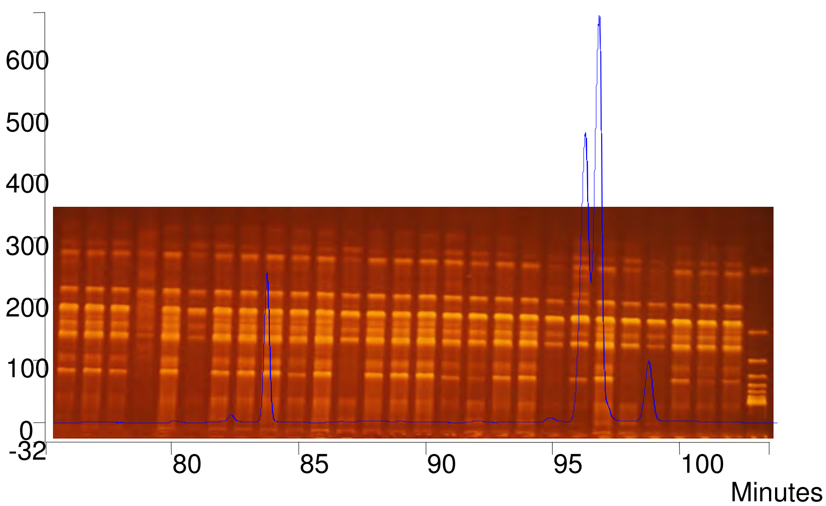
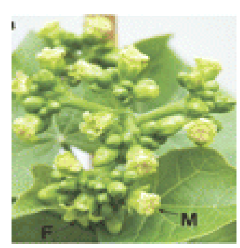
Addis Ababa University
School of Graduate Studies
Faculty of Science

Phenotypic and Molecular Characterization of Physic Nut (*Jatropha curcas* L.) Populations in Ethiopia





By Dereje Hailu

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ACRONYMS

bp –Base Pairs

ISSR – Inter Simple Sequence Repeat

PCR – Polymerase Chain Reaction

DNA – Deoxyribonucleic Acid

A – Adenine

G – Guanine

C – Cytosine

T – Thymine

rDNA – Ribosomal DNA

cpDNA – Chloroplast DNA

AMOVA – Analysis of Molecular Variance

CV – Coefficient of Variance

R^2 – Coefficient of Determination

GC – Gas Chromatography

Abstract

Genetic diversity and fatty methyl esters profile of *Jatropha curcas* represented by fourteen populations (eleven from Ethiopia, two from Kenya and one from Tanzania) were characterized. Twelve different phenotypic traits and five ISSR primers were used to characterize the genetic diversity whereas Gas Chromatography fingerprints were used to describe the fatty methyl ester profiles. A total of 140 individuals (10 individuals per population) were planted using randomized complete block design in three replications at Shewa Robit. Significant variation among the regions and among the populations at the age of 43 weeks were observed for height, leaf length, leaf width, average leaf area, leaf dry matter, length of leaf from width to base and apex . On the other hand, root collar diameter, branch numbers per plant and grain yield were not significantly differed both among regions and populations. Seeds collected from different populations varied significantly with respect to 1000-seeds weight, kernel oil content and seed oil content. The Chiro population performed the best with respect to root collar diameter, height, branch number per plant and first year grain yield (304.19 kg per hectare). The first year grain yield of Mersa, Jeweha, and Assossa population were 247.73, 227.45, and 226.89 kilo gram per hectare, regarding to 1000-seeds weight (540.44 g) and seed oil content (37.4 %). Shewa Robit population was the second superior genotype regarding seed oil content but the grain yield of this population was below the average. When oil yield productivity per hectare is considered, Chiro, Assossa, Mersa and Jeweha populations performed better than others. Growth traits were positively correlated with leaf and agronomic traits except plant height versus average leaf dry matter. Grain yield showed positive correlation with growth traits whilst negative correlations were observed with leaf and agronomic traits. Seed oil content was associated negatively with growth and leaf traits whereas significant positive correlation was observed with agronomic traits. Amplification of genomic DNA using five ISSR primers produced 71 DNA fragments and all fragments were found to be polymorphic. Number of amplified fragments varied from nine for primer (818) to seventeen for primer (873) with an average of 14.2 fragments per primer. Percent of polymorphism within population ranged from 21.43 (Kenya II) to 80.39 (Arbaminch). The Shannon diversity index varied from 0.243 (Kenya II) to 0.759 (Arusha / Tanzania). The Nei's gene diversity (h*), Shannon information index (I*), total gene diversity (Ht) and estimated gene flow were 0.273, 0.431. 0.272 and 0.785, respectively. Analysis of Shannon diversity index, Nei's genetic diversity and AMOVA revealed that most of the genetic diversity was due to variation within population but differentiation among population was also significant. Biodiesel from 14 populations of *Jatropha curcas* comprised six fatty methyl esters in common (16:0, 16:1, 18:0, 18:1, 18:2, and 18:3). Saturated fatty methyl ester varied from 19.04 % (Mersa) to 28.91%

(Arbaminch) with an average of 22.69%. The unsaturated fatty methyl ester ranged from 71.09% (Arbaminch) to 80.96% (Mersa) with an average of 77.33%. Biodiesel from Mersa and Assossa populations exhibited higher monosaturated fatty methyl ester. *Jatropha curcas* populations of this study exhibited higher genetic diversity (particularly, at DNA level) than most of previous studies. Some of these populations produced satisfactory grain yield. Moreover, seed oil content and fatty acid profiles are comparable with most former studies.

Key words: *Jatropha curcas*, genetic diversity, phenotypic traits, fatty acid, biodiesel

1. INTRODUCTION

Fossil fuels (oil, coal and natural gas) are the largest world wide primary energy (energy for transportation and electricity) sources pursued by renewable energy sources (biomass, wind, solar, water, and nuclear energy). Sheehan *et al.* (2001) indicated that about 64% of the global electricity power was produced from fossil fuels in 2000. Nowadays, the demand for these energies is increasing. Hoggwijk (2004) projected the world primary energy needs increase as 55% for years between 2005 and 2030 with an average rate of 1.8% per year. He further indicated that developing countries contribute 74 % of this augment. In spite of this need increase, the resource of fossil fuels is getting exhausted and will result in price augmentation and consequently in abrupt global economic crisis (Heinberg, 2003). Therefore, searching for alternative energies that substitute and/ or extend appropriate use of fossil energy sources is important.

The combustion of fossil fuels release greenhouse gases. This brings about change of atmospheric chemistry and results in global climate change (Lundahl, 1995; Junferng and Kirk, 2007). The energy sector alone has accounted for more than half of the anthropogenic climate changing through the enhancement of greenhouse gas effects (Anonymous, 1990). This makes the energy sector the largest single source of greenhouse gasses. Thus, to reverse anticipated consequence of climate change (enormous loss of economic and natural capital), it requires a transition in energy supply from greenhouse gasses emitting (fossil fuels) with serious promotion of renewable eco-friendly energy sources.

Bioenergy (biodiesel, ethanol, methanol and biogas) is an alternative to petroleum-based diesel fuel (Mittelbac and Remschmidt, 2004; Konthe, 2005). Biodiesel (fatty methyl and/ or ethyl ester) is a renewable liquid fuel that is derived from plant oil and animals fat. It performs very similar to low sulfur petroleum-based diesel in terms of power, torque and fuel efficiency (Tickell, 2000). Relative to petrol-diesel emission, biodiesel emission contains less particulate matter, carbon monoxide and polycyclic aromatic hydrocarbons (McDonald and Spears, 1997; Sharp *et al.,* 2000; Graboski *et al.,* 2003) and consequently, desirable to mitigate climate change. Sheehan (1998) indicated that biodiesel can reduce emission of carbon monoxide by 50% and carbon dioxide by 78% on a net life cycle base. It also can reduce particulate emissions by around 50% when weighted against fossil source diesel (Beer *et al.,* 2004).

Several oil crops have been evaluated for their biodiesel potential. Some of these plants are sunflower (Schlautman *et al.,* 1986), soybean (Yong, 1998), cotton seed (Alhasan *et al.,* 2005), rapeseed (McDnnel *et al*, 2006), and *Jatropha curcas* (Gubitz *et al.*, 1999). Based on ecological, economical and social features *Jatropha curcas* has been identified as the most suitable oil bearing plant for biodiesel (Gubitz *et al.*, 1999; Achten *et al.*, 2007). It can grow in marginal and waste lands with no possibility of land use competition with food production and give reasonable yields (Shekhawat *et al.,* 2007). *Jatropha curcas* oil is very suitable for trans-esterification to convert it into biodiesel (Azam *et al.,* 2005). The cetane number (measure of ignition quality) of *Jatropha curcas* oil is better than rapeseed and ground nut (Vaitlingom and Liennard, 1997; cited in Knothe, 2008). After planting, *Jatropha curcas* gives fruit in a year and give seeds for 50 consecutive years (Shekhawat *et al.,* 2007).

Jatropha curcas was debated on as species of narrow genetic diversity (Ram *et al.,* 2007; Pamidiamarri *et al.,* 2008; Basha and Sugatha, 2009). However, significance difference among populations, provenances and accessions were reported for its productivity (yield per area and seed oil content (Ginwal *et al.,* 2004; Rao *et al.,* 2008). Despite numerous potential of *Jatropha curcas* biodiesel production in Ethiopia, information is lacking for *Jatropha curcas* populations growing in Ethiopia particularly, regarding to their productivity, genetic diversity and oil fatty acid profiles. Such basic information is needed to design appropriate breeding program and to select superior genotypes to use as seed source. Hence, the present study was conducted with the following objectives.

Objectives of the Study

General Objectives

The general objective of the study is to generate basic information on genetic diversity of *Jatropha curcas* populations growing in Ethiopia and to describe fatty methyl esters profiles of biodiesel of these populations.

Specific Objectives

1. To characterize the populations of *Jatropha curcas* using quantitative traits

2. To evaluate the genetic diversity of *Jatropha curcas* populations using ISSR marker

3. To describe fatty methyl esters profile of biodiesel of from *Jatropha curcas* populations collected from Ethiopia

2. LITERATURE REVIEW

2.1 Scientific Classification and Nomenclature

According to Dehgan and Webster (1979), the taxonomic hierarchy of physic nut (*Jatropha curcas* L.) is:

Kingdom	*Plantae*
Phylum	*Magnoliophata*
Class	*Magnoliopsida*
Order	*Malpighiales*
Family	*Euphorbiaceae*
Genus	*Jatropha*
Species	*curcas*

2.1.1 The Family Euphorbiaceae

The euphorbiaceae family is mostly monocieous herbs, shrubs, trees and sometimes succulent and cactus–like. It is the sixth largest families of plant kingdom with about 300 genera and 7,500 species (Watson and Dallwitz, 1992). The family occurs mainly in tropics, with the majority of the species in the Indo-Malayan region and tropical America. There is also a large variety of euphorbiaceae in tropical Africa. However, euphorbia has also many species in non tropical areas such as the Mediterranean region, the Middle East, Southern United State of America and South Africa.

Cytogenetic study of 36 species that belong to 18 genera of euphorbiaceae in Thailand revealed the existence of diversity in chromosome numbers between and within genera (Soontorn and Chaiyasut, 1999). The family is believed to be polyphyletic having at least two base number (Hans, 1973) X = 7 and X = 13. The other base numbers encountered in the family are X = 6, 8, 9, 10, 11, 12, 15, 16, 17 and 18 showing the taxonomic complexity of the family (Hassal, 1976). As much as 48% of the known taxa exhibit polyploidy. Soontorn and Chaiyasut, (1999) indicated that most species had very small chromosomes ranging from 1.0 to 3.33µm. The family comprises both bi-nucleate (II) and tri-nucleate (III) pollen (Grady and Earlene, 1972). The most important economical crops of the family include cassava (*Manihot esculenta* Cratz.), rubber tree (*Hevea* *brasiliensis* Mull.Arg), caster bean (*Ricinus communis* Linn.) and *Jatropha curcas* Linn. (Mabberley, 1987).

2.1.2 The Genus *Jatropha*

The genus *Jatropha* is a morphologically diverse genus and belongs to tribe *Joannesieae* of *Crotonoideae* in the euphorbiaceae family and contains 170 known species (Dehgan, 1984). The genus has wide geographical distribution in seasonally dry tropics. The genus can be either monocieous or dioecious and are found in different forms such as woody trees, shrubs and sub shrubs. Dehgan and Webster (1979) revised the subdivision made by Pax by 1910 based on similarities and differences in growth morphological attributes of reproductive structures and distinguished two sub-genera (*Curcas* and *Jatropha*) of the genus. The sub-genus *Jatropha* includes all African (except for two species), Indian (except for one species), South American and two relic North American species. The sub-genus *Curcas* includes all of the Mexican, one Costa Rican, two African, and one Indian species. From their result these authors postulated that Physic nut (*Jatropha curcas* L.) to be the most primitive form of the genus *Jatropha*. Dehgan and Schutzman (1994) analyzed hierarchical cluster of 77 New World *Jatropha* species and the result indicated that most cladogram show concordance with Dehgan and Webster (1979).

Dehgan (1984) studied the evolutionary specialization of the genus *Jatropha* and postulated that evolution was proceeded toward specialization in vegetative structure, culminating in a facultative annual growth habit in sub-group *Jatropha* and in rhizomatous shrub habit concomitant with polyploidy (2n = 4x = 44) in Mozinna (sub-group *Curcas*).These changes were associated with reduction in reproductive structures in both subgenera. The evolutionary trends of the monotelic inflorescence (Toll, 1964; cited in Dehgan and Webster 1979) showed formation of highly symmetrical compound, dichasium in subgroup *Jatropha* which resulted from primary branching of the main-florescence, and was reduced to a single pistilate flowers. In subgroup *Curcas* however, inflorescences were drastically reduced to a few (or solitary) terminal or lateral flowers together with a gradual change from monoecy to dioecy.

2.1.3 Nomenclature of *Jatropha curcas*

The genus name derived from two Greek words '**Jatros**' (doctor) and '**trophe**' (food) which is to mean medicinal plant. When the botanist Carl Von-Linne (1753) first classified the plants he gave it the botanical name "*Jatropha curcas"*. Thus, Linne had realized the potential of this plant for medicinal purposes. Because of its wide range of distribution and multipurpose uses there are more than 200 different names given for this species (Dehgan and Schutzman 1994). Some of the common names include: Physic nut and Purging nut (English), Purgreen noot (Dutch), Purgueira (Portuguese), Pinoncillo (Mexico), Ayderke (Amharic), Abatamuluk (Oromifa) and Shanshmuk Nesro (Gumiz). All these names designate either medicinal value or stress tolerance ability of the species.

2.2. Description of *Jatropha curcas* L.

2.2.1. Botanical Description

Puangpaka and Thaya (2003) conducted an experiment on the karyology of five *Jatropha* species including *J. curcas* in Thailand. Their finding indicated that *Jatropha curcas* has chromosome numbers of $2n = 2x = 22$ and a basic chromosome number of $x = 11$. The result of this study revealed that meiotic configuration of *Jatropha curcas* chromosome was found to be similar with *Jatropha multifida.* Carvalhoa *et al*. (2008) found that the same number of metacentric and sub-metacentric chromosomes in *Jatropha curcas* and base composition of 38.7% GC. Carvalhoa and his colleagues also indicated that the chromosomes are relatively small size (ranges from 1.24 to 1.7µm) which is in the same size range as that of rice. They also further indicated an average genome size (1C) value to be 416 Mbp which is smaller than that of other species of euphorbiaceae that were reported to vary between 1.3 and 28.6 pg (Arumuganathan and Earle, 1991; Bennett *et al.,* 2000). This is relatively small to plant genome (Zonneveld *et al.,* 2005) and could make *Jatropha curcas* an attractive candidate for genome sequencing.

Jatropha curcas L. is a small tree or a large shrub which can achieve a height of 5 meter and rarely can attain a height of 10 meter under favorable conditions. The plant show articulated growth, with a morphological discontinuity at each increment. It is drought resistant species and known as an arid and a semi-arid plant species (Heller, 1992). *Jatropha curcas* is a deciduous plant and the stem has smooth gray bark which exudes white watery latex during injury. *Jatropha* leaves have narrow lobes and are arranged on stem and branches in an alternate manner. The length and width of the leaves highly vary and morphologically it is similar to *Ricinus communis* L. leaves. It grows mainly primary and secondary branches which are arranged alternately.

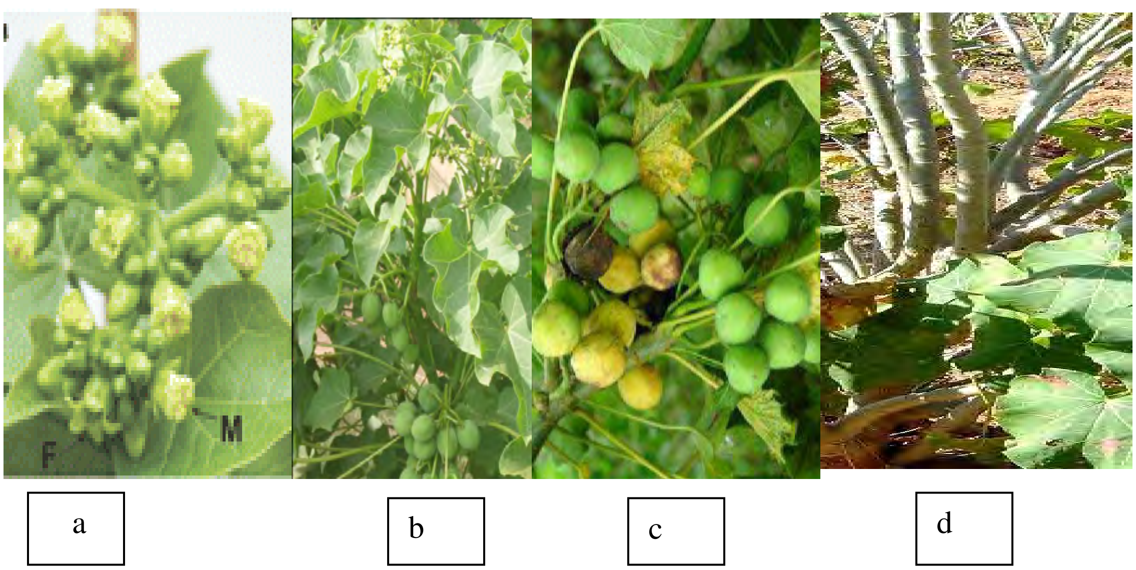


Fig.1 Above ground part of the *Jatropha curcas* (a) Inflorescences (b) Leaf shape and arrangement (c) fruits (d) stem and branches

Jatropha curcas is monoecious with unisexual flowers formed at the terminal of branches and leading stem (Deng, *et al.,* 2008). Dehgan and Webster (1979) reported occasional existence of hermaphrodite plant with incomplete growth of pistil. The inflorescences are complex and possess main and co-florescence with paracladia and botanically described as a cyme type. Deng and his associates designated that the male flowers are numerous (80- 90%) and occupies the subordinate position of the inflorescences. Ten stamens are arranged in two distinct whorls of five each in a single column in the androecium's, and in close proximity to each other. In the gynoecium's, three slender styles are connate to about two-thirds of their length, dilating to massive bifurcate stigmata (Heller, 1992). In the female flowers, the ovary bulges and the stamens degenerate, whereas in the male flowers, the stamens grow normally but the ovary is absent (Deng *et al.,* 2008)

Pollination of the *Jatropha curcas* is by insects. However, the hermaphrodite flowers can be self pollinating. Heller (1992) observed that staminate flowers open later than pistilate flowers in the same inflorescence. However, Munch (1986) did not observe this chronological order in Cape Verde. It seems that such mechanism is influenced by environment. Therefore, both genotype and environment can affect pollinating mechanism. The flower inflorescence yields a bunch of ovoid fruits which mature into yellowish color fruits ready for harvesting. The blackish thin-shelled seeds are 2cm long and 1cm thick, oblong in shape and resemble small castor seeds (Rao *et al*., 2008).

Jatropha curcas can be grown both from seeds and cuttings. It grows five roots when it is propagated by seeds, one central and four peripheral. A tap root is not usually formed by vegetatively propagated plants (Knoblike, 1989) and such plantations have lower production period. Recently tissue culture propagation is used especially in multiplication of high oil yielding genotypes. *Jatropha curcas* starts producing seeds within 12-18 months but reaches its maximum productivity level after 4 to 5 years (Henning, 1996). At maturity Becker and Makkar (2000) reported 2- 3 tons of seeds/ha in semi-arid areas, although yields of 4 to 5 tons/ha are routinely achievable under more favorable (wetter) conditions (Matsuno *et al.,* 1984; Foidl *et al.,* 1996). With oil content of approximately 35% (Henning, 1996), this equates to an average yield of approximately 1.75 tons of oil per hectare. Recently, the high yield of seed from the tree (5 tons /ha/year) and the high oil content of its seeds through genetic modification attracted global attention for the development of *Jatropha curcas* as a source for bio-fuel (Openshaw, 2000; Chen *et al.,* 2006; Li *et al.,* 2008).

2.2.2 Origin and Distributions of *Jatropha curcas* L.

A number of researchers have attempted to define the origin of *Jatropha curcas,* but the source remains controversial. Martin and Mayeux (1984) proposed Brazil (Ceara State) as center of origin but with out giving any arguments. However, other scholars indicated Mexico and Central America as center of origin to physic nut (Wilbur, 1954; Aponte, 1978). This was supported by the information provided by many collectors on the herbarium labels of many herbarium specimens collected from Mexico, Central America and Caribbean regions. Information of herbarium specimens of many collectors in Mexico and Central America indicated that they were from natural forest whereas no specimens or data indicating natural stand in Asia and Africa. *Jatropha curcas* was spread by Portuguese traders as a valuable hedge plant via the Cape Verde Island and Guinea Bissau (Henning, 2008) to countries in Africa, Asia, and India (Jepsen *et al.,* 2006). In Ethiopia it is grown as hedge plant in the low land part of the country. Although it had been introduced recently in all visited area during my seed collection (personal communication), it is found in Southern, Northern, Western, Eastern and Central low lands of the country.

2.2.3 Ecological Description of *Jatropha curcas* L.

Jatropha curcas is a succulent that sheds its leaves during the dry season and therefore, best adapted to arid and semi-arid conditions. The areas where it had been collected in the center of origin and from where the material, were collected for provenance trials show average annual temperatures well above 20 ^{0}C and up to 28 ^{0}C (Foidl *et al.,* 1996). In Ethiopia it is grown up to 1650 meter above sea level (Personal observation) and reported to grow up to 1700 meter above sea level at the Fogo (Kiefer, 1986). In Ethiopia, almost at all visited areas during seed collection *Jatropha curcas* is grown around the home garden and farm lands to use for life fence. These growing areas are fertile and can be used to grow food crops and fruit trees. Further expansion of *Jatropha curcas* at its present growing areas compete land for food crop production and livestock grazing. It grows on well -drained soils with good aeration whereas on vertisol it is affected by root-rot and cracking during rain and dry season respectively (Wang *et al.*, 2007). It is well adapted to marginal soils with low nutrient content and can also grow on gentle slop but not economically profitable to plant on slope that exceed 30% (Wang *et al.,* 2007). Dehgan and Schutzman, (1994) reported that *Jatropha curcas* occurs in dry areas whereas lacking from the moist Amazon region. The current distribution of physic nut show that introduction has been most successful in drier region of tropics with an average annual rainfall of 300 mm and 1000 mm. Munch (1986) reported that physic nut even withstand years with out rainfall in Cape Verde. The average annual rainfall required for optimal yield is between 800 mm and 1200 mm (Wang *et al.,* 2007). Based on experiences elsewhere, for reasonable fruit production the minimum required annual rainfall for *Jatropha curcas* is around 600 mm (Benge, 2006).

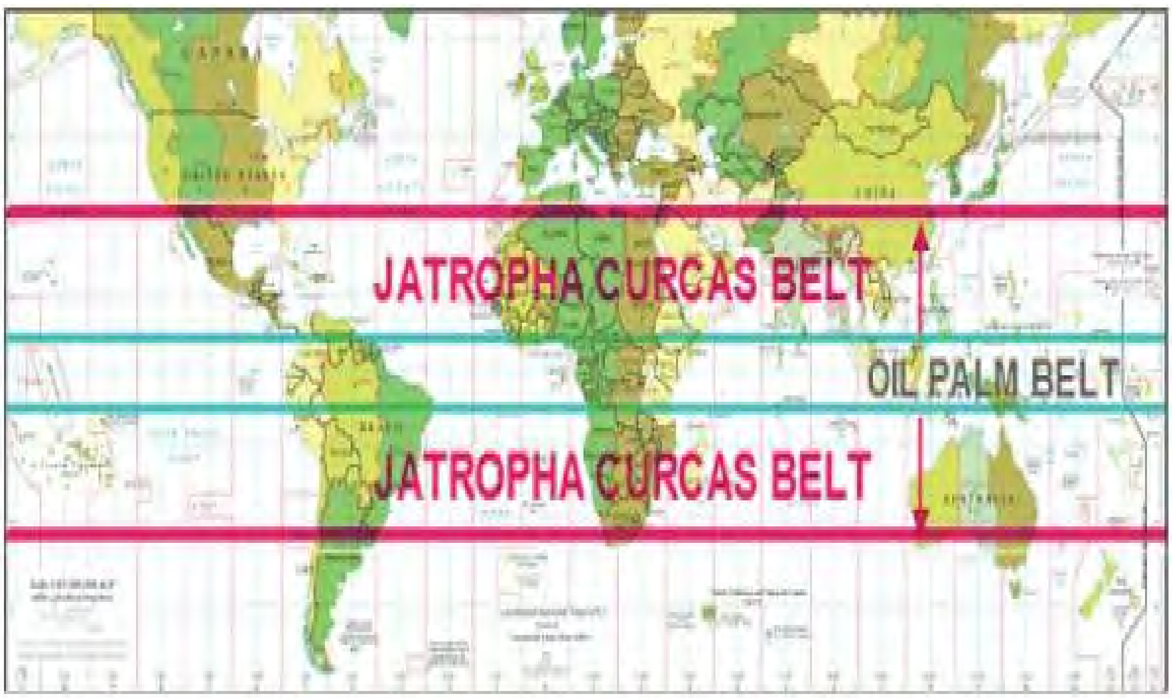


Figure 2 - Indication of the most suitable climate conditions for the growth of *Jatropha curcas* L (30°N, 35 °S) and Oil palm (*Elaeis guinensis* Jacq. (4°N, 8 °S).
Source: adopted from Wang *et al.*, 2007

2.3 Toxicity of *Jatropha curcas* L.

Various biologically active substances have been isolated and characterized from different parts of *Jatropha curcas.* Some of these substances have medicinal value while others are anti-nutrients. Major anti-nutrient substances identified from *Jatropha curcas* seed include, phorbol esters (Gubitza *et al.,* 1999), trypsin inhibitors (curcin), phytates, saponins and lectins (Martinez-Herreraa *et al.,* 2006).

Phorbol esters (Phorbol-12-myrstate 13-acetate) have been identified as the major toxic component in *Jatropha curcas* (Makkar *et al.,* 1997; Makkar and Becker, 1998). Hirota *et al.* (1998) also isolated from *Jatropha* seed a new type of phorbol ester, which has a macrocyclic dicarboxylic acid diester structure. They are bioactive diterpene derivatives that have a multitude effects in cells (Goel *et al.,* 2007). Diets containing *Jatropha* with 1.5 to 2mg/g of phorbol esters have been found to cause suppression of feeding, lesions on the skin, weight loss and death in both fish and rat (Goel *et al.,* 2007). The threshold level at which phorbol esters caused adverse effects was 15 ppm (15µg per gram) in the diet where by a level higher than of 31 µg per gram of extract in the diet resulted in lower average metabolic rate, increase fecal mucus production and rejection of feed (Becker and Makkar, 1998).

A toxic protein was isolated from *Jatropha curcas* seed designated as curcin and it is ribosome inactivating protein (Juan *et al.,* 2003). Juan and his colleagues reported that curcin has antitumor activity and this is related to N-glycosidase action, which cleaves the N-glycosidic bond of adenine A_{4234} of 28s rRNA. This makes ribosome unable to bind the elongation factor 1 or 2, consequently arresting protein synthesis (Endo *et al.,* 1987).

There is an edible genotype of *Jatropha curcas* that exclusively grown in Mexico (Becker and Makkar, 1998). Makkar *et al.* (1997) did an experiment on antinutrient constitutes of 18 different provenances of *Jatropha curcas* from West and East African, North and Central American and Asian countries. The major identified antinutrients in toxic and non toxic varieties are summarized in Table 1.

Table 1. Important antinutrientsin seed meal of edible and non-edible *Jatropha curcas* varieties

Important Antinutrient	Toxic Variety	Edible Variety
Phorbol ester (mg/g kernel)	2.79	0-0.11
Total phenols (% tannic acid equivalent)	0.36	0.22
Tannins (% of tannic acid equivalent)	0.04	0.02
Phytates (% in dry matter)	9.40	8.90
Saponins (% diosgenin equivalent)	2.60	3.40
Trypsin inhibitor (mg trypsin inhibited per gram sample)	21.3	26.5
Lectins (ml/g of meal that produced haemaggutination per ml of assay media	102	051

Sources: Makkar *et al.*, 1997, Makkar and Becker, 1998

According to Makkar and Becker (1998), *Jatropha curcas* seed meal treatment by heat reduce significantly the amount of lectins and trypsin inhibitors. The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the phorbol ester in Jatropha to 75% (Hass and Mittelbach, 2000).

2.4 Use of *Jatropha curcas* L.

Jatropha curcas is a drought resistant multipurpose perennial plant. The plant consists of the stem (woody part), the leave, the root and other reproductive organs that have one or more use. The seed of *Jatropha curcas* is the most useful part of the plant. From the seed oil, a number of individual fatty acids, or combinations of fatty acids, could be used as key intermediates in the manufacturing of different products (Pratt *et al.* 2002). These includes,

i. Fatty chlorides used in the making of detergents and soap
ii. Fatty alkanolamides for laundry softeners
iii. Fatty esters for cosmetics, pharmaceuticals and candles
iv. Methyl stearates used for lubricating oils and greases

v. Fatty amines for metal working, paints and varnishes

vi. Quaternary ammonium chlorides for plastics and printing inks

vii. Methyl esters are used in the formulation of detergents, cosmetics and lubricants

In addition to these products different part of the plant have one or more uses. The plant can serve as living fence (hedge), green manure, fertilizer (oil cake), source of energy, medicine and source of important genes of interest traits.

2.4.1 Living Fence (Hedge)

Jatropha is an excellent hedging plant generally grown in most part of world as live fence for protection of agricultural fields against damage by livestock as unpalatable to most domestic animals. It also serves as wind break both at homestead and agricultural field. The use of *Jatropha* as biofence has been reported as cost effective and lifelong compared to wire fence (Kumar and Sharma, 2008).

2.4.2 Green Manure and Fertilizer

Cultivation of *Jatropha curcas* resulted in 11% average increase in mean weight diameter of the soil and 2% increase in soil macro-aggregate turnover (Chaudhary *et al.,* 2008) and regression analysis showed a significant correlation between organic carbon and mean weight diameter. Such soil structure recovery under cultivation of *Jatropha curcas* implies a sustainable improvement in the surface integrity of these soils, which will ensure more water infiltration rather than runoff and erosion. Such improvement of soil structure increase activity of microorganisms that improve soil synthesis. Furthermore, in a green manure trial with rice in Nepal, the application of 10 tones of fresh physic nut biomass resulted in increase grain yield (Sherchan *et al.,* 1989). *Jatropha* seed cake which is a by product of oil extraction, is a potential as fertilizer and biogas production (Staubmann *et al.,* 1997; Gubitz *et al.,* 1999). The seed cake is rich in protein, phosphorus and calcium compared to Neem oil cake and cow manure and it is an excellent source of plant nutrients (Kumar and Sharma, 2008). Phorbol esters in the seed cake can kill harmful insects to the target crops (Makkar and Becker, 1998)

2.4.3 Energy Source

Different parts of *Jatropha curcas* can be used as source of energy. Common energy forms that can be acquired from different part of *Jatropha curcas* include, biomass energy (log and charcoal), biogas (hydrocarbons of fruit and seed shells) and biodiesel (oil and methyl ester of the oil). Of all these fuel energy generated from *Jatropha,* energy from the seed is paramount and has attracted global attention. The seeds of *Jatropha curcas* contain significant amount of oil and hydrocarbon (Augustus *et al.,* 2002) to produce biodiesel (from the oil) and ethanol from hydrocarbon. According to Augustus and his colleagues the gross heat value for the *Jatropha curcas* seed with 10% moisture content was 20.85 MJ/kg and for the oil was 37.83 MJ/kg. They also reported the gross heat value of hydrocarbon would be about 40.63 MJ/kg. Martinez-Herreraa *et al. (*2005) reported that the gross energy of kernels range from 31.1 to 31.6 MJ/kg of dry matter. Utilization of these energies for different purpose mainly for transportation sector is growing up from time to time and research activities to add value toward this goal have been a focus of many nations and scientists.

Engine tests with *Jatropha curcas* oil were done in Thailand and demonstrated satisfactory engine performance (Takeda, 1982). *Jatropha* oil can be used as fuel diesel engines directly and/or by blending it with methanol (Gubitz *et al.,* 1999). The direct use of *Jatropha* oil for biodiesel require some modification of petroleum engine injector while esterified *Jatropha* oil can be used directly without any modification of petroleum engine injector (Takeda, 1982). Such modification can be attained via trans-esterification that modifies chemical and physical properties of the crude oil and bring these properties in the range and/or near the range of petroleum diesel.

2.4.4 Medicinal Use

Jatropha curcas is widespread in arid and semi-arid tropical region of the world and has been used as traditional flak medicine in many countries. It is a source of several secondary metabolites of medicinal significance. The leaf, fruits, latex and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that exhibit wide ranging medicinal properties that make it pharmaceutically important (Debnath and Bisen, 2008). According to these authors the plant product exhibit anti-bacterial and anti-fungal activities. Phorbol esters of *Jatropha curcas* have mollucidial and insectidal properties (Gubitta and Trabic, 1999). These authors also isolated anti-inflammatory substance and wound healing enzymes. Adebwale and Andedire (2006) evaluated *Jatropha* oil for anti- ovipositional activity and long term protective ability of treated cowpeas against the seed beetle. The result of this investigation indicated that *J. curcas* oil has anti-oviposition and offers a 12 - week protection for treated seeds since there was neither seed damage nor adult insects emerging from the treated cowpea seeds. The seed oil can be applied to treat eczema and skin disease and to soothe rheumatic pain (Heller, 1996).

2.4. 5 Bio-safety of *Jatropha curcas* Biofuel

With the shortage of fossil fuel and rising of global temperature, consumption and demand for energy is increasing in both developed and developing countries (Pimentel *et al,* 2008). Current biofuel production mainly uses the first generation feedstock as these materials are readily available. Corn, soybean, wheat, oil palm, rapeseed and sugar cane are the major biofuel feedstock currently used in biofuel production. These feedstocks are used widely for human consumption shares the same resources to produce food crops. This become a challenge to global food security and particularly, to those countries which are food insecure (Pimentel *et al,* 2008). On the other hand, productions of these feedstocks require fertile land and intensive agricultural inputs. This also competes reserve agricultural land and exerts pressure on forest and woodland resources. Consequently, this causes land use change which results in greenhouse gas emission more than fossil fuel.

Use of non-edible feedstocks (such as *Jatropha* oil) production of second generation biofuels can solve limitations of first generation biofuel. *Jatropha curcas* can grow on degraded land and gives desirable yield. The plant is resistant to many biotic (pest, disease) and abiotic (drought, high temperature, nutrient deficiency and salinity) environmental factors. The fungal association of the plant root, high response of the plant to its seed cake and high soil nutrient turnover from sheded leaves during dry season makes the plant best candidate of biofuel feed stock on degraded lands. Thus, if properly designed production of biofuel from *Jatropha curcas* is ecological, socially and economically feasible.

2. 5. Plant Genetic Resources and Tools for Assessment

Genetic variability is the actual differences among and within any biological taxon level. The taxon level can be individuals, populations, and species and even above species taxonomic groups. Genetic variation can be described as having three major components including (i) genetic diversity (refers to amount of genetic variability), (ii) genetic differentiation (refers to distribution of genetic variability within and among taxa groups) and (iii) genetic distance (refers to amount of genetic variation between pairs of taxa groups). High genetic diversity increases survival of a species under different catastrophes whereas low genetic diversity decreases the adaptability of in variable and changing environments (Hamric, 1994; Young *et al.,* 1996).

The analysis of diversity within and among these levels of biological organization involves the use of different genetic markers. A genetic marker can be defined as (a) a chromosomal landmark or allele that allows for the tracing of spacing region of DNA or a specific pieces of DNA with a known position on genome. King and Stansfield (1990) define it as a gene whose phenotypic expression is usually easily described, used to identify an individual or a cell that carries it or as a probe to mark a nucleus chromosomes or a locus. Nowadays, genetic markers are used in both basic plant research and plant breeding to characterize plant germplasm, for gene isolation, marker-assisted introgression of favorable alleles, production of improved varieties (Henry, 2001), and to obtain information about the genetic variation of populations.

Basically, there are two classes of traits to measure genetic variations. These are (a) traits controlled by many gene loci (polygenic traits) and (b) traits controlled at the single gene level (genetic markers). Therefore, to fully characterize level and pattern of genetic variation in a species, and its evolutionary causes, studies involving both class of traits are necessary. There are three classes of genetic markers. These are morphological characters, biochemical and molecular markers (Doveri *et al.*, 2008; Semagn *et al.*, 2006)

2.5.1 Morphological Markers

Morphological characters measure quantitative traits which are controlled by many gene loci. These characters are influenced by environmental factors and consequently special breeding programs and experimental design are needed to distinguish genetic variation from phenotypic variation. Morphological variability due to qualitative traits are mainly governed by single or a few genes mutation can actually be more pronounced than the extent variation by some markers (Levi *et al.,* 2001). Consequently, certain groups of plants (cultivars, subspecies, or species) appear to be considerably better defined through analysis of qualitative morphological traits rather than by DNA markers (Olsson *et al.,* 2000).

2.5.2. Biochemical Markers

The utility of the biochemical traits as gene markers in population genetic studies was first recognized by Lewontin and Hubby (1966) in their investigation of drosophila and by Harris (1966) in his survey of human population. In higher plants, isozymes were first used as gene markers in population surveys and breeding program by Allard and co-workers (Brown and Allard, 1969; Marshall and Allard, 1969). Since then it has been in the study of pollination biology (Simpson, 1982), verification of controlled crosses (Adams *et al.,* 1997), to measure pollen contamination in seed orchards (El-Kassaby and Davidson, 1990) estimation of mating systems (Yoursy and Ritland, 1998) measure of genetic variation, in biosystematics, gene conservation and to measure the effect of domestication (Adams, 1983).

2.5.3. Molecular Markers

With the advent of molecular markers, a new generation of markers has been introduced over the last three decades, which have revolutionized the entire scenario of biological sciences. It enabled detection of changes in DNA sequence. The major DNA markers evolved in the last three decades have been summarized in Table 2 below.

Table 2. Classes and chronological evolution of DNA markers

Class	Acronym	Nomenclature	Reference
Hybridization	**RLFP**	Restriction Fragment Length Polymorphism	Grodzicker *et al.,*1974
PCR-based	**VNTR**	Variable Number Tandem Repeat	Jeffreys *et al.,* 1985
PCR-based	**ASO**	Allele Specific Oligonucleotides	Saiki *et al.,* 1986
PCR-based	**OP**	Oligonucleotides Polymorphism	Beckman, 1988
PCR-based	**AS-PCR**	Allele Specific Polymerase Chain Reaction	Landegren *et al.,* 1988
PCR-based	**SSCP**	Single Stranded Conformational Polymorphism	Orita *et al.,* 1989
PCR-based	**STS**	Sequence Tagged Site	Olsen *et al.,* 1989
PCR-based	**STMS**	Sequence Tagged Microsatellite Site	Bechmann and Soller, 1990
PCR-based	**OFLP**	Oligo-Amplified Fragment Length Polymorphism	Lee *et al.,* 1990
PCR-based	**RAPD**	Randomly Amplified Polymorphic DNA	Williams *et al.,* 1990
PCR-based	**AP-PCR**	Arbitrarily Primed Polymerase Chain Reaction	Welsh and McClelland, 1990
PCR-based	**DAF**	DNA Amplification Fingerprinting	Caetano-Annolles *et al.,*1991
PCR-based	**RLGS**	Restriction Landmark Genome Scanning	Hatada *et al.,*1991
PCR-based	**SSR**	Simple Sequence Repeat	Akkaya *et al.,* 1992
PCR-based	**CAPS**	Cleaved Amplified Polymorphic	Akopyanz *et al.,* 1992

		Sequence	
PCR-based	**DOP-PCR**	Degenerate Oligonucleotide Primer-PCR	Telenius, 1992
PCR-based	**MAAP**	Multiple Arbitrary Amplicon Profiling	Caetano-Annolles *et al.,*1993
PCR-based	**SCAR**	Sequence Characterized Amplified Region	Paran and Michelmore, 1993
PCR-based/ Hybridization electrophoresis	**SNP**	Single Nucleotide Polymorphysim	Jordan and Humphries, 1994
PCR-based	**SAMPL**	Selective Amplification Of Microsatellite Polymorphic Loci	Morgante and Vogel, 1994
PCR-based	**ISSR**	Inter-Simple Sequence Repeat	Zietkiewicz *et al.,* 1994
PCR-based	**ASAP**	Allelic Specific Associated Primers	Gu *et al.,* 1995
PCR-based	**AFLP**	Amplified Fragment Length Polymorphisms	Vos *et al.,* 1995
PCR-based	**CFLP**	Cleaved Fragment Length Polymorphisms	Brow *et al.,* 1996
PCR-based	**ISTR**	Inverse Sequence-Tagged Repeat	Rohde, 1996
PCR-based	**DAMD-PCR**	Directed Amplification Of Minisatellite DNA-PCR	Bebeli *et al.,*1997
PCR-based	**S-SAP**	Sequence-Specific Amplified Polymorphism	Waugh *et al.,* 1997
PCR-hybridization	**RBIP**	Retrotransposon-Based Insertional Polymorphism	Flavell *et al.,* 1998
PCR-based	**IRAP**	Inter-Retrotransposon Amplified Polymorphism	Kalendar *et al.,* 1999
PCR-based	**REMAP**	Retrotransposon-Microsatellite Amplified Polymorphism	Kalendar *et al.,* 1999
PCR-based	**TE-**	Three Endonuclease Aflp	Vander Wurff *et al* 2000

	AFLP		
PCR-based	**IMP**	Inter-Mite Polymorphism	Change *et al.,* 2001
PCR-based	**SRAP**	Sequence-Related Amplified Polymorphism	Li and Quiros, 2001
PCR-based	**TRAP**	Target Region Amplification Polymorphism	Hu and Vick, 2003
PCR-based	**TBP**	Tubulin-Based Polymorphism	Bardini *et al.,* 2004

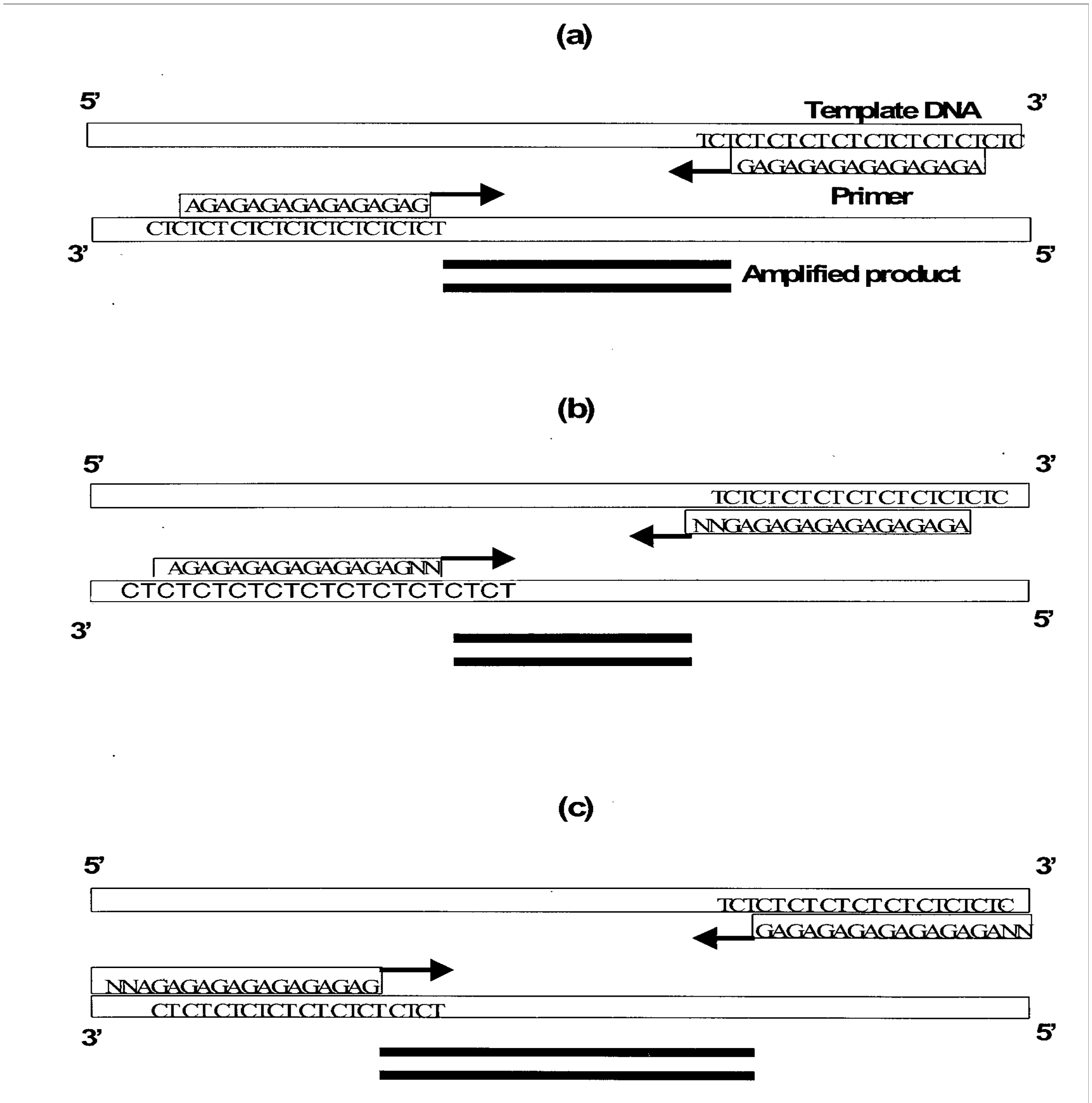
Source: adopted from Doveri *et al.* (2008)

Based on their genomic abundance, level of polymorphism, and detection of all their alleles' microsatellite, ALFP and ISSR are preferred.

2.5.4. Inter Simple Sequence Repeats (ISSR)

Inter simple sequence repeats (ISSR) are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. The technique uses microsatellites as primer in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes. The microsatellite repeats used as primer for ISSRs can be di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide. The primers used can be either unanchored (Meyer *et al.,* 1993) or more usually anchored at 3'or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.,* 1994).

The main advantage of ISSR is that no prior sequence data for primer construction are needed. Because the analytical procedures include PCR, only low quantities of template DNA are required (5-50 ng per reaction). Furthermore, ISSR are randomly distributed throughout the genome. Like RAPDs, dominant inheritance, and homology of co-migrating amplification products are the main limitations of ISSRs (Semagn *et al.,* 2006). However, Fang and Roose (1997) reported a reproducibility level of more than 99 % after performing remediable test for ISSR markers by using DNA samples of the same cultivar growing in different locations, DNA extracted from different edge leaves of the same individual and by performing separated PCR runs. Because of the multi-locus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone strain identification and taxonomic studies of closely related species. In addition, ISSR are considered useful in gene mapping studies (Zietkiewicz *et al.,* 1994; Gupta *et al.,* 1994; Godwin *et al.,* 1997).



Adopted from: Reddy et al., 2002

Fig.3 ISSR-PCR: A schematic representation of a single primer (AG)8, unanchored (a), 3'-anchored (b) and 5'-anchored (c) targeting a (TC)*n* repeat used to amplify inter simple sequence repeat region flanked by two inversely oriented (TC)*n* sequences. (a) Unanchored (AG)*n* primer can anneal anywhere in the (TC)*n* repeat region on the template DNA leading to slippage and ultimately smear formation (b) (AG)*n* primer anchored with 2 nucleotides (NN) at the 3' end anneals at specific regions on the template DNA and produces clear bands (c) (AG)*n* primer anchored with 2 nucleotides

Table 3. Levels of discrimination provided by the major molecular marker techniques currently used

Method	Suitable for Analyses		
	Above Genus Level	Between Species	Within Species
Allozymes	-	+	+
PCR-based DNA techniques			
- Sequencing nuclear DNA Genes (e.g., rDNA)	++	+/-	-
- Noncoding DNA (e.g., STS)	+/-	++	+
Multilocus nuclear DNA markers			
- AFLP	-	+	++
- ISSR	-	+	++
- RAPD	-	+	+
- SRAP	-	+	+
Single-locus nuclear DNA markers			
- Microsatellite	-	-/+	++
- SCARs	-	-/+	++
- SNPs	+	+	+
Chloroplast markers			
- cpDNA-CAPS	+	++	+
- cpDNA gene sequences	++	+	-
- cpDNA noncoding DNA sequences	+/-	++	+
- cpDNA microsatellites	-	+/-	++
Hybridization-based DNA techniques			
- Mini- and microsatellite probes	-	+/-	++
- RFLP probes	+/-	++	++
- RAMPO	-	+	+

Rating: ++, highly useful; +, useful; +/– useful in some cases; –, generally not useful

Source: Adopted from Weissig *et al.,* 2005

3. MATERIALS AND METHODS

3.1 Sampling of *Jatropha curcas* Populations and Planting Materials

Primary information were collected with respect to *Jatropha curcas* distribution in Ethiopia and eleven populations were selected for the experiment by means of random stratified sampling approach. Additional three populations (two from Kenya, one from Tanzania) were included in the study. For selected *Jatropha curcas* populations from Ethiopia reconnaissance surveys were made on the population status and 10 individuals were identified by random stratified sampling approach approximately at the distance of 100 meter to 1 kilometer (in case of Sawula population) from each others depending on the area coverage topography of the populations and presence of *Jatropha.* From each, identified individual seeds were collected from 50 capsules in separate plastic bag and labeled. For *Jatropha* population from abroad ¼ kilo gram of seeds were obtained from Green Biofuel Company from their Adama field site.

Table 4. Altitude and respective region of sampled *Jatropha* populations

No	Population	Altitude (m) asl	Region Code	Geographic location
1	Babile	1430	2	East Hararge
2	Chiro	1650	2	West Hararge
3	Doni	1520	2	Arsi
4	Sawula	1250-1320	3	South Ethiopia
5	Arbaminch	1200	3	South Ethiopia
6	Assossa	1680	4	West Ethiopia
7	Pawe	1150	4	North West Ethiopia
8	Metema	1000	4	North Ethiopia
9	Shewa Robit	1250	5	North Shewa
10	Jeweha	1150	5	North Shewa
11	Mersa	1550	5	South Wollo
12	Kenya I	------	1	Kenya
13	Kenya II	------	1	Kenya
14	Arusha	------	1	Tanzania

N.B: East Ethiopia (region code 2), South Ethiopia (region code 3), North and North West Ethiopia (region code 4), Central Ethiopia (region code 5) and other east African countries (region code 1).

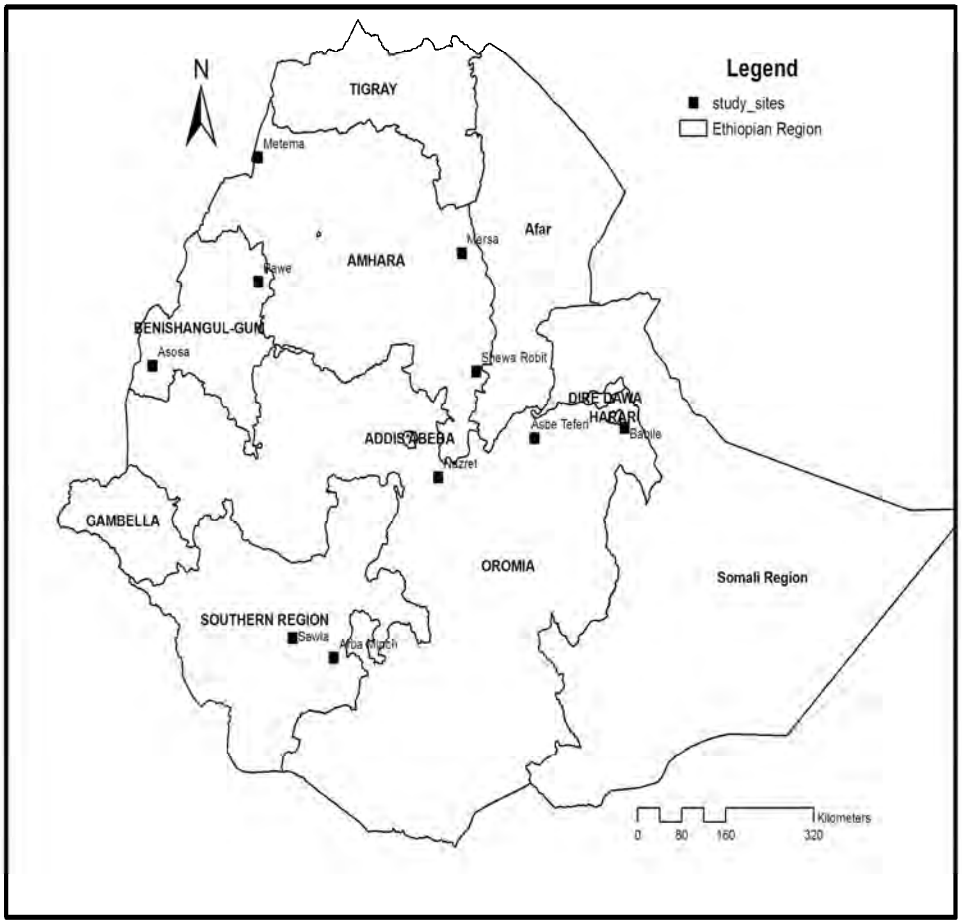


Fig. 4 Geographical location *Jatropha curcas* population of the study from Ethiopia and field experiment site

Note. Jeweha is situated at 15 kilo meter north of Shewa Robit near to the main road from Addis Ababa to Dessie and Mekele; Asebe Tefer is the former name of Chiro

3.2 **Description of Experimental Site**

The on-station research experiment was conducted at Shewa Robit, which is located in the Rift Valley. It is situated at about 225 kilo meter north east of Addis Ababa near the main road from Addis Ababa to Dessie and Mekele. Because it is the highland bordering of the Rift Valley, the study area is characterized by deep, moderately weathered dark reddish brown soil of clay loams which is Rift Valley volcanic soil. The physiochemical analysis of soil of the experimental site indicates pH 7.58, organic carbon 1.38%, available phosphorus 21.54 ppm, total nitrogen 0.13% and carbon nitrogen ratio 11. The climate of the experimental site is characterized by semi-arid eco-climatic zone. The rain fall in the area is mainly mono-modal in the period from July to August and some times small rain shower can exist between March and April. The average annual rain fall of the area is 760.20mm whereas the temperature varies from 15 to 30 ^{0}c.

3.3 Experimental Design

A total of six seeds representing each individual were randomly picked and planted in randomized complete block design in three replications. Twenty-five seeds were planted in each plot in five rows (5m X 5m) at one meter distance both within and between rows. Two meter and one and half meter spacing were used between blocks and plots respectively. Individuals were planted systematically in each plot.

3.4 Data Collection

Different systems were applied to generate different data from representative individuals. Data of growth traits such as root collar diameter, and total plant height were collected from all 10 representative individuals after every 30 days while number of branches for these 10 representatives' individuals was collected at the age of 11th months of the experiment. During this period five fully matured leaves were collected from six randomly selected representatives of each plot. For each leaf, lamina length, leaf width at the widest point, lamina length from the widest width, and lamina length from base to widest width were measured. Average dry matter was calculated after drying the leaves for 24 hours at 130 ^{0}c in oven dry. Finally, leaf surface area were estimated by equation A = (0.84 lamina length X leaf width at the widest pint)$^{0.99}$ which was developed by Severino *et al.,* (2007) for *Jatropha curcas* leaf area estimation.

3.5 Leaf Sampling for DNA Analysis, DNA Extraction and Test Gel

From the second replication of the experiment, six individuals of each population were selected randomly. A young leaf was collected from each selected individuals and dried by silica gel in plastic bag separately. From each collected leaf a sample of 100mg sample was taken and genomic DNA was extracted based on a modified protocol developed by Borsch *et al.* (2003).

Agaros gels with 0.98 % for test gel were prepared and 2µl genomic DNA of each sample was loaded with 6µl loading dye. Then the loaded gels were electrophoresed at a constant voltage of 100V for 1 ½ hour. The electrophoresed gels were stained in distilled water containing ethidium bromide for 30 minutes and distained in distilled water for the same period. The pictures of the gels were taken by digital camera adjusted with BioDocAnalyze. Finally, from two duplicate extractions of each sample those with high band intensity were selected for PCR.

3.6 Primer Selection and Optimization

A total of 25 ISSR primers were obtained from University of British Colombia and were tested based on their background information provided by Gupta *et al.,* (2008) and Wie *et al.,* (2007) on *Jatropha curcas.* After caring out several combination of the PCR components finally, a mixture of 12.3 µl double distilled water (ultra pure), 5.6 µl DNTPs (1.25mM), 2.6 µl reaction buffer (10x), 2.5 µl $MgCl_2$ (@5U/µl), 0.6 µl ISSR primer (25mM) and 0.4 µl Taq DNA polymerase(20pm/µl) with 1 µl template DNA were used at respective annealing temperature of each primer. From the 25 ISSR primers used on the template DNA only seven of them were showed PCR products. Finally we repeated the PCR on the similar manner to check reproducibility of the primers and five primers were found to be reproducible. The five primers were used to amplify all samples and the generated data were used to analysis genetic diversity of *Jatropha curcas* populations under study.

Table 5. List of primers used in the optimization processes. Primers amplified the template DNA and reproducible represented by (A), primers amplified but no reproducible (B) and primers those could not amplify represented by (C)

Primer Name	**Sequences**	**Ann. To (^{0}c)**	**Result**	**Primer Name**	**Sequences**	**Ann. To (^{0}c)**	**Result**
808	(AG)8C	48	B	845	(CT)8RG	48	B
809	(AG)8G	48	B	870	(TGC)6	48	C
811	(GA)8C	48	C	872	(GATA)4	45	C
813	(CT)8T	45	C	**873**	**(GACA)4**	**48**	**A**
815	(CT)8G	48	C	877	(TGCA)4	48	C
818	**(CA)8G**	**48**	**A**	878	(GGAT)4	48	C
820	(GT)8C	48	C	879	(CTTCA)3	45	C
826	**(AC)8C**	**48**	**A**	884	HBH(AG)7	48	C
834	**(AG)8YT**	**48**	**A**	886	VDV(CT)7	48	C
835	(AG)8YC	48	C	892	TAGATCTGAT ATCTGAATTCC	45	C
840	(GA)8YT	48	C	895	AGAGTTGGTAG CTCTTGATC	45	C
843	(CT)8RA	45	C	900	ACTT(C)4ACAG GTTAACACA	45	C
844	**(CT)8RC**	**48**	**A**				

Note. Y and R represents DNA bases (Y = C or T and R = A or G)

3.7 Oil Extraction and Esterification

One hundred gram seed of each population was taken from each replication and the seed coats were removed manually. The kernel of each population reweighed and homogenized using mortar and pestle separately. From crushed kernel 3g powder was sampled and extracted by Soxtec System Ht 1043 Extraction Unit. The sample was heated at 110 ^{0}C for 20 minutes with hexane. Then, the oil was allowed to drop for 30 minutes at the same temperature. The solvent was evaporated for five minutes at this temperature and the oil samples were dried at 100 ^{0}C in oven for 30 minutes. Finally, the oils were cooled in desiccators and were weighted.

Biodiesel was prepared using the method standardized by Gupta (1994). Ten milliliter crude oil samples were obtained from extracted oil of each population and heated for 30 mints at 60 ^{0}C in water bath. Potassium hydroxide dissolved in methanol at weight ratio of 1:100 (one gram of potassium hydroxide for 100 gram crude oil) and the methanol potassium hydroxide solution was added to the heated crude oil samples in the ratio of 1:6 (methano-potassium hydroxide to crude oil). The mixture was heated for 2 hours and was allowed to cool for 24 hours. Then, the supernatant was collected and washed by double distilled water to remove impurities and made ready to use in Gas Chromatography. One micro liter esterified oil was sampled and dissolved in 10µl di-chloromethane. Finally, for each population 0.5µl micro liter of this solution was sampled and analyzed for its fatty methyl ester profiles using 3800 Gas Chromatography unit equipped with DB5 column of 0.53 mm x 25 m. The GC was operated at 220 ^{0}C, 2.0 retention times and 1.5 flow rates.

3.8 Data Analysis

Growth traits including root collar diameter, total plant height and number of branches were subjected to mean analysis at regional and population level using SAS system (Version 8.1 computer program). Using the same analysis tool mean analysis of agronomic traits (1000-seeds weight, yield per hectare, and seed oil content) among populations was analyzed. Different phenotypic traits growth, leaf and agronomic traits were subjected to correlation analysis.

3.9 Scoring and Analysis of ISSR Data

Since ISSR is a dominant marker the presence and absence of bands (fragments) were used as a character. Amplified bands were scored for presence (1) or absence (0) and for ambiguous (?). These data were subjected to different computer software to generate different information. Shannon-Weaver Diversity Index (H) was calculated to measure intra-population diversity (Hpop), total species diversity (Hsp) as well as inter-population diversity (Hsp-Hpop/Hsp) (Lewontin, 1972).

To calculate genetic diversity for each population, number of polymorphic loci and percent of polymorphism POPGEN version 1.32 computer software (Yehe *et al.,* 2006) was used. Areliquin version 3.01 (Excoffier *et al.,* 2006) was used to develop AMOVA in order to calculate variation among and within population. NTSYS version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al.,* 1999) software was used to calculate Jaccard's similarity coefficient. The unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and to compare the individual genotypes and generate dendrogram using NTSYS-pc version 2.02 (Rohlf, 2000). The neighbor Joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotypes clustering using Free Tree 0.9.1.50 software (Pavlicek *et al.,* 1999). Moreover, to examine the pattern of variation among individual samples a principal coordinate analysis was carried out based on Jaccard's similarity (Jaccard, 1908) using PAST software version 1.18 (Hammer *et al.,* 2001) Statistica soft. Inc., 2001).

4. RESULT

4.1 Phenotypic Traits

4.1.1 Phenotypic Trait Variability of *Jatropha curcas* Populations

The analysis of mean performance of growth and leaf traits of 14 *Jatropha curcas* populations collected from five regions were significantly different among regions except for root collar diameter (6.61) and number of branches (4.53) at age of 43 weeks. Total plant height (184.84) and average leaf dry matter (7.86) were significant at 0.05 probability level whereas all the remaining analyzed leaf traits were significant at 0.005 probability level (Table 6).

Mean performance analysis of populations indicated that population performance was significantly different for growth, leaf and agronomic traits except for root collar diameter (6.6), number of branches per plant (4.53) and yield (178.08kg) per hectare. Population performance for oil seed content were significantly different at 0.05 probability level whereas all studied leaf traits, total plant height and 1000-seeds weight were significantly different at 0.01 probability level (Table 6).

Table 6. Analysis of growth, leaf and agronomic traits of *Jatropha curcas* seeds collected from fourteen population representing five regions

Taxa level	Parameters	d.f	Sum square	Mean square	Root MSE	Mean	CV (%)	R^2	P- value
Among Region	**Growth traits**								
	Root collar diameter at age of 43 week (R43WAP)	4	9.56	2.39	1.66	6.61	25.11	0.94	0.4833
	Total plant height at age of 43 week (H43WAP)	4	20005.54	5001.54	41.78	184.84	22.60	0.39	0.0237
	Number of branch at age of 43 week (NB43WAP)	4	7.43	1.85	1.12	4.53	24.74	0.39	0.2336
	Leaf traits								
	Lamina length (LL)	4	35.79	8.95	1.14	15.02	7.56	0.26	0.0001
	Lamina width (LW)	4	27.60	6.91	1.20	16.64	7.20	0.20	0.0016
	Lamina length from widest width to tips (LWT)	4	20.68	5.17	0.96	12.53	7.68	0.22	0.0005
	Lamina length from base to widest width (LBW)	4	4.16	1.04	0.50	2.48	2.39	0.17	0.0046
	Average leaf dry matter (LDM)	4	32.40	8.10	1.56	7.86	19.81	0.14	0.0140
	Average leaf area (ALA)	4	18713.00	4678.35	28.28	199.74	14.16	0.23	0.0004
	Agronomic traits								
	Grain yield per hectare	4	40193.08	10048.27	60.26	178.08	33.83	0.72	0.0148
	1000-seed weight	4	24392.24	1626.15	23.11	479.46	4.82	0.64	0.404
	Oil content (%)	4	18.29	4.57	0.53	34.71	5.27	053	0.2728

Among Population	**Growth traits**								
	Root collar diameter at age of 43 week (R43WAP)	9	44.09	4.90	1.66	6.60	25.11	0.39	0.0718
	Total plant height at age of 43 week (H43WAP)	9	55325.71	6147.30	41.78	184.85	22.60	0.40	0.0004
	Number of branch at age of 43 week (NB43WAP)	9	83.18	9.24	3.12	4.53	24.74	0.35	0.4849
	Leaf traits								
	Lamina length (LL)	9	8.56	6.20	0.90	15.02	6.02	0.58	0.0001
	Lamina width (LW)	9	65.85	5.07	1.04	16.64	6.23	0.47	0.0001
	Lamina length from widest width to tips (LWT)	9	13.22	1.02	0.40	2.47	16.05	0.54	0.0001
	Lamina length from base to widest width (LBW)	9	45.90	3.53	0.83	12.53	6.61	0.49	0.0001
	Average leaf dry matter (LDM)	9	79.70	6.13	1.44	7.86	18.26	.0.36	0.0017
	Average leaf area (ALA)	9	44264.67	3404.97	23.19	199.74	11.61	0.54	0.0001
	Agronomic traits								
	1000- seeds weight	9	17060.9	1895.65	23.13	479.46	4.82	0.61	0.0048
	Grain yield per hectare	9	96546.84	10727.43	84.07	178.08	47.28	0.41	0.1902
	Percent of seed oil content (weight base)	9	76.82	8.54	1.78	34.71	5.13	0.52	0.0215

The mean performances of root collar diameter among regions varied from 6.84 to 6.47 centimeter whereas mean performance of total plant height was ranged from 196.96 to 177.56cm. Seeds collected from Region 2 attained top performance both in total plant height and root collar diameter growth. The lowest growth for these traits was scored for seeds samples collected form region 4 and region 5, respectively. The highest mean performance for all investigated leaf traits were recorded for seed source from region 1 whereas lowest mean performance of these traits was recorded for seed source from Region 3 (Table 7).

Duncan's multiple range test /analysis/ of *Jatropha curcas* populations mean performance indicated that the first two highest mean performances for growth traits were attained by seeds collected from *Jatropha curcas* population growing around Chiro and Jeweha, respectively. The lowest mean root collar diameter (5.94cm) and total plant height (161.30cm) were recorded for *Jatropha curcas* seeds collected from population growing around Mersa. Kenya II seed sources were superior for all investigated leaf trait mean performance whereas Mersa population was inferior for all described leaf traits. Despite growth and leaf traits, *Jatropha curcas* population mean performance was inconsistent regarding agronomic traits. Maximum first year grain yield per hectar was attained by Chiro population (304.2 kg) followed by Mersa population (247.7 kg). The minimum grain yield was recorded for Kenya II population (125.3 kg). *Jatropha curcas* population from Assossa was superior for both 1000-seed weight (540.44g) and seed oil content (37.4%) whereas Kenya I seed source was inferior to these traits with mean performance of 452.2 gram and 31.8% respectively (Table 8).

Table 7. Mean performances of growth and leaf traits of *Jatropha curcas* among regions.

Location	Agronomic traits			Growth traits			Leaf traits					
	Yield per hectare	S. O. %	10^3-seed Weight	Collar Dia.	Height	Branch number	LL	LW	LWT	LBW	LDM	ALA
Region 2	199.08a	34.7ab	467.93bc	6.83a	196.95a	4.59a	14.94b	16.93a	12.41a	2.54ab	7.65b	201.65ab
Region 5	207.63a	34.4ab	481.7abc	6.47a	186.92ab	4.37a	14.98b	16.43a	12.40a	2.54ab	7.90ab	196.50b
Region 1	143.92ab	33.8b	488.47ab	6.58a	179.52b	4.84a	15.84a	17.32a	13.06a	2.78a	8.85a	219.00a
Region 4	195.27a	35.1ab	493.41a	6.52a	177.55b	4.94a	15.23ab	16.68a	12.93a	2.28c	7.76ab	202.58ab
Region 3	127.73b	35.9a	458.98c	6.53a	183.25ab	3.66a	13.64c	15.45b	11.52b	2.10c	6.79b	168.60c
Mean	**174.726**	**34.78**	**478.098**	**6.60**	**184.85**	**4.53**	**15.02**	**16.64**	**12.53**	**2.48**	**7.86**	**199.74**

Notice:

- Number of branch at age of 43 week (NB43WAP),Total plant height at age of 43 week (H43WAP), Seed Oil content (%) (S.O.%),1000-seed weight, Grain yield per hectare Average leaf area (ALA), Average leaf dry matter (LDM), Lamina length from base to widest width (LBW), Lamina length from widest width to tips (LWT), Lamina width (LW) Lamina length (LL)
- Region1 (Kenya I, II and Arusha), Region 2 (Babile, Chiro and Doni), Region 3 (Arbaminch, Sawula), Region 4 (Assossa, Pawe and Metema) and Region 5 (Shewa Robit, Mersa and Jeweha).Regions with the same letter/s/ are not significantly different in performance for respective trait/s/
- Standard deviation indicated in section 8 table 18.

Table 8. Mean performance of 14 *Jatropha curcas* populations for growth, leaf and agronomic traits

	GROWTH TRAITS					LEAF TRAITS				AGRONOMIC TRAITS		
population	D43WAP (cm)	H43WAP (cm)	NB43 WAP	LL (cm)	LW (cm)	LBW (cm)	LWT (cm)	LDM (g)	ALA (cm2)	Grain yield (kg)	Weight 1000 seeds (g)	Seed oil content (%)
Chiro	7.32a	210.70a	5.3a	15.35bcd	17.33abc	2.51bcd	12.88bc	7.35bc	211.93bcd	304.19a	459.33c	34.42abcd
Jeweha	7.00ab	204.03a	4.9a	15.51bcd	16.88bcd	2.61bc	12.95bc	7.07bc	208.89bcd	227.45a	486.00bc	34.23abcd
Shewe Robit	6.99ab	195.43abc	4.8a	15.36bcd	16.88bcd	2.81b	12.40c	7.98bc	206.36bcd	147.69a	495.78bc	36.41ab
Pawe	6.90ab	192.6abc	5.3a	16.36ab	17.38abc	2.26cdef	14.06a	8.83ab	226.71ab	191.68a	469.56c	33.27bcd
Doni	6.74ab	179.77abc	4.3a	14.65bcd	16.65bcd	2.50bcd	12.15c	7.38bcd	194.84def	166.99a	477.78bc	34.43abcd
Arusha	6.66abc	186.30abc	4.8a	14.67cde	15.75de	2.05def	12.63bc	7.55bcd	183.85def	207.02a	484.89bc	33.83bcd
Kenya II	6.65abc	179.77abc	4.7a	17.05a	18.30a	3.38a	13.65ab	10.35a	248.25a	125.27a	518.29ab	35.82ab
Metema	6.51abc	182.57abc	4.6a	14.70cde	16.3cde	1.95ef	12.76bc	7.58bcd	188.39de	167.24a	470.22c	34.76abcd
Babile	6.44abc	185.07abc	4.1a	14.81cde	16.80bcd	2.61bc	12.20c	8.21bc	198.17cde	126.08a	466.67c	35.35abc
Kenya I	6.42bc	172.50abc	4.9a	15.80bc	17.91ab	2.90b	12.90bc	8.65abc	224.80abc	138.49a	462.22c	31.82d
Arbaminch	6.42bc	187.87abc	3.9a	12.90f	15.12e	1.82f	11.06d	5.98d	155.55f	126.97a	453.95c	36.10ab
Sawula	6.28bc	178.63abc	3.4a	14.38de	15.78de	2.38bcde	11.98cd	7.60bcd	181.62def	128.95a	464.00c	35.71abc
Assossa	6.14bc	163.43c	4.9a	14.38e	15.78cd	2.38bc	11.98cd	7.60cd	181.64de	226.89a	540.44a	37.40a
Mersa	5.94c	161.30c	3.3a	14.05f	15.52de	2.2cdef	11.85cd	7.65bcd	174.26ef	247.73a	463.33c	32.37cd
Mean	**6.6330**	**185.900**	**4.5**	**15.012**		**2.47**	**12.53**	**7.86**	**199.74**	**178.08**	**479.46**	**34.71**

Notice: Populations with the same letter/s/ are not significantly different in performance for respective trait and Standard deviation indicated in section 8 table 18.

4.1.2 Correlation among Different Quantitative Traits of *Jatropha curcas*

The Pearson correlation analysis of *Jatropha curcas* phenotypic traits showed different patterns of association among traits. Some traits were positively correlated some other traits were correlated negatively. An overall correlation coefficient ranged from 0.38 to 0.98. Correlation coefficient indicated that the investigated leaf and growth traits were positively correlated. But, this positive correlation was not significant. Growth traits were positively correlated to each other and the relationship were significant at 0.01 probability level except for correlation between number of branches and total plant height (Table 9). All correlations among leaf traits were positive and significant at 0.001 probability level.

The agronomic traits were negatively correlated with majority of growth and leaf traits. But none of these negative correlations were significant at $p = 0.05$. Grain yield of the first year per hectare was negatively correlated with other investigated agronomic traits; however, this negative correlation was not significant. Other agronomic traits were positively correlated and these correlations were significant between 1000-seeds weight and seed oil content at 0.05 probability level. Strong relationships were observed among kernel oil content, seed kernel content and seed oil content. The analyzed correlations among these traits were highly significant at 0.001 probability level. The maximum correlation coefficient (r) was 0.92 between kernel oil content and seed oil content

All investigated leaf traits were positively correlated with growth traits except leaf dry matter versus total plant height. These positive correlations were significant for number of branch versus lamina length, width, and average leaf area at 0.05 probability level.

Table 9. Correlation coefficients among various traits of *Jatropha curcas*

Traits	D43 WAP	H43 WAP	NB43 WAP	LL	LW	LDM	ALA	Grain yield	10^3-S. W.	Seed kernel	K.O.%	S.O. %
D43 WAP	*											
H43 WAP	0.9274 0.0001	*										
NB43 WAP	0.6559 0.0010	0.4053 0.1505	*									
LL	0.4296 0.1252	0.1338 0.6483	0.6547 0.0110	*								
LW	0.3716 0.1908	0.1077 0.7139	0.6847 0.0069	0.9179 .0001	*							
LDM	0.1637 0.5759	-0.0760 0.7960	0.2933 0.3088	0.9002 .0001	0.7838 0.0009	*						
ALA	0.4028 0.1532	0.1168 0.6908	0.6684 0.0090	0.9831 .0001	0.9732 .0001	0.8738 .0001	*					
Grain yield	0.2748 0.3416	0.2551 0.3786	0.2879 0.3182	-0.106 0.7176	-0.1287 0.6610	-0.3587 0.2078	-0.1348 0.6459	*				
10^3s.W	-0.0482 0.8700	-0.2288 0.4313	0.3740 0.1877	0.3514 0.2178	0.3102 0.2804	0.2475 0.4034	0.3350 0.2416	-0.0462 0.8758	*			
S.K.%	-0.1789 0.5404	-0.0879 0.7656	-0.1074 0.7276	-0.2311 0.4266	-0.0899 0.7599	-0.2560 0.0378	-0.1704 0.5601	-0.3808 0.1792	0.5099 0.0657	*		
K.O.%	-0.1505 0.6075	-0.0737 0.8022	-0.19386 0.5066	-0.2607 0.3680	-0.3351 0.2414	-0.2668 0.3564	-0.2956 0.3048	-0.0096 0.9738	0.5046 0.0657	0.6911 0.0062	*	
S. O.%	-0.1821 0.5331	-0.0931 0.7514	-0.1595 0.7514	-0.2667 0.7514	-0.2337 0.4220	-0.2840 0.3250	-0.2540 0.3807	-0.2049 0.4821	0.5558 0.0390	0.9146 .0001	0.9241 .0001	*

Notice: S.K.% (seed kernel content), K.O.% (kernel oil content), and S.O.% (seed oil content), 10^3-S.W (1000 seeds weight), Number of branch at age of 43 week (NB43WAP), Total plant height at age of 43 week (H43WAP), Average leaf dry matter (LDM), Lamina length from base to widest width (LBW), Lamina length from widest width to tips (LWT), Lamina width (LW) Lamina length (LL) the upper figures represents Pearson correlation coefficient where as the lower figure represents the p-value.

4.1.3 Clustering analysis of the Populations based on Quantitative Traits

Twenty two different quantitative traits (including fatty acid proportions) were used to construct UPGM dendrogram. The coefficients of similarity of fourteen populations under investigation ranged from 14.41 to 146.54. The highest similarity was observed between Doni and Metema and followed by populations from Sawula and Arbaminch (Table 10). The population dendrogram grouped the populations into four major classes. Group I comprised two subgroups (Ia and Ib) each with four and three populations respectively. Group II encompassed five populations whereas group III and V each contained only one population (Fig. 5)

Table 10. Quantitative trait similarity coefficients of 14 *Jatropha curcas* populations

	Arbaminch	Arusha	Assossa	Babile	Chiro	Doni	Jeweha	KenyaI	KenyaII	Mersa	Metema	Pawe	Sawula	Sh.Robit
Arbaminch	**													
Arusha	8.972	**												
Assossa	1.361	7.079	**											
Babile	2.704	8.617	1.272	**										
Chiro	1.819	1.052	1.238	1.809	**									
Doni	5.332	4.322	9.183	4.359	1.411	**								
Jeweha	1.109	3.410	7.057	1.056	8.070	6.453	**							
KenyaI	5.180	8.818	1.229	3.355	1.710	4.918	9.912	**						
KenyaII	1.032	1.432	1.450	8.337	2.312	1.067	1.527	8.109	**					
Mersa	1.264	6.170	8.230	1.267	8.552	9.048	6.427	1.211	1.873	**				
Metema	4.957	4.667	9.446	4.308	1.42	1.44	6.985	4.886	1.1239	8.49	**			

					8	1				5				
Pawe	8.193	5.092	8.968	7.177	1.16	4.24	4.684	5.608	1.189	8.18	4.647	**		
					8	1				0				
Sawula	1.688	8.531	1.264	1.964	1.81	4.67	1.080	4.617	9.658	1.21	4.188	7.85	**	
					4	5				5		1		
ShewaRob	5.492	6.570	9.787	3.886	1.61	3.06	8.094	4.696	8.035	1.15	4.048	5.60	4.788	**
it					7	8				9		4		

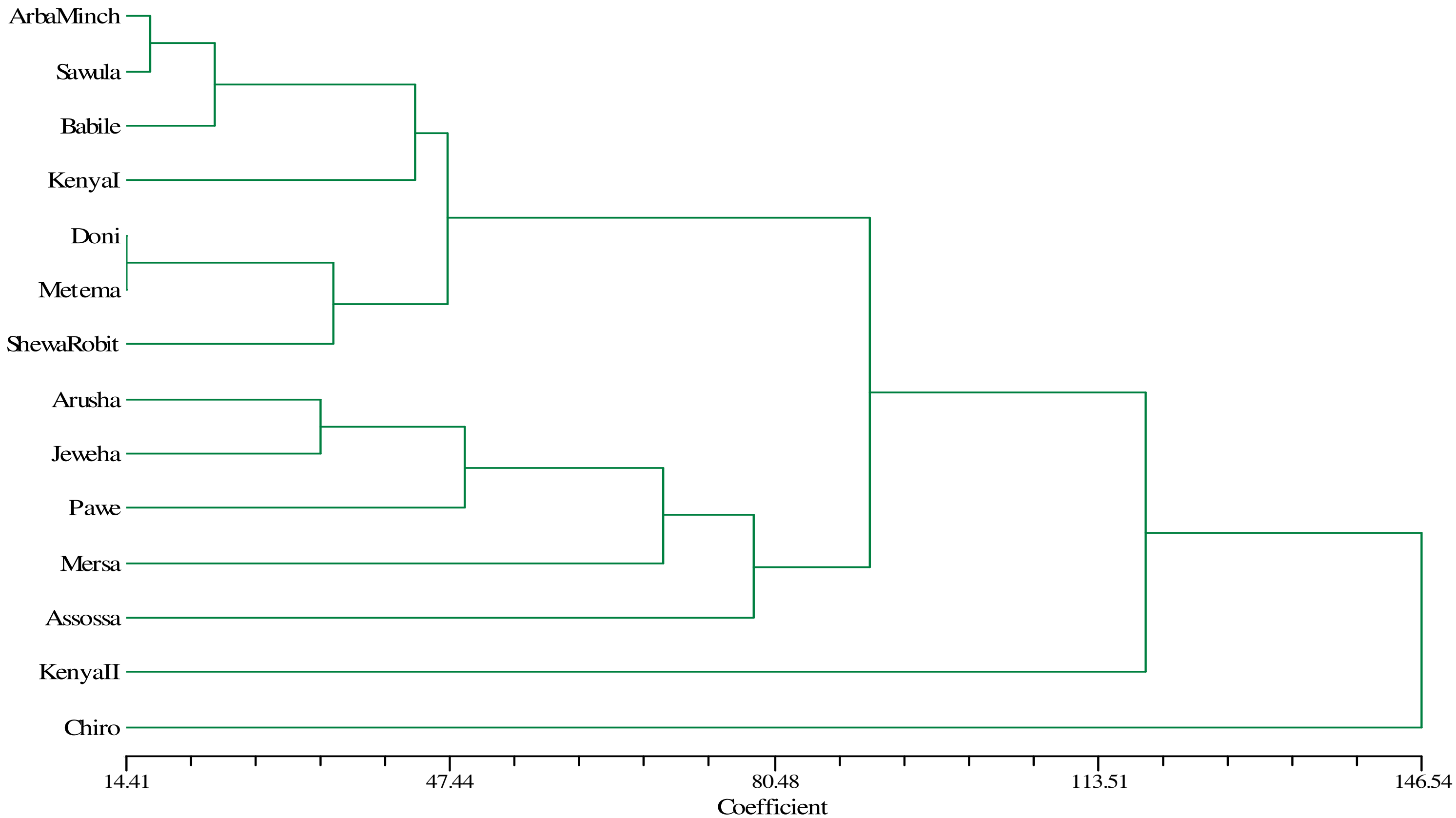


Fig 5. Dendrogram of fourteen *Jatropha curcas* populations developed based on quantitative traits

4.2 Genetic Diversity of *Jatropha curcas* Populations

4.2.1 ISSR Marker Profiles

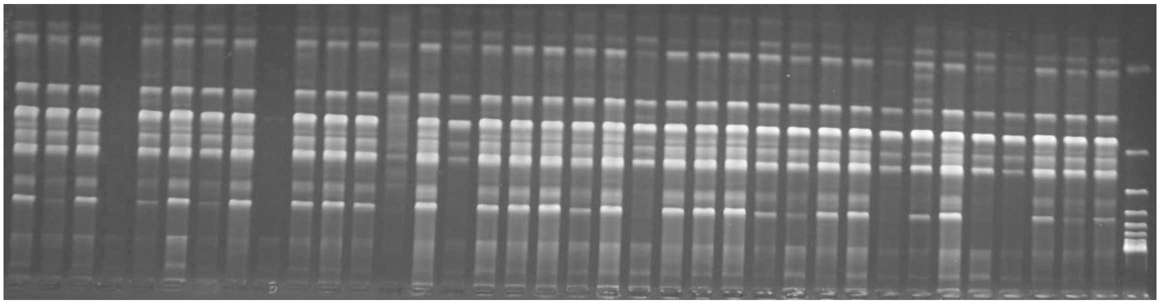
The total number of amplified products was 71 DNA fragments /bands/ ranging from 250 base pairs to 3000 base pairs and all of the amplified products were polymorphic. Maximum and minimum number of bands were generated by 873 (17) and 818 (9) primers, respectively. Average amplified bands per primer were 14.20 (Table 11). The studied primer sequences were composed of four di- and one tetra repeat sequences. Both primers showed 100% polymorphism. Highest diversity was detected by primer 826 (0.661) and followed by primer 873 (0.564). The lowest Shannon-weaver Diversity Index was revealed by primer 818 (0.458).

Table 11. Summary of details of five ISSR primers and amplified bands of all DNA samples as observed from 80 individuals of *Jatropha curcas* populations

ISSR Primers	NAB	NMB	NPB	PP	Shannon Diversity Index	Estimated Band Size		Repeat Motifs
						Max.	Min.	
818	9	0	9	100	0.458	1250	500	**Di- nucleotides**
826	14	0	14	100	0.661	3000	400	**Di- nucleotides**
834	16	0	16	100	0.550	2500	250	**Di- nucleotides**
844	15	0	15	100	0.462	2750	250	**Di- nucleotides**
873	17	0	17	100	0.564	2250	400	**Tetra- nucleotides**
Average	14.2	0	14.2	100	0.539	2550	360	

** Number of amplified bands (NAB), Number of monomorphic bands (NMB), Number of polymorphic bands (NPB), Percent polymorphism, (PP)*

Fig. 6 ISSR bands generated from *Jatropha curcas* individuals using primer 873. Different letters represented different populations whereas number 1-6 represents individuals of a population.



4.2.2. Inter-Population Genetic Diversity

The value of Nei's genetic diversity and Shannon Information Index of 14 populations were 0.273 and 0.431 respectively. The total gene diversity (Ht) was 0.272, whereas within population diversity (Hs) was found to be 0.160. The mean coefficient of gene differentiation (Gst) was 0.389. Ratio of Hs/Ht (59 %) indicated the more genetic diversity resided at the population level (Table 12).

Table 12. Analysis of gene diversity in subdivided populations

Methods of Analysis	Parameter Estimated	Mean	**St.Dev.**
Nei's Gene Diversity	Nei's genetic diversity (h*)	0.273	0.141
	Total gene diversity (Ht)	0.272	0.020
	Within population diversity (Hs)	0.160	0.010
	Proportion of among population diversity (Gst)	0.389	------
	Gene flow (Nm*)	0.785	------
Shannon-Weaver Diversity Index	Shannon-Weaver Diversity Index at species level(Hsp)	0.545	------
	Within population genetic diversity (Hpopn)	0.445	------
	Proportion of population diversity to species diversity (Hpopn/Hsp)	0.83	------
	Proportion of among population diversity to species diversity	0.17	------

4.2.3 Intra-Population Genetic Diversity

Maximum polymorphic loci were recorded for *Jatropha curcas* population sampled from Arbamich area (41) and followed by *Jatropha curcas* sampled from Sawula (36) which is closer to Arbaminch. The polymorphic percent of these two populations were 80.4 and 67.9 respectively. Kenya I and Kenya II populations exhibited the lowest level of variability with 11 and 13 polymorphic loci and 20.7 and 21.4 percent polymorphism, respectively. This may be due to the fact that Kenya I and II are highly selected. Average gene diversity was highest within Arbaminch population (0.309 $\pm$ 0.1867) and was lowest within Kenya II population (0.094 +/- 0.0611). Population from Arusha attained the highest Shannon-Diversity Index (0.759) and followed by Jeweha population (0.632) (Table 13). The Arusha population seems to be less selected compared to the Kenyan populations.

Table 13. Summary of intra-population genetic diversity information with all primers

Populations	NPL	PP	AGDOAL	S.W. D.I
Kenya II (A)	12	21.43	0.094 ± 0.0612	0.243
Babile (B)	16	32.00	0.151 ± 0.0947	0.346
Jeweha (N)	33	36.36	0.281 ± 0.7126	0.632
Assossa (F)	22	47.82	0.169 ± 0.0632	0.378
Doni (C)	19	29.69	0.131 ± 0.0818	0.261
Sawula (D)	36	67.92	0.252 ± 0.1527	0.521
Arbaminch(E)	41	80.39	0.309 ± 0.1867	0.580
Pawe(G)	19	50.00	0.254 ± 0.1769	0.470
Shewa Robit (H)	13	26.00	0.123 ± 0.0785	0.320
Mersa(I)	13	23.21	0.107 ± 0.0687	0.278
Metem(J)	19	30.65	0.124 ± 0.7763	0.267
Kenya I (K)	11	20.75	0.132 ± 0.0939	0.517
Chiro(L)	14	24.56	0.119 ± 0.0756	0.655
Arusha(M)	20	44.44	0.184 ± 0.115	0.759
Average	**20.57**	**38.23**		**0.44**

Notice: NPL (number of polymorphic loci), PP (percent of polymorphism), AGDOAL (average gene diversity over all loci) and S.W.D.I (Shannon-Weaver diversity index)

4.2.4 Analysis of Molecular Variance (AMOVA)

The AMOVA provided corroborating evidence for the genetic structure obtained from Nei's genetic diversity and Shannon-Weaver Information index estimates. The total variance analyzed without considering the regional distribution of the population was 15.19% among population and 84.81% within populations. When regional distribution of populations was considered, the variance distributions were 1.66%, 13.72 % and 84.61% shared by among groups, among population within groups and within population respectively (Tables 14 and 15).

Table 14. Analysis of molecular variance (AMOVA) of *Jatropha curcas* populations based on distance method: Pair wise difference Regional clustering is avoided

Source of variation	d.f .	Sum of squares	Variance components	Percentage of variation
Among populations	13	92.642	0.63113 Va	15.19
Within populations	67	232.583	3.524Vb	84.81
Total	80	325.225	4.155512	100
Fixation Index	*FST : 0.1551*			

N.B. (Va) represents variance components among populations, (Vb) represent variance components due to within populations variation

Table 15. Analysis of molecular variance (AMOVA) of *Jatropha curcas* populations based on distance method: Pair wise difference when populations cluster in respective regions

Source of variation	d.f .	Sum of squares	Variance components	Percentage of variation
Among groups	4	31.77	0.069Va	1.66
Among populations within groups	9	60.87	0.57Vb	13.72
Within populations	67	232.52	3.52Vc	84.61
Total	80	325.23	4.155512	100.00
Fixation Index	*FST : 0.1551*			

N.B. (Va) represents variance components due to among groups, (Vb) represent variance components due to among populations within groups and (Vc) within populations

4.2.5 Genetic Similarity

Based on Jaccard's similarity coefficient among population similarity ranged from 0.622 to 0.885. The highest similarity was observed between Chiro and Kenya I populations (0.885). It was followed by similarity between Kenya I versus Doni and Sawula versus Kenya I with 0.874 and 0.868 similarity coefficient respectively. The maximum genetic distance was observed between population from Pawe and Arbaminch followed by Jeweha and Assossa with similarty coefficient of 0.622 and 0.627, respectively (Table 16).

Table 16. Jaccard's coefficient of similarity

Poplation	Kenya II	Babile	Doni	Sawula	Arbam.	Assossa	Pawe	Shewa Robit	Mersa	Metema	Kenya I	Chiro	Arusha	Jeweha
Kenya II	****													
Babile	0.8242	****												
Doni	0.8746	0.84502	****											
Sawula	0.7794	0.75522	0.8333	****										
A.Minch	0.7763	0.73700	0.7568	0.7167	****									
Assossa	0.8348	0.78750	0.7899	0.7002	0.6972	*****								
Pawe	0.7743	0.71052	0.7706	0.7894	0.6224	0.8051	*****							
Sh.Robi	0.745	0.7604	0.763	0.725	0.7368	0.7254	0.772	*****						

t	0	1	4	4			7							
Mersa	0.850	0.8333	0.861	0.801	0.7194	0.8130	0.802	0.771	****					
	2	3	3	6			6	5						
Metema	0.836	0.8059	0.804	0.786	0.7359	0.8006	0.825	0.806	0.8490	*****				
	8	7	8	9			0	7	0					
Kenya I	0.827	0.8227	0.826	0.867	0.6787	0.7788	0.817	0.790	0.8559	0.8504	****			
	1	2	2	8			2	8	3					
Chiro	0.804	0.8322	0.850	0.803	0.7558	0.7836	0.823	0.790	0.8448	0.8187	0.884	****		
	7	9	4	4			8	6	2		7			
Arusha	0.820	0.7543	0.787	0.733	0.6733	0.7507	0.738	0.726	0.8089	0.7994	0.768	0.795	****	
	0	3	0	1		2	3	9	8		5	3		
Jeweha	0.703	0.7018	0.719	0.793	0.6372	0.6276	0.778	0.699	0.7477	0.7708	0.853	0.741	0.729	**
	3	0	2	4		9	8	6	4		3	2	9	

4.2.6 Clustering Analysis Based on ISSR Data

A UPGM dendrogram produced using the Jaccard's similarity coefficient between populations and individuals are illustrated in Figures 7 and 8, respectively. The population dendrogram clustered seven populations in the same cluster (**I**). This cluster comprised two sub-groups (Ia, Ib) each with four and three populations respectively. Sub-group Ia comprised Kenya II, Doni, Mersa and Babile populations whereas Ib sub-group comprised Sawula, Kenya I and Chiro population. Populations from North West part of the country were clustered together (II). The remaining populations (Assossa, Arusha, Shewa Robit, Jeweha, and Arbaminch) were clustered each as separate class (III, IV, V, VI and VII) (Figure 7).

The Jaccard's coefficient among individuals ranged from 0.2 to 1.00. Dendrogram of individuals divided the genotypes in to six major clustered groups (I, II, III, IV, V, VI, VII, a) whereas some individuals were formed a separate class alone (Figure 7). Individuals' dendrogram revealed that individuals of a population were clustered with individuals from different populations. However, group V comprised only individuals from Shewa Robit populations. In group I some individuals from population A and from population F were found to be more similar and made a separate sub-group. Similarly, individuals from population M (Arusha sample) and N (Jeweha sample) were more related to each other and were clustered in a separate sub-group under group II (Figure 8).

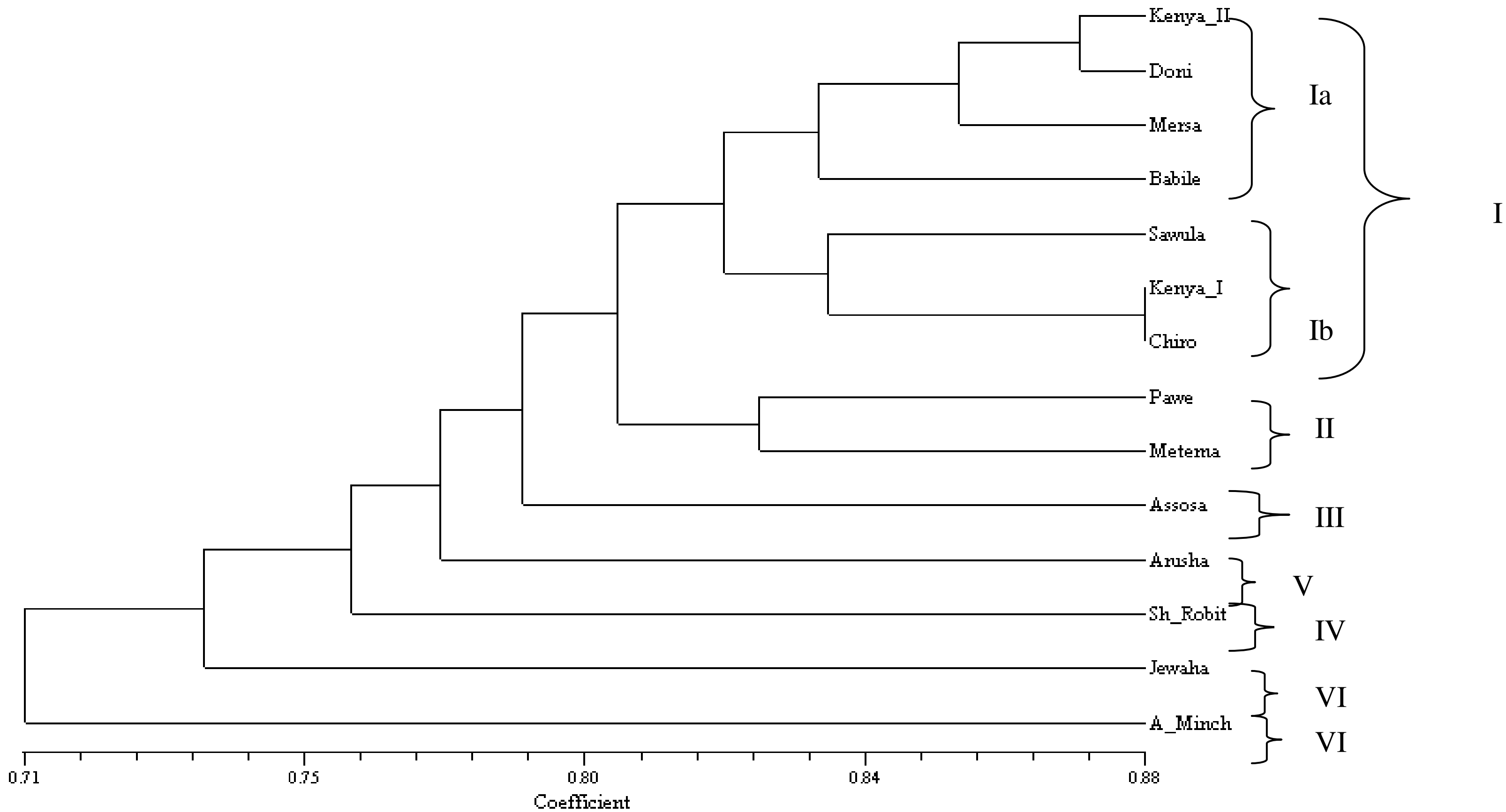


Fig. 7 Dendrogram of the fourteen *Jatropha curcas* populations constructed from presence and absence of ISSR data

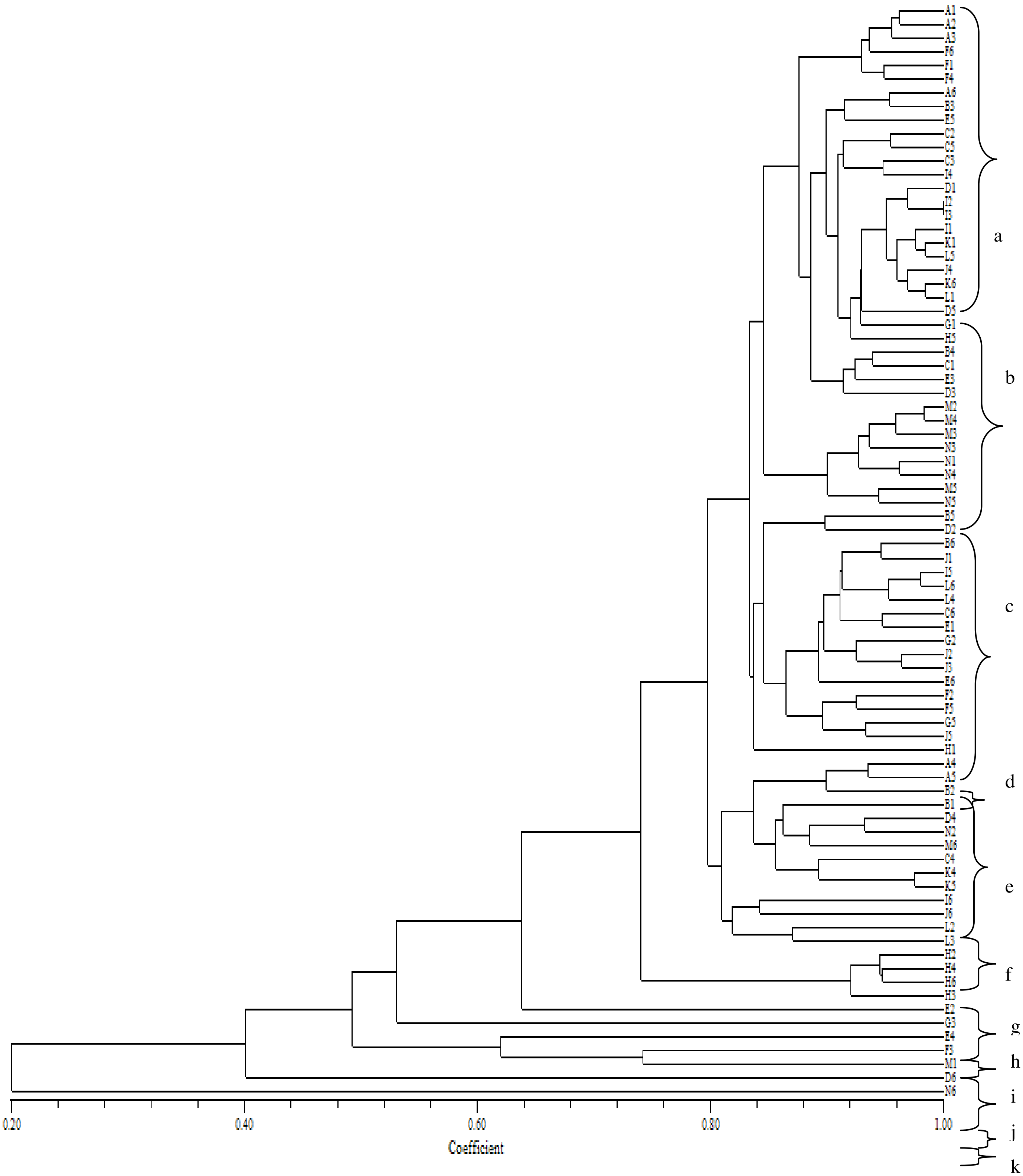


Fig. 8 Dendrogram of the 80 individuals of *Jatropha curcas*

4.2.7 Principal Coordinate Analysis (PCO)

All data generated by five ISSR primers were subjected to PCO analysis using Jaccard's coefficient of similarity. The three coordinates of the PCO with eigenvals of 18.69, 8.57 and 5.65 and variance of 7.33%, 3.36% and 2.22%, respectively were used to cluster individuals into coordinates. On two dimensional coordinate individuals were laid between -0.2 and 0.2 of first coordinate and -0.2 and 0.3 of the second coordinate. Most individuals were clustered into six groups while some individuals such as M1, G2 and K4 were found outside of these clusters. On three dimensional coordinate individuals was not form any group and they clustered intermingling.

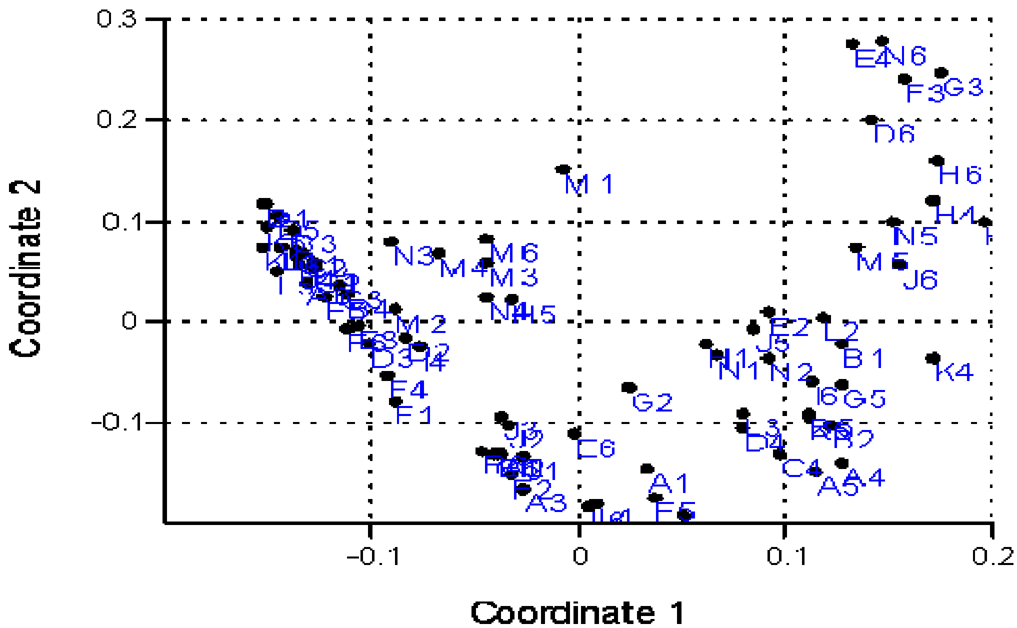


Fig. 9 Two dimensions coordinate analysis of 80 individuals of *Jatropha* populations

Notice: letter A to N represents populations (as described in page 43) and the affix numbers (1-6) represents individual samples within population

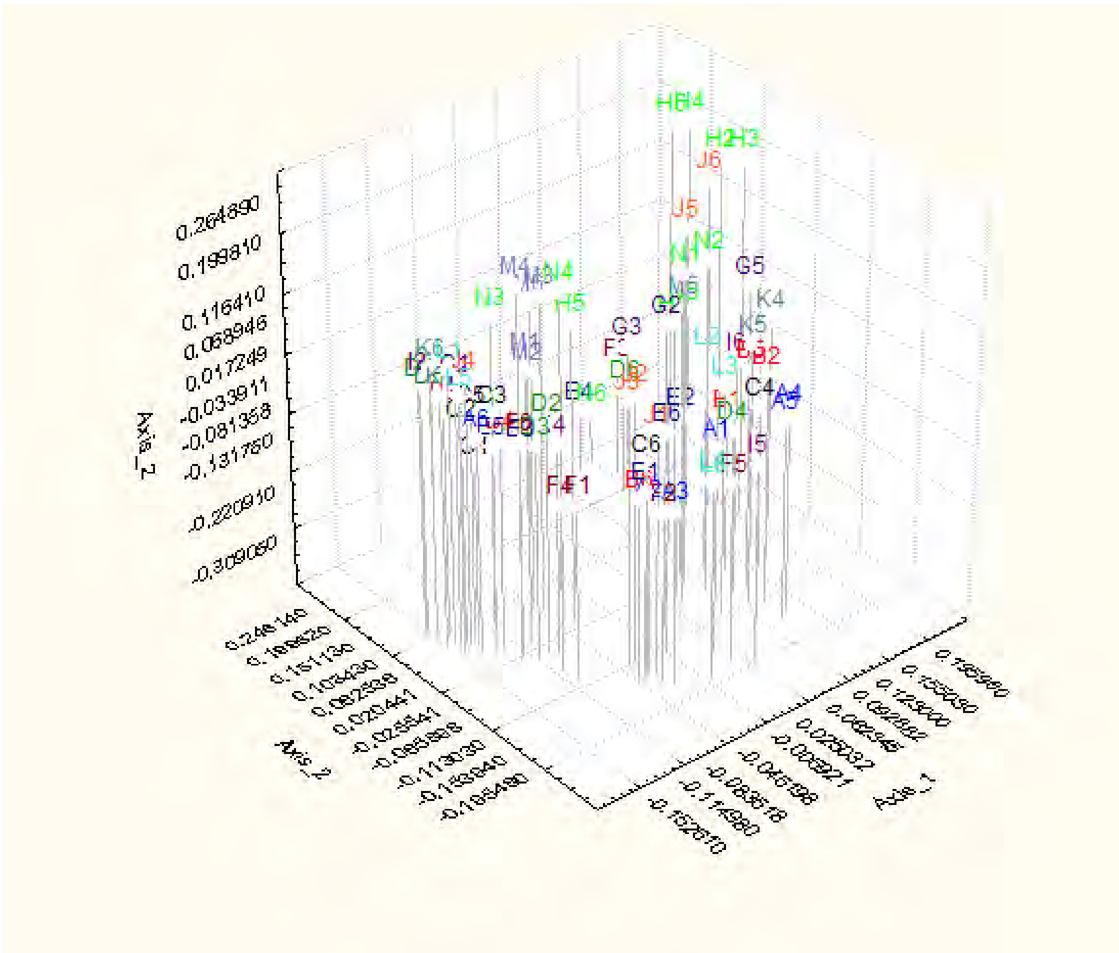


Fig. 10 Three dimensions coordinate analysis of 80 individuals of *Jatropha* populations

Notice: letter A to N represents populations (as described in page 43) and the affix numbers (1-6) represents individual samples within population

4.3 Fatty Methyl Ester Profile of *Jatropha curcas* Oils

Six different types of fatty methyl esters (methyl palmitoleate, methyl palmitate, methyl stearate, methyl oleate, and methyl linoleate and methyl linoleneate) were identified from *Jatropha curcas* biodiesel (transesterifed *Jatropha* oil). Methyl esters from all fourteen populations in common contained these six fatty methyl esters but in different proportions. Percentage of saturated fatty methyl esters varied from 19.04% (Mersa population) to 28.9% (Arbaminch population) whereas unsaturated fatty acids ranged from 71.09% (Arbaminch population) to 81.44% (Kenya II population). The maximum composition of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate and linoleneate were found to be 22.81% (Arbaminch), 6.68 % (Pawe), 48.39% (Kenya II), 33.05% (Doni) and 8.99% (Kenya II) respectively (Table 17). The maximum monosaturated fatty methyl ester was observed for Mersa population (49.01) and followed by Kenya II (48.39) and Assossa population (47.6).

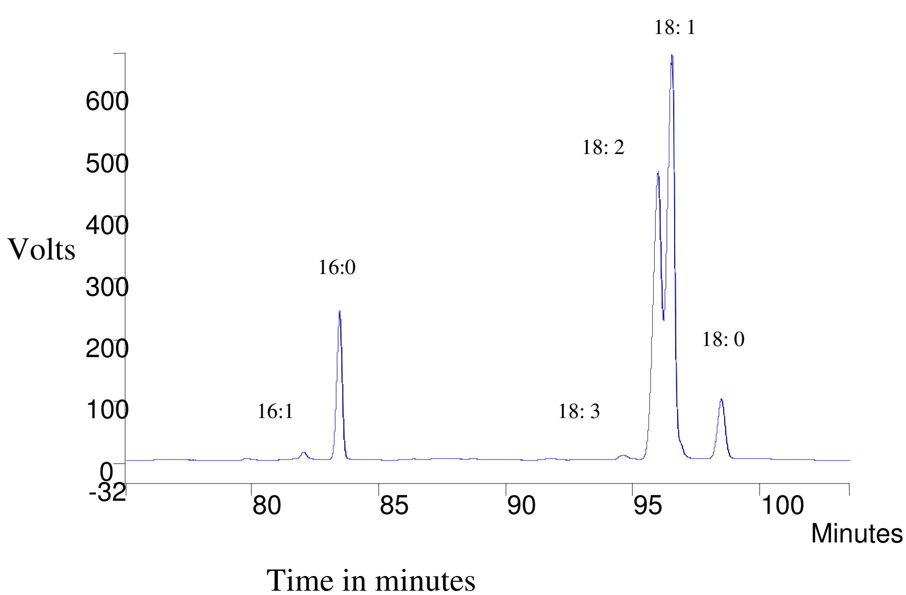


Fig. 11 GC peaks show band pattern of *Jatropha curcas* methyl ester profile

Table 17. Fatty methyl esters profiles of fourteen *Jatropha curcas* populations

population	Percent of Fatty methyl esters									
	16:1	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**SA.**	**USA..**	**PUS**	**MSA.**
Babile	1.15	16.91	6.82	43.52	31	0.8	23.73	76.47	31.8	44.67
Chiro	2.01	18.88	5.15	43.06	26.32	4.57	24.03	75.96	30.89	45.07
Doni	0.9	15.15	5.43	44.53	33.05	0.94	20.58	79.42	33.99	45.43
Sawula	2.34	19.51	5.68	42.1	26.02	4.34	25.19	74.8	30.36	44.44
Arbaminch	3.86	22.81	6.1	38.95	20.74	7.54	28.91	71.09	28.28	42.81
Assossa	1.68	14.76	5.83	45.92	27.65	4.16	20.59	79.41	31.81	47.6
Pawe	1.74	16.64	6.68	43.48	28.7	1.77	23.32	75.69	30.47	45.22
She.Robit	1.54	18.87	6.64	42.78	28.74	1.6	25.51	74.66	30.34	44.32
Mersa	1.1	13.75	5.29	47.91	29.81	2.14	19.04	80.96	31.95	49.01
Metema	0.46	15.55	4.74	46.26	32.42	0.73	20.29	79.87	33.15	46.72
Kenya I	2.34	14.56	5.65	44.06	28.18	5.16	20.21	79.74	33.34	46.4
Kenya II	0	16.41	5.18	48.39	24.06	5.99	21.63	79.39	30.05	48.39
Arusha	1.27	15.4	4.47	45.41	31.83	1.64	19.87	80.15	33.47	46.68
Jeweha	1.97	19.75	5.05	42.17	27.68	3.3	24.8	75.12	30.98	44.14
Average	**1.59**	**17.06**	**5.62**	**44.18**	**28.3**	**3.19**	**22.69**	**77.33**	**31.49**	**45.77**

N.B (16:1) fatty methyl palmitoleate, (16:0) fatty methyl palmitate, (18:0) fatty methyl stearate, (18:1) fatty methyl oleate, (18:2) fatty methyl linoleate, (18:3) fatty methyl linoleneate, (SA.) saturated fatty methyl ester, (USA) unsaturated fatty methyl ester, (PUS) polyunsaturated fatty methyl ester and (MSA) monounsaturated fatty methyl ester

5. DISCUSSION

5.1 Quantitative Traits Variations

5.1.1 Morphological Diversity of *Jatropha curcas* and Their Inference

Morphological characters of plants such as diameter, height and number of branches are described as plant growth traits (Givnish, 1997). The variance analysis of these parameters of 14 *Jatropha curcas* populations both among regions and populations demonstrated similar patterns. Root collar diameter and number of branches at age of 43 weeks after planting (43WAP) were not significantly different both among regions and among populations whereas total plant height was significantly different at ($P < 0.05$) both among region and populations. This result is in agreement with the observation by Rao *et al.,* (2008) among eleven *Jatropha curcas* accessions. Ginwal *et al.* (2004) conducted an experiment on phenotypic trait variation of ten *Jatropha curcas* seed sources. The result of their experiment revealed the existence of significant difference among seed sources for total plant height, root collar diameter and number of branches. Gohil *et al.* (2008) observed significant difference among *Jatropha curcas* genotypes in respect of all growth traits. From his adaptation trial of *Jatropha curcas and Jatropha gossypifolia,* Heller (1992) observed good variation among provenances for plant height and number of branches per plant. However, Sukarin and his associates (1987) did not observe any morphological differences among 42 clones of *Jatropha curcas* originated from different part of Thailand. Such inconsistent results indicate that growth traits of *Jatropha curcas* are highly affected by genetic and environmental interaction. In other aspect, the significant difference in height growth among populations may result from adjustment of the populations to their specific environmental condition via natural selection. The mean root collar diameter and number of branches of the current study attained at age of 43 weeks are higher than the reported mean value *Jatropha curcas* from other seed sources at 24 months after planting (Ginwal *et al.,* 2004) whereas the mean value of the total plant height is almost similar.

Variance analysis of six different leaf traits and three agronomic traits of fourteen *Jatropha curcas* populations indicated significant differences except for grain yield per hectare. As indicated in Table 6, all leaf traits were significantly different at ($p < 0.01$) both among regions and populations. Except average leaf dry matters the rest of the investigated leaf traits were still significantly different at $P < 0.0001$ among populations. This result is in accordance with the former study by Ginwal *et al.* (2004). Such variation in leaf traits among populations may vary in photosynthetic efficiency of the genotypes (Givnish, 1997). Leaf size can also influence the amount of chloroplast DNA (Shaver *et al.,* 2008) where lipid synthesis of the higher plant is initiated (Brows *et al.,* 1990). Gohil and Pandya (2008) noticed that *Jatropha curcas* populations were also significantly vary with respect to their leaf number per plant. Result of the present study indicated that populations from eastern part of Ethiopia are superior in all leaf traits whereas populations from Region 3 were found to be inferior in all leaf traits. Among fourteen populations, Kenya II population demonstrated maximum mean values of all leaf traits whereas the minimum mean vales of all studied leaf traits were observed for Arbaminch population. Such genetic differences in leaf traits of populations from different localities were observed for many perennial plants (Borazan and Mehmet, 2003). Givnish (1979) reported the adaptive value of small leaves in water stressed, water-logged and nutrient poor environments. This indicates that the variation is due to adaptation of populations to different environmental conditions and it indicates a possibility of using different population under different environmental conditions.

Variance analysis of 14 *Jatropha curcas* populations indicated that populations were significantly different in the percent of oil content and 1000-seeds weight at $P < 0.05$ and $P < 0.005$, respectively. This result is in agreement with previous works (Heller, 1992; Ginwal *et al.,* 2004; Kaushik *et al.,* 2007; Rao *et al.,* 2007; Sunil *et al.,* 2008; Akbar *et al.,* 2009). The significant difference of oil content and 1000-seeds weight among different populations and accessions were also reported for other oil crops such as peanut and *Brassica* species (Jogloy *et al.*, 2005; Zhang and Zhou, 2006). These implies that different genotypes do perform differently and evaluation of all available populations for their performance with respect to oil content before starting breeding program will result in better successes. The mean value of seed oil content varied from 31.82 % (Kenya I) to 37.40% (Assossa). In some of the former studies carried out by different scholars it ranged from 23% to 42% (Ferrao *et al.,* 1982; Ginwal *et al.,* 2004; Rao *et al.,* 2008). The present result is comparable with most oil content reported by these authors. This indicate that oil content of *Jatropha curcas* populations growing in Ethiopia are comparable with the oil contents of *Jatropha curcas* of some other genotypes growing in other part of the world.

Grain yield of the first year ranged from 125.27 kilogram per hectare (Kenya II) to 304.19 kilogram per hector (Chiro). Jones and Miller (1992) reported that in the first year *Jatropha curcas* provide 6% of its potential yield at maturity. Based on this, grain yields of the present populations are anticipated to range from 2.1 tons to 5.07 tons per year per hectare. The projected grain yield for the first three top performed populations is comparable with grain yield reported by Matsune *et al.* (1984) and Foidl *et al.* (1996). It is possible to infer that the populations under present study are comparable in their quantitative trait diversity with previous studies and this may resulted from adjustment of the populations to their specific environmental heterogeneity. Furthermore, each population may have their own important traits under specific condition and considering each population and individuals within population is crucial in breeding program.

5.1.2 Correlation of Quantitative Traits

The phenotypic traits of this study demonstrated different mode of correlation varied from unfavorable correlation to significant favorable correlation. Such information of correlation is required to obtain the expected response of other characters when selection is applied to the joint character of interest in breeding programs (Falconer, 1989). The correlation result of this study revealed that significant positive correlation among growth parameters at $P < 0.002$ except for correlation between number of branches and total plant height. Similarly, significant positive correlation at $P < 0.001$ was observed among all leaf traits. To improve oil productivity of *Jatropha curcas* investments, it requires to breeding towards boosting grain yield per hectare and improving seed oil content. Hence, those phenotypic traits having strong positive or negative correlations with these traits would be of interest in the breeding program and assist the breeders in plus tree selection.

The grain yield of the present study had positive association with all studied growth traits whereas negative association was observed with all the remaining phenotypic traits. The positive association was stronger with number of branches than any other traits. This implies that breeders should look for genotypes with higher number of branches during selection process.

Seed oil content was positively correlated with kernel oil content, seed kernel content and 1000-seed weight. This association was significant at $P < 0.05$ with 1000-seed weight and highly significant with seed kernel content and kernel oil content at $P < 0.0001$. This result is in harmony with previous findings of different *Jatropha curcas* populations and accessions (Ferrao, 1984; Heller, 1992; Ginwal *et al.,* 2004). Several such positive associations between oil content and 1000-seeds weight were noticed for other oil crops such as *Brassica napus*, peanut and sunflower (Jogloy *et al.,* 2005; Ahmad *et al.,* 2006; Iqbal *et al.,* 2009). This positive interdependency between 1000-seed weight and seed oil content will be an important indicator to select superior genotype for further breeding purpose. The negative correlation of grain yield and seed oil content imply that the breeding program can focus towards either oil productivity per unit area or developing pure line for high grain yield and seed oil content and crossing these pure lines.

5.2 Genetic Variability

5.2.1 ISSR Marker Loci Diversity of *Jatropha curcas* and Its Implication

The five ISSR primers amplified an average of 14.2 loci per primer and all primers were polymorphic which is higher than previous studies (Subramanyam *et al.,* 2008 (12.5, 75.2%); Gupta *et al.,* 2008 (5.3, 84.26) using RAPD primers for 40 and 13 *Jatropha curcas* accessions, respectively. The latter authors reported an average of 5.8 amplified bands per primer and 76.54% of polymorphism using ISSR primers for the same experiment. Polymorphism of this study was also higher than polymorphism reported using ISSR primers for nine natural populations of *Jatropha curcas* growing in China (Wei *et al.,* 2007). Loci assessed by current study were more polymorphic than previous result reported among *Jatropha* species including *Jatropha curcas* (Ram *et al.,* 2007; Pamidiamarri *et al.,* 2008; Basha and Sugatha, 2009). This high polymorphism may indicate high genetic diversity and mixed mating system that favor xenogamous than geitonogamous (Solomon and Ezranam, 2002) exhibiting within population diversity which may be favored either by the population biology or the environment.

The Nei's genetic diversity (h*), Shannon difersity index and total genotype diversity among population (Ht) of this study were 0.273, 0.431 and 0.272 respectively. All these values are higher than the results reported for 13 *Jatropha curcas* accession*s* studied using RAPD and ISSR primers reported by Gupta *et al.* (2008). Populations under present study showed higher total genetic diversity than some tropical trees including *Euterp edulis* reported by Cardoso *et al.* (2000) and *Cratylia argentea* reported by Andersson *et al.* (2006). The mean Shannon-Weaver diversity index of the studied 14 population of *Jatropha curcas* populations was 0.539 and this value is higher than reported values for tropical tree species of natural stand such as *Tamarindus indica* (Ousmane *et al.,* 2007).

The intra-population polymorphic percentile of present study varied from 20.75% (Kenya II population) to 80.39% (*Jatropha curcas* growing around Arbaminch) and it is higher than reported by Wei *et al.,* (2007) that ranged from 15.98% to 45.56%. Polymorphic percentiles of *Jatropha curcas* population growing around Babile, Assossa, Sawula, Arbaminch, Pawe, and Arusha were higher than polymorphism reported for all populations in previous studies (Kumar *et al.,* 2009; Sunil *et al.,* 2008) using RAPD and AFLP markers, respectively. The average gene diversity of these populations was higher than average gene diversity of eleven natural populations of *Euterpe edulis* (Cardoso *et al.,* 2000). Within population, Shannon-Weaver diversity index varied from 0.243 to 0.759 for Kenya II and Arusha populations, respectively. This large variation in intra-population variability, may result from population demography (population size, density), environmental variability (biotic such as pollinators and abiotic stress), and sampling of individuals for experiment (particularly in the case of populations out of Ethiopia). These factors can affect the extent of outcross mating, and genetic drift.

ISSR result of the present study indicate that, although *Jatropha curcas* had been introduced in the recent past, it exhibited higher genetic diversity than most reported diversity from different part of the world. This may be due to adaptation to a highly diversified environment of the country and from environmental factors that favor pollinators. It may also be due to the fact that the introduced *Jatropha curcas* is from different regions that pulled variability to the country at different time.

5.2.2 Genetic Differentiation among Population of *Jatropha curcas* and Their Possible Causes

Analysis of the ISSR markers using various methods of analysis (Nei's genetic diversity analysis, Shannon-Weaver diversity index and analysis of molecular variance (AMOVA) revealed the same pattern of genetic structure of *Jatropha curcas* populations. The overall degree of estimate of genetic differentiation was (Gst = 0.389) and this is higher than the average genetic differentiation (Gst = 0.32) for dicotlydons (Nybom, 2004). This differentiation values are also higher than genetic differentiation reported for *Shorea leprosula* and *Shorea paruifolia* (Cao *et al.,* 2006) in Indonesia. But it is less than genetic differentiation of highly differentiated populations of endangered species (Song and Thomas, 2007) and previous repot for 13 *Jatropha curcas* genotypes (Gupta *et al.,* 2008). According to an AMOVA analysis, a significant genetic differentiation among populations was estimated (Fst = 0.1551) whereas most genetic variation was within population. Similarly, analysis of Shannon-Weaver diversity index indicated that about 83% of the genetic variation observed in *Jatropha curcas* populations was due to within population variation. Although, within population variation was remarkably higher than among populations' variation, the Nei's genetic diversity, AMOVA and Shannon-Weaver diversity index indicated that the populations exhibited high degree of among population differentiation (Chaiyasist *et al.,* 1995; Chen *et al.,* 2009).

The genetic structure of plant populations is the result of several interacting factors, including life history, genetic drift, mating system, environmental heterogeneity, and gene flow (Hogbin and Peakall, 1999). Nybom and Bartish (2000) estimated genetic differentiations among populations (Fst) using RAPD markers. They found that proportion of genetic diversity among populations were 59%, and 19%, for selfing and mixed breeding systems respectively. Hamrick and Godt (1989) using allozyme data in selfing species reported that 51% of the genetic variations were partitioned among populations while in out-crossed and mixed mating system it was reduced to 10-22%. Hence, mating system could be one possible cause for higher proportion of within population diversity than among populations of this study.

Populations of the present study were sampled from heterogeneous environments. The pressure exerted by natural selection in different ecological environment drives the populations to adaptive divergence (Endler, 1986; Schluter, 2000). Some empirical studies indicated that the magnitude of this divergence is constrainted by gene flow between environments (Hendry *et al.,* 2002; Nosil and Crespi, 2004). Although *Jatropha curcas* is a recently introduced species, the roles of natural selection and gene flow have to be important for the significant genetic differentiation among populations. On the other hand, the small population size that lead to genetic drift and the vegetative (commonly used) reproduction systems of *Jatropha curcas* may contribute for the significant population differentiations.

5.2.3 Geographical and Genetic Distance

Genetic distance quantifies the degree of differences between two individuals or group of individuals (whether these are population or even species). Ideally, a genetic distance method provides values that vary between zero (when all markers are shared between two individuals or populations) and one (when no marker is shared between individuals or populations). Jaccard's genetic similarity coefficients of the fourteen *Jatropha curcas* populations varied from 0.622 to 0.884. The maximum genetic similarity was observed between *Jatropha curcas* populations growing around Chiro and population obtained form Kenya (Kenya I). The UPGM dendrogram also revealed these two populations to be closely related than any others. The genetic similarity coefficient between Kenya II and Doni indicated that these two populations were the second most similar populations and followed by similarity coefficient between Kenya I and Sawula.

Some *Jatropha curcas* populations of the current study such as Kenya I, Kenya II, Mersa, and Sawula were not clustered in the phenogram as per their geographical locations. To find out the possible reasons we looked into possibilities that allowed exchange of genetic materials among these populations. We came up with two possible cases. In the first case, the seed obtained from Green Biofuel Company (particularly for Kenya I and Kenya II) were harvested from an experiment established at Melkasa Agricultural Research Center with other accessions. In this experiment some accessions were randomized in RCBD whereas some other accessions were arranged in opportunistic way due to their own reasons. Plots of Kenya II population were adjacent to Waki Teyo (around Melkasa), Sodere, Meieso, and Chefa (geographically near to Mersa) populations. Plots of Kenya I population were adjacent to Sodere, Meieso, Cheffa and Sawula populations. Because *Jatropha curcas* is a mixed mating species, there could be exchange of genetic material (pollen) among these adjacent populations and consequently population that was considered as Kenya I in this study probably introgrested with genes from Meieso, Sodere, and Sawula populations. Thus, it was clustered with populations from Doni (near to Melkasa) and Mersa which is geographically near to Cheffa. On other hand, population considered as Kenya II probably shared some genes of Waki Teyo, Sodere, Meieso and Cheffa and showed strong genetic similarity.

In the second case, the high similarity between Sawula population and populations from eastern part of the country was due to its ancestral relationship. *Jatropha curcas* was first introduced in 1966/67 to Goffa area (Sawula) by Colonel Demeke Teseme who governed the Goffa district during this period (personal communication). According to this information Teseme brought some *Jatropha curcas* cuttings from Hararge region and planted in public nursery. Afterward the Goffa farmers expanded *Jatropha curcas* around their farmland using planting materials from this nursery. In addition to these reasons some of the Ethiopian *Jatropha* populations and Kenyan population may had common origin.

Populations from Pawe and Metema were clustered in the same group (II) and this indicate that their UPGM pattern (the genetic distance) as per geographical distance. The rest of populations (Assossa, Arusha, Shewa Robit, Jeweha, and Arbaminch), each was clustered as separate class. This indicates genetic distinctness of the populations and absence (few) of gene flow of the populations. Probably they may be from different ancestor or introduced from different source materials. In the breeding program, consideration of individuals those acquainted traits of interest from such different populations may increase genetic diversity.

5.3 Fatty Acid Profiles of *Jatropha curcas* Oil

5.3.1 Fatty Acid Composition of *Jatropha curcas* Oil

Fatty acids are molecules of lipid and derivative compounds that are synthesized in nature via condensation of malonyl-Coenzyme A units by a fatty acid synthetase complex. The fatty acids of plant origin generally contain even number of carbon atoms in straight chains, with a carboxyl group at one extremity and with double bond/s/ of the cis configuration in specific position. In higher plants, it rarely has more than three double bounds. It also may contain wide variety of functional groups such as *trans* double bonds, epoxy, hydroxyl, and ether groups.

Saturated fatty acid composition of *Jatropha curcas* oil extracted from fourteen populations varied from 18.01 to 28.94 % for oil extract from Kenya II and Arbaminch population respectively. Saturated fatty acid of different *Jatropha curcas* populations of the previous works were in this range (Chhetri *et al.,* 2008; Akbar *et al.,* 2009; Diwani *et al.,* 2009). Palmitic acid (16:0) was the dominant saturated fatty acid of *Jatropha curcas* oil and it varied from 13.75 to 22.81% of the extracted oil. Edem (2002) and Diwani *et al.,* (2009), respectively determined that 14.2 % and 18.22 % of *Jatropha curcas* oil was palmitic acid. The stearic acid composition of *Jatropha* oil of the present study ranged from 4.47 % to 6.82 % which is in agreement with result of these authors. Although it could not be detected in this study, trace amount of myristic fatty acid was reported in *Jatropha curcas* oil (Foidl *et al.,* 1996)

Unsaturated fatty acids were found to dominate the fatty acid profile of *Jatropha curcas* oil. They ranged from 71.09% (Arbamich) to 80.96% (Mersa). The mono-saturated acids (16:1 and 18:1) which are referred to as ideal fatty acid for biodiesel (Knothe, 2008) make the higher proportion of unsaturated fatty acids of *Jatropha* oil and followed by polyunsaturated fatty acids specifically (18:2 and 18:3). This is comparable with previous studies (Gubitz *et al.,* 1999). Oleic acid was the most dominant fatty acid that varied from 38.95% to 47.91% and followed by linoleic acid which ranged from 20.74% to 33.05%. Prior studies also reported that these two fatty acids were dominant in *Jatropha curcas* oil (King, 2009; Gubitz *et al.,* 1999). However, the described proportion of these two fatty acids varied and their proportion has inverse relationship in the former studies (Chhetri *et al.,* 2008). For instance, in one case the reported proportions of oleic acid and linoleic acid were 28.46 % and 48.18 % (Diwani *et al.,* 2009) respectively while in other cases, the proportion of oleic acid ranged to be 44.7 % and linoleic acid was 32.8 % (Akbar *et al.,* 2009). The linolenic acid proportion in this study ranged from 0.8% to 7. 54 %. The proportion of this fatty acid in some *Jatropha curcas* populations were higher than the results described in previous studies (Knothe, 2008; King, 2009; Gubitz *et al.,* 1999). Such high proportion of polysaturated fatty acid especially linolenic acid negatively affect oxidative stability of biodiesel (Knothe, 2008; Akbar *et al*., 2009).

5.3.2 Fatty Acid Profile versus Biodiesel Quality

The qualities of biodiesel are based on physiochemical characteristics (criteria) of methyl or ethyl esters. The fact that, fatty esters are the smallest molecules of these compounds (methyl and ethyl esters), types and proportion of fatty esters determine the physical and chemical properties (quality) of biodiesel (Browse, 1991). The values of these physical and chemical properties help to make decision on the quality of the biodiesel by comparing them with specified standards. Therefore, composition (profile) of fatty acid can determine the ability of particular oil to meet the specified criteria (standard). In particular, some properties of biodiesel such as cetane number (CN), cold-flow and cloud point properties, kinetic viscosity, and oxidative stability are significantly influenced by fatty acid profile (King *et al.,* 2009). Unfortunately, no one single fatty methyl esters is ideal when matched against all the designated standards but oils containing high level of oleate and palmitoleate (16:1 and 18:1) are desirable (Knothe, 2008). Since oils of all *Jatropha curcas* populations included in this study are dominated by monosaturated fatty esters, may be these genotypes are preferable for production of biodiesel of desirable properties.

The cetane number is a measure of delay in the combustion of the fuel from ignition. It is the most important factor of biodiesel. The minimum required value of cetane number is 47 by ASTMD6751 and 51 by EN14214 (biodiesel standard for United State of America and European Union, respectively). Higher CN value, improve engine performance until the value become 54.5 while above this value performance progress is not detected (Bamgboye and Hansen, 2008). Knothe (2008) noticed that the CN value of biodiesel increase with an increase in chain length and saturation. Similarly, Bamgboye and Hansen (2008) indicated that biodiesel with higher oleic acid composition has high CN value. Hence, *Jatropha* biodiesel from Kenya II, Mersa, Assossa, and Metema populations is expected to have higher CN value.

5.4. Target Traits of *Jatropha curcas* for Improvement

Oil yield of oil seed crops is determined by some agronomic traits such as grain yield per hectare and oil content of the seeds. These agronomic traits are influenced by growth traits such as number of branches, plant height and environmental factors. Traits such as high seed yield, high oil content, hard stem, seed toxicity, synchronous flowering, reducing thickness of testa and increasing biosynthesis of desirable fatty acids are the important target traits to be improved. The improvements of these traits will realize great contribution of *Jatropha curcas* in the energy sector.

The development of high yielding crop varieties through plant breeding has significantly increased (Evenson and Gollin, 2003). Improvement in seed yield could be achieved in a number of ways. *Jatropha curcas* is monoecious and has a male female flower ratio of 29:1 (Raju and Ezradanan, 2002). Rao and his associates (2008) indicated a correlation between female flower ratio and yield. They also noted that this trait was highly heritable. Therefore, increasing the ratio of female flower will increase seed yield productivity of *Jatropha curcas.* Yield increase in a number of plant species have also been obtained through the modification of plant architecture (Sakamoto and Matsaka, 2004). Increasing the number of branches on *Jatropha curcas* may lead to increase in number of inflorescences and ultimately the number of seeds per plant. This can be achieved through appropriate silvicultural practice and plant architecture modification. Parthiban *et al* (2009) from their interspecific hybridization experiment identified progenies that produced an average of 1.4 kilogram seed per plant at third year of establishment which is 500% higher than the loca*l Jatropha* seed yield at the same age (200-300 gram per plant).

Increment in oil content of *Jatropha curcas* seeds can be achieved by different methods. Parthiban *et al.* (2009) conducted an interspecfic hybridization experiment of *Jatropha curca*s with eight other Jatropha species. Their result indicated that hybridization of J*atropha curcas* with *Jatropha intanjiorensis* exhibited increased oil content and seed yield. The hybrid clonal progenies of BC1F1 at the third year resulted in high seed yield and high oil content (17.95- 55.26%). Increasing the oil content in seed can be achieved also by altering the expression level of enzymes in the triacylglycerol biosynthetic pathway. The regulation of seed development and triacylglycerol biosynthesis in a number of studies indicated that the possibility of increasing oil content of seed via manipulation of level of key regulators of seed oil accumulation. For example, disruption of the homeobox gene GLBRA2led to increased oil content in *Arabidopsis (*Shen *et al.,* 2006) and an over expression of soybean transcription factors GmDOF4 and GmDOF11 in *Arabidopsis* have also been shown to result in an increased seed oil content (Wang *et al.,* 2007).

The result of this experiment and former studies indicated high variation of seed oil content among population (Rao *et al.,* 2007; Sunil *et al.,* 2008; Akbar *et al.,* 2009). Kaushki *et al.* (2007) observed that high heritability and genetic gain for *Jatropha curcas* oil content. This can lead to the deduction of opportunity to improve seed oil content of this plant through conventional breeding system.

6. Conclusion and Implication for *Jatropha* Use

The quantitative traits of 14 *Jatropha curcas* populations have revealed moderate diversity. The populations were different with respect to leaf, agronomic and total plant height traits. However, the grain yield among populations although highly varied but statistically was not significant. This does not mean that populations under the present study have equal productivity. Populations under present study have ample oil but breeding program is required to produce elite genotypes with high oil content. Besides improving the existing genotype in the country introduction of superior genotypes is important.

The ISSR marker clearly showed that populations under present study exhibited higher genetic diversity than most former reported results. Some *Jatropha curcas* populations growing in Ethiopia yielded optimum first year grain yield and oil content. Moreover, biodiesel from these populations were characterized by mixture of six fatty methyl esters. The proportions of these esters of some populations indicated that *Jatropha* populations growing in Ethiopia would have high-quality physical properties such as ignition quality and oxidation stability. This entail the populations under present study are of potential for further breeding program and populations with interest of traits should be considered equally. Furthermore, the large within population diversity direct the focus of breeder to be at individual trees.

7. Recommendations and Further Research Needs

This study was conducted at single location. Since the gene by environment interaction highly affect the performance of the genotypes, similar experiments have to be carried out under different environmental conditions. The experiment was conducted at station only for a year. Some populations performed better than others and superior genotypes in terms of oil productivity were reported from some countries. These genotypes have to be evaluated under field condition for more years by selecting promising trees. Environmental factors such as light intensity and temperature can affect oil content of seed and its fatty acids proportion. Particularly, the proportions of fatty acids determine physical properties of biodiesel. Hence, describing physical properties of biodiesel for the superior genotype under different environmental condition is highly required. Identification of markers for high grain yield and high oil content genotypes will be an important research gap. Different enzymes control fatty acid synthesis pathways in oil crops. Identification of genes that are rate limiting and responsible for the synthesis of desirable fatty acids for biodiesel of *Jatropha curcas* and modifying the pathways would be an additional imperative research gap.

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Table 18. Simple statistics for 14 quantitative traits of 14 *Jatropha curcas* populations

Variable	Mean	Std. Dev	Sum	Maximum	Minimum
R43WAP	6.63	0.389	92.863	7.320	5.946
H43WAP	185.0	14.558	2603.00	211.700	161.300
NB43WAP	4.52	0.639	63.333	5.33	3.33
Grain yield	178.08	59.207	2493.00	304.186	86.100
Seed kernel %	61. 09	1.315	855.300	63.200	58.900
Kernel oil %	56.96	1.332	797.440	59.170	53.210
Seed oil %	34.80	1.438	487.360	37.395	31.819
1000-seed weight	479.46	24.442	6712.00	540.44	453.968
LL	15.01	1.016	210.233	17.050	12.900
LW	16.64	0.918	232.970	18.00	15.120
LWT	2.47	0.411	34.653	3.383	1.820
LBW	12.53	0.787	175.443	14.066	11.060
LDM	7.86	1.010	110.046	10.350	5.980
ALA	199.74	23.822	2796.00	248.247	155.549

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