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A comparative study of genetic relationships among and within male and female genotypes of dioecious Jojoba (*Simmondsia chinensis* L. Schneider) using RAPD and ISSR markers

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ABSTRACT

The female plants of dioecious plant species, jojoba (*Simmondsia chinensis* L. Schneider), is popularly known for its seed oil (jojoba oil), which is of great economic importance in industry, medicine and food. The sex of the seedling remains indistinguishable until the first flower buds appear which may take up to four years. Two types of arbitrary molecular markers, RAPD and ISSR were employed for genetic diversity analysis and identification of sex at an early/seedling stage in jojoba. A total of 756 (732 polymorphic) and 363 (339 polymorphic) bands were detected using 72 RAPD and 48 ISSR primers, respectively. The salient features of RAPD and ISSR marker data analyzed using dendrogram constructed using UPGMA from a genetic-similarity matrix and Mantel test are as given below: (i) Both RAPD and ISSR analysis showed high level of genetic diversity (96 and 91.4%, respectively) among the 39 jojoba genotypes studied; (ii) In RAPD analysis, most of the genotypes formed small clusters based on their sex, but dendrogram based on ISSR data showed a more-complicated genetic variation pattern with interspersed male and female genotypes; (iii) RAPD and ISSR marker data-sets showed low and positive correlation (Mantel test, r = 0.348, $p < 0.01$). As many as ten RAPD primers amplified eleven specific bands, which appeared exclusively in male plants at a frequency ranging from 25% to 62.5%. Similarly, three RAPD primers amplified bands unique to females, at a frequency of 53.3% to 73.3%. Validation of these sex-specific markers using other known male and female plants confirmed their sex specificity as well as reproducibility of these markers. Thus, a set of two or more of male or female specific markers could differentiate between the sexes of jojoba with more than 90% accuracy.

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Jojoba (*Simmondsia chinensis* L. Schneider), the sole species in the family Simmondsiaceae, is an evergreen dioecious perennial angiosperm, native to sonoran desert (southwest Arizona, Northern Mexico and Baja California). It is a tetraploid with X = 13, perhaps a product of allopolyploidization (Lee *et al*., 1985). The shrubs grows well at extreme temperatures, ranging from 120^{0}F (48^{0}C) to 16^{0}F (-9^{0}C) and in marginal lands affected by drought and salinity which are unproductive for conventional crops. Jojoba oil is of economic significance in industry (lubricants and varnishes), medicine (antibiotic production and as a treatment of skin disorders) and food (oil substitute) (Benzioni and Forti, 1989; Verschuren, 1989). Jojoba is an outcrossing crop (Kohorn *et al*., 1994) and lacks practical key for sex identification and diversity assessment.

The sex of the jojoba plant remains indistinguishable until the onset of flowering, which takes at least 3-4 years of growth. There is little or no sexual dimorphism as observed in most of the dioecious flowering plants (Llyod and Webb, 1977). The genetic basis of sex determination is extremely diverse in dioecious plants and several distinct genetic mechanisms underlying dioecy have been reported (Durand and Durand, 1990; Negrutiu *et al*., 2001). Of the 1,41,620 dioecious species, only a tiny fraction has evolved sex chromosomes and still lesser exhibiting heteromorphic sex chromosomes (Ming and Moore, 2007), which provide cytological key for sex diagnosis. In some other dioecious species, such as asparagus (Uno *et al*., 2002), sex is determined by single or multiple autosomal nuclear loci. In contrast, the sex in wood sorrel (*Rumex acetosa*) and Hops (*Humulus lupulus*) is influenced by a dosage compensation mechanism (Ainsworth *et al*., 1995). Yet, the influence of epigenetic factors such as environmental conditions and phytohormones are known to control sex determination in some other dioecious plant species such as *Mercurialis annua* (Louis, 1989).

The mechanism of sex-determination in dioecious jojoba still remains to be understood. In jojoba, aberrant sex-ratios are known to be associated with environmental factors (Cole, 1979). In jojoba, where distinguishable heteromorphic chromosomes have not been identified (Lee