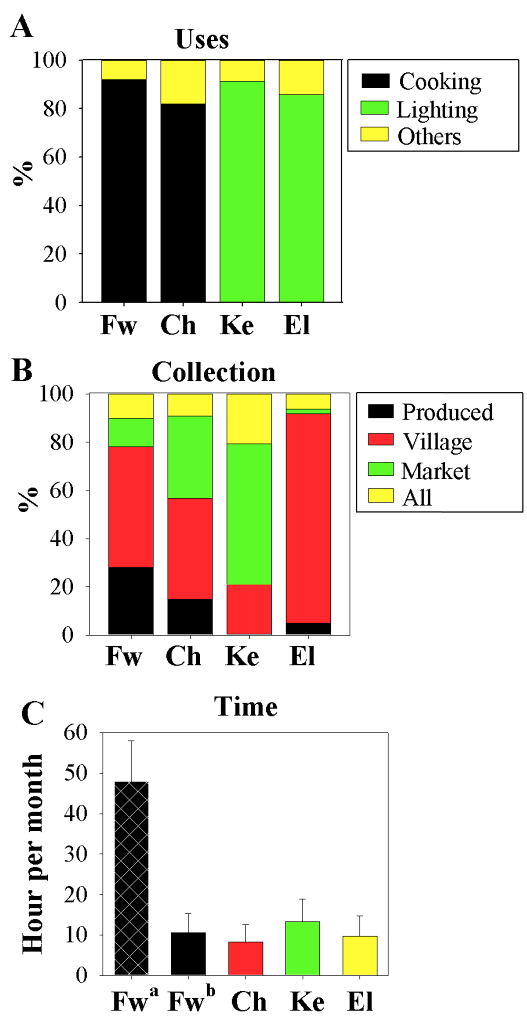

Figure 4. a) Additive percentages of energy source potential uses. b) Additive percentages of energy sources collection types. c) Average (±S.D.) monthly time spent to the source per month. (Fw = firewood, Fw[a] = harvested firewood, Fw[b] = purchased firewood Ch = charcoal, Ke = Kerosene, El = Electricity).

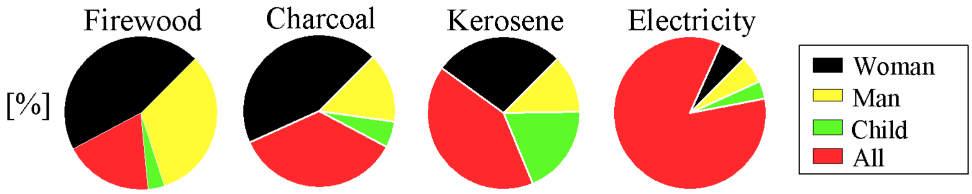

Figure 5. Percentages of responsibility to collect or buy the different energy sources.

As emerged from the PRA analysis, *J. curcas*, called *Baanyemaasim* or Flower, was already known among local communities in Ghana. Anyway, since the benefits of *J. curcas* were not well known, only 9% of the interviewees cultivated it and used *J. curcas* to provide shade for animals (44%), to protect the fowl from hawks (39%), as a live-fencing (13%), for medical proposes and as snake repellent. With respect to the agronomic practices already applied to *J. curcas* cultivation, around half of the farmers who cultivated *J. curcas* weeded the land before planting and applied fertilizer, 40% carried out regular weeding during the rainy season, 80% carried out regular pruning, and none of the farmers performed activities against pest and diseases. The yield was very low and absolutely non-competitive. Fruits were harvested manually with rubber gloves, and seeds were stored in rubber bags after chemical applications. There were not prejudices for its cultivation, and local farmers expressed the willingness to be engaged in *J. curcas* system, although they did not present a wide and complete knowledge about its by-products. Eighty-eight percent of the interviewees knew that it is possible to obtain biodiesel from *J. curcas* seeds and 12% knew that it is possible to obtain soap, but none of them used these products. On the contrary, 16% of the interviewees used regular *J. curcas* leaves, roots, and stem, powdered or water boiled, to treat fever, stomach pains, headache, ringworm, or toothache. Additionally, *J. curcas* sap was used by children to mend books. The availability of lands was not a limiting factor for a family, which potentially could extend the cultivation for the total replacement of traditional fuels with *J. curcas* energy products. On the contrary, the main hurdle for the *J. curcas* diffusion and sustainability was represented by the lack of a complete knowledge about its uses and potentialities (Pretty et al., 2011).

4. SUSTAINABILITY OF *J. CURCAS* SYSTEM

The assessment of socio-economic and environmental sustainability of *J. curcas* plantations presented in this study allows delineating some benefits and controversial aspects that *J. curcas* can lead to rural Ghanaian communities. This study has been performed in the context of the project "Use of *Jatropha* plant to improve sustainable renewable energy development and create income-generating activities: an integrated approach to ensure sustainable livelihood conditions and mitigate land degradation effects in rural areas of Ghana (GHAJA)", implemented for six years (2009-2015), within the European Commission (EC) "Environment and sustainable management of natural resources, including energy" programme (EuropeAid). In 2010, the project involved seven rural communities of the West Mamprusi District (Northern Region, Ghana), providing the know-how and allocating financial resources for the realization of smallholder *J. curcas* plantations on abandoned farmland and for the provision of the equipment required for *J. curcas* oilseed and by-products production. According to the key requirements for sustainable intensification in African agriculture proposed by Pretty *et al.* (2011), this project is being able to: (i) provide scientific input practices combined with appropriate agro-ecological and agronomic management, (ii) improve farmer knowledge and know-how, (iii) enhance farmers' capacity to add value through their own business development, and (iv) focus on women's educational and agricultural technology needs.

4.1. Environmental sustainability

Low soil fertility is widely recognized as a major obstacle to improve agricultural productivity in sub-Saharan Africa (Oluyede et al., 2011). From the environmental sustainability point of view, the cultivation of *J. curcas* can lead environmental benefits, including the reduction of deforestation and soil degradation. *J. curcas* ability to grow on poor quality soils, combined with its capacity to improve soil physical conditions and reduced soil erosion, makes this tree an excellent biological system for the reclamation of degraded soils, such as abandoned farmland (Openshaw, 2000). The development of a deep taproot, functioning as an efficient nutrient circulation pump, permits to extracts mineral and nutrients leached down and releases them to the surface through the leaf or fruit shed, forming mulch nearby the base of the tree (Kumar and Sharma, 2008). In this context, the fertilization of *J. curcas* plantations with the by-products obtained from oil extraction, such as seed cake, should be an essential action in order to allow an effective soil reclamation. Practically, the nutrient net removal from the soil due to the fruit production and harvesting should be compensated by fertilizing *J. curcas* plantations with 100 kg of seed cake per year. This value is calculated considering: (i) seed cake composition (nitrogen (N) 3.82-6.40 % per dry matter weight, phosphorus (P) 0.9-2.9 % per dry matter weight, and potassium (P) 0.95-1.75 % per dry matter weight) and (ii) nutrient net removal (estimated from the fruit nutrient composition and equals to 14.3-34.3 kg N, 0.7-7.0 kg P and 14.3-31.6 kg K per ha, considering a seed yield of 1,000 kg per ha) (Jongschaap et al., 2007). Over a long period, intercropping or agro-forestry systems can be considered additional benefits for the local populations.

J. curcas oil (604 kg), husk (1,744 kg), and seed cake (800 kg), excluding soap production and fertilization, when used as combustible fuel for cooking or lighting, could replace 23% of firewood, 23.5% of the charcoals, or 100% kerosene used by the interviewees (Table 2 and Figure 6).

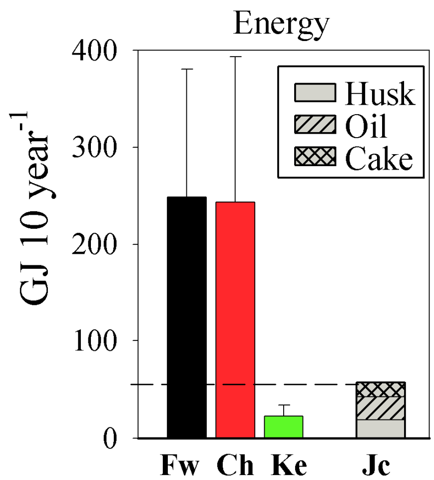

Figure 6. Average (±S.D.) of the total energy of firewood (Fw, black), charcoal (Ch, grey), and kerosene (Ke, dark grey) consumed for cooking and lighting over a period of 10 years and estimated average of the total energy of *J. curcas* energy products (Jc, white - oil, husk, and seed cake) produced from 1 acre plantation over a period of 10 years.

Over a period of 10 years, the replacement of 23 % of firewood avoids the use of 2,863 kg of firewood (of a total of 12,421 kg) per family. The replacement of 23.5% of charcoal avoids the use of 1,590 kg of charcoal (of a total of 6,757 kg) per family. The 492 kg of kerosene used by interviewees in 10 year can be easily replaced with the *J. curcas* products. For the replacement of the total consumption of traditional fuels, a family, mostly composed by 6-15 people, should cultivate about 9 acres. Generally, the harvesting of firewood takes place through an intensive tree pruning (20-33 kg per tree). Unfortunately, it has not been possible to find in literature the value of annual productivity of the most common tree species present in the Ghanaian savannah. In order to calculate the actual reduction of deforestation due to the GHAJA project, further investigations are needed.

4.2. Socio-economic sustainability

The introduction of community-based *J. curcas* plantations could be able to modify the tasks and activities within the household, changing the responsibilities and labour divisions between men and women. Abandoned farmlands are, in fact, frequently owned by women, and they often represent the sole lands that women can have access to (Rossi and Lambrou, 2008). Furthermore, the responsibility to collect or buy firewood and charcoal is mostly in charge of women (Figure 4), while the agricultural works are mainly performed by men. As a consequence, *J. curcas* plantations and energy products could increase the value of women land properties and reduce their labour activities, generating socio-economic impacts not predictable, and analysable only in the long-term.

The comparison between the firewood or charcoal system and *J. curcas* energy product system has been carried out considering both the time spent and the total costs incurred for the provision of these different energy sources. In order to properly compare the production of *J. curcas* energy products and firewood collection, it is important to independently analyse the interviewees (31%) who harvested firewood from those (67%) who purchased it. Considering the time spent by interviewees over a period of ten years, the time requested for carrying out both all the agricultural practices on the standard *J. curcas* plantation and the oil extraction activities has been calculated and amounts to 1,400 h (175 days/8 h per day, as shown in Table 1). This figure is comparable to time spent to harvest 2,863 kg of firewood (1,435 h±358). On the other hand, the time requested for *J. curcas* energy products (1,400 h) is higher than the time spent to purchase 2,863 kg of firewood (315 h ±138) or to collect 1,590 kg of charcoal (254 h ±129), implying an additional working time of 13-15 days per year for the production of *J. curcas* energy products. However, this result does not consider the following: (i) in the first and second years, when *J. curcas* plants are not productive yet, the total time spent for the plantation management is equals to 10 and 5 days per year respectively (Table 1), (ii) the more efficient work organization, mostly concentrated at the end of the rainy season or during the drought season, and (iii) the time saved by the farmers, when *J. curcas* trees are intercropped with food crops, for the carrying out of the common agricultural practices. In an economy of scale, the spent time to carry out certain agronomical activities for *J. curcas*, such as weeding and fertilising (6 days per year), could be taken up by the standard agronomical food crops practices. Although the availability of lands is not a limiting factor, if the overall consumption of traditional fuels (12,421 kg of firewood; 6,757 kg of charcoal; 492 kg of kerosene) would be totally replaced with *J. curcas* energy products, 9 acres per family would be necessary. This hypothesis will entail an additional time equals to more than 3 months per year per family for carrying out both *J. curcas* agricultural practices and oil extraction activities.

As regards *J. curcas* energy products, costs were spitted into costs in charge of project and of the interviewees. The project costs covered all the machinery and infrastructures and they have been completed funded by EuropeAid program (Table 4).

Table 4. Project cost for *J. curcas* machinery, facility, and seeds.

	Unit price (GHS)	Number	TOT (GHS)
Decorticator + engine	4,350	7	30,450
Expeller + engine	5,350	7	37,450
House facility	9,000	7	63,000
Cooking stove	60	402	24,120
Seed	2 (per kg)	185 (kg)	370
TOT			**155,390**

The interviewees costs were the cost of one person employed both in the *J. curcas* plantation management and in oil, husk, and seed cake production over a period of 10 years. Soap was made using 5 kg of *J. curcas* oil and a solution of sodium hydroxide (approximately 150 g of sodium hydroxide with 0.750 l of water for 1 l of oil). In order to produce 50 bars of soap of 100 g each, the interviewees would need to work for 2 days and spend around 20 GHS (10 GHS for 1 kg of sodium hydroxide, 5 GHS for soap mould, and 5 GHS for wrapping paper). Since in Ghana the average price of one bar of soap is 1-2 GHS, the 50 bars of soap could be sold, with a net profit of 30-80 GHS.

The cost of one person employed in the *J. curcas* plantation and production of its energy products, calculated in 844 GHS per 10 years, is in line with the range of expenditures made by each family for firewood (harvested 646-1,075, purchased 519-823) or charcoal supply (497-935), if the saving or the potential profit (30-80 GHS) associated with the *J. curcas* soap production are considered (Table 5).

Table 5. Range of cost for firewood (harvested or purchased), charcoal, and *J. curcas* energy products. For firewood and charcoal, both the cost of the combustible (replaced by *J. curcas* energy products) and the labour cost of one person in charge of the energy source collection over a period of 10 year are considered. For *J. curcas* energy products, the cost of one person employed in the *J. curcas* plantation management and in the oil, husk, and seed cake production over a 10 year period are considered.

Energy source	**Material cost per 10 year**	**Person cost per 10 year***	**TOT GHS**
Harvested Firewood**	-	646-1,075	646-1,075
Purchased Firewood**	414-552	105-271	519-823
Charcoal***	423-705	74-230	497-935
J. curcas	-	840	840

*0.6 GHS per hour
**Considering the cost of the 23% of used firewood
***Considering the cost of the 23.5% od used charcoal

It is worth mentioning that the real cost of purchased firewood and charcoal includes both material and labour costs, while the real cost of *J. curcas* energy products includes only the labour costs. For this reason, this *J. curcas* system does not need to have an actual capital, but only the availability of labour force. In conclusion, if all the costs related to *J. curcas* productive chain are considered (around 22,200 GHS per community), the implementation of the above mentioned activities results economically sustainable for a Ghanaian rural community only if the costs for the *J. curcas* plantation setting and for the oil production equipment are in charge of external aid, such as the EuropeAid cooperation project (Table 4). Moreover it is difficult to achieve a *J. curcas* energy system without inputs in term of know-how, organisation and equipment.

5. CONCLUSIONS

Sustainability potentialities and criticalities of the considered *J. curcas* system have been illustrated, highlighting some of its socio-economic and environmental benefits for rural communities. Nevertheless, the gains achieved with *J. curcas* plantations totally depend on the cultivation system, with high differences between community-based *vs* large-scale plantations, leading the system from sustainable to unsustainable.

Results suggest that community-based *J. curcas* initiatives for local use, such as smallholder and decentralized *J. curcas* plantations on poor quality soils, can be seen as an opportunity for positively contributing to rural livelihoods in Ghana. The considered *J. curcas* scenario includes the production of 5 kg of soap per year, the fertilization of *J. curcas* plantations with 100 kg of seed cake per year, and the possible replacement of the traditional energy sources (firewood, charcoal, or kerosene) generally used by the interviewees with oil, husk, and seed cake. The cultivation of 1-2 acres per family is able to ensure access to sustainable and affordable energy sources, considerably reducing the collection of firewood, and partially modifying the tasks and activities of the household members.

Large-scale monoculture *J. curcas* plantations for biodiesel production might distort local economy and social system, even exacerbating gender inequalities: men and women might have different employment opportunities and conditions on plantations and might be exposed to different work-related health risks, possibly contributing to the socio-economic marginalization of women (Rossi and Lambrou, 2008). Additionally, if *J. curcas* competes for fertile lands with food crops, as generally happens in large-scale plantation for biodiesel production, it would lose its environmental sustainability and advantages (Achten et al., 2010).

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Chapter 3

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

Abstract

In the last decades, *Jatropha curcas* has become popular thanks to its wide capabilities and plethora of uses, including biodiesel production. Two key factors in *J. curcas* production chain are seed germination and seedling early growth. This study aimed to investigate the effects of different pre-sowing treatments on germination behaviour and to assess the growth and vigour of the seedlings through the measurements of growth parameters, in order to identify the best 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 Ghana and subjected to five different pre-sowing treatments: (i) control; (ii) soaking in 30 °C water for 24 hours; (iii) hammer shell cracking; (iv) warm stratification at 37 °C for 24 hours; (v) hammer shell cracking plus warm stratification at 37 °C for 24 hours. Amongst the seventeen indices considered in the experiment (six germination indices and eleven growth rate indices), results revealed that the tested pre-sowing treatments influenced much more seed germination than seedling growth. Warm stratification treatment enhanced seed germination and promoted seedling growth as compared to the other tested treatments. Further research is needed to both test other different *J. curcas* pre-sowing treatments and investigate the interaction between seed storage methods and pre-sowing treatments, with the aim at finding the optimal factor combination to increase both seed germination and seedling growth.

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

1. 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., 2010). Nowadays, *J. curcas* grows in tropical and subtropical regions in a wide range of climatic conditions from semiarid to humid

(Achten et al., 2010). 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 1-2 million hectares of *J. curcas* yearly planted at global level (Contran et al., 2013). *J. curcas* seeds contain about 25-35% of oil, 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 (Contran et al., 2013).

Besides the economic value derived from *J. curcas* oil and its derived products, *J. curcas* strength as a crop derives from its 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 reclaim marginal land or abandoned farmland (Contran et al., 2013).

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, since the scarcity of scientific researches. *J. curcas* is still a (semi-)wild undomesticated plant. Its basic agronomic needs are not thoroughly understood, the growing and management practices are poorly documented, and the environmental effects have not been investigated yet (Moncaleano-Escandon et al., 2013). Only few studies on propagation of *J. curcas*, representing a critical stage in the plant life cycle, have been carried out so far (Islam et al., 2009; Windauer et al., 2012). *J. curcas* can be propagated by vegetative (cuttings) and generative (seeds) methods. Seeds can be used either to raise seedlings in nursery or to be sown directly in the field. These practices favour the development of trees with a deep taproot, more drought resistant and with more longevity as compared to those obtained by cuttings (Contran et al., 2013).

One of the major issues of *J. curcas* seed germination relies on its poor germination rate, which is mainly due to the presence of a hard and water impermeable seed coat, which impedes water absorption and causes a physical dormancy (Baskin and Baskin, 1998). Despite the seed dormancy is a strategy to assure the survival of the species, it is also a factor limiting propagation. In order to enhance germination percentage, seeds can be 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.

There are only few 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 hours before sowing, showed a significantly higher germination percentage than control in all the different genotypes tested in the experiment. Windauer et al. (2012) tested the effects of different temperatures (from 15 °C 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 technique to induce seed dormancy-breaking (Wang and Hanson, 2008). No study was found in literature on the effects of other pre-sowing treatments on the growth of *J. curcas* seedlings.

The aim of this study was to investigate the effects of different pre-sowing treatments on germination behaviour and to assess the growth and vigour of the seedlings through the measurements of growth parameters (Cornelissen et al., 2003), 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.

2. Materials and methods

2.1. Experimental set up

The experiment was performed in a growth chamber of the Agricultural Department of the University of Sassari (Italy) and carried out on *J. curcas* seeds of the 'Indian' cultivar. This cultivar has been chosen in the study, since it is one of the most common cultivar used in Ghana and India and largely adopted in both small-scale extensive plantations, promoted by cooperation projects, and large-scale intensive plantations, operated by multinational companies. *J. curcas* seeds were collected in October 2011 in Tamale (Ghana Yendi road Farm, Northern Region of Ghana) and stored at 18 (±2) °C 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 hours; (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 hours, 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 hours; (v) hammer shell cracking plus warm stratification at 37 °C for 24 hours, 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 hours. Germination test was carried out in a growth chamber at 28 °C, under a 8/16 light/dark regime at 400 μmol m^{-2} s^{-1}. A completely randomised design with four replications of ten seeds per replication was used. Ten seeds per treatment were tested for each replication. 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/m^3) and fully irrigated with a total of 55 ml of distilled water during the first two weeks of the experiment.

2.2. Measurements

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 on 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 of germinated seeds were separated into cotyledons, leaves, stem, and (washed) roots and 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 transversal area), and (iv) root (length, diameter, fresh and dry weight, and transversal section area). Dry weight was measured when samples at 100 °C reached a constant weight (around 48 hours). Total and transversal section areas were measured by an Area Meter (LI-3100C Area meter, Licor).

Measured data allowed calculation of several parameters related to germination process and seedling growth, as reported in Table 1.

2.3. Statistical analyses

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test). Percent values were arcsine-square root transformed prior to analysis. Analysis of variance (ANOVA) was applied to test the effect of Treatments. For the ANOVA of seedling growth parameters, the statistical unit was the single seedling. A Tukey HSD test was applied to compare the above effects between homogeneous groups. Tests of significance were made at a 95% confidence level. Analyses were processed using STATISTICA 6.0 Package for Windows (StatSoft 2001, Tulsa, OK).

Table 1. Germination process and seedling growth parameters.

Variable	Formula	References
Germination process		
Germinability (G) [%]	G = (Ng / N) * 100	-
True leaf expansion (Tl) [%]	Tl = (Nl / G) *100	-
Emergence rate (Er) [day^{-1}]	Er = [ΣNd / (Σ D * Nd)] *100	Kotowski (1926)
Emergence index (EI) [day^{-1}]	EI = Σ (Nd / D)	AOSA (1983)
Mean emergence time (MET) [day]	MET = (Σ D * Nd) / ΣNd	Ellis and Roberts (1981)
Seedling vigour index (SVI) [cm %]	SVI = [(Ls+Lr) * 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) [mm^2 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) [mm^2 mg^{-1}]	SLA = Al /DWl	Cornelissen et al. (2003)
Leaf dry matter content (LDMC) [mg g^{-1}]	LDCM = DWl / FWl	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 area (SRA) [mm^2 mg^{-1}]	SRA = Ar /DWr	-
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 [day]; N = total number of seed; Ng = total number of germinated seeds; Nd = number of seeds germinated on day D after sowing; Nl = number of expanded true leaf when seed is germintaed; N5 = number of seeds germinated on day 5 after sowing; N15 = number of seeds germinated on day 15 after sowing; Ls = average stem lenght (cm); Lr = average root lenght (cm); Ac = cotyledon area [mm^2]; DWc = cotyledon dry weight [mg]; FWc = cotyledon fresh weight [g]; Al = leaf area [mm^2]; DWl = leaf dry weight [mg]; FWl = leaf fresh weight [g]; DWs = stem dry weight [mg]; FWs = stem fresh weight [g]; Ar = root area [mm^2]; DWr = root dry weight [mg]; DWc+l+s = cotyledons plus stem plus leaf dry weight [g].

3. Results

The statistical analysis revealed that the tested pre-sowing treatments have various effects on different germination parameters of *J. curcas* seeds. In particular, germinability, 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).

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

	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 vigour index	1.9^{ns}

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

More in detail, water soaking treated seeds showed the lowest percentage of germinability (7.5%) ($p \leq 0.05$, 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 ($p \leq 0.05$, Figure 1). Furthermore, the highest value in emergence rate (24 day^{-1}) was found in warm stratification treated seeds, which showed higher emergence rate compared also 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). The effect of water soaking treatment on emergence rate, emergence index and mean emergence time was not possible to be calculated, due to a very low germination rate of seeds (Figure 1).

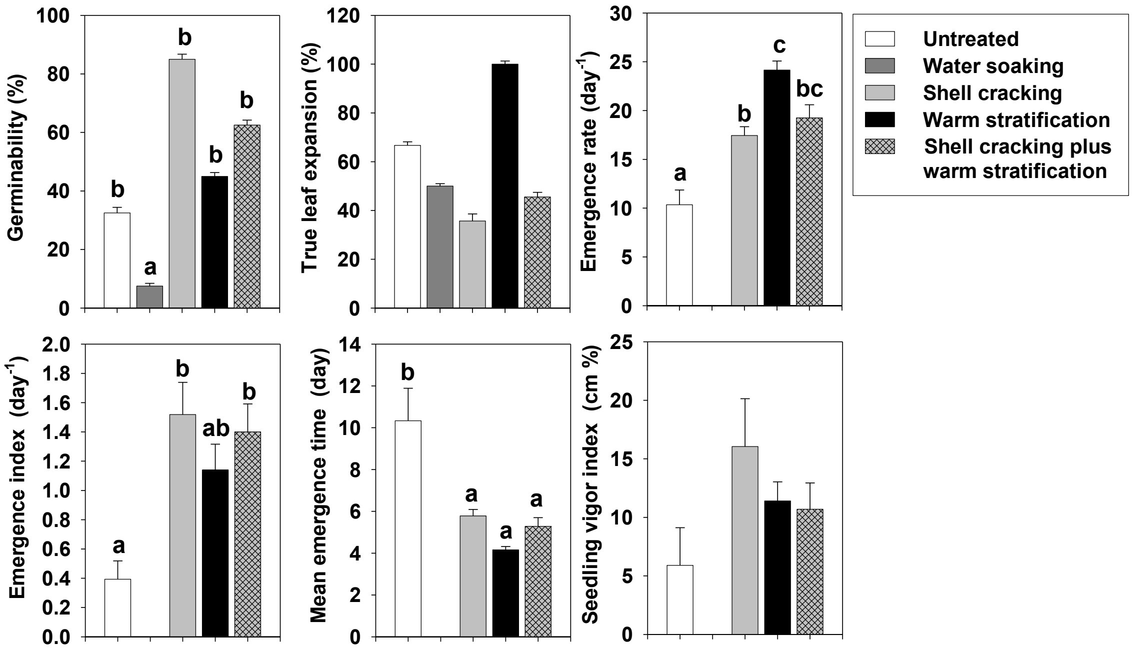

Figure 1. Germinability, true leaf expansion, emergence rate, emergence index, mean emergence time, and seedling vigour index of *J. curcas* seeds subjected to different pre-sowing treatments: untreated control, soaking in 30 °C water for 24 hours, shell cracking, warm stratification at 37 °C for 24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

After 35 days from the sown, amongst the eleven growth parameters, only cotyledon size, leaf size, and specific root length showed significant differences among treatments (Table 3).

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

	Treatment
d.f.	4
Cotyledon size	6.2***
Specific cotyledon area	0.5^{ns}
Cot. dry matter content	1.7^{ns}
Leaf size	6.6***
Specific leaf area	1.9^{ns}
Leaf dry matter content	0.9^{ns}
Stem specific density	0.4^{ns}
Stem dry matter content	1.6^{ns}
Specific root area	1.6^{ns}
Specific root length	3.6*
Root:shoot ratio	2.1^{ns}

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

Water soaking and warm stratification treated seedlings showed the highest cotyledon size, which is statistically 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 a significant difference as compared with control, shell cracking and shell cracking plus warm stratification treated seedlings (Figure 2). Shell cracking plus warm stratification treated seedlings showed the highest specific root length values amongst the five tested pre-sowing treatments, and also highlighted a statistically significant difference when compared to water soaking treated seedlings (Figure 2).

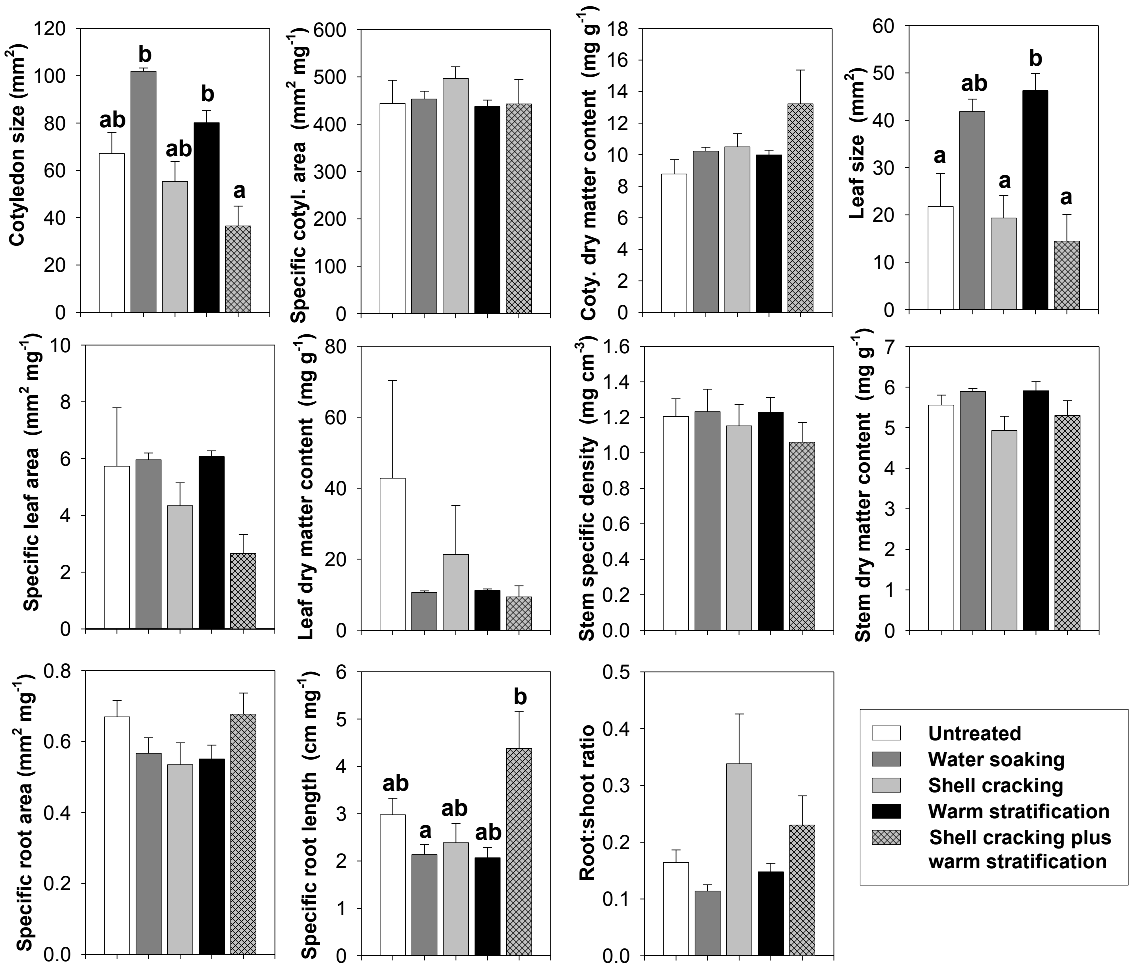

Figure 2. Cotyledon size, specific cotyledon area, cotyledon dry matter content, leaf size, specific leaf area, leaf dry matter content, stem specific density, stem dry matter content, specific root area, specific root length, and root:shoot ratio of *J. curcas* seeds subjected to different pre-sowing treatments: untreated control, soaking in 30 °C water for 24 hours, shell cracking, warm stratification at 37 °C for 24 hours, and shell cracking plus warm stratification at 37 °C for 24 hours. Values represent means ±S.E (N=4). When Treatment factor is significant, different letters show significant differences among treatments (Tukey HDS test, $p \leq 0.05$).

4. CONCLUSIONS

J. curcas is a plant with several both economical and ecological potentialities, which are still far from being achieved, due to a poor knowledge on agronomical, management and environmental aspects of this species (Contran et al., 2013). Amongst these different issues, which need to be deeply investigated, seed storage and aging and seed physical dormancy represent the main critical factors influencing *J. curcas* seeds germination and seedlings early growth for this species (Moncaleano-Escandon et al., 2013; Duong et al., 2013; Islam et al., 2009). *J. curcas* seeds have a viability period less than six months and an increasing of storage temperature quickens seed germination potential loss (Moncaleano-Escandon et al., 2013). Additionally, *J. curcas* seed germination potential rapidly decreases with unfavourable climatic and edaphic conditions and improper seed storage techniques (Duong et al., 2013). Negussie et al. (2013) showed that no spontaneous regeneration was observed in land adjacent to *J. curcas* plantations and primary seed dispersal was limited, predominantly under the canopy of the mother plant. The highest germination success occurs when seeds were buried artificially at 1–2 cm depth, even if this does not occur under natural conditions. For this reason *J. curcas* can be considered as a non aggressive invader plant species when grown under natural conditions. (Negussie et al., 2013).

Currently, there is a lack of knowledge on the effects of pre-sowing *J. curcas* seed treatments on seed germination and growth of seedlings. This study focused on *J. curcas* seed germination and seedlings early growth, which are considered one of the main important factors in *J. curcas* production chain (Islam et al., 2009). The effects of five different seed pre-sowing treatments on germination and growth of *J. curcas* seedlings have been investigated, in order to understand which treatment could better increase seeds germination rate and at the same time guarantee the best performance in term of growth and development of the seedlings. In effect, a vigorous and well-established seedling is able to bear periods of drought, which often occur in arid and semi-arid areas, guaranteeing the future survival and development of the plant.

Results indicated that the considered treatments influenced much more seed germination than seedling growth, the latter characterised by having high variability and no significant differences from the control. 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 significantly reduction of the emergence time. We were expecting a much higher performance of soaking seeds in water at 30 °C for 24 hours, since Islam et al. (2009) demonstrated that *J. curcas* seeds soaked in water had a 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. Dry matter and 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 significant expansion of both cotyledon and primary leaf size. Furthermore, in shell cracking plus water stratification treated seedlings, an antagonistic effect on the aboveground part of the seedlings between mechanical scarification treatment and warm stratification treatment was observed, while an additive effect on root system growth was found (Figure 2).

Amongst the tasted treatments, warm stratification treatment should be preferred to the other treatments since it expressed the best performance on seed germination and promotes the seedling growth. However, this treatment requires a certain degree of investment, due to the fact that a growth chamber is needed 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. As a consequence, scarification treatment, allowing a higher root growth, could be a replicable and economic solution to be promoted. 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 at finding the optimal combination of these two factors, which could increase both seed germination and seedlings growth parameters. In fact, *J. curcas* seed has a very short period of viability and high seed storage temperatures, which are common in tropical areas where *J. curcas* is planted, strongly speed up the loss of seed germination (Moncaleano-Escandon et al., 2013).

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Chapter 4

Physiological responses of *Jatropha curcas* seedlings to severe soil drought stress

Abstract

The rising demand of water and land resources for food production, the growing energy request, and the uncertain impacts of the climate change on drought and flood occurrence make biofuel production a key issue for development, particularly in arid and semi-arid regions. A possible winning strategy could be to encourage the production of biofuel without competing for land and water resources needed for food production. The use of drought-tolerant *Jatropha curcas* has been proposed for the economic exploitation of non-cultivated and marginal areas. However, there are still knowledge gaps on the evaluation of the agronomic performance of *J. curcas* in response to environmental stresses, especially in terms of growth and seed yield traits.

In general, drought adaptation depends on the severity of the water deficit, and two different water use strategies may be employed by wood plants: strategies of drought avoidance or drought tolerance. The aim of this study is to investigate physiological responses, in term of growth and photosynthesis, of *J. curcas* seedlings exposed to a severe soil drought stress. The effects of 26 days of water deficit on growth, water relations, leaf gas exchange, and chlorophyll fluorescence of 2 (YO) and 3 (OL) months old *J. curcas* seedlings have been investigated. Results suggested that in *J. curcas* seedlings drought avoidance and drought tolerance are not mutually exclusive. Furthermore, the work demonstrated that YO and OL seedlings implemented different stress responses.

Key words: Physic nut, water stress, gas exchange, chlorophyll *a* fluorescence, water content, biomass allocation

1. Introduction

Jatropha curcas L. is a deciduous large shrub or small tree, with a life expectancy of up 50 years (Heller, 1996). In the last decades, *J. curcas* has become popular thanks to its wide capabilities and plethora of uses. *J. curcas* seeds contain about 25-35% of oil, which can be extracted and converted in biodiesel. Oil can be used also as cooking/lighting fuel, medicine, bio-pesticide, and for soap making and oil extraction by-products can be used as organic fertilizer, combustible fuel, or for biogas production (Contran et al., 2013). Furthermore, *J. curcas* strength as a crop derives from its capability to be cultivated under semi-arid and poor quality soil conditions, without potentially competing with food production for land use (Fairless, 2007; Ye et al., 2009).

J. curcas grows in tropical and sub-tropical regions, with cultivation limits at 30°N and 35°S. *J. curcas* requires mean annual temperatures between 18°C and 28°C (with optimal values around 26-27°C), average minimum temperatures above 8-9°C, indicating a clear lack of tolerance to frost, and average maximum temperatures between 35°C and 45°C (Trabucco et al., 2010). Natural suitable climatic conditions were found with annual precipitation above 900 mm, with an optimum at 1500 mm (Trabucco et al., 2010), and more humid environmental conditions result in a higher productivity (Maes et al., 2009). Even though *J. curcas* is not naturally present in regions with arid and semi-arid climates, its high ecological adaptability allows *J. curcas* to be cultivated in a wide range of climatic conditions from semiarid to humid (annual rainfall varying from 300 mm to 3,000 mm) (Maes et al., 2009). As a result, the climatic conditions at the plantations are often different from those of the natural distribution and the major cultivation areas are characterized by high evaporative demand and low water availability (Maes et al., 2009). Consequently, *J. curcas* often faced soil drought stress and excessive salinity.

Soil drought is one of the most important limitations to photosynthesis (Tezara et al., 1999), resulting in lower growth rates and productivity and causing serious socio-economic and environmental losses. There is now substantial consensus that the increase in diffusive resistances to CO_2 at stomata and/or mesophyll level under mild water stress (Flexas et al., 2002, 2004) or the alterations of photosynthetic metabolism under severe water stress, such as a decline in Rubisco activity and impairment of ATP synthesis (Tezara et al., 1999), directly cause down-regulation of photosynthesis (Lawlor and Tezara, 2009). Another biochemical limitation of photosynthesis is the accumulation of sugars and other osmolytes in leaves, which might exert a modulation on photosynthesis by a negative feedback mechanism (Drodzova et al., 2004). Drought impact on photosynthesis can also occur as a secondary effect, namely oxidative stress (Chaves et al., 2009). The accumulation of sugars suggests that soil drought affect plants through the formation of reactive oxygen species (ROS) (Mittler, 2006). When photosynthesis is limited by stomatal closure (e.g. to avoid water loss under drought), plants must dissipate excess light energy by down-regulating photosynthetic electron transport through the down-regulation of PSII activity. This leads to an increase in photo-excited compounds that results in the generation of singlet oxygen (Apel and Hirt, 2004). Soil drought thus results in oxidative stress, which needs to be compensated by the antioxidant system or other defence mechanism in order to avoid injury (Rennenberg et al., 2006).

Only recently, the scientific community has begun to investigate *J. curcas* responses, in term of biomass production and partitioning, plant-water relationships, leaf gas exchange, and osmotic adjustment, to limited water availability conditions (Maes et al., 2009a; Achten et al., 2010; Pompelli et al., 2010; Silva et al., 2010, 2010a; Krishnamurthy et al., 2012; Díaz-López et al., 2012; Kesava Rao et al., 2012; Matos et al., 2012; Silva et al., 2012; dos Santos et al., 2013; Sapeta et al., 2013). *J. curcas* is believed to be drought resistant (Maes et al., 2009a; Silva et al., 2012); anyway the physiological mechanisms behind the high drought resistance of *J. curcas* are scarcely described, and recent researches have shed a new light on the water relations and water requirements of *J. curcas* (Achten et al., 2010; Kesava Rao et al., 2012; dos Santos et al., 2013; Sapeta et al., 2013). *J. curcas* is considered a species with a high water use efficiency (Maes et al., 2009a) and water use assessment of *J. curcas* plantations in the semi-arid tropics indicated a monthly water use varying from 10 mm to 140 mm, depending on crop phenophase, environmental demand and water availability (Kesava Rao et al., 2012). Probably drought stress triggers a coordinate down-regulation in the photosynthesis (photochemistry and carboxylation phases), which could be modulated by accumulation of sugar and of osmotically active solutes (Silva et al., 2012; dos Santos et al., 2013; Sapeta et al., 2013). The energy excess at PSII level is dissipated by non-photochemical mechanisms associated with enhancement in photorespiration, restricting photo-damages (Silva et al., 2010a; 2012). In parallel, the antioxidant enzymatic protection was beneficial for oxidative damage protection (Pompelli et al., 2012; Silva et al., 2012). Additionally, *J. curcas* has several leaf traits in common with other stem succulent deciduous trees (Maes et al., 2009a), where stem water is a possible reserve for the fresh leaf flushing as well as for keeping these leaves active for several weeks after the start of the dry season (Chapotin et al., 2006). As do other stem succulent species with green stems, *J. curcas* probably has no a pure C3-metabolism, but rather a CAM-metabolism in the succulent stem, with leaves shifting from C3-

metabolism to the more water-efficient CAM-metabolism under drought (Maes et al., 2009a). Anyway, the metabolism of J. curcas deserves further attention.

The aim of this study is to investigate physiological responses, in term of growth and photosynthesis, of *J. curcas* seedlings exposed to a severe soil drought stress. The effects of 26 days of water deficit on growth, water relations, leaf gas exchange, and chlorophyll fluorescence of 2 and 3 months old *J. curcas* seedlings have been investigated. The study has been conducted because an improved understanding is essential in order to adopt competitive strategies for enhancing *J. curcas* production. Additionally, the peculiar characteristics of *J. curcas* and its resistance mechanisms to drought stress make this plant an important object of study in the investigation of plant responses to abiotic factors.

2. Material and methods

2.1. Experimental design

The experiment was performed in the greenhouse facility located at the Campo Didattico-Sperimentale Mauro Deidda (Dipartimento di Agraria of Università degli Studi di Sassari) at Ottava (SS), Italy (40°46'47''N; 8°28'34''E, elevation 221 m asl). Air temperature and humidity inside the greenhouse were recorded automatically every 2 hour from June to October 2011 with a thermohygrograph (Modello Siap, Bologna, Italy) (Figure 1).

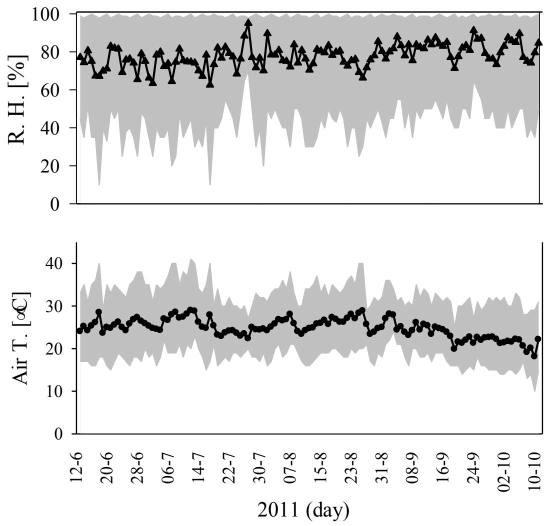

Figure 1. Daily mean relative humidity (R.H.) and daily mean air temperature (Air T.) in 2011 during the experimental period in the greenhouse facility. Grey areas indicate daily minimum and maximum values of R.H. and Air T.

The experiment was carried out on *J. curcas* seeds of the 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°C (±2) until the start of the experiment.

J. curcas seeds were sown in platou (5 cm diameter and 6 cm height), filled with peat amendment substrate. Sowing was carried out on 16^{th} June (OL – older seedlings) and on 25^{th} July (YO – younger seedlings). After 20 days from the germination, when the first leaf completely expanded, a total of 40 seedlings (20 OL seedlings and 20 YO seedlings) were transplanted in pots (40 cm diameter and 60 cm height), filled with 25 kg of substrate (2/4 of filtered soil, 1/4 of potting compost and 1/4 of agriperlite). Until the beginning of the treatment, seedlings had been regularly irrigated with 1,000-1,500 ml of water every 2 days, in order to keep up the soil water above field capacity.

On 12^{th} September 2011, after 2 (OL) and 1 month (YO) from transplanting respectively, seedlings were exposed to treatments: watered (W, control treatment) or unwatered (D, soil drought treatment). In control treatment, seedlings have been regularly irrigated with 1,000-1,500 ml of water every 2 days, in order to keep up the soil water above field capacity. On the contrary, soil drought treatment was imposed by withholding water.

Pots were arranged in a completely randomised design. On 4^{th}, 8^{th}, 12^{th}, 19^{th}, and 26^{th} day from treatment beginning, leaf and soil water content, biometric, gas exchange, and fluorescence measurements were performed on 6 seedlings per treatment.

On 26^{th} day from treatment beginning, biometric destructive measurements were carried out on 5 seedlings per treatment.

2.2. Soil and leaf water content

Before transplanting, substrate field capacity weight (sFW, measured in saturated substrate where downward movement of water has virtually ceased) and substrate dry weight (sDW) were measured, and field capacity (FC) of each pot was calculated as FC=(sFW-sDW)/sDW*100. At each measurement date, a cylinder of 238.5 cm^3 of substrate was collected in each pot between 11:00 and 12:00. The sampling was always performed between seedling and pot edge, in order to avoid edge effect, and shifting the area of sampling. The substrate was immediately replaced, adding in each pot an equal cylinder of 238.5 cm^3 of substrate collected from a pot subjected to the same water treatment.

Samples were weighted (fresh weight, FW) and then, after oven drying at 100°C, when samples reached a constant weight (around 72 h), weighted again (dry weight, DW). Substrate water content (SC) was calculated as: SC=(FW-DW)/DW*100. Substrate water potential (Ψs) was calculated through an empirical relationship between substrate water content (SC) *vs* substrate water potential (Ψs). The relation was determined by a water-content *vs* water-potential curve constructed by using a pressure plate device (Richard's pressure plate apparatus).

Measurements were performed on six randomly selected substrate samples in a pressure range from 0.02 to 1.5 Mpa, founding the relation $\Psi s = 81.607 * e^{(-0.3118 * SC)}$ (R^2=0.88).

Two leaf discs of 14.5 cm^2 from one mature leaf were collected between 11:00 and 12:00 from each plant and at each measurement date. 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 (around 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 (Ds), number of leaves (Nl), number of fallen leaves (Nfl), and number of secondary branches (Nb) were measured at each measurement date. According to Achten et al. (2010), total above dry biomass (AB) was calculated through the allometric relationship $AB=0.029*Ds^{2.328}$, where Ds was the basal stem diameter. The accuracy of allometric relationship was evaluated 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 beginning, seedlings were separated into leaves, stem, and (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), stem transversal area (As), root length (Lr), root diameter (Dr), root fresh (FWr), root dry weight (DWr), and root transversal area (Ar). Dry weights were measured when samples at 80°C reached a constant weight (around 48 hours). Total and transversal area were measured by an Area Meter (LI-3100C Area meter, Laicor). Stem volume was measured by cutting the stem into smaller sections and immerging it in a graduated cylinder (500 ml). The amount of displaced water was assumed to be equal to the volume of the stem section. From the collected data, according to Cornelissen et al. (2003), several parameters related to seedling growth were calculated: specific leaf area (SAl=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 area (SAr=Ar/DWr) [mm^2 mg^{-1}], specific root length (SLr=Lr/DWr) [mm mg^{-1}], root:shoot ratio (R:S= DWr/(DWl+DWs)).

2.4. Gas exchange and fluorescence measurements

Light-saturated net photosynthesis (A_{max}) and stomatal conductance to water vapor (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-cm^2 Parkinson leaf cuvette, which controlled leaf temperature (ambient ± 1 °C), leaf-to-air vapor pressure deficit (ambient ± 0.2 kPa), saturating light (1500 ± 20 µmol m^{-2} s^{-1}) and carbon dioxide (CO_2) concentration (380 ± 10 µmol mol^{-1}). Preliminary light curves showed that light at 1,500 µmol m^{-2} s^{-1} was saturating.

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 rising transient was induced by saturating red-actinic light (1,500 µmol m^{-2} s^{-1}, peak at 650 nm, duration 1 s). Data acquisition was recorded for 1 s, starting from 10 µs after the onset of illumination.

The values of Fo, ground fluorescence yield in the dark-adapted state (when all reaction centres of PSII are considered open) and Fm, maximal fluorescence yield in the dark (when all reaction centres of PSII are considered closed), were collected. Maximum quantum yield for primary photochemistry (Fv/Fm) was calculated as (Fm-Fo)/Fm (Maxwell and Johnson, 2000).

Additionally, the quantitative analysis of the polyphasic fast fluorescence rise transient, called JIP-test (Strasser and Strasser, 1995), allowed to calculate several biophysical and phenomenological expressions which quantify the stepwise flow of energy through the photosystem two (PSII): performance index per absorption flux (PI_{abx}), electron transport probability (ψ_o), quantum yield for electron transport (ϕE_o), quantum yield for energy dissipation (ϕD_o), effective antenna size of an active reaction centre (ABS/RC), maximal trapping rate of PSII (TR_o/RC), electron transport in an active reaction centre (ET_o/RC), and effective dissipation of an active reaction centre (DI_o/RC) (see Contran et al., 2009 for parameter definitions). Analysis of the transient was performed with *Biolyzer 3.06* software (by Ronald Maldonado-Rodriguez, Bioenergetics Laboratory, Geneva, CH).

2.5. Statistical analyses

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's Test). On data collected at treatment beginning (time 0), a t-student test was applied to compare the effect of seedling Age (OL *vs* YO). On data collected 26 days from treatment 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 compare the above effects 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 is the seedling. Analyses were processed using STATISTICA 6.0 Package for Windows (StatSoft 2001, Tulsa, OK).

3. Results

From June to October, daily mean air temperature inside the greenhouse facility was 24.7°C (±2.2), with minimum value of 17.8°C (±2.2) and maximum value of 33.1°C (±3.2) and daily mean relative humidity was 77.8% (±6.2), with minimum value of 42.0% (±9.0) and maximum value of 99.1% (±0.6) (Figure 1). Average substrate field capacity of pots was 28.2% (±1.13), equivalent to the substrate water potential of -0.014 MPa (±0.004).

In watered treatment, seedlings have been regularly irrigated and substrate water content was maintained above field capacity with a substrate water potential of -0.25 MPa (±0.07) (Figure 2). On the contrary, in soil drought treatment, after 8 days from treatment beginning, the substrate water potential reached the value of -1.5 MPa, corresponding to the conventional permanent wilting point, that is the threshold values for water availability in the soil (Tolk 2003), and then it drastically decreased until the value of -14.5 MPa (±2.5), when only hygroscopically bound water remains

(Fig. 2). Nevertheless 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 ($p>0.05$, Figure 2). Additionally, no differences were observed between leaf RWC of OL seedlings (older, 3 months old) and YO seedlings (younger, 2 months old) ($p>0.05$, Figure 2).

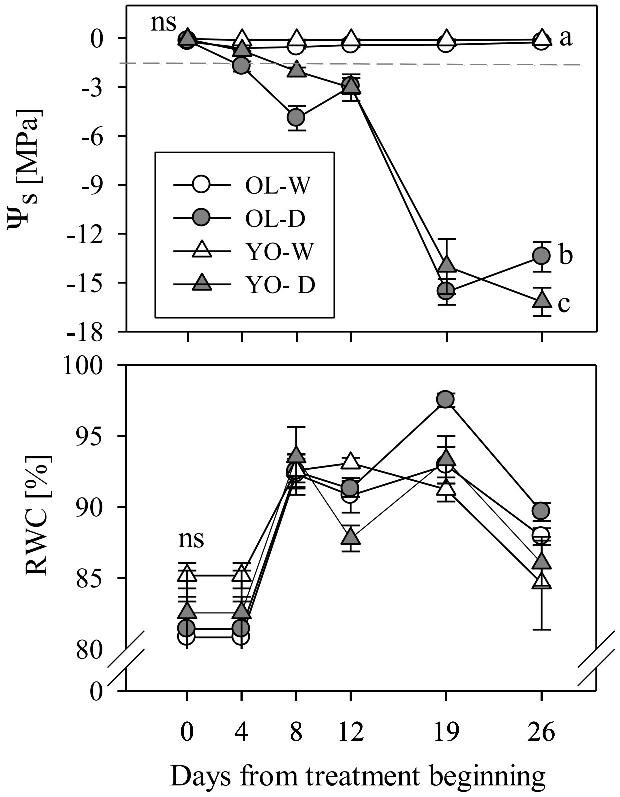

Figure 2. Substrate water potential (Ψs) and leaf relative water content (RWC) of *J. curcas* seedlings Old (OL - Circles) or Young (YO – Triangles) and exposed to Watered (W – White symbols) or soil Drought (D – Grey symbols) treatments (N=6). Values represent means ±S.E. The dashed line indicates the value of the substrate permanent wilting point (Ψs = -1.5 MPa). At treatment beginning (day zero), stars show significant differences between OL and YO seedlings (t-student, N=6, *≤0.05, **≤0.01, ***≤0.001, and ns>0.05 not significant). On 26° day, different letters show significant differences among groups (OL-W, OL-D, YO-W, and YO-D), if group factor is significant (Tukey HDS test, N=6, p ≤ 0.05).

At treatment beginning (day zero), all the biophysical 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 ($p<0.001$, Figure 3).

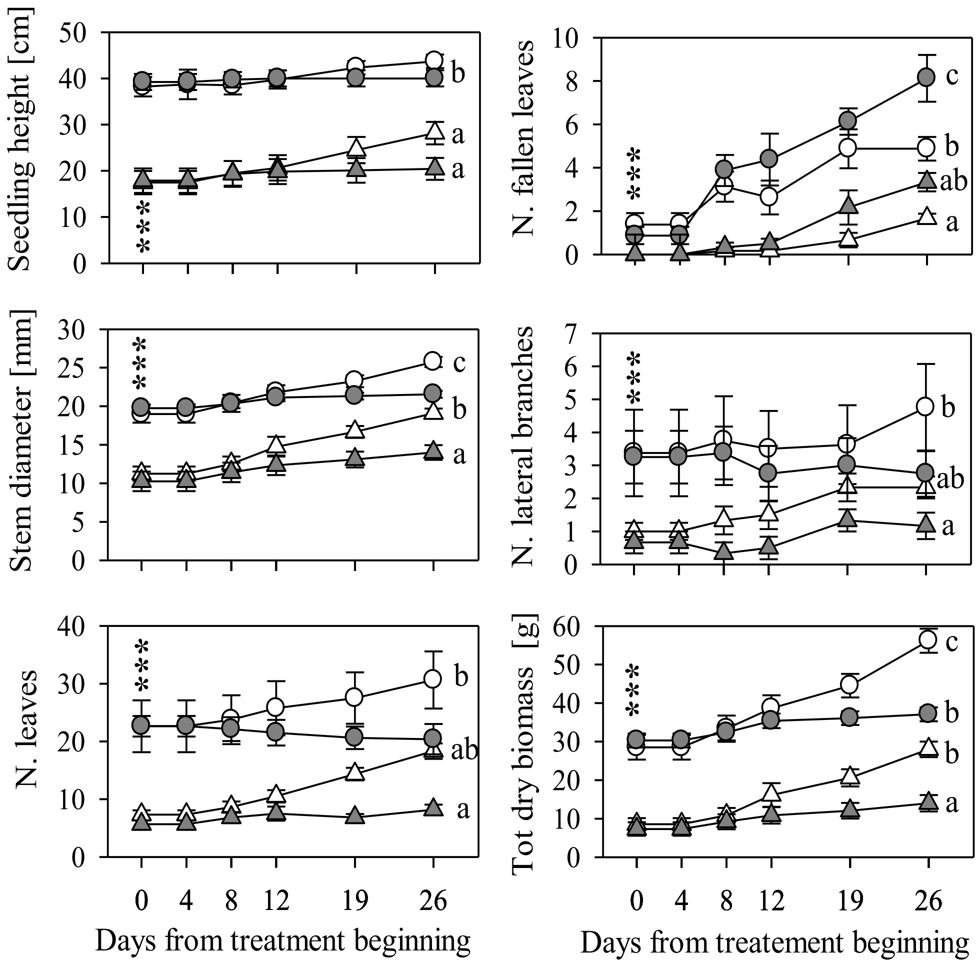

Figure 3. Seedling height, stem basal diameter, number of leaves, number of fallen leaves, number of secondary branches, and total above dry biomass of *J. curcas* seedlings Old (OL - Circles) or Young (YO – Triangles) and exposed to Watered (W – White symbols) or soil Drought (D – Grey symbols) treatments. Values represent means ±S.E. (N=6). At treatment beginning (day zero), stars show significant differences between OL and YO seedlings (t-student, N=6, *≤0.05, **≤0.01, ***≤0.001, and ns>0.05 not significant). On 26° day, different letters show significant differences among groups (OL-W, OL-D, YO-W, and YO-D), if group factor is significant (Tukey HDS test, N=6, p ≤ 0.05).

On the contrary, gas exchange parameters (A_{max} and G_w) measured at the beginning of the treatment were higher in YO seedlings than in OL seedlings ($p<0.05$, Fig. 5, with the exception of midday A_{max}. In regard to the chlorophyll *a* fluorescence parameters measured at the beginning of the treatment, midday Fv/Fm and morning PI_{abx} were higher in OL seedlings than in YO seedlings ($p<0.01$, Figure 5); morning ψ_o and ϕE_o were higher in OL seedlings than in YO seedlings ($p<0.05$, data not shown);

while morning ϕD_o, ABS/RC, TR_o/RC and DI_o/RC were lower in OL seedlings than in YO seedlings (p<0.05, data not shown).

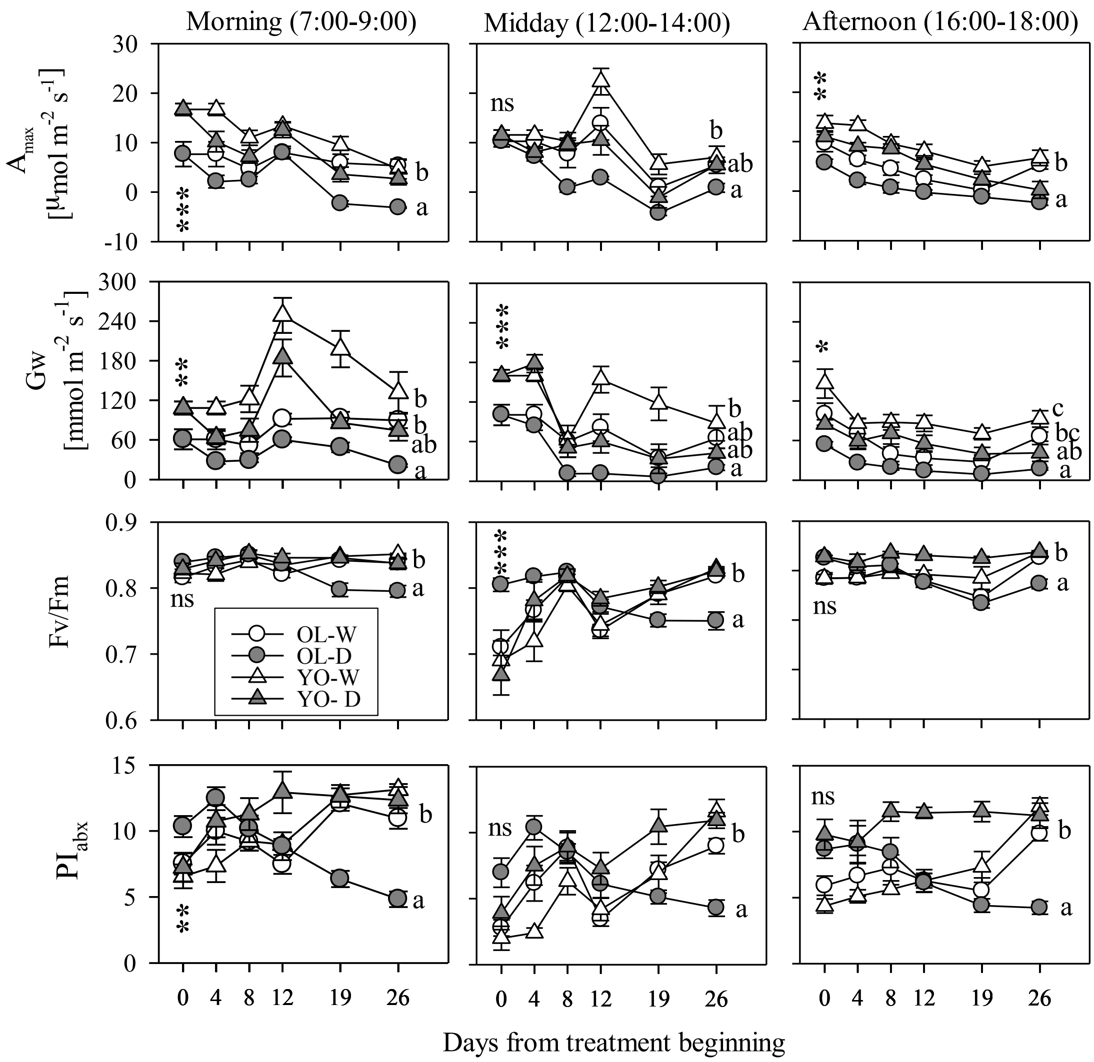

Figure 5. Light-saturated net photosynthesis (A_{max}), stomatal conductance to water vapor (G_w), maximum quantum yield for primary photochemistry (Fv/Fm), and performance index per absorption flux (PI_{abx}), measured in the morning, at midday, and in the afternoon, of *J. curcas* seedlings Old (OL - Circles) or Young (YO – Triangles) and exposed to Watered (W – White symbols) or soil Drought (D – Grey symbols) treatments. Values represent means ±S.E. (N=6). At treatment beginning (day zero), stars show significant differences between OL and YO seedlings (t-student, N=6, *≤0.05, **≤0.01, ***≤0.001, and ns>0.05 not significant). On 26° day, different letters show significant differences among groups (OL-W, OL-D, YO-W, and YO-D), if group factor is significant (Tukey HDS test, N=6, p ≤ 0.05).

During the experiment, both in YO and OL seedlings, watered seedlings grew regularly, while unwatered seedlings maintained their initial level (Figure 3).

After 26 days of soil drought stress, watered YO seedlings reached, in terms of growth, unwatered OL seedlings, as shown by the trend of stem diameter, number of leaves, number of lateral branches, and total dry biomass (Figure 3). On the contrary, despite of the strong intensity of soil drought stress, both in OL and YO seedlings, drought stress influenced only stem diameter and total dry biomass, but it did not influenced seedlings height, number of leaves and number of lateral branches (Figure 3).

The number of leaves of YO and OL watered seedlings was not significantly different compared with the number of leaves of YO and OL unwatered seedlings, and unwatered OL seedlings lost more leaves than watered OL seedlings (Figure 3). Anyway, both in OL and YO seedlings, total leaf area was significantly lower in unwatered seedlings than in watered seedlings, and leaf dry matter content was significantly lower in unwatered OL seedlings than in watered OL seedlings (Figure 4). Additionally, YO and OL unwatered seedlings had higher specific leaf area and root:shoot ratio, compared to the correspondent watered seedlings, and unwatered YO seedlings had also higher specific root area than YO watered seedlings (Figure 4). In regards to the parameters calculated on the basis of destructive measurements, between watered and unwatered seedlings were not observed other significant differences in both OL and YO (Figure 4).

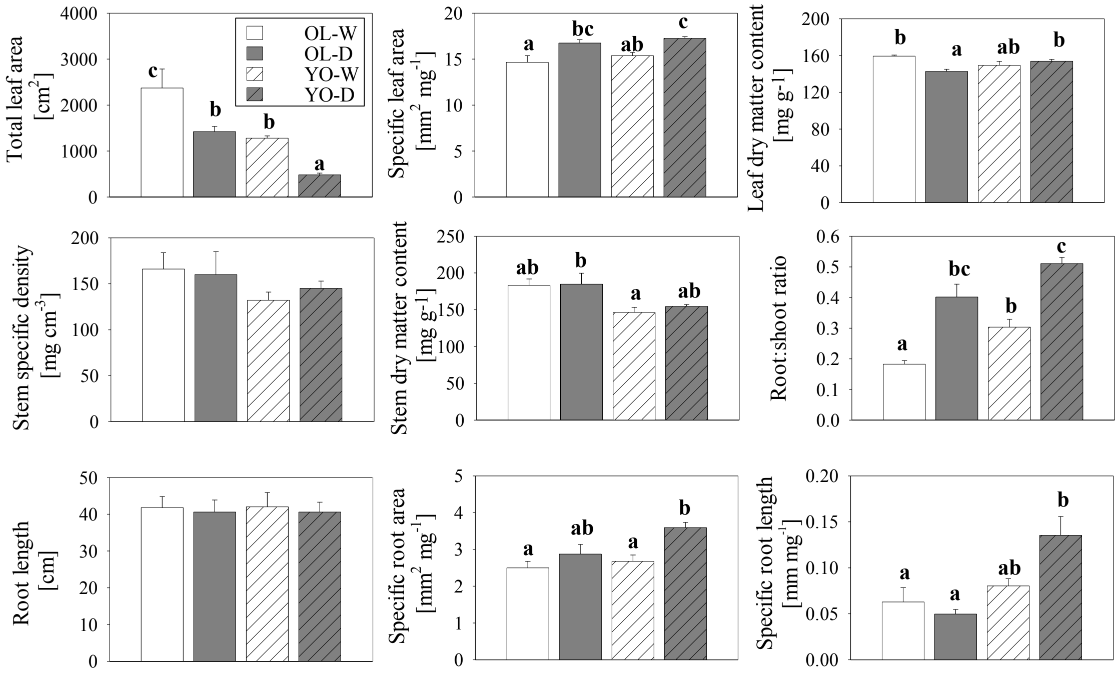

Figure 4. Total leaf area, specific leaf area, leaf dry matter content, stem specific density, stem dry matter content, root:shoot ratio, root length, specific root area, and specific root length of *J. curcas* seedlings Old (OL – no-line patterned columns) or Young (YO – line patterned columns) and exposed to Watered (W – White columns) or soil drought (D – Grey columns) treatments. Values represent means ±S.E. (N=5). When group factor (OL-W, OL-D, YO-W, and YO-D) is significant, different letters show significant differences among groups (Tukey HDS test, N=6, p ≤ 0.05).

During the experiment, morning and midday A_{max} and G_w of YO seedlings, both watered and unwatered, reached the values of morning and midday A_{max} and G_w of watered OL seedlings (Figure 5). The latter, in fact, has remained constant throughout the whole period (ANOVA resuts for time factor for watered OL seedlings: p>0.05, Figure 5). Instead, morning A_{max} and G_w of OL unwatered seedling were significantly reduced by the stress and reached A_{max} negative values after 2 weeks of treatment (Figure 5). In regards to afternoon gas exchange, only in watered YO seedlings A_{max} and G_w reached the values of A_{max} and G_w of watered OL seedlings (Figure 5), while afternoon A_{max} and G_w of unwatered seedling, both OL and YO, were significantly reduced by the stress (Figure 5). Chlorophyll *a* fluorescence parameters (with the exception of ET_o/RC) were significantly influenced by soil drought only in OL seedlings, while in YO seedlings there were not differences between control and treated seedlings (Figure 5 and Figure 6). At all hours of the day, Fv/Fm, PI_{abx}, ψ_o, and ϕE_o were lower in OL unwatered seedlings than in watered OL seedlings, while ϕD_o, ABS/RC, TR_o/RC and DI_o/RC were higher in OL unwatered seedlings than in watered OL seedlings ($p<0.05$, Figure 5 and Figure 6).

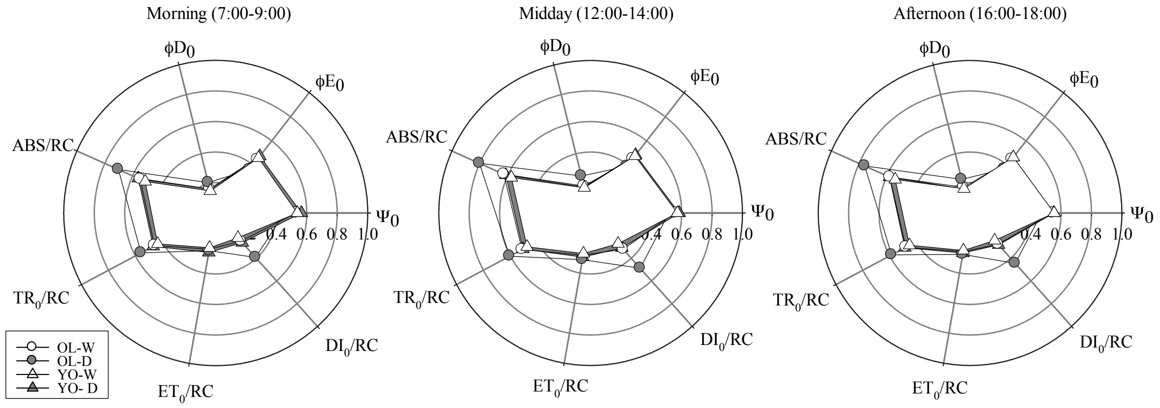

Fig. 6. Electron transport probability (ψ_o), quantum yield for electron transport (ϕE_o), quantum yield for energy dissipation (ϕD_o), effective antenna size of an active reaction centre (ABS/RC), maximal trapping rate of PSII (TR_o/RC), electron transport in an active reaction centre (ET_o/RC), and effective dissipation of an active reaction centre (DI_o/RC), measured after 26 days from treatment beginning in the morning, at midday, and in the afternoon, of *J. curcas* seedlings Old (OL - Circles) or Young (YO – Triangles) and exposed to Watered (W – White symbols) or soil Drought (D – Grey symbols) treatments. Values represent means (N=6).

4. CONCLUSIONS

The rising demand of water and land resources for food production, the growing energy request, and the uncertain impacts of the climate change on drought and flood occurrence make biofuel production a key issue for development, particularly in arid and semi-arid regions (Powell et al., 2012; Contran et al., 2013). A possible winning strategy could be to encourage the production of biofuel without competing for land and water resources needed for food production (Kesava Rao et al., 2013). For this reason, the use of drought-tolerant crops has been proposed for the economic exploitation of non-cultivated and marginal areas (Behera et al., 2010). The biofuel production from *J. curcas* plantation has seen by researchers, policy makers and industries a possible way to implement this strategy. *J. curcas* is a non-edible oil crop, it could be grown on marginal and abandoned lands not suitable for crop production, and, being drought resistant, it allows to enhance rainwater use efficiency (Ye et al., 2009; Contran et al., 2013). However, there are some concerns regarding the cultivation of *J. curcas* for biofuel production: the climatic conditions at the plantations are often different from those of the *J. curcas* natural distribution but little is known about physiological traits and the adaptation capacity of *J. curcas* to changing and more adverse climate conditions (Fairless, 2007; Maes et al., 2009). These knowledge gaps restrict our capacity to properly evaluate and predict the agronomic performance of *J. curcas* in response to environmental stresses, especially in terms of growth and seed yield traits (Kheira and Atta, 2009; Sapeta et al., 2013).

Soil drought is one of the most important environment factors affecting agricultural productivity around the world, and water availability affects plant growth and yield, especially in arid and semi-arid regions, where plants are often subjected to long periods of drought (Hessine et al., 2009). Therefore, the knowledge of *J. curcas* physiological mechanisms involved in responses to soil drought generates considerable interest. In this study, we would like to investigate physiological responses, in term of growth, water relations, leaf gas exchange, and chlorophyll fluorescence of *J. curcas* seedlings exposed to a severe soil drought stress. Since *J. curcas* germination percentage is very low, the best generative propagation technique is the transplanting of pre-cultivated seedlings. Field activities in arid or semi-arid areas have shown that age of seedling and time of transplantation become of crucial importance due to drought stress periods (Contran et al., 2013). For this reason, in order to improve our knowledge on *J. crucas* responses to soil drought, the experiment was performed on 2 and 3 months old seedlings.

The results of this study evidence that *J. curcas* seedlings have an efficient adaptive mechanism to avoid a severe drought stress, by maintaining a good leaf water status (Figure 2). This strategy is also associated to a rapid growth reduction and an impairment of photosynthesis by means of an effective moderately stomatal closure (Figure 5). During the drought stress period, the soil water potential of unwatered pots remained under the conventional threshold values for water availability in the soil (permanent wilting point), fixed at $\Psi s = -1.5$MPa, for 18 days (Tolk, 2003). It means that only hygroscopically bound water remains and seedlings have no available gravitational water. Anyway, the permanent wilting point is crop and climate specific and, to a limited extent, plants are able to actively lower their root water potential in order to obtain more water from soil. In general, xerophytes can lower the root water potential to, at most, -6MPa (Larcher, 2003). Probably, even *J. curcas* seedlings have reduced their root water potential, since, the leaf relative water content of *J. curcas* seedlings was maintained at level of watered seedlings, even under extended severe stressing conditions (Figure 2).

On the contrary, the growth of unwatered seedlings was drastically stopped by soil drought, without any differences between OL and YO seedlings, so that in 26 days, watered YO seedlings reached the development of unwatered OL seedlings, in terms of stem diameter, total dry biomass, leaf area, and leaf dry matter content (Figure 3 and Figure 4). Although drought treatment did not influenced the total number of leaves, independently of seedling age, results indicated that, under drought conditions, seedlings reduced their leaf size, with important consequences for the leaf energy and water balance (Figure 4).

Even though species in resource-rich environments tend to have larger specific leaf area than those in environments with resource stress, the increase of unwatered *J. curcas* specific leaf area indicated that seedlings reduced their investment in structural leaf defences and short leaf lifespan, but maximized the photosynthetic rate. Actually, specific leaf area is, in many cases, a good positive correlate of its potential relative growth rate or mass-based maximum photosynthetic rate (Cornelissen et al., 2003). Both in YO and OL seedlings, soil drought stress increased the ratio between root and shoot (Figure 4).

Anyway, results suggest that YO and OL seedlings implemented different stress responses. In OL seedlings the increase of root:shoot ratio was due more to a reduction of leaf dry matter content and to an higher leaf abscission than to a greater allocation of resources in the roots (Figure 4). On the contrary, in YO seedlings, the increase of root:shoot ratio was due both to a decrease in leaf weight and to an higher root size (Figure 4). The highest specific root area and length of unwatered *J. curcas* seedlings suggest 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 (Cornelissen et al., 2003). Even though this strategy allows a faster root elongation rate, which results in higher nutrient and water uptake, the production of thinner root reduces the penetration force on soil, the withstand of low soil moisture, and the water transport rate within the root. The latter three are key characteristics for seedlings that should grow on marginal soils in arid and semi-arid regions.

After 26 days of treatment, soil drought induced an afternoon down-regulation of photosynthesis by an increase in diffusive resistances to CO_2 at stomata level in both YO and OL *J. curcas* seedlings. Anyway, YO and OL seedlings differed even in the photosynthetic response to soil drought, since prolonged soil drought stress resulted more damaging in OL seedlings. We hypothesise that this difference depends on the initial condition of photosynthetic activity. At the beginning of the experiment, 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), caused by a higher stomatal closure (Figure 5). Actually, 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 ψ0, ϕE0, data not shown), which leads to a reduced need to dissipate excess of energy (low ϕD0, TR0/RC and DI0/RC, data not shown) (Contran et al., 2009). 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 (Mittler, 2006). In this situation, the already low values of gas exchange and the lack of preparation in dealing with problems, such as excess energy dissipation and ROS production, have made OL seedlings more sensitive to soil drought. The further reduction of stomatal conductance, even in the early hours of the day, caused by soil drought and the consequently considerable reduction of net photosynthesis led to a strong alteration of photosynthetic metabolism in OL seedlings (Figure 5). This resulted in a down-regulating photosynthetic electron transport through the down-regulation of photosystem II activity (Figure 6), possibly caused by integrity loss, and in a subsequently reduction of the maximum quantum yield for primary photochemistry at all hours of the day (Figure 5) (Apel and Hirt, 2004; Rossini et al., 2013).

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. In general, drought adaptation depends on the severity of the water deficit, and two different water use strategies may be employed by wood plants: strategies of drought avoidance or drought tolerance (Passioura, 1982). Both strategies involve diverse physiological and biochemical mechanisms which enable a plant to grow and survive under drought conditions. Nevertheless, these strategies are not mutually exclusive and, in practise, plants may combine a range of response type (Levitt, 1980).

Despite the recent studies on the *J. curcas* responses to limited water availability, the physiological mechanisms behind the high drought resistance of this species are still scarcely understood. Although all researchers confirm the high *J. curcas* drought resistance, there is no clear agreement on the mechanisms involved in the resistance. Maes et al. (2009a), Achten et al. (2010), and Sapeta et al. (2013) found that *J. curcas* is a species with a clear drought avoidance strategy in its leaves and several plant-water relations in common with deciduous stem succulent trees. Generally, the main mechanisms carried out by drought avoidant trees involve stomatal regulation, extensive root system, high capacity for water transport from roots and leaves, high leaf mass area ratio (Levitt, 1980; Larcher, 2003). *J. curcas* has an embolism avoidance strategy and the stem water is reserved for the fresh leaf flushing as well as for keeping these leaves active for several weeks after the start of the dry season (Maes et al., 2009a; Achten et al., 2010). The stem water was also found not to play a role in maximizing stomatal conductance, which is generally low and decreases during the day (Krishnamurthy et al., 2012). *J. curcas* root architecture facilitates the exploration of deeper soil horizons, allowing a better water access in semiarid environments (Reubens et al., 2011). On the contrary, the mechanisms triggered in response to drought, as described by Silva et al. (2010a; 2012), Pompelli et al. (2010), and dos Santos et al. (2013), suggest that *J. curcas* tree activates drought tolerance strategy. Generally, the main mechanisms carried out by drought tolerant trees involve osmotic adjustment, antioxidant system, compatible solutes and high resistance to xylem cavitation (Levitt, 1980; Larcher, 2003). *J. curcas* tolerance to drought is associated to the ability to maintain water in leaf and root tissues, combining effectively osmotic adjustment with stomatal control mechanisms, in order to allow a continuous growth (Silva et al., 2010a; 2012). Drought stress probably triggers a coordinate down-regulation in the photosynthesis (photochemistry and carboxylation phases), which could be modulated by accumulation of both sugar and osmotically active solutes (Silva et al., 2012; dos Santos et al., 2013; Sapeta et al., 2013). In parallel, the antioxidant enzymatic protection was beneficial for oxidative damage protection (Pompelli et al., 2012; Silva et al., 2012).

The present work suggests that in *J. curcas* seedlings these two water use strategies are not mutually exclusive. Drought significantly reduced leaf area, aboveground biomass and relative growth rate, increased specific leaf area and ratio between root and shoot, but had no effect on leaf water content. Our results confirm that *J. curcas* is a low stem density species and stem functions as a water reservoir, probably activated by osmotic adjustment. Leaves were not immediately shed after the seedlings were confronted with drought. Net photosynthesis was partially affected by drought stress, due to reduced stomatal conductance. However, our results highlight that mechanisms of drought response are highly influenced by seedling age. OL seedlings, probably less acclimated to excess energy, did not present an efficient mechanism for protection against drought-induced oxidative stress, as suggested by photosystem II integrity loss (Strasser and Strasser, 1995). On the contrary, YO seedlings implemented mechanisms of tolerance, through the activation of excess energy dissipation mechanisms or, probably, antioxidant system. Results support the hypothesis that *J. curcas* is appropriate for cultivation in areas with limited water availability or period of soil drought.

Anyway, before promoting *J. curcas* use as a source of biofuel, particularly in developing countries, it is necessary to fill some knowledge gaps on the best agronomic conditions for an economically and socially sustainable yield. In addition to a deeper understanding of response mechanisms to soil drought, the mechanisms of *J. curcas* drought resistance should be investigated in combination with other co-occurring constraints, such as heat stress and salinity, in order to promote its cultivation.

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Conclusions

J. curcas is a tree with several both economical and ecological potentialities, which are still far from being achieved, due to a poor knowledge on agronomical, management and environmental aspects of this species (Contran et al., 2013). The review (Chapter 1) traced and summarized each phase of *J. curcas* productive chain, from sowing to bio-diesel production, in order to logically organize the knowledge around *J. curcas* system and to assess its weaknesses and potentialities, highlighting the knowledge gaps which need still to be deeply explored. Amongst these knowledge gaps, the thesis focused basically on three main issues, which are considered the most critical factors for the promotion of *J. curcas* in international cooperation projects implemented in arid and semi-arid areas of developing countries. The examined aspects have been the following:

(i) socio-economic and environmental sustainability of community-based *J. curcas* initiatives for local use, such as smallholder and decentralized *J. curcas* plantations, such as those promoted by GHAJA international cooperation project (Chapter 2);

(ii) effects of different pre-sowing treatments on germination behaviour and growth of the seedlings (Chapter 3);

(iii) physiological responses, in term of growth and photosynthesis, of *J. curcas* seedlings exposed to a severe soil drought stress (Chapter 4).

With reference to Chapter 2, the sustainability potentialities and criticalities of the *J. curcas* system set up in the framework of the European Union GHAJA project was assessed. This study carried out in the project implementation area (West Mamprusi District, Northern Region of Ghana) demonstrated that the gains achieved with *J. curcas* plantations totally depend on the cultivation system, with high differences between community-based *vs* large-scale plantations, leading the system from sustainable to unsustainable. The obtained results confirmed that community-based *J. curcas* initiatives for local use, such as smallholder and decentralized *J. curcas* plantations, based on small plantations in marginal lands or on intercropped agro-forestry systems, can be seen as an opportunity for positively contributing to rural livelihoods of the seven rural communities involved in GHAJA project. The study, carried out through participatory methods (e.g. individual interviews, focus group discussions, questionnaires, resource mapping, and rankings), stated that the cultivation of 1 acre per family with *J. curcas* could be able to ensure access to sustainable and affordable energy sources, considerably reducing the collection of firewood, and partially modifying the tasks of the household members, thus reducing rural women heavy-labour activities. More in detail, the *J. curcas* scenario considered in the study included the production of 5 kg of soap per year, the fertilization of *J. curcas* plantations with 100 kg of seed cake per year, and the possible replacement of 23% of firewood (main energy source used by rural communities of Ghana) with 604 kg of oil, 1,744 kg of husk, and 800 kg of seed cake obtained over a period of 10 years and used as combustible fuel for cooking or lighting. In this sense, the integration of *J. curcas* in different agro-forestry systems proved to be a powerful mean of sustainable development in rural areas of Ghana, guaranteeing both socio-economic and environmental sustainability in the long-term.

As far Chapter 3 is concerned, the described experiment tasted the effects of five different seed pre-sowing treatments on germination and growth of *J. curcas* seedlings, in order to understand which treatment could better increase seeds germination rate and at the same time guarantee the best performance in term of growth and development of the seedlings. In effect, a vigorous and well-established seedling is able to bear periods of drought, which often occur in arid and semi-arid areas, guaranteeing the future survival and development of the plant.

Results indicated that the considered treatments influenced much more seed germination than seedling growth, the latter characterised by having high variability and no significant differences from the control. Amongst the tasted treatments, warm stratification treatment should be preferred to the other treatments since it expressed the best performance on seed germination and promoted the seedling growth. However, this treatment requires a certain degree of investment, due to the fact that a growth chamber is needed 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. As a consequence, scarification treatment, allowing a higher root growth, could be a replicable and economic solution to be promoted. 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. Another aspect to be investigated could be the interaction between seed storage methods and pre-sowing treatments, with the aim at finding the optimal combination of these two factors which could increase both seed germination and seedlings growth parameters. In fact, *J. curcas* seed has a very short period of viability and high seed storage temperatures, which are common in tropical areas where *J. curcas* is planted, strongly speed up the loss of seed germination.

Regarding the experiment described in Chapter 4, it aimed to investigate physiological responses, in term of growth, water relations, leaf gas exchange, and chlorophyll fluorescence of *J. curcas* seedlings exposed to a severe soil drought stress. The experiment was performed on 2 and 3 months old *J. curcas* seedlings since field activities in arid or semi-arid areas have shown that age of seedling and time of transplantation become of crucial importance due to drought stress periods. The results of this study evidenced that *J. curcas* seedlings have an efficient adaptive mechanism in response to a severe drought stress, by maintaining a good leaf water status and partially reducing the seedling growth. Anyway, results suggested that younger and older seedlings implemented different stress responses, and older seedlings resulted more sensitive to soil drought. Furthermore, the study suggested that *J. curcas* seedlings adopt both strategies of drought avoidance and of drought tolerance when exposed to prolonged periods of water deficit and these strategies are not mutually exclusive. However, our results highlight that mechanisms of drought response are highly influenced by seedling age. Results supported the hypothesis that *J. curcas* is appropriate for cultivation in areas with limited water availability or period of soil drought. In addition to a deeper understanding of response mechanisms to soil drought, the mechanisms of *J. curcas* drought resistance should be investigated in combination with other co-occurring constraints, such as heat stress and salinity, in order to promote its cultivation.

In conclusion, the studies undertaken within the present doctoral dissertation confirmed that community-based initiatives on *J. curcas* plantation could positively contribute to the livelihoods of rural communities living in arid and semi-arid areas of developing countries, if based on small plantations in marginal lands or on intercropped agro-forestry systems. However, it is still necessary to fill some knowledge gaps and much more research is required for guaranteeing a full socio-economic and environmental sustainability of *J. curcas* used as a trigger of rural development in Sub-Saharan Africa.

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