Jojoba Seed: Phenotypic within Plant Variability in Wax Content and Composition

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ABSTRACT

Data are presented on the wax content and composition and seed weight of 7 individual plants in Aguanga, California. Mean wax content was 48.5% and average range 17.6 percent units. Mean seed weight was 0.38 g and mean range 0.43 g. The range in seed weight wax content and composition among seeds within single plants was comparable in magnitude to that found among single plant means. It appears that most of that variability is due to environmental factors. Thus, selection for these traits should be based on single plant performance; propagation should not be based on analyses of single seeds harvested in bulk from several plants. Correlations between fatty acids and alcohols among seeds within plants were the same as those found among single plant samples. Seeds of jojoba can be cut in half transversely without impairing germination of the basal half including the embryo axis. The upper half can be used to obtain a wax sample for single-seed analyses.

INTRODUCTION

One of the major factors which will determine if jojoba [*Simmondsia chinensis* (Link) Schneider] will become a commercial crop is the development of superior cultivars with high seed and wax yield and desirable wax composition for as many industrial applications as possible. Since genetic advance under selection depends on the amount of genetic variability available in the parental breeding material, it would be very helpful to jojoba breeders to know how much genetic variability exists within the various natural populations of jojoba from which they obtain their basic parental seedstocks. The components of phenotypic variability are best identified when populations or individuals of known genetic make up are available, and when the mode of inheritance of the characteristics studied is known. No such information is available for any of the commercially significant characteristics of jojoba and little is known about the genetic make up of the existing natural populations. The data reported in this study represent an attempt to arrive at some preliminary conclusions on the components of phenotypic variability in jojoba seed selections available at the University of California in Riverside. In the course of these studies a technique was developed for analyzing jojoba seeds without destroying their viability. With this technique, seeds with valuable compositional characteristics can be germinated to produce progeny and make their genes available for further breeding.

No information is available on the genetic determination of the quantity and quality of wax in wax-bearing seeds. In spite of the possibility that the biosynthesis of waxes is totally different from that of triglycerides, a jojoba breeder might need to keep in focus some of the basic findings reported on the inheritance of oil quality and quantity in oilseeds. Of particular significance might be the following two basic genetic principles found to be valid in practically all oil crops: (a) the oil content of a seed is determined by the genotype of the maternal plant on which the seed is developing; (b) the fatty acid composition of the oil is determined largely by the genotype of the seed itself, especially in seeds which, like jojoba, have only traces of endosperm tissue. It would be of particular interest to breeders to establish whether these principles are also valid for jojoba.

In view of the fact that jojoba is dioecious all jojoba seed is "hybrid." If the above two principles were valid in the case of jojoba, individual seeds developing on a given maternal plant could vary in wax composition because of genetic reasons if they received mixtures of pollen from male plants carrying different genes controlling particular fatty acids and alcohols. The same seeds, however, would not exhibit any genetic variability for wax content since they all came from the same maternal plant. In the case of individual jojoba plants of native populations, variability in wax content and quality among seeds of each plant arising from environmental sources is superimposed on the genetic variability present. Major sources of environmental variability could be: (a) date of pollination of individual flowers, (b) position of seed on a branch and of that branch in the plant, (c) mega- and microclimatic conditions around each developing seed, (d) number of seeds per flower as well as number of flowers per axil. When seeds bulked from several maternal plants are studied, variability from genetic and environmental sources among plants is added to the within-plant variability described earlier. The above concepts were kept in view in the interpretation of the data obtained in this project whose objective was to study the amount of variability in wax content and composition in individual seeds harvested from single plants from the native population of jojoba in Aguanga, CA.

MATERIALS AND METHODS

One of the major needs of this study was to develop a nondestructive method of determining the quantity and quality of wax in single seeds of jojoba. Previous work with wideline nuclear magnetic resonance (NMR) proved that this method is quick and accurate in determining the wax content of single seeds (1). What was to be further developed, therefore, was a nondestructive method of obtaining a wax sample, after the completion of the NMR determinations, from individual seeds for chemical analysis.

Two techniques were tried. One consisted of cutting off part of the seed for analytical purposes and germinating the remainder to obtain plant progeny of the seed analyzed. The part of the seed cut off, in one treatment, consisted of the one cotyledon carefully excised without damaging the embryo axis that remained attached to the other cotyledon (Fig. 1). The excised cotyledon was crushed in a tissue grinder with 5 cc of petroleum ether. The petroleum ether was taken up with a pipette stoppered with glass wool at the tip to prevent solids from entering the pipette and was transferred to 12 x 1 cm test tubes. The wax was recovered in these test tubes by evaporating the petroleum ether at room temperature by blowing nitrogen gas in each of them. The wax was then hydrolyzed by ethanolysis and the resulting acid and alcohol ethyl esters were analyzed by gas liquid chromatography (GLC) as described in an earlier report (2). In another treatment, the seed was cut transversely in two halves (Fig. 1). The basal half of the seed carrying the embryo axis was germinated while the opposite half was crushed as previously described to obtain a wax sample for GLC analysis. Germination of these half seeds was done in

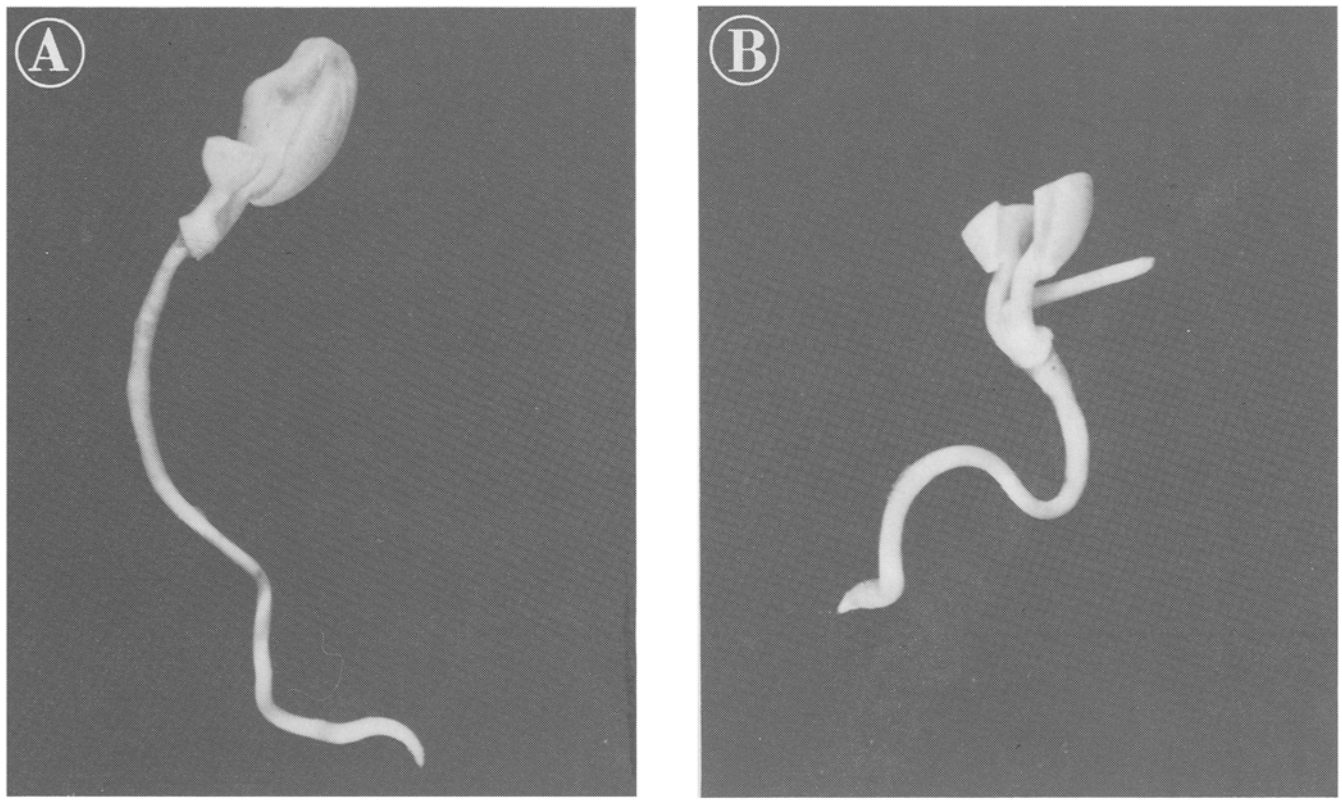

FIG. 1. Germinating seeds of jojoba with one cotyledon excised (A) or with both cotyledons cut off transversely at midpoint of long seed axis (B).

petri dishes placed in germinators set at 27 C constant.
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The other technique of obtaining a wax sample consisted of immersing individual whole seeds in a lipid solvent at room temperature in test tubes for periods ranging from 1-7 days. Upon termination of the immersion period, the seed was tested for germination as previously described, while the solvent was evaporated by blowing nitrogen gas in each of the test tubes, kept at the boiling-point temperature of the respective solvent used in each case. Solvents used were petroleum ether, ether, hexane, chloroform, acetone, benzene, ethanol and carbon tetrachloride.

The first of the half-seed techniques described above was used to obtain samples of wax from 7 samples of 200 seeds each randomly taken from a bulk sample of seeds harvested from each of 7 plants in Aguanga, CA. Data recorded on these seeds were seed weight, wax content, fatty acid and fatty alcohol composition.

RESULTS

Seeds with either one entire cotyledon or with half of each of the two cotyledons excised germinated as successfully as whole seeds. No adverse effects were observed on later plant development because of the removal of the cotyledonary tissue. Paired samples of wax extracted from each of the two cotyledons of over 200 seeds had the same fatty acid and alcohol composition. The same was true when comparisons were made between the two halves of each of over 200 seeds cut transversely. Cutting the seed transversely is less likely to damage the embryo axis comapred to removing one of the two cotyledons; therefore, this type of sectioning is preferable. Thus, a nondestructive method of determining the wax content and composition of individual seeds of jojoba would consist of two steps: first, determining the wax content of each seed by NMR; second, cutting the seed in two halves transversely and using the half with embryo axis for germination and the other half to obtain a wax sample for GLC analysis.

When whole seeds were immersed in lipid solvents to obtain a wax sample the following observations were made. After 8 days of immersion, the amount of ether-extracted wax corresponded to 3.9% of the original weight of the seed prior to immersion. The other solvents extracted the following quantities, respectively: chloroform and benzene 2.2%, petroleum ether 1.5%, carbon tetrachloride and hexane 0.8%, acetone and ethanol 0.1%. Ethanol, in particular, extracted additional seed constituents, other than wax, which were not identified. The wax extracted by the various solvents had the same composition except in the case of acetone and ethanol. Wax extracted by the latter had a significantly higher 18:1 acid content and a significantly lower 20:1 acid content. Solvent-soaked seeds retained amounts of hexane, chloroform, benzene, and carbon tetrachloride corresponding to 1.5-2% of the original seed weight even after drying for 48 hr at 60 C.

Much greater amounts of wax could be extracted from the seed if a small area of seed coat tissue were peeled off or even if the seed were merely "nicked" (Fig. 2). Such treatments of the seed were abandoned, however, because they lowered seed viability to a point where the method could no longer be called nondestructive.

The nondestructive seed analysis technique was used to develop information on the wax content and composition of single seeds harvested from individual plants from the native jojoba population in the Aguanga, California, area.

The frequency distribution for wax content and seed weight of 200 single seeds of one of the single plants studied (no. Ag 306) is shown in Figures 3 and 4. The range in wax content was from 40.9-55.8%, the mean 48.5% and the co-efficient of variation 5.6. Similar values and frequency distribution were obtained from 200 seed samples from each of 6 other individual plants: the mean wax content of these 7 plants was 48.5% and the average range 17.6 percent units. The range in weight of individual seeds from

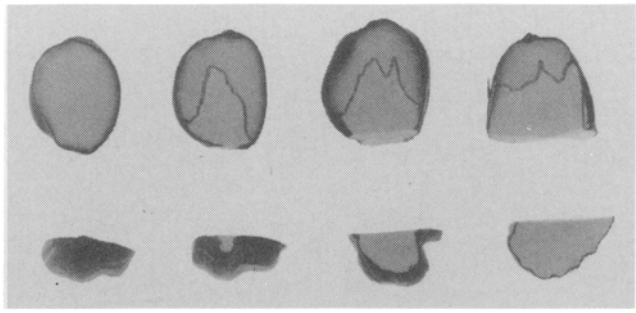

FIG. 2. Inbibition of petroleum ether by jojoba seed following 7 day immersion. Far left seed entire, others with sections of the seed removed. Line traced on seed to indicate depth of inbibition.

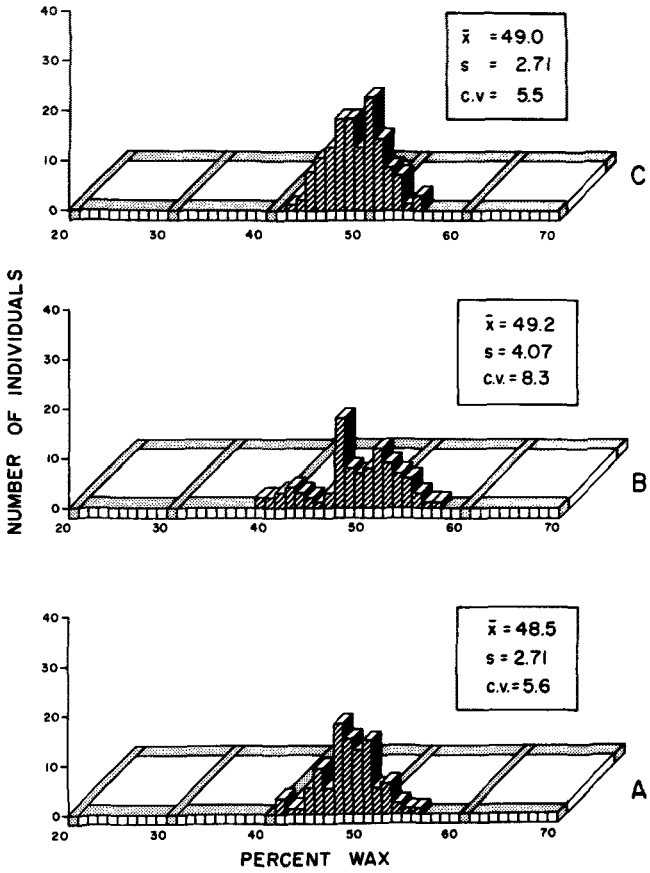

FIG. 3. Variability in percent wax content of individual seeds of jojoba from: (A) one plant (no. Ag 306) in Aguanga, Calif. (B) a bulk sample from several plants in Jacumba, Calif., and (C) a bulk sample for several plants in Aguanga, Calif.

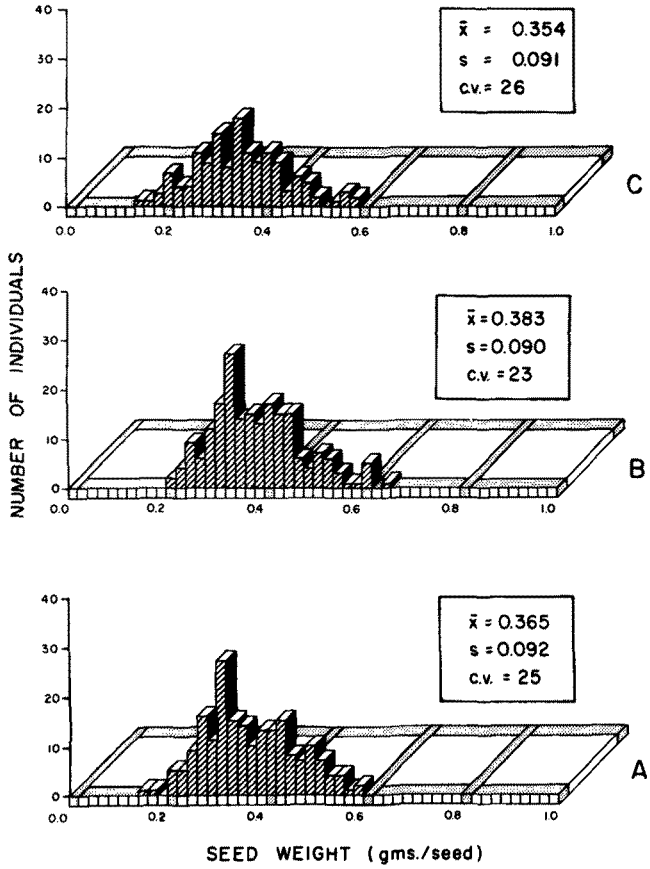

FIG. 4. Variability in seed weight of individual seeds of jojoba from: (A) one plant (no. Ag 306) in Aguanga, Calif., (B) a bulk sample from several plants in Jacumba, Calif., and (C) a bulk sample from several plants in Aguanga, Calif.

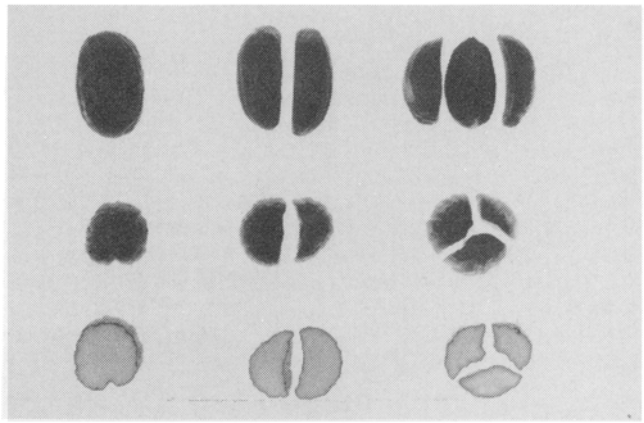

FIG. 5. Shape of jojoba seed when 1 (left), 2 (center), or 3 (right) seeds per fruit develop.

plant Ag 306 was 0.14-0.60 g, with a mean of 0.36 and a coefficient of variation of 25. Similar values and frequency distributions were obtained from the seed of the 7 plants which had a mean weight of 0.38 g per seed and a mean range of 0.43 g. The correlation between wax content and seed weight was positive but it was significant only in 4 of the 7 single plants studied. Observations on seed morphology were made later to elucidate the reasons for this inconsistency. Jojoba fruits are spherical or cylindrical in shape and may potentially produce 3 seeds each. In some cases 2 of the 3 seeds abort at an early stage of development and only a single cylindrical or spherical seed per fruit reaches maturity. When only 1 seed aborts, two mature seeds per fruit develop which, together, have approximately the size and shape of a single seed, if there were only one seed developing per fruit. Each of these twin seeds is about half as heavy as the latter and crescent shaped in cross section (Fig. 5). Thus a seed may be small simply because it is a member of a pair of twin seeds or, less often, of a triplet of seeds, irrespective of other genetic and environmental factors that affect seed size. Due to variable numbers of seeds per fruit, to study the effects of seed size on wax content, the following procedure was followed. The seed of each of 20 plants was divided into 2 groups on the basis of their origin as single or twin seeds per fruit. Each of the two groups was further subdivided into 2 subgroups, one consisting of large and one of small seed, on the basis of single seed weight (seeds were classified as large or small depending on whether their single seed weight was above or below the respective group mean single seed weight). Wax content

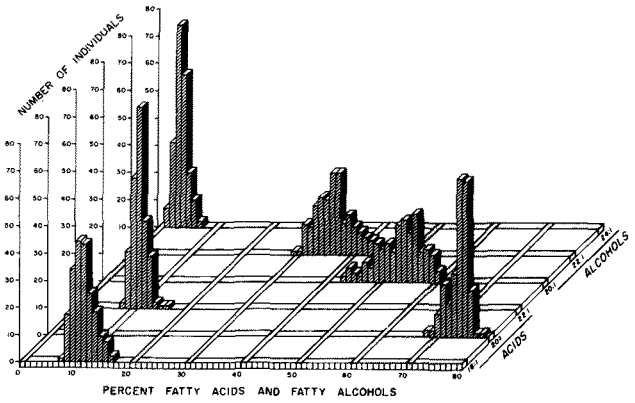

FIG. 6. Variability in fatty acids and alcohols in individual seeds of jojoba plant no. Ag. 306.

determinations of 30 seed samples from each of the 4 subgroups of seed from each of 20 plants taken at random indicate that the mean wax content of the 20 single-large seed samples (52.6%) was significantly higher ($P<0.05$) then that of the single-small seed samples (49.3%). Mean seed weight of the large seed samples was 0.54 g and of the small 0.30 g. Similarly, the mean wax content of the twin-large seeds (51.2%) was significantly higher ($P<0.05$) than that of the twin-small seeds (47.4%). Seed weight of the twin-large seed was 0.36 g, of the twin-small seeds, 0.19 g. The difference between the mean wax content of the single-large seed samples and the twin-large seed samples was not significant. In summary, wax content was correlated with seed size within each of the two groups studied, i.e., the single and twin seed groups. The inconsistency observed in the correlation between seed weight and wax content might be due to the fact that when seeds were analyzed no discrimination was made between single- twin- or triple-seeds. Large twin seeds, although weighing only about 60% as much as single-large seeds, had the same wax content as the latter.

In addition to the frequency distribution for wax content and seed weight of single seeds from a given plant, Figures 3 and 4 show the frequency distributions for the same characteristics in a bulk sample of 200 seeds obtained from several dozen plants in the Aguanga area, and in a similar bulk seed sample obtained from the area of Jacumba, CA. The range of variability for the two traits studied is of very similar magnitude in all 3 samples. Since in the single plant sample we are dealing with environmental variability only (assuming that seed wax content is not modified by pollen effects), but in the other two samples with both genetic and environmental, these figures indicate that environmental variability makes up the major portion of phenotypic variability observed in this case.

Figure 6 shows the frequency distribution of percent fatty acid and alcohol composition of the same 200 seed sample obtained from single plant no. Ag 306. Alcohols 20:1 and 22:1 appear to have a much wider range than the 3 fatty acids and alcohol 24:1. The fatty acid and alcohol composition of the other two bulk seed sampels from Aguanga and Jacumba gave essentially comparable frequency distributions which are omitted here. In a previous study (3) it was reported that variability in wax composition among plants in the Aguange population was small. Consequently, the pollen effects, if any, on wax composition would also be expected to be small. Thus one would be led to postulate that the restricted variability in composition, too, comes mostly from environmental factors. Compositional uniformity in this case is an asset in terms of marketing the wax extracted from seed harvested from the Aguanga population.

TABLE I

Simple Correlation Coefficients Between Fatty Acids and Alcohols in Single Seeds of One Jojoba Plant[a]

	Fatty Acids			Alcohols	
	18:1	20:1	22:1	20:1	22:1
Fatty acid 20:1 (%)	−.79[b]				
Fatty acid 22:1 (%)	−.64[b]	−.11			
Alcohol 22:1 (%)	.75[b]	.08	−.92[b]		
Alcohol 22:1 (%)	−.77[b]	.07	.89[b]	−.89[b]	.88[b]
Alcohol 24:1 (%)	−.65[b]	−.09	.78[b]	−.85[b]	.88[b]

[a]Based on 200 single seed analyses

[b]Significant, $P<0.01$.

Table 1 shows simple correlation coefficients between fatty acids and alcohols in 200 single seeds of jojoba from plant no. Ag 306. Identical correlations were obtained in the other 6 single seed samples of 200 seeds each. The signs and significance relationships of these coefficients are identical to those reported when mean wax composition of single plants was studied (3). These correlations could be of considerable help in biochemical studies for the establishment of the pathways that regulate the production of wax esters. It is of particular interest to note that acid 20:1 is significantly correlated with acid 18:1 only.

The results discussed have certain implications in relation to selection procedures in jojoba. Phenotypic variability in seed wax content and quality and in seed size within plants is of comparable magnitude as among plant means for the same traits (3). Thus, selection for these characteristics would be most efficient if seed from superior plants, on the basis of single plant performance, was used for propagation and further breeding. Selecting superior single seeds from bulked samples obtained from harvesting large numbers of plants could result in slow progress, if any. Finally, comparisons for wax content would be more dependable if seeds of the same type were compared (i.e., single, twin, or triplet).

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