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Sex determination of young nursery Jojoba (*Simmondsia chinensis* L.) plants using morphological traits in semi arid areas of Voi, Kenya

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Abstract
Jojoba (*Simmondsia chinensis* L.) is a dioecious desert shrub that produces highly valued oil for cosmetics and lubrication. Most of the existing plantations have low yields due to high ratio 1:1 of the males compared to the females. However, there is no existing morphological trait method for distinguishing sex at an early age in Jojoba. Use of morphological traits for identifying sex at the juvenile stage is economical and more practicable for field workers hence more preferred than the molecular marker technique. To overcome this problem, two experiments were carried out with an objective of identifying sex of young Jojoba plants using morphological traits. The first experiment was set up using nursery seedlings whereas the second used rooted cuttings. The experimental design was a Randomized Complete Block Design (RCBD) consisting of 8 treatments replicated 3 times. The treatments consisted of 4 males and 4 females per replicate which were selected using stratified random sampling. Foliage morphological data was collected from both the seedlings and rooted cuttings according to a modified Jojoba descriptors procedure. The nursery seedlings were raised in an open nursery whereas those of the rooted cuttings were carried out in a polythene sheet tunnel. These experiments were carried out from February to August 2012. Data was analyzed using SAS statistical package whereas means were separated by Least Significant Difference (LSD). The results for seedlings showed that foliage morphological traits for single leaf area in male seedlings (4.4 cm^2) were significantly higher ($p \leq 0.05$) compared to the female seedlings (3.2 cm^2). However, all the other foliage variables did not show any significant difference although male seedlings were greater in leaf length, leaf width, number of leaves, total leaf area and leaf area/0.3m relative to the female seedlings by 13%, 14%, 19%, 63% and 69% respectively. The females were superior in only leaf shape index by 1.4% compared with the males. On the other hand, rooted cuttings showed a similar pattern with those of seedlings although there was no significant difference in all the morphological traits measured. However, males showed higher foliage growth compared with the females in leaf length, leaf width, single leaf area, number of leaves and total leaf area by 37%, 43%, 95%, 155% and 458% respectively. The results indicate that single leaf area can be used for sex differentiation in juvenile Jojoba plants hence recommended at the nursery stage in order to determine the right planting ratio of male to female of 1: 10 respectively in the field for maximum stand production. Further research is recommended for a longer period to identify other foliage traits useful for sexing of Jojoba nursery plants.
Keywords: Jojoba plants, sex determination, morphological traits

Introduction

Over 80% of Kenya is composed of arid and semi arid lands (ASALs) with only a few crops being grown mainly for subsistence purposes (KARI, 2009). They experience frequent drought leading to crop failure hence overdependence on food relief (Barrow, 1996). In recent years, there has been considerable interest in using ASALs more productively by promoting crops which can tolerate these conditions such as *Jatropha curcas* (Ngethe, 2007) and Jojoba (*Simmondsia chinensis*) (Thagana *et al*., 2004). These are multipurpose crops, and have a potential use for rehabilitation as well as provision of income to the poor communities.

Jojoba is a high value shrub growing in the arid areas (NAS, 1984) and it is a promising cash crop for the arid-lands throughout the world. It is a native shrub of Sonoran desert of USA and Mexico (Gentry, 1958). It grows on coarse, light and medium textured well drained sandy soils with marginal fertility and acidic to alkaline pH of 5-8 (Undersander *et al*., 1990). It tolerates saline environments with low rainfall (220-400 mm per year) and high optimum temperatures between 27-33^{0}C but is susceptible to frost below -3^{0}C (Stephens, 1994).

Jojoba produces nuts with 45-55% of its weight as oil. The oil from Jojoba is similar to that obtained from Sperm Whale which is threatened with extinction (Hogan and Bemis, 1983). It is used in cosmetics, lubricant industry, pharmaceuticals (Muthana, 1981; Amarger and Mercier, 1996; Ward, 2003), electronics and computer industries (Undersander *et al*., 1990). A Jojoba stand can be in production for 100-200 years depending on management (Martin, 1983) and has a deep rooting habit (Forster and Wright, 2002). On average, one mature Jojoba bush can yield 2 kilogrammes of seed per year which in-turn gives 1 litre of oil after extraction. The seed production per hectare (ha) is 2 to 3 metric tonnes which is equated with 124 whales (Ward, 2003). Being dioecious, a seeded plantation of Jojoba has high seed variability (Inoti *et al*., 2015), genetic heterogeneity and low average yields (Benzioni, 1997). Seed raised seedlings give a ratio of 1:1 (male to female) in the field leading to low production. The recommended ratio is 1:10 (male to female) in order to obtain maximum yields per ha (Undersander *et al*., 1990). Most studies of dioecious species have focused on mature individuals (Gehring, 1990) and very little work is reported on cuttings or seedlings at the nursery stage. Similarly, there is no existing morphological trait method for distinguishing sex at an early age in Jojoba (Ince and Karaca, 2011).

In natural stands, no genetic information is available and phenotypic selection is used to identify individuals with potentially high genetic value. The main criterion for selection index is based on growth parameters such as height, crown diameter and volume (Balocchi, 1990; Thagana *et al*., 2009) as well as those characteristics of stem form and branching habit that show up early in life (Longman, 1993). Data is available on mature stands of dioecious plants showing that females allocate higher resources to reproduction than the males leading to lower vegetative growth in the former (Nicotra *et al*., 2003; Zunzunegui *et al*., 2005; Barrett and Hough, 2013). However, Wheelwright (2012) reported that females of *Ocotea tenera* compensated for higher costs of reproduction and diminished photosynthetic capacity by producing larger leaves. Kohorn (1995) did field assessment of mature plantations and found that female Jojoba plants had larger leaves and more branches whilst males were taller than females but these parameters were, also, influenced by the environment.

Use of morphological traits for identifying sex at the juvenile stage is economical and more practicable for field workers hence more preferred than the molecular marker technique which is more expensive and restricted to large scale plantations and research centres. El-Baz *et al*. (2009) has reported work on characterization of mature female Jojoba bushes using morphological traits. Clonal propagation of elite individuals of known sexuality is necessary to ensure that the plants in commercial plots will be highly productive (Chaturvedi and Sharma, 1989).

Jojoba has come at a time when there are dwindling natural resources and increased concern for the environment (Tremper, 1996). However, there are certain challenges that need to be addressed and these include; low yields, multiplication and determination of sex at early stages. An experiment was setup with an objective of using morphological traits to identify sex of juvenile Jojoba nursery seedlings and rooted cuttings. This study was aimed at solving the problem providing the right planting ratio of 1: 10 (male to female) in the field for maximum production.

Objectives

The objectives of the trial were to study: i) The morphological traits for identifying sex in nursery Jojoba seedlings, ii) The morphological traits for identifying sex in rooted Jojoba cuttings

Materials and methods

Site description

The research was conducted in Rukinga Wildlife Works, Maungu, Kenya, where Jojoba bushes have been established. This site is located about 30 km away from Voi urban centre in the coastal province. It lies in semi arid savannahs with an annual rainfall of 596 mm and an altitude of 892 meters. Soils are moderately fertile with sandy loam and gravel texture and pH of 5-7. Temperatures average at 26^0C with moderate humidity of 59% (Thagana *et al*., 2003; TTDP 2008).

Experimental designs and sampling procedures

The experimental design was a Randomized Complete Block Design (RCBD) consisting of 8 treatments replicated 3 times (Gomez and Gomez, 1984).

Identification of sex using morphological traits in young nursery seedlings

Nursery seedlings were raised in the nursery from February to August 2012 (Plate 1). The potting media used consisted of sand and farm yard manure (FYM) in the ratio of 2:1 respectively. The large seeds were selected and directly sown in potted polythene bags measuring 12.5 cm width by 20 cm length. A few nursery seedlings started flowering at 6 months due to water stress (with male to female ratio of 5:1) and this enabled selection and isolation of the males and females for the experiment. A total of 4 males and 4 females were randomly selected to constitute each replicate.

Plate 1: **A fully grown Jojoba seedling raised in an open nursery**

Identification of sex using morphological traits in nursery rooted cuttings

The treatments consisted of 4 males and 4 females per replicate. Stratified random sampling was used to select 4 female as well as 4 male bushes from which 40 stem cuttings were collected for each sex per replicate from the clean weeded bushes. Each treatment constituted a row of 10 potted plants per replicate. Therefore, there were 8 rows per replicate which were made up of 4 males and 4 females. The treatments were independently and randomly allocated in each replicate. For rooted cuttings, the experiment was carried out in a polythene sheet tunnel which was 1 m wide and 50 cm high (Plate 2), while the nursery seedlings were raised in an open nursery.

Plate 2: Polythene sheet tunnels for propagation of Jojoba cuttings under *Delonix elata* tree for shade.

Stem cuttings consisting of 5 nodes each were harvested from the middle portion of the crown using a sharp secateur sterilized by use of methylated spirit. These were collected at the dormant stage, 4-5 weeks after onset of rains. The cuttings were collected in the morning or late in the afternoon. After harvesting, they were placed inside a polythene bag and misted before transporting them to the propagation sheet.

Rooted cuttings were propagated in the polythene sheet tunnel (Plate 2) for a period of 5 months for rooting and sprouting. The potting media used was sterilized sand. A rooting Plant Growth Regulator (PGR), IBA+ boric acid was used. A volume of 5000 mgL^{-1} IBA+ 15.5 mgL^{-1} boric acid was prepared and placed in a container. Freshly harvested twigs were then quickly dipped in the PGR for 10 seconds and then planted immediately into the potted polythene bags. These containers were then left in the polythene sheet tunnel where humidity (80-95%), temperature (23-28^{0}C) and watering (at 4 day interval) were regulated. Routine procedures involved watering, misting (3 times per day) and weeding. Humidity was regulated by misting and slightly opening the ends of the sheet tunnel. Morphological data was collected from both the rooted Jojoba cuttings and seedlings according to modified Jojoba descriptors procedure outlined by El-Baz *et al*. (2009) (Table 1). Three seedlings were randomly sampled for each sex per treatment and the variables scored were as outlined in El-Baz *et al*. (2009) procedure. These rooted cuttings were raised between April 2012 and August 2012.

Table 1: Morphological descriptors for sex identification modified from El-Baz *et al*. 2009

Morphological character	Description
1) Height (cm)	Main shoot measured from the ground level to the tip.
2) Node density	Main shoot counted from the top to a certain length in cm. Node density= number of nodes in length A divided by length of A.
3) Shoot number	Number of shoots or sprouts per plant.
4) Shoot length (cm)	Average length of all the shoots. The results were represented as an average shoot length per seedling. Shoot length= total length of shoots divided by number of shoots.
5) Number of internodes per shoot	The number of internodes per shoot.
6) Internode length (cm) and thickness (mm)	The internode length and thickness of the second internode from the shoot base were measured and expressed in cm and mm by using a ruler and a veneer caliper respectively.
7) Number of leaves per shoot	The number of leaves per shoot was counted.
8) Single leaf area (cm^2)	A representative sample of three leaves from shoots on seedlings of each replicate was chosen from the second or third leaf from the top of the shoots. These were then traced on a graph paper and the number of squares inside the leaf were counted and computed to give the area.
9) Total leaf area per shoot (cm^2)	The estimation of leaf area was done according to the following equation: Total leaf area= single leaf area x number of leaves.
10) Leaf area/0.3 m of vegetative growth (cm^2)	It was calculated using the following equation: Leaf area /0.3 m of vegetative growth= Total leaf number multiplied by leaf area divided by shoot length which is the multiplied by 100.
11) Shape index of leaf per shoot	The shape index of leaf per shoot was calculated using the following formula and second or third leaf from the top were measured: Shape index of leaf (mm)= Leaf length (mm) divided by leaf width (mm).
12) Root number	Number of roots per plant.
13) Root length (cm)	Complete root length of the main root from the root collar to the tip.
14) Fresh shoot biomass (g)	Weight of the fresh above ground mass from the root collar to the shoot tip.
15) Fresh total plant biomass (g)	Weight of both fresh root and shoot biomass.

Data analysis

Analysis of variance (ANOVA) was performed on the measured variables using SAS statistical package (SAS, 1996) while the means were separated using Least Significance Difference (LSD) at $p \leq 0.05$.

Results

Use of morphological traits in identifying sex in juvenile nursery seedlings of Jojoba

Shoot morphological characteristics did not show any significant difference for male and female sex for young Jojoba nursery seedlings (Table 2). However, male seedlings showed higher growth in internode length, number of shoots and total fresh plant biomass relative to female seedlings by 16%, 10% and 1.6% respectively. Similarly, female seedlings showed greater performance in number of internodes, node density, shoot length and height compared with male seedlings by 6.3%, 4.3%, 3.1% and 2.1% respectively. High node density and number of internodes in females indicates stunted growth while long internodes translate to faster growth in males.

Table 2: Shoot morphological characteristics of juvenile male and female Jojoba seedlings

Sex	Height (cm)	Number of shoots	Shoot length (cm)	Number of internodes	Internode length (mm)	Node density	Total fresh plant biomass (g)
Male	29.2	5.5	19.3	12.7	19.6	0.47	25.7
Female	29.8	5.0	19.9	13.5	16.9	0.49	25.3
Mean	29.5	5.2	19.6	13.1	18.2	0.48	25.5
CV	7.2	17.6	10.0	3.2	41.5	4.75	15.0
LSD	7.4	3.2	6.9	1.5	26.6	0.08	13.4
$P \leq 0.05$	NS	NS	NS	NS	NS	NS	NS

Key: NS= No significant difference among the means in each column

Foliage morphological traits showed single leaf area for male seedlings (4.4 cm^2) to be significantly higher ($p \leq 0.05$) compared to the female seedlings (3.2 cm^2) (Table 3). However, all the other foliage variables did not show any significant difference although male seedlings were greater in leaf length, leaf width, number of leaves, total leaf area and leaf area/0.3m relative to female seedlings by 13%, 14%, 19%, 63% and 69% respectively. The females were superior in only leaf shape index by 1.4% compared with the males. The results show that male seedlings were superior in foliage traits relative to female seedlings with single leaf area showing significant differences.

Table 3: Foliage morphological characteristics of juvenile male and female Jojoba seedlings

Sex	Number of leaves	Leaf length (cm)	Leaf width (cm)	Leaf shape index	Single leaf area (cm^2)	Total leaf area (cm^2)	Leaf area/0.3M
Male	71.3a	3.4a	1.6a	2.17a	4.4a	310.0a	1627.3a
Female	59.7a	3.0a	1.4a	2.20a	3.2b	190.7a	965.7a
Mean	65.5	3.2	1.5	2.18	3.8	250.3	1296.5
CV	21.8	9.0	12.8	1.89	7.5	19.7	30.3
LSD	50.2	1.0	0.7	0.14	1.0	173.3	1377.9

Means with the same letter in each column are not significantly different to each other at $p \leq 0.05$

Results indicated in Table 4 show that root morphological traits did not show any significant difference for male and female sex for young Jojoba nursery seedlings. However, female seedlings showed higher growth in all the root variables measured compared with the male seedlings. Females showed higher values compared with males in root length, root collar diameter, number of roots and root to shoot ratio by 1.9%, 2%, 6.6% and 8.3% respectively.

Table 4: Root morphological characteristics of juvenile male and female Jojoba seedlings

Sex	Root collar diameter (mm)	Root length (cm)	Number of roots	Root to shoot ratio
Male	5.07	36.6	24.1	1.2
Female	5.17	37.9	25.7	1.3
Mean	5.10	37.2	24.9	1.25
CV	7.0	11.9	19.4	8.6
LSD	1.3	15.5	17.0	0.4
$P \leq 0.05$	NS	NS	NS	NS

Key: NS= No significant difference among the means in each column

Use of morphological traits in identifying sex in juvenile rooted Jojoba cuttings

Male rooted cuttings did not show any significant difference compared with the female cuttings in all the variables measured (Table 5). However, males were superior in root collar diameter, root length, height, internode length and height of new growth by 3%, 5%, 25%, 30% and 92% relative to the females, whereas, females were higher in number of roots and root to shoot ratio by 14% and 30% respectively compared with males.

Table 5: Shoot and root morphological characteristics of juvenile rooted Jojoba cuttings

Sex	Height (cm)	Height of new growth (cm)	Internode length (mm)	Root collar diameter (mm)	Root length (cm)	Number of roots	Root to shoot ratio
Male	13.8	10.2	25.3	3.1	30.2	36.0	2.3
Female	11.0	5.3	19.5	3.0	28.8	41.0	3.0
Mean	12.4	7.8	22.4	3.05	29.0	38.5	2.7
CV	41.3	7.3	24.2	9.7	17.9	53.4	37.1
LSD	60.9	6.7	64.4	3.5	61.5	235.5	11.0
$P \leq 0.05$	NS	NS	NS	NS	NS	NS	NS

Key: NS= No significant difference among the means in each column

Foliage traits in Table 6 for rooted cuttings did not show any significant differences in the entire variables measured. However, males were higher relative to females in leaf length, leaf width, single leaf area, number of leaves and total leaf area by 37%, 43%, 95%, 155% and 458% respectively.

Table 6: Foliage morphological characteristics of juvenile rooted Jojoba cuttings

Sex	Number of leaves	Leaf length (mm)	Leaf width (mm)	Leaf shape index	Single leaf area (cm^2)	Total leaf area (cm^2)
Male	14.0	35.6	15.0	2.4	3.7	56.4
Female	5.5	26.0	10.5	2.5	1.9	10.1
Mean	9.8	30.8	12.8	2.45	2.8	33.3
CV	32.6	2.8	11.0	12.3	8.4	11.8
LSD	40.0	10.4	16.8	3.5	2.9	51.6
$P \leq 0.05$	NS	NS	NS	NS	NS	NS

Key: NS= No significant difference among the means in each column

Discussion

Literature on use of morphological traits to identify sex before flowering in plants is quite limited. However, several authors have reported studies in mature plants (Culley *et al*., 2005; Wheelwright *et al*., 2012; Barrett and Hough, 2013; Teitel *et al*., 2015) and also genotype/clonal differentiation within similar sex (Benzioni, 1997; Tobares *et al*., 2004; El-Baz *et al*., 2009; Nderitu *et al*., 2014). There are no reported studies at present on the use of morphological traits for sexing in young seedlings of Jojoba. However, a few studies have been reported by some authors to characterize crops such as vanilla (Mantengu *et al*., 2007) and papaya (Reddy *et al*., 2012) before the flowering stage. Leaf morphology has been used to identify Papaya sex at seedling stage where male leaves are 3-lobed while females are 5-lobed (Reddy *et al*., 2012). The males were slower growing compared to females in papaya.

In the present study, both male and female seedlings and cuttings performed fairly equally in the overall growth since the male seedlings were compensated by the higher foliage growth while the female seedlings by root growth. This shows that there are no clear morphological characters that can be used to identify sex of juvenile Jojoba seedlings except the single leaf area.

Hoffman and Alliende (1984) reported different male trees/shrubs species having larger size and faster growth rate (*Fragaria chiloensis)* and greater biomass (*Laretia acauli*, *Lithrea caustic* and *Peumus boldus)* than females. These studies are in agreement with the present study but they are based on mature plants. Studies on *Baccharis halimifolia*, a dioecious shrub by Krischik and Denno (1990) showed that males possessed longer shoots and more tender leaves, grew faster and flowered and senesced earlier than female plants. The tenderness of leaves made males to be herbivore targets by beetles than females hence resulting to a more female bias in the field.

The results of this study corroborate with other studies by Kohorn (1994) on Jojoba in California who stated that females were smaller than the males. Kohorn (1995) also reported that females had larger leaves than males which are divergent to this study. In xeric sites, males have smaller leaves and more compact canopies than females whereas in more mesic sites, populations of Jojoba do not differ in vegetative morphology (Kohorn, 1994). Spatial segregation of sexes associated with microhabitat differences is common in dioecious tree species (Zhang *et al*., 2010).

Studies by Dawson and Bliss (1989) on *Salix arctica* in different sites, reported that males showed faster growth rate in dry site while females were faster in wet site. This may change the sex ratio in the field depending on the adaptive ability (Hultline *et al*., 2008), since males are more adapted for warmer climates (Zhao *et al*., 2012). Dioecious plants are vulnerable to change in population size and structure, thus sensitive to habitat fragmentation through human or livestock encroachment (Yu and Lu, 2011).

Male plants of perennial species are either larger, grow faster, have more ramets or have higher biomass than female plants though the growth rate could be habitat dependent (Jing and Coley, 1990). Gao *et al*. (2010) reported that climatic sensitivity in male and female trees of dioecious species is different, yet this difference is not stable through time. Elevated $C0_2$ concentration and increased air temperature caused males to increase leaf expansion rate leading to higher biomass accumulation than females (Zhao *et al*., 2012).

However, the current study was conducted in a semi arid environment, hence this accounts for some differences in the findings. This shows that Jojoba can display some level of phenotyphic plasticity which is the ability to develop different phenotypes in response to environmental conditions (Winn, 1996), is heritable and plays an important role in species evolutionary strategy (Agrawal, *et al*., 2008).

Allocation differences in dioecious species have been found in species that are markedly dimorphic, such as *Simmmondsia chinensis* (Kohorn *et al*., 1994) as well as in *Siparuna grandiflora* that is less obviously dimorphic (Oyama and Dirzo, 1988). Several studies have demonstrated that if there are no mechanisms to compensate for resource allocation to reproduction, males achieve greater growth than females (Lloyd and Webb, 1977; Popp and Reinartz, 1988; Garcia and Antor, 1995; Forero-Montana and Zimmerman, 2010; Zhao *et al*., 2012; Barrett and Hough, 2013).

Morphological studies on 24 female Jojoba genotypes by El-Baz *et al*. (2009) found considerable variability among all parameters studied ranging from seed, leaves, node, shoot, flowers and plant height. These variations observed in the morphological parameters were principally due to genotype differences. Many authors found significant variability in different morphological traits of productive characteristics, such as node density, branching, leaf shape and area, seed size and weight and wax content per seed (Yermanos and Duncan, 1976; Hogan *et al*., 1980; Nagvi and Ting, 1990; Gaber *et al*., 2007; El-Baz *et al*., 2009; Inoti *et al*., 2015). The natural degree of variability was found to be enormous, and this gives a geneticist a huge range of possible gene combinations for future selection and clonal improvement of Jojoba.

Previous studies on growth characteristics between males and females of dioecious plants have shown that females are smaller than the males (Hoffman and Alliende, 1984; Vasiliauskas and Aarsseen, 1992) and the females grow more slowly (Jing and Coley, 1990; Cipollini and Whigham, 1994; Reddy *et al*., 2012). This study is consistent with studies on *Schiedea salicaria* which showed evidence of sexual dimorphism where males had higher mass based photosynthetic rate and specific leaf area than females (Culley *et al*., 2005).

Work by Lambers *et al*. (1998) noted that females had thicker and denser leaves leading to lower specific leaf area, which could potentially slow their growth rate. Studies on *Silene latifolia* by Gehring and Monson (1994) and Larporte and Delph (1996) agrees with this study since higher leaf size and leaf area lead to higher photosynthesis and more carbon accumulation in males than females of dioecious plants. These differences are thought to exist because females allocate more resources to reproduction than males and therefore should have fewer resources for vegetative growth (Wilson, 1983). Other research works by Li *et al*. (2007) on *Hippophae rhamnoides*, a deciduous shrub in Southwest China showed that females had a higher specific leaf area than males along an altitudinal gradient which contradicts the current findings.

Further work by Cepedo-Cornejo and Dirzo (2010) on Neotropical palms reported that asymmetrical allocation to reproduction by females may lead to reproduction-growth tradeoff, where female plants grow less than male plants, but invest more in defense and thus experience lower herbivory than male plants. Studies in *Corema album*, a dioecious plant in Iberian Peninsula, showed that reproductive effort was 3 times higher in females than in males or hermaphrodite plants (Zunzunegui *et al*., 2005).

Male plants of perennial species are either larger, grow faster, have more ramets or have higher biomass than female plants though the growth rate could be habitat dependent (Jing and Coley, 1990). Dudley (2008) stated that comparisons of short term studies can be misleading because correlations of fitness and growth to physiological traits varies widely among plant species, environmental conditions and life stages. Hence long term growth studies rather than short term physiological studies are recommended. The present study demonstrates sex-based differences in growth at the nursery stage. However, similar work by Nicotra (1999) reported studies on *Siparuna grandiflora*, a dioecious shrub on the vegetative traits for sex-based differences in both cuttings and mature plants.

Results on pre-reproductive males showed larger leaves and leaf area than females while mature females allocated less biomass per unit stem length than males (Nicotra, 1999). These findings are in agreement with this study. Females allocate more biomass to reproduction and the latter has negative effect on the growth of females but not in males. Other authors have identified a wide range of dioecious species with pattern of growth equivalent despite reproductive allocation (Gehring and Linhart, 1993; Ramadan *et al*., 1994).

Females of *Ocotea tenera* compensated for higher costs of reproduction and diminished photosynthetic capacity by producing larger leaves (Wheelwright *et al*., 2012). Jing *et al*. (2008) reported that net photosynthetic rate of female ginkgo was significantly higher than males. Similarly, studies on *Rumex hastatulus*, a wind pollinated annual plant, provides support for high male reproductive costs (Teitel *et al*., 2015) which is contradictory to the present study. The variation could probably be explained by the fact that the current study used a long-lived shrub.

The results will provide a cheap and practical method of identifying sex in Jojoba at the nursery stage in order to achieve the recommended ratio of 1:10 of male to female for increased productivity in ASALs. The technique will be useful to farmers, researchers and policy makers.

Conclusion

Foliage traits for the males of Jojoba cuttings and seedlings were superior relative to the females. Single leaf area was found to be significantly superior for the males as compared to the females hence used to determine sex in Jojoba seedlings.

Single leaf area morphological trait may be used for sexing of Jojoba seedlings especially after stressing of the seedlings. Morphological studies can be improved in future by sampling at juvenile and mature stages. More research is recommended to identify other foliage traits for sex differentiation in Jojoba as well as determining the level of water stress that can trigger flowering in nursery seedlings.

Further research should be carried out for a longer period of 9-12 months in the nursery on normal non stressed seedlings after which they can be stressed by reducing the watering regimes in order to identify their sexes. The current study was carried out for a period of 7 months. A further delay in the nursery through use of bigger pots up to 4 litres can help to identify sex by allowing flowering to occur before field planting, although this option is more expensive.

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