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Environmental influence on the physico-chemical and physiological properties of *Jatropha curcas* seeds

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Abstract. The present study investigated the influence of two Brazilian environments on the physico-chemical and physiological properties of *Jatropha curcas* L. (physic nut) – an oilseed plant of great potential in biodiesel production. Fruits and seeds of plants grown in the Brazilian Atlantic rainforest (AF) and in the semiarid climate of Caatinga (SA) were compared. Fruits from the SA were longer, wider and heavier than those from the AF. In contrast, the AF seeds were larger and heavier than the SA seeds. Soluble sugars, protein, relative water content and electrical conductivity of the SA seeds significantly exceeded that of the AF seeds by 28%, 23%, 32% and 94%, respectively. Seeds of the AF had a significantly greater percentage germination (17.5%) and needed less time to germinate (10%) than did the SA seeds. Shoot dry biomass of seedlings grown from the AF seeds significantly exceeded that of the SA by 18.5%. Results suggest this pattern may be due to the higher carbon storage (i.e. sugars, proteins and oil) in the AF than SA seeds. Further studies are warranted to verify whether the differences observed between the seed sources investigated in the present study could be due to varietal or biotype factors.

Introduction

Jatropha curcas L. (physic nut or purging nut) (Euphorbiaceae) is a drought-resistant shrub or small tree growing up to 6 m in height. It is native to tropical America and the Caribbean. It is highly toxic (Abdu-Aguye *et al*. 1986; Germosén-Robineau 2005). Although it is cultivated in Central and South America, South-east Asia, India and Africa for ornamental, medicinal, environmental and economic purposes (Heller 1996), it is also listed as a weed in Brazil, Fiji, Honduras, India, Jamaica, Panama, Puerto Rico and Salvador (Holm *et al*. 1979). *J. curcas* can thrive in several climatic zones, with annual precipitation between 250 and 1200 mm (Openshaw 2000; Achten *et al*. 2008). It is well adapted to arid and semiarid conditions and has low fertility and moisture demands, and it can also be grown on moderately saline, degraded and eroded soils (Abou Kheira and Atta 2009).

In Brazil, it occurs naturally from the states of Maranhão to Paraná, in sandy and infertile areas, and is recommended for cultivation on poor, degraded soils as a multipurpose tree. The plant can be used to prevent and/or control erosion and in-land reclamation. It can be grown as a live fence, especially to contain or exclude farm animals, and it can be planted as a commercial crop. Various parts of the plant are of medicinal value; its bark contains tannins and the flowers attract bees, and thus the plant is potentially useful in honey production; its wood and fruit can be used for numerous purposes, including fuel (Openshaw 2000).

Current interest in the commercialisation of *J. curcas* is related to the production of oil from its seeds, which is then used as a biofuel (Foidl *et al*. 1996; Openshaw 2000; Achten *et al*. 2008). Jatropha seeds contain viscous, non-edible oil that is used as a substitute for diesel (hence called biodiesel). Biodiesel production from the oil of jatropha seeds has become a booming business. The oil produced by this crop can be easily converted to liquid biofuel that meets American and European standards (Tiwari *et al*. 2007). Additionally, the press cake can be used as a fertiliser, and the organic waste products can be digested to produce biogas (CH_4) (Srivastava and Prasad 2004).

Because of the great importance of *J. curcas* seeds to the production of biodiesel, there is an increasing market for seeds. To obtain a continuous and efficient production of oil, it is important to have plants that produce large quantities of fruits and seeds of good quality. However, there is no standardisation of the type of seed used or germination tests of seed vigour suited to assess and ensure good seedling quality and field performance. Ecological understanding of the relationship among these characteristics and the performance of seeds in wastelands is of great practical interest.

Among the ecological factors that can affect plant performance, seed size is one over which the producer may have some degree of control (Wulff 1986). Variability in seed size may affect germination, dispersal, seed–water relations and the ability to emerge from different depths of sowing, and thus may affect several aspects of seedling establishment (Wulff

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1986). Consequently, evaluation of seed quality is of fundamental importance and great value in programs of seed production.

Variation in seed size has often been correlated with environmental factors. Both within and among species, a larger seed size has been associated with many factors. Of these factors, dryness is among the most relevant (Wulff 1986). These correlations in other plant species have led to the assumption that in *J. curcas*, seedlings from larger seeds may have a higher rate of root extension than seedlings from smaller seeds, and this in turn results in greater tolerance to drought and other stressors (Karrfalt 2008). Many of these assumptions, however, have not been tested experimentally in *J. curcas*.

The present study evaluated the influence of the Brazilian environment on length, width, total biomass, volume, biochemistry and germination characteristics of *J. curcas* fruits and seeds.

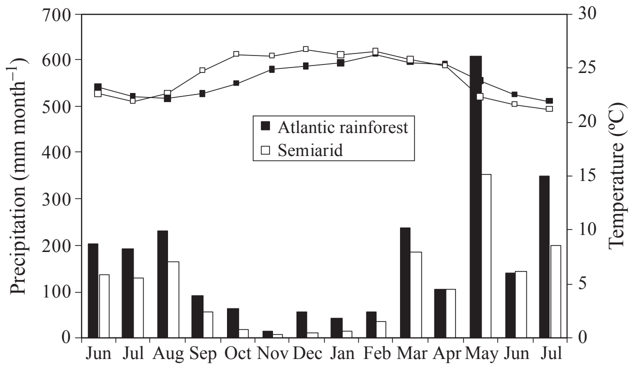

Fig. 1. Precipitation (bars) and temperature (lines) registered in 2007–2008 in the Atlantic rainforest (AF; black symbols) and the semiarid (SA; open symbols) regions of Brazil. Source: Agritempo (http://www.agritempo.gov.br/agroclima/sumario?uF=AL).

Materials and methods

Area description

The semiarid Caatinga area (SA) occupies the region between the Amazon forest (south of the equator) and the Atlantic rainforest (AF). It covers ~10% of the Brazilian arid and semiarid region. The Caatinga climate and its seasonality are related to climatic oscillations (Monteiro *et al*. 2006). The average regional annual rainfall is under 800 mm and is distributed entirely during a short rainy season (i.e. May–August), resulting in xerophytic and caducifolious vegetation (Queiroz 2006).

The AF is the second-richest neotropical biome and one of the most endangered in the world (Myers *et al*. 2000). It extends into tropical and subtropical regions. The wide longitudinal range leads to differences in forest species composition because of the decreasing rainfall away from the coasts. The rain falls between April and August. The coastal areas receive large amounts of rain year-round, with rainfall reaching more than 4000 mm year^{-1}, whereas inland forests receive ~1000 mm year^{-1} (Câmara 2003). These geographical characteristics, combined with a large altitudinal range, have favoured high diversity and endemism (Myers *et al*. 2000).

In the AF and SA, the historical average rainfall is 1481 and 902 mm month^{-1}, respectively. Between June 2007 and July 2008, the total rainfall was 2381 and 1548 mm in the AF and SA regions, respectively (Fig. 1). The temperature does not significantly vary throughout the year; however, it was on average higher in the SA than the AF region (24.3 ± 2.1 and 23.9 ± 1.5, respectively, Fig. 1).

Four hundred seedling, originated from seeds of a commercial plantation were transferred to SA region, located in the semiarid region (09°32′13″S, 36°38′01″W, 240 m a.s.l.) and the same number were transferred to AF region, located in Rio Largo city (09°28′42″S, 35°51′21″W, 39 m a.s.l.) in March 2003. In May 2008, seeds originated from the new plantation grown in each region were collected from at least 20 different plants for seed analysis (see below).

Allometry of fruits and seeds

Random fruits of *J. curcas* were collected manually from populations in the two locations described above, dried under shade at ambient temperatures for 2–3 days and then stored at 4 ± 2°C until use. For four subsamples, each containing either 30 fruits or 160 seeds, the length and width of fruits and seeds were measured with a caliper, with an accuracy of ±0.01 mm, and the total biomass and volume of fruits and seeds were determined. The water content of the seeds was quantified in four subsamples, each containing 30 g of seeds, as described in Vertucci (1993). To determine the biomass of 1000 seeds, 10 subsamples of 100 seeds were weighed with a precision of 0.001 g in accordance with the International Seed Testing Association (Karrfalt 2008). To determine the volume of fruits and seeds, three subsamples of with 15 fruits or 50 seeds were immersed in 250 mL of distilled water, and the displacement volume was measured with a graduated tube.

Imbibitions of seeds

Water uptake was measured in four replicates of 25 seeds from each location that were subjected to hydration under conditions of zero osmotic potential, as described in Bittencourt *et al*. (2004). Seeds were imbibed for 120 h. In the first 14 h, the samples were weighed every 2 h, and thereafter every 24 h. The seeds were removed from the imbibition solution, reweighed and then returned to a new solution under conditions of zero osmotic potential. The results were expressed in g H_2O g^{-1} dry weight, representing the amount of water absorbed by the seeds during the entire course of imbibition.

Biochemical analysis of seeds

Three seeds from each location were thoroughly ground with a cold mortar and pestle in an ice bath, until no fibrous residue could be seen in a 50% (v/v) ethanol solution (Trethewey *et al*. 1998) for soluble-sugar analysis, and in Stitt buffer (Armengaud *et al*. 2009) for proteins. Soluble sugars and proteins were analysed colorimetrically by the methods of Dubois and Bradford, respectively (Dubois *et al*. 1956; Bradford 1976). The oil contents of the seeds were analysed using the methodology described by Ahmad *et al*. (1981). All analyses were performed in triplicate.

Seed germination and seedling growth

Four subsamples of 100 seeds were planted in 180-cm^3 plastic bags filled with a mixture of soil, sand and organic matter

(1 : 1 : 1), at 0.5 cm below the soil surface, in accordance with the International Seed Testing Association (Karrfalt 2008). Seeds were considered to have germinated on the substrate when they produced a normal seedling, with all the essential structures well developed (Ranal and Santana 2006). The substrate was rehydrated with distilled water when necessary. Germinated seeds were counted daily for 20 days. At the end of the 20-day period, the germination times and germination synchrony were recorded and expressed in days and bits, respectively (Ranal and Santana 2006). Physiological characteristics of the seedlings were determined by germination rate, seedling height, accumulated shoot and root biomass, and leaf area.

Electrical conductivity (EC) of the leachate from seeds was determined on five subsamples of 50 seeds. Seeds were placed in plastic cups with 75 mL distilled water and kept in a germinator at 25°C. After 24 h, the EC of the leachate was determined with a conductivity meter and the mean values are expressed in $dS\,m^{-1}\,g^{-1}$ fresh weight, as described in Marcos-Filho (1998).

Statistical analysis

The results were analysed with a mixed-model ANOVA, and the means were compared using the Newman–Keuls test (SNK). Analyses were performed in Statgraphics Plus Version 5.1 (StatPoint Inc., Herndon, VA, USA).

Results

Fruit size and seed weight

Significant ($P < 0.001$) differences in length, width and biomass were detected between the fruits of *J. curcas* obtained from the AF and those obtained from the SA region (Table 1). Fruits collected from the AF region were significantly ($P < 0.001$) smaller than those collected from the SA region. Length, width and biomass of fruits obtained from the AF region were 9%, 3% and 8% smaller than those from the SA region (Table 1).

Similarly, significant ($P < 0.001$) differences in the length, width, biomass and volume were detected between the seeds obtained from the AF region and those obtained from the SA region (Table 1). In contrast to fruit size, seeds from the AF region were significantly ($P < 0.001$) larger (8%) and heavier (37%) than those from the SA region (Table 1).

Soluble sugars, proteins and oils

There were highly significant ($P < 0.001$) differences between the AF and the SA seeds in the soluble sugar, protein, oil and relative water contents and the EC (Table 2). The soluble sugar, protein and relative water contents and the EC of the SA seeds were greater, on average by 28%, 23% 32% and 94%, respectively, than those of the AF seeds (Table 2). In contrast, the oil content of the SA seeds was significantly lower (12%) than that of the AF seeds (Table 2).

Table 1. Biometric characteristics of fruits and seeds of *Jatropha curcas* from the Atlantic rainforest (AF) and the semiarid (SA) regions (Alagoas, Brazil)

The values are the average ± s.d. of four replicates of either 30 fruit, or 160 seeds. $^{***}P \leq 0.001$; n.s., the means are not significantly different (Newman–Keuls test, $P = 0.05$)

Parameter	AF	SA	Significance
Fruits			
Length (mm)	32.40 ± 0.32	35.41 ± 0.47	***
Width (mm)	29.27 ± 0.54	30.28 ± 0.17	***
Biomass (g)	13.21 ± 0.62	14.34 ± 0.36	***
Volume (cm^3)	14.46 ± 0.57	15.53 ± 0.10	n.s.
Seeds			
Length (mm)	16.64 ± 1.02	15.28 ± 0.07	***
Width (mm)	10.51 ± 0.38	9.85 ± 0.03	***
Biomass (g)	0.63 ± 0.11	0.46 ± 0.01	***
Biomass of 1000[A] seeds (g)	683.13 ± 12.75	512.73 ± 8.71	***
Volume (cm^3)	0.80 ± 0.01	0.47 ± 0.05	***

[A]10 subsamples of 100 seeds.

Seed imbibition

Jatropha curcas seeds hydrated rapidly, regardless of the size. In the first 14 h of imbibition, the seeds reached 0.41 g $H_2O\,g^{-1}$ fresh weight (Fig. 2). This rapid absorption of water characterised the first phase of the imbibition process and was a consequence of the matric potential of the dry seed tissues. In the first 24 h, the speed of imbibition of SA seeds was higher than that of the AF seeds. However, after 14 h, the imbibition rate decreased continuously and, at 120 h, reached 0.53 and 0.57 g $H_2O\,g^{-1}$ fresh weight for the AF and SA seeds, respectively (Fig. 2). The curves of absorption for seeds in both regions were biphasic.

Seed structure

Seeds of *J. curcas* have a hard, brittle and resinous integument. Under the shell of the seed is a white film that covers the almond. The white albumen is abundant and rich in fatty acids and contains the embryo, which comprises two large-plane cotyledons (Fig. 3*B*). The sequential stages of germination and seedling development are shown in Fig. 3*C*–*G*. A white and slender root appears as the seed increases in size and ruptures (Fig. 3*C*). Usually, five roots are formed, with one being central and four peripheral (Fig. 3*D*). The peripheral roots, i.e. lateral roots, develop from the taproot. Concomitant with the formation of

Table 2. Total soluble sugars, protein, oil content, relative water content and electric conductivity of *Jatropha curcas* seeds collected from the Atlantic rainforest (AF) and the semiarid (SA) regions (Alagoas, Brazil)

The values are the average ± s.d. of three replicates of three seeds each. $^{*}P \leq 0.05$, $^{***}P \leq 0.001$ (Newman–Keuls test, $P = 0.05$)

Parameter	AF seeds	SA seeds	Significance
Total soluble sugars ($g\,kg^{-1}$ DW)	13.62 ± 0.42	17.49 ± 0.32	***
Proteins ($g\,kg^{-1}$ DW)	50.09 ± 2.29	61.57 ± 3.74	*
Oil content ($g\,kg^{-1}$ DW)	314.00 ± 5.87	275.03 ± 5.85	***
Relative water content ($g\,kg^{-1}$ FW)	154.14 ± 1.94	203.58 ± 7.60	***
Electric conductivity ($dS\,m^{-1}\,g^{-1}$ FW)	0.65 ± 0.04	1.26 ± 0.06	***

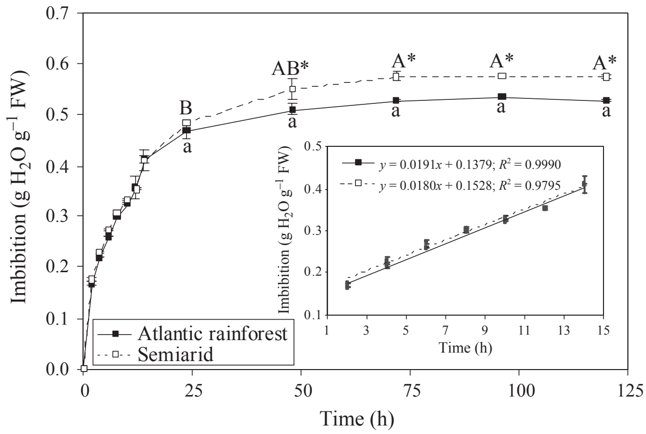

Fig. 2. Imbibition curves of *Jatropha curcas* seeds collected from the Atlantic rainforest (AF; black symbols) and the semiarid (SA; open symbols) regions of Brazil. The initial logarithmic phase of imbibition is given in the inset for the seeds of both regions. The values represent the average ± s.d. of four replicates of 25 seeds. Different letters and asterisks indicate a statistically significant ($P \leq 0.05$) difference in the means between the two localities (Newman–Keuls test).

the root system is the development of the hypocotyl, which is a pale and greenish-white structure (Fig. 3*E*). The cotyledons are fully attached to the endosperm, which is relatively thick and white. Germination is completed in ~15 days, after which the seedling is functional and physiologically normal (Fig. 3*G*).

Seed germination and seedling growth

The germination rate, duration and synchrony, and the shoot dry biomass were significantly ($P < 0.05$) affected by the location of the seed source (Table 3). The germination rate of the AF seeds exceeded that of the SA seeds by 17.5%, and the AF seeds took significantly less time (10%) to germinate than did the SA seeds (Table 3). The germination synchrony was more acute in the SA seeds than in the AF seeds (Fig. 4). Germination was complete in ~15 days, with seedlings being functional and physiologically normal thereafter (Fig. 3*G*). The shoot dry biomass of the seedlings grown from seeds obtained from the AF location significantly exceeded that of the seedlings grown from seeds obtained from the SA by 18.5% (Table 3).

Discussion

Jatropha curcas seeds from the SA region imbibed more water than did the seeds from the AF region. Other studies on agricultural crops have indicated that seeds with a higher protein content usually imbibed more water; in contrast, seeds with a higher oil content usually imbibed less water (Copeland and McDonald 1995). Our study showed that in the first 24 h of imbibition, the water content of the seeds increased by 50%. This suggests that even with high relative humidity, the seeds of *J. curcas* absorb water at a considerable speed (Vertucci and Leopold 1984). This observation was more acute in the AF than SA seeds. Similar observation was reported in some leguminous seeds (Hsu *et al.* 1983). It is likely, that the 120 h during which the study was conducted was insufficient to allow entry into Phase III of the imbibition, as proposed by Bewley (1997), and as evidenced from the finding that there was no protrusion of the radicle during the experiment. This same pattern was also found in asparagus seeds (Bittencourt *et al.* 2004) in which the first protrusion of radicle and the imbibition Phase III required at least 168 h.

Our results showed that the fruits collected from the SA region were larger and heavier than the ones collected from the AF region. However, fruit size did not translate into larger seeds. We believe that these variations in fruit and seed size were influenced by the characteristics of the region of cultivation because the plants from which the fruits and seeds were collected originated from the same seedbank.

Studies reported elsewhere have suggested that smaller seeds can influence positively (Edward and Hartwig 1971; Beckert *et al.* 2000), negatively (Hsu *et al.* 1983; Souza 1996; Thomas and Costa 1996), or have no effect (Pompelli 2006), on the rate of seed germination water imbibition, seedling vigour, growth and the resulting plant productivity (Krzyzanowski *et al.* 1991). However, seedlings from larger seeds may have an advantage over those from smaller seeds in both seedling establishment and physiological characteristics (Wulff 1986).

Our study showed that the AF seeds were more competitive than the SA seeds because they were more developed and physiologically more competent. Apparently, the SA seeds had higher biological activity than AF seeds, which may have contributed negatively to seed germination, because the leachate from the seeds can haul the solutes (i.e. sugars, proteins, amino acids) important for germination. The SA seeds had higher concentrations of soluble sugars and proteins, and this also may have contributed to low germination rates, as previously reported by Miguel and Cícero (1999).

Sugars represent an important source of energy and carbon skeletons for plant growth and development, and they also act as signalling molecules whose transduction pathways influence developmental and metabolic processes (Chen *et al.* 2006). Sugars negatively affect seed germination and early seedling development (Arenas-Huertero *et al.* 2000). However, sugar supplements are related to the levels endogenous hormones, such as ABA and GA, which are key internal regulators of seed germination (Gibson 2005). The SA seeds exhibited high quantities of soluble sugars and proteins. Therefore, it seems that glucose and other sugars exert their inhibitory effects via biosynthesis, degradation or signalling pathways for these hormones to a greater extent in the SA than in the AF seeds.

Success in crop production requires that seeds germinate and resultant seedlings emerge quickly and uniformly (upper germination synchrony), so that water, light and nutrients can be used with maximum efficiency. Our study showed that the percentage of SA seeds that germinated was significantly lower than that of AF seeds; however, SA seeds were more synchronous. We believe that AF seeds showed more vigour, partly because of decreased germination time, as described for other species (Maguire 1962). This finding may be a consequence of the seed size, where larger seeds contain conditions more favourable to germination and seedling growth. Present results suggest that seed size is an intrinsic attribute of the seed, although these characteristics can be modulated and then changed by climate. In general, the seeds of *J. curcas* resulted in normal seedlings with sound physiological traits.

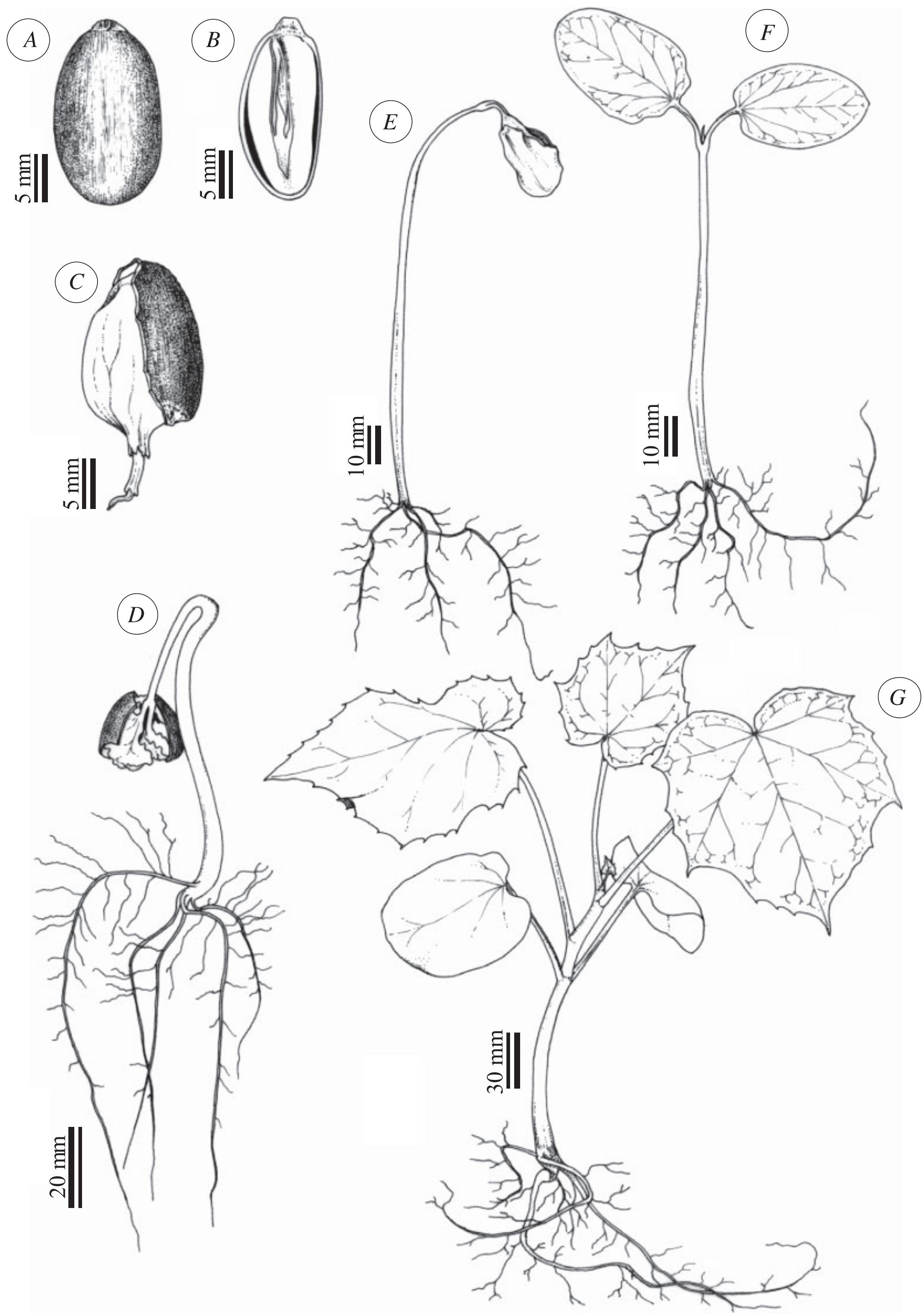

Fig. 3. Sequential processes of germination of *Jatropha curcas*. (*A*) Seed with wrap intact. (*B*) Seed cut to halves, showing the embryo inside. (*C*) Early stages of germination; radicle protrusion. (*D*) Formation of gain plumule and the development of white and branching roots. (*E*) Full expansion of the gain plumule and the early opening of the cotyledons. (*F*) Expansion of cotyledons and elongation of the seedling. (*G*) Seedling fully formed, with an expansion of the first true leaves, after 15 days.

The characteristics of biomass allocation evaluated in the present study allowed us to conclude that the AF seeds are more vigorous and of better quality than the SA seeds. This finding is in agreement with Dan *et al*. (1987), who suggested that biomass partitioning between shoots and roots is a very important feature when evaluating the physiological quality of different groups of seeds. In general, plants that can allocate more biomass to the underground parts than to the shoots may be advantageous

Table 3. Germination characteristics and seedling growth of *Jatropha curcas* from the Atlantic rainforest (AF) and the semiarid (SA) regions (Alagoas, Brazil)

The values are the average ± s.d. of four replicates of 100 seeds each. *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; n.s., the means not significantly different (Newman–Keuls test, $P = 0.05$)

Parameter	AF seeds	SA seeds	Significance
Germination rate (%)	62.93 ± 2.63	53.57 ± 1.65	**
Germination time (days)	7.77 ± 0.21	8.64 ± 0.17	**
Germination synchrony (bits)	2.43 ± 0.07	2.14 ± 0.10	*
Plant height (cm)	14.36 ± 0.23	14.98 ± 0.24	n.s.
Shoot dry biomass (g)	0.96 ± 0.02	0.81 ± 0.02	***
Root dry biomass (g)	0.12 ± 0.01	0.11 ± 0.01	n.s.
Shoot : root ratio	8.37 ± 0.48	7.72 ± 0.36	n.s.
Total leaf area (cm^2)	176.82 ± 3.42	169.49 ± 2.25	n.s.

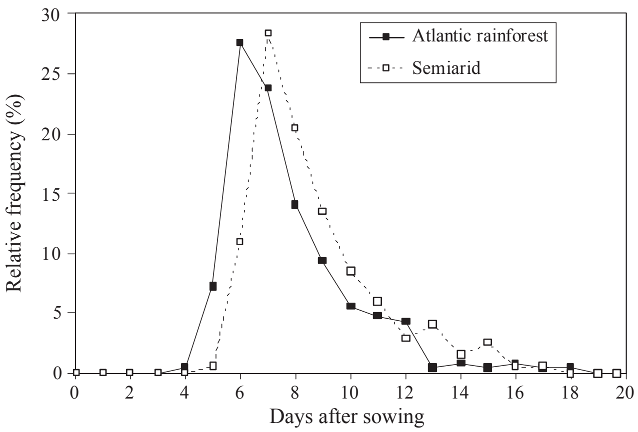

Fig. 4. Relative frequency of seed germination of *Jatropha curcas* seeds collected from the Atlantic rainforest (AF; black symbols) and the semiarid (SA; open symbols) regions (Alagoas, Brazil). The values represent the average of four replicates of 100 seeds each.

in a more arid climate, because their roots are able to withstand a greater water intake to compensate for the possible higher transpiration.

Despite recent reports that *J. curcas* is a species adapted to conditions of water shortage, it is likely that the characteristics of the AF region are more favourable for the development of this species than are the climatic characteristics of the SA region (Pompelli *et al*. 2010). It is also likely that the seeds produced in these two areas of Brazil (the AF and SA regions) have resulted from development of two biotypes or varieties. Further research is warranted to investigate the possibility of existence of biotypes or varieties as has been reported in Mexico (Delgado and Parado 1989) and other sister species such as *J. gossypiifolia* in Australia (Bebawi *et al*. 2007) and North America (Dehgan 1982).

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