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Characterization of the Oleaginous Potential of *Jatropha curcas* in Burkina Faso: Study of Accessions Resistance to Fungal Pathogens, Seed Traits and Molecular Diversity

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Abstract: The development of the *Jatropha* sector is limited by the lack of adequate plant genetic resources and data on local genotypes. However, knowledge of the characteristics of local accessions can help to identify suitable genotypes and/or identify varietal improvement paths for sustainable biofuel production. In order to characterize the local genotypes of *Jatropha curcas* in Burkina Faso, seeds of a collection from 40 plantations of the different climatic zones of the country were used to assess the accessions resistance to fungal pathogens, seeds oil and germination and molecular diversity. The results revealed a high variability in accessions resistance to fungal pathogens, seeds oil content and germination depending on the accessions. These variations of seeds oil content, seeds germination capacity and accessions resistance to fungal pathogens could been explained by genetic factors. This hypothesis is confirmed by genetic parameters which showed a strong heritability of the studied characters. Indeed, outside the diameter of the necrosis, the study exhibited high phenotypic and genotypic coefficients of variation and high heritability in broad sense. The study also revealed positive correlations between resistance parameters and seed oil content on the one hand and between these parameters and germination capacity on the other hand. There are good opportunities to improve accessions resistance to pathogens, seeds oil content and germination capacity. However, the evaluation of molecular diversity based on 20 microsatellites markers showed low genetic diversity. The high phenotypic variability observed in seed traits and resistance of accessions contrasts with a low level of genetic diversity of accessions. This study constitutes an important contribution to the characterization of local genotypes in order to identify the best genotypes for improvement of seeds traits and accessions resistance to fungi in a breeding program.

Keywords: Jatropha, Oil Content, Genetic Diversity, Resistance, Pathogens

1. Introduction

In tropical and subtropical countries, *Jatropha curcas* is used as a biofuel crop. Among the oil-bearing tree species [1, 2], J*atropha curcas* is sought because of its drought hardiness, rapid growth, easy propagation, low cost of seeds, high oil content, small gestation period, wide adaptation, good production on rich and degraded soils and the optimum plant size that makes the seeds collection more convenient [1, 3]. Worldwide, introduction of *J. curcas* for various purposes has had limited success due to pests and diseases, unreliable seed and oil yields and low economic returns [4, 5]. The propagation of Jatropha through quality plant material is today

a major challenge. It is propagated normally through seeds or vegetative cuttings. However, plants propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than plants propagated by seeds [6]. Many problems (poor seed viability, low germination, scanty and delayed rooting of seedlings) are associated with *Jatropha curcas* propagation through seeds [6, 7]. Indeed, *Jatropha curcas* seeds are usually unreliable in terms of germination rates which varies from 10-95 per cent [7]. The seed yields, accessions resistance to pathogens and seed oil content are variable and generally weak, reducing its economic potential and making its cultivation a risk business [1].

Therefore, genetic improvement and development of superior genotypes with high quality of seeds germinations, seeds high oil content, resistance to pests and diseases are essential for a sustainable biodiesel production [8]. The potential areas of genetic improvement of *J. curcas* are the increased in seed oil content, seeds germination capacity and genotypes resistance to pathogens [2]. Genetic improvements of *J. curcas* can be based on morphological traits expressed in the existing local population [9]. Indeed, regarding the large distribution of the specie, it is expected large variability of seeds traits and accessions resistance to pathogens [10]. Burkina Faso has a large climate gradient from North to South. It covers dry and hot climates in the Sahelian zone to humid climates in the south Sudanian. This climatic gradient is a suitable option for the assessment of variations in *Jatropha* germplasm. Some local genotypes may have the best adaptation to climatic and soil conditions and the key for the success of any genetic improvement program depends in the availability of genetic variability for the desired traits [8]. The improvement work must begin with the evaluation of the local genotypes. *J. curcas*, which is also highly cross-pollinated species, is expected to contain wide genetic variability with a high potential for the breeding of genotypes with superior traits [5]. However, very few studies have been published on the variation of seeds germination, oil content, resistance of local accessions to pathogenic fungi and on the determinism of this variation. Most of these studies have focused on the morphological traits of plants, [11], fruits [12] and the morpho-metric of seeds traits [10, 11]. However, knowledge of the relationship between seeds oil content variation, germination and resistance of accessions to pests and diseases can optimize breeding and improvement programs [13]. In this research study, we investigated the seeds oil content and germination capacity of local accessions of *J. curcas* from Burkina Faso and their resistance against three fungal species. In order to establish the probable determinism of this variability, the molecular variability of local accessions was study.

2. Material and Methods

2.1. Plant Material

The plant material is constituted by seeds of 40 genotypes of *J. curcas* collected from different sites (plantations and hedges) spread over the 3 climatic zones of Burkina Faso: Southern Sudan zone, the Northern Sudan zone and the Sahelian zone (sub-Sahelian and Sahelian). These seeds were collected from *J. curcas* plants at least 5 years old and were used to investigate seeds oil content and germination capacity, to sow Jatropha seeds in a greenhouse in order to study the resistance of accessions to fungal pathogens and to assess their molecular diversity. The characteristics of the different collecting sites are presented in table 1.

Table 1. *Characteristics of the collecting plantations and hedges.*

Climatic zone Age of plantations	Number of hedges		Number of plantations		Total
	5 ≤ 9	10 ≤ years	5 ≤ 9	10 ≤ years	
Northern soudan	2	1	10	0	13
Sahelian	0	0	2	0	2
Sub sahelian	3	0	2	0	5
South soudan	1	0	14	5	20

2.2. Fungal Material

The fungal material is composed of 3 fungal pathogens of *J. curcas*: *Curvularia Lunata*, *Fusarium oxysporum* and *Lasiodiplodia theobromae*. These fungi come from the plant clinic laboratory of Nazi BONI University and have been isolated and characterized by [14].

2.3. Seeds Traits Variation Assessment

2.3.1. Seeds Oil Content

Seeds oil content was determined by Soxhlet method described by Sama *et al*. [15]. Six (6) hours extraction with petroleum ether as extraction solvent were performed. The extracted oil is recovered after solvent evaporation at 40°C under reduced pressure using a rotavapor. The extracted seed oil was weighed. The amount of oil in seeds was calculated and expressed as percentage (%) by following formula:

$$\text{Seeds oil content (\%)} = \frac{Oil\ weight * 100}{sample\ weight}$$

2.3.2. Seeds Germination Capacity

Seeds germination capacity of local accessions was assessed in the greenhouse. Three lots of ten (10) seeds of each accession randomly selected were sown in plastic pots of about 1 litter capacity at the rate of one seed per pot. Each pot contained a mixture of sand, compost and organic manure in 3/1/1 (v/v/v) proportion. For each treatment, the seeds were surface sterilized with 2% of sodium hypochlorite solution for 1/2 minute and then washed 2 to 3 times with distilled water and seeds were then sown. The water supply was made every day in each pot during the experiment. The

number of germinated seeds per accession was recorded daily for 3 weeks. The germination capacity of the seeds was calculated according to the following formula:

$$\text{Germination Capacity (\%)} = \frac{Number\ of\ germinated\ seeds * 100}{Total\ number\ of\ sown\ seeds}$$

2.4. Accessions Resistance Investigation

2.4.1. Seedling and Experimental Design

The researches were performed in the green house of the plant clinic laboratory of the Nazi BONI University. The experiment was carried out in a completely randomized block design divided in 3 treatments with three replicates. The three treatments are: a negative control treatment with any treatment, a positive control treatment treated with sterilized water and a test treatment in which the plants have been inoculated by the pathogenic fungus. The same design was adopted for each genotype and for the tree fungal species. Seeds of the tested genotypes were sown in plastic pots according to the method described by Sama *et al*. [14]. Each pot contains a mixture of sand, compost and organic manure in the proportions 3/1/1. This mixture was previously sterilized at 120°C for four (04) hours. Plants were maintained in the greenhouse and watered every day.

2.4.2. Fungal Pathogens Cultivation and Inoculum Production

Pathogenic fungi were cultivated according to the method described by Setti *et al*. [16]. Isolates of each pathogen was grown on Potato Dextrose Agar (PDA) medium for 10 days at 22°C. Conidia of 10 days were collected by adding 10 ml of sterilized water and the concentration of suspension after filtration was adjusted to 2. 10^6 conidia.ml^{-1}.

2.4.3. Plants Inoculation

The plants were inoculated on the 30th day according to the method described by Hernández-Cubero *et al*. [17] after rubbing the carborandum on the leaves to cause micro-injuries on the leaves. Inoculated plants were observed daily and the resistance parameters were measured. The experiment was followed for 14 days. The inoculated leaves were observed daily until the symptoms of diseases appeared.

2.4.4. Measurement of Resistance Parameters

The resistance parameters of the tested genotypes estimated were incubation period (I.P), necrosis diameters and (N. D) and frequency of successful inoculations (F. S. I.). The incubation period was expressed in days and can be definite as the period between the day of inoculation and the day of appearance of a visible reaction on the leaf of the inoculated plant. The diameter of necrosis was expressed in millimeter and was measured using a caliper. The number of successful inoculations is the ratio of the number of plants showing disease symptoms per test and per genotype.

2.5. Genetic Diversity Assessment

2.5.1. DNA Extraction

The young leaves of the seedlings from the nursery previously set up in the greenhouse were used for the study of molecular diversity. DNA was extracted from fresh young leaves of *Jatropha curcas* using MATAB (Mixed Alkyl Trimethyl Ammonium Bromide) as extraction buffer following the method described by Ouattara *et al*. [5] with minor modifications. 100 mg of fresh leaves were ground and homogenized in 1700 µL of MATAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 25 mM EDTA, 2% MATAB, 1% PEG 6000, 0.5% sodium sulphite; pH8) and incubated at 65°C. After 1 h, 500 µL of chloroform-isoamyl (24:1) were added in each micro tube. The micro tubes were mixed for 5 minutes by inverting and the mixture was centrifuged at 13,000 rpm for 10 minutes. The chloroform step was repeated and the aqueous phase was transferred carefully into a clean microtubes. The DNA was precipitated with 450 µL of cold isopropanol. The micro tubes were mixed by inverting the tubes until a ball is formed. The mixture is then centrifuged at 13000 rpm for 5 minutes and the supernatant was removed. The pellets DNA were washed with 450 µL of cold ethanol 70% then centrifuged at 12000 rpm at 4°C for 5 minutes and dried until the ethanol has evaporated completely. 5 µL of RNase is added and the mixture incubated over a 45 minute to 1-hour period and the extracted DNA is suspended in 75 µL TE 0.1X buffer or sterile distilled water and stored at -20°C until used. The concentration and purity of the DNA samples were evaluated using a Nanodrop and by running the samples on a 0.8% agarose gel based on the intensities of bands by comparison with the Lambda DNA marker as standard and diluted in 1X TE to a concentration of 5 ng/µL.

2.5.2. Microsatellite Primers

Primer pairs specific to 20 microsatellites containing sequences of *J. curcas* were used. They were selected considering their high level of polymorphism reported by different authors (Table 2). The forward of each of the 20 primer pairs used is presented in table 2.

Table 2. *Sequences of used SSR primers.*

Number	Locus	Repeat motif	Primer sequence	Tm (°C)	Number of reported alleles	References
1	JCT17	$(GA)_6$…$(GA)_{11}$ $(GT)_{21}$	F: TCTCTCATTGTTGCGCTGTC R: TAACAAGTCCTCCCCCTCCT	60.0	3	[5]
2	JCT15	(A)22…(CT)10	F: AATTCTCTTTCCGCGATCCT R: CGTAGACCTTCCAACAGCAA	60.0	3	[18]
3	JCT27	$(CT)_{17}$	F: GCCATTAGAATGGACGGCTA R: TGCGTGAAGCTTTGATTTGA	60.0	3	
4	Jcms21	$(CA)_7$	F: TAACCTCTTCCTGACA	43.0	3	[13]

Number	Locus	Repeat motif	Primer sequence	Tm (°C)	Number of reported alleles	References
			R: ATAGGAAATAAGAGTTCAAA			
5	JMDB04	$(AT)_7$	F: CTTCTTTCCCTGTGGCTTTG R: ACCTCTCCCTTTTGGTCTGC	52.0	2	
6	JCT16	$(GT)_{11}$	F: GCCTCCAGCATCTTTCAATC R: AACAATCCCCATTCCTCCTC	60.0	2	
7	JCT27	$(CT)_{17}$	F: GCCATTAGAATGGACGGCTA R: TGCGTGAAGCTTTGATTTGA	60.0	2	
8	Jcds24	$(CA)_5$ $(TA)_8$ $(CA)_4$… $(TA)_3GA$ $(TA)_4$	F: GGATATGAAGTTTCATGGGACAAG R: TTCATTGAATGGATGGTTGTAAGG	51.0	11	
9	Jcps20	$(CA)_2$…$(CA)_4$	F: ACAGCAAGTGCACAACAATCTCA R: TACTGCAGATGGATGGCATGA	55.0	9	[7]
10	jcds41	$(CA)_6$ $(TA)_2$	F: AACACACCATGGGCCACAGGT R: TGCATGTGTGCGGGTTTGATTAC	60.0	5	
11	jcps9	$(TG)_{12}$ $(GA)_{22}$	F: GTACTTAGATCTCTTGTAACTAACAG R: TATCTCTTGTTCAGAAATGGAT	60.0	4	
12	JcSSR3	$(GA)_{24}$	F: GACGGGAAAATATATGGCTA R: GTTTTCAGGATTGAATGCTCT	51.0	6	
13	JcSSR4	$(AG)_{20}$	F: CGCAATCCCATTTTTATTAT R: GATGGGCTCTTTGTAGCTTTT	55.0	6	[11]
14	JcSSR8	$(AC)_{17}$	F: ATGTCTCTTTTCCATGTCCAA R: TCAACCCACCCTCATATAAACC	60.0	4	
15	JcSSR26	$(CA)_{18}$	F: CATACAAAGCCTTGTCC R: AACAGCATAATACGACTC	55.0	4	[18]
16	phi299852	AGCC	F: CAAGTGCTCCGAGATCTTCCA R: CGCGAACATATTCAGAAGTTTG	52	8	
17	bnlg1614	(AG)15	F: CCAACCCACCCAGAGGAGA R: AGCGGGCGAGATCTTCAT	58	6	
18	phi053	GCT	F: ATTCCGACGCAATCAACA R: TTCATCTCCTCCAGGAGCCTT	54	6	[19]
19	phi032	CCG	F: CCGGCAGTCGATTACTCC R: CGAGACCAAGAGAACCCTCA	54	6	
20	phi109275	AGCT	F: CGGTTCATGCTAGCTCTGC R: GTTGTGGCTGTGGTGGTG	54	5	

2.5.3. PCR Amplification

The reaction mixture was 25µL and included 3 µL of DNA (10 ng / µL), 2.5 µL of 10X buffer, 2 µL of dNTP (2.5 mM), 2 µL of $MgCl_2$ (25 mM), 0.05 µL of each primer, 0.2 µL of 0.5 U Taq polymerase and 15.2 µL of ultra-pure water. The amplification was performed with a thermocycler. The PCR program used is as follows: 37 cycles including one (1) DNA pre-denaturation cycle at 95°C for 1 min followed by a set of 35 cycles with denaturation for 30s at 95°C, hybridization at 55°C for 30s and extension for 30 s at 72°C. A final phase containing only a 5 min extension to 72°C.

2.5.4. PCR Products Revelation

The PCR products were migrated on an agarose gel (1% gel) for 30 min at 135 V and visualized under UV light. 1 kb DNA Ladder has been used as a molecular marker to determine the molecular weights and sizes of the different bands.

2.6. Statistical Analyses

Analysis of variance (ANOVA) was carried out using version 2016 of XL-STAT on the seed traits and the resistance parameters in order to access the genotype effect for all traits. The graphs were performed using version 6.01 of GraphPad Prism. Phenotypic and genotypic coefficients of variance were estimated according to the method described by Zongo *et al*. [20] to quantify the genetic variance among the genotypes, the heritability in broad sense and genetic advance as a percent of mean.

3. Results and Discussion

3.1. Variation in Seeds Oil Content and Germination Capacity

The seeds oil content and germination capacity of the different accessions of *J. curcas* are shown in table 3. The results showed that seeds oil content and germination capacity varied significantly among the accessions. Seed oil content expressed as a dry weight percentage ranged from 33.83 ± 1.65 to 57.21 ± 2.44% for the J1 and J7 accessions, respectively. The seeds germination capacity of the different accessions expressed as percentage of germinated seeds also varied significantly from 5 to 95% with an average of 58.54%. The lowest germination capacity was recorded at accession J37 while the highest value was recorded at accession J39. In addition, the variation of seeds oil content and germination of genotypes is not depending to their geographical distribution. Indeed, the seeds of genotypes of the same climatic zone have often shown different characteristics.

3.2. Response to Fungal Pathogens Inoculation in Leaves

Seedling of local 40 genotypes of *J. curcas* reaction

against fungal pathogens are shown in table 3. Data about the seedling reaction showed a range of responses depending both accessions and pathogens. Among the 40 accessions tested, the inoculated leaves of 20 accessions exhibited yellowing and necrosis on the inoculated zones while those of others accessions did not present any symptoms of disease. The resistance parameters were also measured. All the resistance parameters measured presented significant variations depending to the genotype. The incubation period varied of 4 to 11.67 days respectively for J21 and J2 accessions. The necrosis diameters of accessions which showed symptoms of diseases ranged from 4.33 to 12.83 mm for J20 and J17 respectively. The percentage of successful inoculations ranged from 22.2 to 88.9% for accessions which showed susceptible response. Based on genotypes reactions, three type of genotypes were identified. The first corresponds to accessions which leaves showed no symptoms of disease with the 3 pathogens. This group was considered as a group of resistant accessions. The second group composed of accessions which leaves have shown symptoms of disease with one or two of the pathogens. In addition, the leaves of these accessions showed lows diameters of necrosis and the numbers of inoculation in a controlled environment. These accessions also have average incubation periods (8.00 to 11.67 days). The third group showed symptoms of disease with all the three pathogens used. In addition, they have relatively short incubation times (4.67 to 8.00 days) with high necrosis diameters (10.97 to 12.82 mm) and successful inoculation percentage (≥ 88%). This group could therefore represent the group of susceptible accessions.

Table 3. *Seeds traits and resistance parameters of the different accessions.*

Accession	Seeds traits		Resistance parameters		
	Seeds oil content (%)	Germination capacity (%)	Incubation period (days)	Necrosis diameter (mm)	Number of successful inoculation (%)
J1	46.317 ^{bcdefghi}	90.000 ^{ab}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J2	33.833 ^{lm}	65.000 ^{abcde}	11.667 ^{ab}	6.443 ^{ab}	44.333 ^{ab}
J3	42.643 ^{bcdefghijkl}	90.000 ^{ab}	14.000 a	0.000 ^{b}	0.000 ^{b}
J4	44.713 ^{bcdefghijk}	20.000 ^{ijk}	8.000 ^{bc}	8.000 ^{ab}	44.333 ^{ab}
J5	31.300 ^{m}	70.000 ^{abcde}	10.667 ^{ab}	7.557 ^{ab}	44.333 ^{ab}
J6	38.417 ^{hijklm}	75.000 ^{abcd}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J7	57.210 ^{a}	50.000 ^{defghi}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J8	48.833 ^{abcd}	60.000 ^{bcdefg}	11.333 ^{ab}	6.333 ^{ab}	44.333 ^{ab}
J9	36.200 ^{klm}	55.000 ^{cdefg}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J10	48.493 ^{abcd}	85,000 ^{abc}	14,000 ^{a}	0.000 ^{b}	0.000 ^{b}
J11	36.567 ^{klm}	55.000 ^{cdefg}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J12	46.317 ^{bcdefghi}	51.667 ^{defgh}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J13	49.667 ^{abcd}	75.000 ^{abcd}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J14	40.853 ^{defghijkl}	65.000 ^{abcde}	11.000 ^{ab}	5.000 ^{ab}	44.333 ^{ab}
J15	39.467 ^{efghijklm}	73.333 ^{abcde}	10.667 ^{ab}	5.000 ^{ab}	44;333 ^{ab}
J16	34.103 ^{lm}	23.333 ^{hijk}	11.110 ^{ab}	5.000 ^{ab}	44.333 ^{ab}
J17	41.170 ^{defghijkl}	43.333 ^{efghij}	5.000 ^{c}	12.827 ^{a}	88.900 ^{a}
J18	50.827 ^{ab}	73.333 ^{abcde}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J19	41.377 ^{cdefghijkl}	16.667 ^{jk}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J20	50.133 ^{abc}	85.000 ^{abc}	14.000 ^{a}	0.000 ^{b}	0.000 $^{\underline{b}}$
J21	36.917 ^{jklm}	70.000 ^{abcde}	11.333 ^{ab}	4.333 ^{ab}	44.333 ^{ab}
J22	39.023 ^{fghijklm}	66.667 ^{abcde}	4.000 ^{c}	12.140 ^{a}	88.900 ^{a}
J23	49.413 ^{abcd}	75.000 ^{abcd}	14.000 ^{a}	0.000 ^{b}	0,000 ^{b}
J24	39.340 ^{efghijklm}	15.000 ^{jk}	8.000 ^{bc}	5.777 ^{ab}	44.333 ^{ab}
J25	31.363 ^{m}	6.667 ^{k}	9.000 ^{abc}	7.000 ^{ab}	44.333 ^{ab}
J26	37.667 ^{ijklm}	10.000 ^{k}	4.667 ^{c}	11.000 ^{a}	88.900 ^{a}
J27	50.433 ^{ab}	85.000 ^{abc}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J28	46.667 ^{bcdefgh}	61.667 ^{bcdef}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J29	50.633 ^{ab}	80.000 ^{abcd}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J30	47.810 ^{bcdef}	55.000 ^{cdefg}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J31	41.933 ^{bcdefghijkl}	70.000 ^{abcde}	11.333 ^{ab}	6.667 ^{ab}	33.333^{ab}
J32	50.707 ^{ab}	30.000 ^{ghijk}	14.000 ^{a}	0.000 ^{b}	88.900 ^{a}
J33	45.643 ^{bcdefghij}	66.667 ^{abcde}	4.667 ^{c}	11.890 ^{a}	44.333 ^{a}
J34	39.187 ^{efghijklm}	12.000 ^{k}	10.333 ^{ab}	6.333 ^{ab}	22.890 ^{ab}
J35	47.407 ^{bcdefg}	30.000 ^{ghijk}	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J36	50.177 ^{abc}	30.000 ghijk	14.000 ^{a}	0.000 ^{b}	0.000 ^{b}
J37	42.393 ^{bcdefghijkl}	5.000 ^{k}	11.000 ^{ab}	6.333 ^{ab}	44.333 ^{ab}
J38	47.967 ^{bcde}	33.333 ^{fghijk}	4.000 ^{c}	10.967 ^{a}	88.900 ^{a}
J39	38.667 ^{ghijklm}	95.000 ^{a}	11.333 ^{ab}	5.000 ^{ab}	33.333 ^{ab}
J40	38.933 ^{fghijklm}	75.000 ^{abcd}	11.000 ^{ab}	5.000 ^{ab}	44.333 ^{ab}
Pr > F (Model)	0.000	0.000	< 0.0001	< 0.0001	< 0.0001
Significaty	Yes	Yes	Yes	Yes	Yes

Values that have different subscript are significantly different according to the Newman-Keuls test at the 5% level.

3.3. Genetic Parameters of Seeds Traits and Resistance Parameters

The genetic parameters have been calculated and results are reported in Table 3. The highest values of phenotypic variation coefficient (CVP) (95%) and genotypic variation coefficient (CVG) (98%) were recorded for seed oil content and incubation period respectively. The highest heritability (0.94) and the highest genetic advance (12.02) were observed for seeds oil content. The lowest genetic gain was found for seed germination capacity (0.01).

Table 4. *Genetic parameters of seeds traits and accessions resistance parameters.*

Source	Coefficient of variation		Heritability in broad sense	Genetic advance	Genetic gain (%)
	Phenotypic	Genotypic			
Seeds oil content	0.95	0.97	0.94	12.02	0.01
Seeds germination	0.81	0.90	0.92	11.31	0.01
Incubation period	0.95	0.98	0.93	6.17	0.02
Necrosis diameter	0.03	0.07	0.90	10.84	0.03
F. S. I	0.83	0.91	0.76	1.22	0.01

3.4. Correlation Between Seeds Traits and Accessions Resistance to Fungi

The Pearson correlation matrix of seed traits (seeds oil content and germination capacity) and the different resistance parameters of the accessions are presented in the table 5. The results showed that the incubation period was significantly and negatively correlated with the necrosis diameter (-0.96) and the frequency of successful inoculation (-0.87). Positive and significant correlation (0.88) was recorded between frequency of successful inoculation and the necrosis diameter parameters.

Table 5. *Pearson correlation matrix of seeds traits and accessions resistance parameters.*

Variables	Seeds oil content	Germination capacity	Incubation period	N. D.	F. S. I.
Seeds oil content	1				
Germination capacity	0.20	1			
Incubation period	0.35	0,35	1		
N. D.	-0.45	-0.31	-0.96	1	
F. S. I.	-0.34	-0.35	-0.87	0,88	1

N. D: Necrosis Diameters; F. S. I.: Number of successful inoculations.

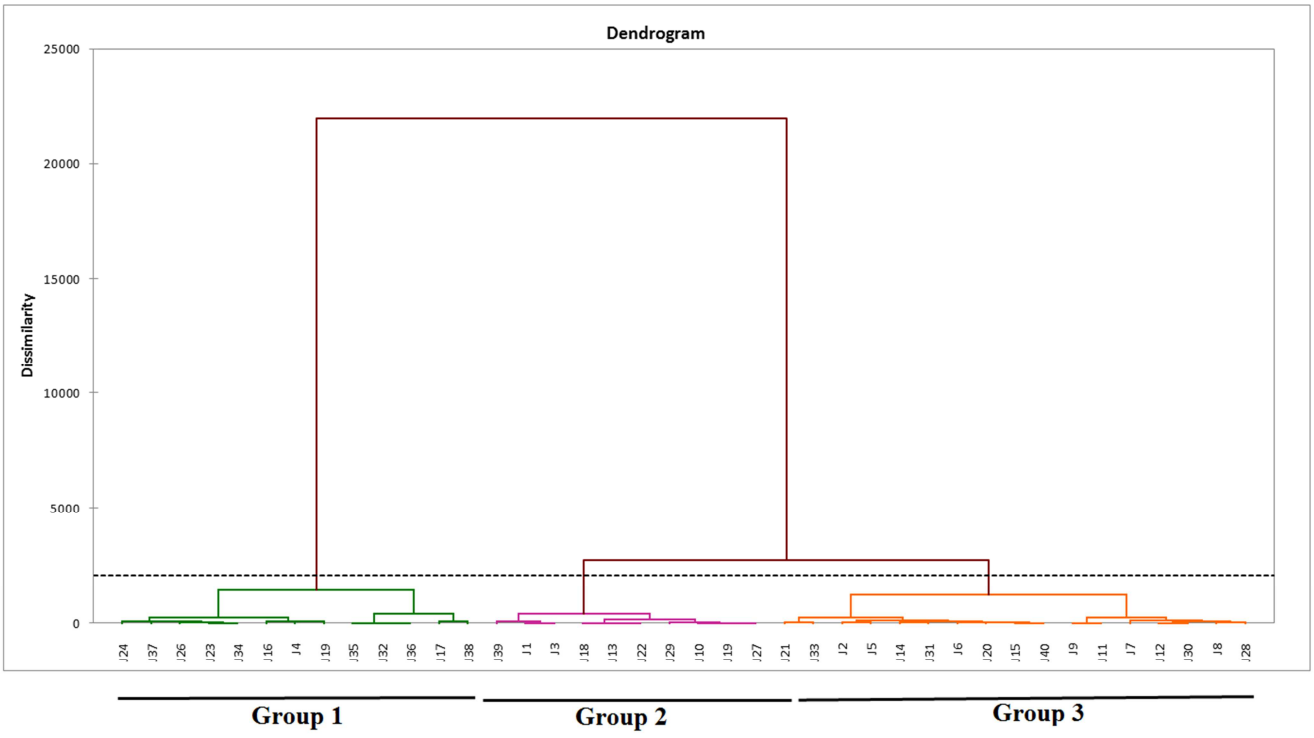

Figure 1. *Hierarchical Analysis Classification on seeds traits and accessions resistance to fungal pathogens.*

Table 6. *Composition and characteristics of the different groups.*

Group	Accessions	seeds oil content	Seeds germination capacity	Incubation Period	Necrosis diameter	Percentage of successful inoculation
1	J1, J3, J10, J13, J18, J19, J22, J27, J29, J39	48,493	85,000	14,000	0,000	0,000
2	J2, J5, J6, J7, J8, J9, J11, J12, J12, J14, J15, J20, J21, J28, J30, J31, J33, J40	40,853	65,000	11,000	5,000	1,333
3	J4, J16, J17, J19, J23, J24, J26, J32, J34, J35, J36, J37, J38	44,713	20,000	8,000	8,000	1,333

3.5. Diversity of Local Genotypes Based on Seeds Traits and Resistance to Fungal Pathogens

Data of accessions resistance to pathogenic fungi, seeds oil content and germination capacity were used to perform the hierarchical ascendant classification (HAC). The results (Figure 1) showed a breakdown of accessions into three groups whose characteristics are presented in table 6. Their characteristics can be summarized as follows: the group 1 is constituted by accessions which have very interesting seed traits (high oil content and high germination capacity) and which did not presented symptom of disease. This group present a high potential for selection and breeding programs. Group 2 presented seed and resistance traits intermediate between group 1 and group 3. Group 2 reassembled the accessions with intermediate features between the two previous groups.

3.6. Molecular Diversity of the Local Accessions

The molecular diversity of the 40 local accessions was assessed using 20 primers microsatellites. Among the 20 primers tested (table 2), only 3 microsatellite primers (JcSSR4, JCT16 and Phi032) showed exploitable polymorphism for the diversity study. A diversity analysis was performed on our study genotypes by using these 3 primers. The figure 2 presented the profile obtained using the primer JcSSR4.

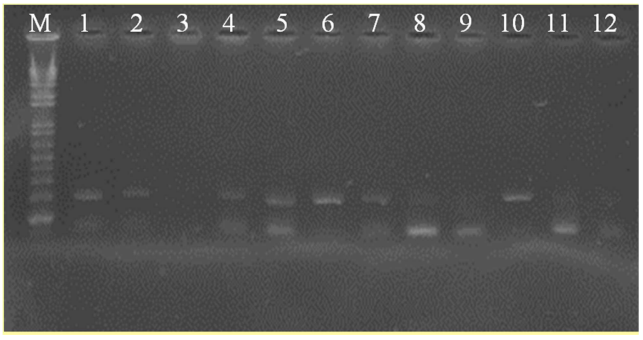

Figure 2. *Molecular analysis of J. curcas germplasm through microsatellite primer JcSSR4.*

The number of the alleles produced by each polymorphic primer was 12.67. The genetic diversity parameters of the three primer microsatellites are shown in table 5. The results show that the diversity ranges from 0.20 for primer PhiO32 to 0.64 for primers JcSSR4 and JCT-16 respectively with an average of 0.49. Observed heterozygosity and expected heterozygosity ranged from 0.13 for Phi032 to 0.45 for JcSSR4 and JCT-16.

Table 7. *Genetic diversity parameters of the three polymorphic primers.*

	JcSSR4	JCT-16	Phi032	Mean
N	12.68 ± 4.33	12.68 ± 4.33	12.67 ± 4.33	12.67 ± 2.17
Na	2.00 ± 0.00	2.00 ± 0.00	1.33 ± 0.33	1.78 ± 1.78
Ne	1.84 ± 0.15	1.84 ± 0.15	1.22 ± 0.22	1.63 ± 0.14
I	0.64 ± 0.05	0.64 ± 0.05	0.20 ± 0.20	0.490 ± 0.10
Ho	0.45 ± 0.05	0.45 ± 0.05	0.13 ± 0.13	0.34 ± 0.07
He	0.47 ± 0.06	0.47 ± 0.06	0.14 ± 0.13	0.36 ± 0.7

N: sample size; Na: number of different alleles; Ne: number of effective alleles; I: Shannon's information index; Ho: observed heterozygosity; He: expected heterozygosity.

Genetic variability analysis between the climatic zone collections showed a low level of heterozygosity variability ranging from 0.24 for the southern Sudanian zone to 0.50 for the sub-Sahelian zone. The genetic variability index varied from 0.35 to 0.66. The lowest value was observed in the southern Sudanian zone. The dissimilarity matrix based on the Nei distances between the different populations showed that the weakest differentiation is observed between the North Sudan zone and the Sub Sahelian zone while the largest difference is observed between those of the South and North zones. To evaluate the intra and inter population variations, a molecular variance analysis (AMOVA) was performed. The results showed a very low variability (1%) between climate zones. Much of the variability (99%) can therefore be defined only between accessions.

4. Discussion

Accessions resistance to fungal pathogens, seeds oil content and germination capacity are important breeding characters that can increase economic potential of *Jatropha curcas*. All the studied parameters exhibited high variations among the accessions in the germplasm used for this study. This high variability of seed traits constitutes an important input for the species improvement programs. These results are in agreement with the findings of [2] and [21] whom observed high variations in the oil content in different *J. curcas* populations in India. Large variation of seeds weight and oil content for *Jatropha* accessions from 21 locations in the southwest Guangxi of China was reported [22]. High variations in *J. curcas* fruits and seeds traits and plants morphological traits depending of accessions respectively in Senegal [5] and in Burkina Faso [10-12] were also observed. Such variations can be explained by genetic variability among plants, biotic interactions (human intervention, pests

and diseases) and abiotic factors (soil properties, temperature, rainfall, nutrient content in soil). The climate factors or ecological conditions (temperature, precipitation, sunshine etc.) could have a significant effect on growth, distribution, productivity, seed yield and oil content of *J. curcas* [22, 23]. If environmental conditions can be evoked to explain the variability of seed oil content, they cannot be evoked in this study because the germination and the plants used for the evaluation of the resistance of accessions to fungal pathogens were carried out under the same conditions (in greenhouse). Only genetic factors could explain the variation in accessions resistance and germinative capacity of the seeds in this study. Indeed, genetic factors have been confirmed to affect seed traits (oil content and morphological traits) and accessions characteristics (plants growth and seeds yield) of *J. curcas* in a multitude of studies [5, 10, 21]. These results are also confirmed by Abdul [24] who reported that conidia only start to germinate 6 to 12 hours after artificial inoculation. Forty-eight hours after inoculation, 95 to 99% of conidia have germinated systematically on all plants but genotypes showed different reactions to infection. Thus, before symptoms appear, conidia germinate but the possibility of penetration into stomata and causing infection varies according to the host genotypes [13, 24]. There is then a more or less long incubation period depending on the genotypes. In susceptible genotypes, there is severe swelling and the first symptoms appear quickly between four and seven days. In tolerant genotypes, invasion is less and symptoms development time can be long (between 7 to 12 days).

The hierarchical ascendant classification (HAC) distinguished 3 types of genotypes basis on recorded data. Among these genotypes, the accessions of group 1 present the best potential for selection programs. The accessions of this group have a good degree of resistance in adult plant and their seeds present high oil seeds content and germination capacity. The accessions could be considered in future breeding programs to improve the natural resistance of *J. curcas*. Accessions of group 2 are tolerant to pathogen and their seeds have lower oil content and capacity than those in Group 1. Those of group 3 have low potential for breeding programs because they include pathogen-sensitive accessions and present low values for germination capacity and seed oil content. Our results are in agreement with those of Draz *et al*. [13]. Their work on wheat genotypes for leaf rust resistance has distinguished different levels of resistance including resistant genotypes, partially resistant and susceptible genotypes. Outside the diameter of the necrosis, the study exhibited high phenotypic and genotypic coefficients of variation for all studied parameters. The variability of parameters estimated in this study are very close to the results of genetic parameters of *J. curcas* previously found [21, 25], whose reported high genetic parameters variations in *Jatropha* seeds traits respectively in India and Senegal. The heritability in the broad sense was high (>70%) for all the studied parameters. Regarding these results, these traits can be considered as the best gain characteristics for *Jatropha* improvement. Similar results have been reported in many species including *J. curcas* [5, 21]. The study also showed correlation between some seed traits and resistance parameters of some accessions. Correlation is one of the important biometric tools that measures the degree and magnitude of association between different traits. In tree improvement programs, a clear understanding of association between different traits have great importance because it illustrates the choice of a character that confirms the appearance or disappearance of another [21]. Similar results have been reported by Lama *et al*. and Tiendrebeogo *et al*. [9, 21] whose works also reported correlations between *Jatropha* seeds traits. These results confirm the good potential of this study in breeding and varietal improvement programs for seed traits and resistance of accessions to fungal pathogens.

However, the study of the molecular diversity of accessions showed a low molecular variability of local accessions. Thus, the high phenotypic variability observed in seed traits and resistance of accessions contrasts with a low level of genetic diversity of accessions. Similar results were also reported [5, 18, 25] in Senegal and Indian *Jatropha* accessions. These results make the genetic determinism of the phenotypic variability speculative. Indeed, a low genetic diversity with a wide distribution in different agro-ecological zones suggests that *J. curcas* has a large ecological plasticity. Ecological plasticity is known as a trait that promotes the adaptation of species to a wide range of environmental conditions through morphological and physiological modifications necessary for the survival of the species [5]. Unfortunately, in this study the influence of ecological factors on the observed variability can be minimized considering that the nurseries for molecular analysis were grown under the same conditions. Therefore, two hypotheses can explain these results. Either the markers used did not reveal the molecular diversity of accessions or the observed variability can be explained by the influence of epigenetic factors. Indeed, the molecular basis of a strong phenotypic variation on a background of low genetic diversity would therefore be due to the effect of epigenetic factors that regulate gene transcription [18, 19]. Such variation often results from the action of environmental factors (temperature, diet, physico-chemical characteristics of the environment, etc.) on phenotypic expression. Epigenetic mechanisms for such variations have also been reported in the case of *Arabidopsis* and *J. curcas*

5. Conclusion

The study exhibited high and significant variation between the accessions of *J curcas* from Burkina Faso. Such variation of seed traits and accessions resistance to fungal pathogens could been explained by genetic factors. This hypothesis is confirmed by the parameters which showed a strong heritability of the studied characters. In addition, the study revealed positive correlations between resistance parameters and seed oil content on the one hand and between these parameters and germination capacity on the other hand.

There are good opportunities to improve the Jatropha accessions resistance to pathogens, seeds oil content and germination capacity. However, the high phenotypic variability observed in seed traits and resistance of accessions contrasts with a low level of genetic diversity of accessions This could be explained by the fact that the markers used in the study could not reveal genetic diversity. This study constitutes a contribution to the identification of the best genotypes for improvement of seeds traits and accessions resistance to fungi in a breeding program.

Conflict of Interest

The authors declare that they have no competing interest.

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