Bot. Bull. Acad. Sin. (1995) **36:** 1–8

Genetic variability and relationships among ten populations of rubber rabbitbrush (*Chrysothamnus nauseosus* ssp. *hololeucus*) determined by RAPD analysis of bulked genomic DNA samples

David R. Gang and Darrell J. Weber[1]

Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602, USA

(Received September 5, 1994; Accepted December 1, 1994)

Abstract. *Chrysothamnus nauseosus* (rubber rabbitbrush) has the potential to be an economically feasible crop. This plant produces significant quantities of secondary chemicals and is a large shrub able to grow in a wide range of environmental conditions. Ten geographically distinct populations of *C. nauseosus* ssp. *hololeucus* were analysed by random amplified polymorphic DNA (RAPD) analysis using bulked genomic DNA. A DNA extraction procedure was modified for use with *C. nauseosus*, and produced ample quantities of good quality DNA for RAPD analysis. Forty 10-mer random primers were used in the analysis of the bulked genomic DNAs. Of these primers, 24 produced RAPD bands in all populations. A total of 330 bands were scored. The banding patterns clearly mirrored the geographical grouping of the rabbitbrush populations, indicating that RAPD can be used in phylogenetic studies of *Chrysothamnus*. A high level of genetic variation was observed in these ten populations: 63.3% of the bands were polymorphic and 18.2% of the total bands were population specific. The high level of genetic variation could be used in breeding programs to increase the value of this shrub as a crop species. RAPD markers are easily identifiable and could be linked to important traits.

Keywords: Bulked genomic DNA; *Chrysothamnus nauseosus*; Interpopulation variation; RAPDs; Rabbitbrush.

Introduction

Renewable energy resources from plants have considerable appeal, particularly if the products can be grown in low cost areas that are not involved in intense agriculture, and because they provide a sustainable system rather than a resource that becomes depleted. An example of such a renewable plant hydrocarbon resource is rubber rabbitbrush, (*Chrysothamnus nauseosus* [Pallas] Britt.) a shrub of the Intermountain West in the United States, southern Canada, and northern Mexico. Its range spreads from New Mexico and northern Baja California in the south to southern Alberta and British Columbia in the north (Anderson, 1984). Rubber rabbitbrush grows in a wide range of environmental conditions from sea level to 3000 m, even under high stress conditions on semi-arid lands and disturbed and saline soils (Weber et al., 1985).

Rabbitbrush produces considerable amounts of secondary chemicals in the form of rubber, resins, and terpenoid compounds (Bhat et al., 1990), the concentrations of which appear to be under seasonal control. The average dry weight, per-plant biomass for plants grown under very favorable conditions is 29 kg (McKell and Van Epps, 1980). The average number of rubber rabbitbrush plants per hectare in a normal population is 2632 (McKell and Van Epps, 1980). Rubber content as high as 7% of the plant dry weight has been reported, with an average molecular weight of 542,641 (Bhat et al., 1990). The highest rubber content (7%) occurs during the summer, when the resin content is the lowest. In the spring and fall, the resin content is the highest (20% to 30% dry weight) and the rubber content the lowest (Hegerhorst et al., 1988). The resin content can make up as much as 35% of the biomass. Buchannon et al. (1978) evaluated over 100 plants as potential hydrocarbon crops, and rubber rabbitbrush rated high—11.5% of the plant is usable hydrocarbons. *Chrysothamnus nauseosus* ssp. *hololeucus* is a common, widespread variety of rabbitbrush that has average rubber and resin production.

It would be valuable to regulate the production of the secondary products in rubber rabbitbrush. Once production of these various hydrocarbons is linked to genetic markers, it should be possible to selectively breed certain lines of rubber rabbitbrush for increased rubber or resin production, making it an economically important agricultural plant capable of being grown even in harsh environments where other crops fail. Before this can be done, it is necessary to determine how much genetic variability exists within the various subspecies of rubber rabbitbrush and to know whether the traits would be heritable to any significant degree.

Random amplified polymorphic DNA (RAPD) has shown great promise in determining genetic variability within species and in showing relationships between populations. This technique has recently been used to

[1]Corresponding author.

investigate a wide variety of plant groups, such as *Lycopersicon*, *Stylosanthes*, *Carica*, *papaya*, *Allium*, *Brassica*, and *Juniperus* (Williams and St. Clair, 1993; Kazan et al., 1993; Stiles et al., 1993; Wilkie et al., 1993; Demeke et al., 1992; Adams and Demeke, 1993). A method of great promise for rapid estimation of variability within species involves bulking the DNAs from members of a population and using the bulked DNA in RAPD reactions. Fu and Pauls (1993) have demonstrated that this procedure correctly identifies relationships between alfalfa populations. Once variability is determined, the bulked DNAs can be used in bulk segregant analysis to screen for markers linked to specific regions of the genome (Tingey and Deltufo, 1993). If this method of DNA analysis is applied to rubber rabbitbrush, it may be possible to identify RAPD markers that segregate with agronomically important traits.

To our knowledge, neither RAPD nor any other genomic DNA analysis work has been attempted on rubber rabbitbrush. The purpose of this research was fourfold: to find a DNA extraction procedure that would work well with a plant as chemically active as *Chrysothamnus*; to tailor RAPD analysis protocols to *Chrysothamnus* DNA to obtain clear and reproducible results; to determine the level of variability within the *hololeucus* subspecies; and to determine genetic relationships between ten geographically distinct populations of subspecies *hololeucus*.

Materials and Methods

Population and Tissue Selection

Ten local breeding populations of *Chrysothamnus nauseosus* spp. *hololeucus* were selected and labeled according to their geographic locations around and near Utah County in central Utah (*see* Figure 1): Provo (PR), Provo Canyon (PC), North Orem (NO), Lehi (LE), Utah Lake (UL), Goshen (GO), Santaquin (SQ), Nephi (NE), Fountain Green (FG) and Spanish Fork Canyon (SC). Leaves were collected from ten randomly selected members from each breeding population—leaves of a wide range of ages were chosen because of the diversity of ages present within each of the populations. The plants selected in each local population were growing within a 10 m radius of each other. The selected plants were tagged with metal disks for future reference. In June 1993, small stems were clipped from actively growing plants and immediately placed on ice. Leaves were plucked from the stems on the day of the clipping and immediately used for DNA extraction.

Population Description

The sample populations were chosen to represent a wide range of environmental and ecological conditions. Many of the populations (PR, PC, LE, GO, NE, and SC) were from roadside, or otherwise disturbed, communities; the rest of the populations (NO, UL, SQ, and FG) were more stable, climax communities. Many of the sites (PC, LE, UL, GO, NE, and FG) had a significant water source, either stream bed or run-off from roads, while the rest (PR, NO, SQ, and SC) occupied sites with no apparent water source other than rain or snowfall. Most of the populations (PR, NO, LE, GO, NE, SC, and FG) were from relatively flat sites; the remaining populations (PC, UL, and SQ) grew on significant slopes. The individuals chosen for DNA extraction represented a wide range of ages, from new seedlings to very old.

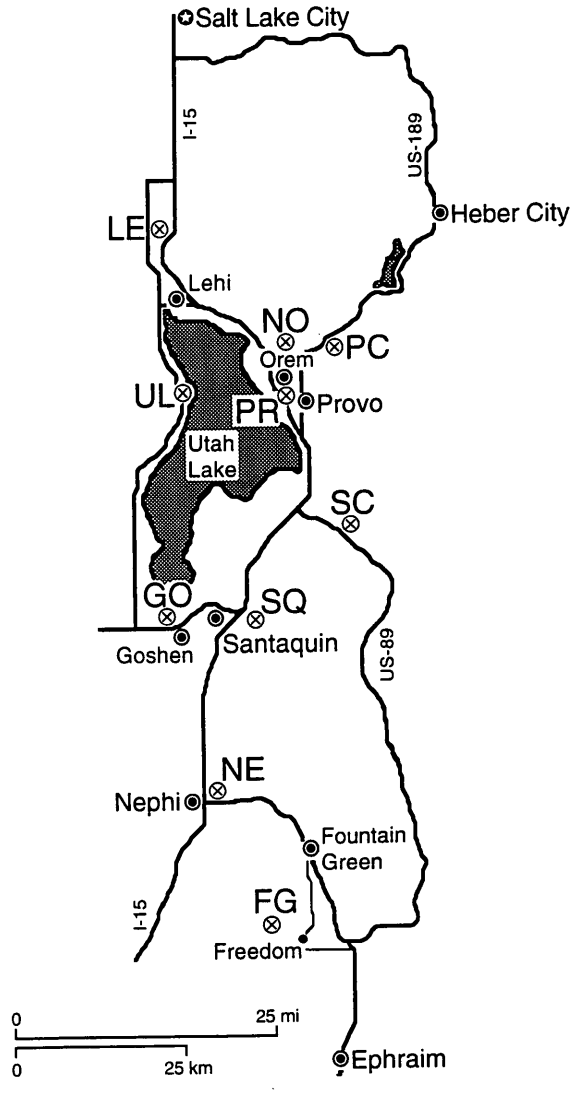

Figure 1. Geographic distribution of the 10 *Chrysothamnus nauseosus* ssp. *hololeucus* populations. Location indicated by population label.

Most polymorphism in a species is concentrated between local populations rather than within them (Hillis, 1987). Hillis (1987) recommends taking a number of small samples throughout the range of the subspecies rather than taking a large sample in a concentrated area. In line with this, ten individuals in each of ten different populations were chosen for this investigation, rather than one hundred individuals from one large population. It was desirable to determine if the differences found between the DNA of individuals or populations could be correlated to some external factors, such as community type or geographical location.

DNA Extraction

First we attempted a traditional SDS DNA extraction of *Chrysothamnus* leaves. The yield was low and a significant amount of time was needed to remove unwanted carbohydrates from the DNA extracts. For this reason, a procedure using CTAB (hexadecyltrim-ethylammonium bromide) was attempted and modified. DNA was extracted from the leaves using a modification of the procedure of Rogers and Bendich (1985). The DNA was extracted separately from each individual and bulked after quantification as described. 160 mg of fresh young leaf tissue from each individual was placed in a mortar, covered with liquid nitrogen, and hand-ground with a pestle until the leaf material began to thaw. One ml of extraction buffer (2% CTAB, 150.0 mM Tris HCl [pH 7.8], 15.0 mM NaEDTA [pH 8.0], 1.05 M NaCl, 0.2% β-mercaptoethanol [added to buffer just prior to extraction]) was immediately added and the mixture was ground until smooth (about 1 min). The ground leaf mixture was transferred to a 1.5 ml microcentrifuge tube and left at room temperature until the samples from all ten individuals from the population were ground. The samples were then incubated in a water bath at 68°C for 1 hour. After incubation, the samples were placed at room temperature for 30 minutes. 100 μl of 3.0 M sodium acetate was then added to each microcentrifuge tube and the tubes were gently inverted for 10 seconds to mix. The mixture was separated in a Beckman Microcentrifuge at high speed for 5 minutes. The supernatant liquid from each sample was transferred to a new 1.5 ml microcentrifuge tube, and the pellets were discarded. An equal volume of chloroform-isoamyl alcohol(24:1) (about 500 μl) was added to each tube, and the tubes were gently inverted for 10 seconds. This mixture was separated in a Beckman Microcentrifuge at 15,850 *g* for 5 minutes. From each tube, the top layer was carefully removed and transferred to a new 1.5 ml microcentrifuge tube. To each sample, an equal volume of 100% isopropanol (about 500 μl) was added, and the tubes were gently inverted for 5 seconds. This precipitated the DNA, which appeared thick and mucus-like in the center of each tube. The precipitated DNA was removed with hooks prepared earlier from capillary pasteur pipets. The DNA was washed in 70% ethanol by dipping the hook into a 0.65 ml microcentrifuge tube containing the ethanol and gently swirling until the DNA looked like cotton—being careful to not to shear the DNA or knock it off the hook. The DNA (still on the hook) was then removed from the tube and dried for about 30 seconds by gently waving the hook in the air. The DNA was dispersed in 150 μl TE Buffer (10.0 mM TE, 1.0 mM NaEDTA [pH 8.0]) in a 0.65 ml microcentrifuge tube) by gently swirling the hook in the solution. The samples were immediately put on ice, where they were kept until quantified.

DNA Quantification and Bulking

The samples were individually quantified using a Hoefer TKO 100 Fluorometer (Hoefer Scientific Instruments, San Francisco) and a Hoechst 33258 dye solution ([Bis benzamide, 2'-(4-hydroxyphenyl)-5-methyl-1-piperazynl]-2,5'-bis-l-H-benzamidazole trihydrochloride pentahydrate, 1 μg /ml in TNE buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.4). DNA concentrations from all individuals were evaluated to see if plant age played a role in the quantity and quality of DNA extracted. Each sample was then standardized to a concentration of 10 ng/μl. Equal amounts of genomic DNA (10 μl of the standardized solutions) from each of the ten individuals in a population were mixed together for the bulked RAPD analysis.

Optimization of PCR Amplification of DNA from Chrysothamnus nauseosus

Before RAPD analysis of the bulked DNAs from the ten rubber rabbitbrush populations was performed, the RAPD protocol was adjusted to optimize the clarity and reproducibility of banding patterns from rubber rabbitbrush DNAs. DNA amplifications were accomplished by the PCR procedure. Unmixed standardized DNA from each of the ten individuals in population PR were used in several concentration gradients to determine the optimum DNA concentration for reproducibility and clarity. The thermal cycling program was optimized for best results. Random primers (Operon Technologies Inc., Alameda, California, USA) that gave no false bands in controls (amplifications containing all reaction elements except for the DNA) and that showed good reproducibility were chosen for the bulked DNA analysis.

DNA Amplification, Separation, and Marker Scoring

RAPD analysis was carried out on the bulked population DNA using the parameters which gave the clearest and most reproducible results in the test runs with population PR. Each 15 μl reaction contained 8 ng rubber rabbitbrush DNA, 1.2 units AmpliTaq® DNA Polymerase-Stoffel Fragment (Perkin Elmer Cetus, Norwalk, Connecticut, USA), 3.5 mM $MgCl_2$, 10 mM KCl, 10 mM Tris-HCl (pH 8.0), 0.4 μM random primer (Operon Technologies Inc., Alameda, California, USA) and 100 mM dNTPs. Each reaction was overlaid with one drop of mineral oil to prevent evaporation. Amplifications were carried out using the PCR procedure programmed as follows: a preliminary 3 min denaturation at 94°C ; 40 cycles of ramp for l min to 94°C (denaturation), ramp for 15 s and hold for 45 s at 35°C (annealing), ramp for 105 s to 72°C (elongation); hold for 7 min at 72°C (termination of elongation); ramp (3 degrees/sec) followed by indefinite hold at 4°C (storage until resolution on agarose gel). The RAPD fragments were resolved by electrophoresis in 1.4% agarose gels containing 1 μM ethidium bromide (International Biotechnologies, Inc., New Haven, Connecticut, USA) alongside pUC 19 (Biosynthesis, Inc.) molecular weight markers. The gels were photographed under UV light using Polaroid® 107c film. The RAPD bands were scored for presence (faint to bright) or absence (no band visible) in each population bulk. Demeke et al. (1992) demonstrated that using both faint and bright bands gave a taxonomic relationship for *Brassica* which

was closer to classical relationships than when using either only the faint or only the bright bands.

Data Analysis

The computer program NTSYS-pc (Exeter Software, Setauket, New York, USA), subroutine SIMINT, was used to calculate coefficients of similarity. The program calculates both Jaccard's (a / [a + b + c]) and simple matching ([a + d] / [a + b + c + d]), and genetic distance (1 - 2a / [2a + b + c]) between each population pair (where a is the number of fragments common to both populations, b is the number of fragments found only in population 1, c is the number of fragments found only in population 2, and d is the number of fragments found in neither population). Cluster analysis, using the unweighted pair group method with arithmetic mean (UPGMA), was performed by the SAHN subroutine of NTSYS-pc on the similarity/genetic distance matrices. The TREE subroutine of NTSYS-pc was used to generate a phenogram from each of the cluster groupings.

Results and Discussion

DNA Extraction and Amplification Procedure Results and Reproducibility

Table 1 lists the population average concentration of DNA per 160 mg fresh rubber rabbitbrush leaves extracted with the procedure outlined in *Materials and Methods* and dispersed in 150 μl TE buffer. Population PR had a much lower average DNA concentration (49 ng DNA / μl stock solution) than any of the other populations because, being the trial population for the extraction procedure, it was not reextracted once the DNA solutions had been obtained. The highest average population concentration obtained from this extraction procedure was 100 ng of DNA per μl of DNA stock solution in population FG. A total average concentration of 79 ng of DNA per μl of DNA stock solution was obtained from this extraction procedure. The age of the plant appeared to have no influence (data not shown) on the quantity or quality of DNA extracted.

Table 1. Average concentration of DNA extracted from 160 mg of *Chrysothamnus nauseosus* ssp. *hololeucus* leaf tissue.

Population	ng DNA per μl soln. (Pop. average)	Std. error
PR	49	7
PC	78	7
NO	91	4
LE	78	7
UL	81	9
GO	70	7
SQ	71	12
NE	75	8
FG	100	10
SC	99	8
All populations	79	3

Several concentration gradients were used to obtain the optimum concentration of *Chrysothamnus* DNA per amplification reaction. The concentrations ranged from 0.5 ng DNA per reaction to 30 ng DNA per reaction—8 ng per amplification reaction showed the clearest banding patterns with the largest number of reproducible bands (data not shown).

The thermal cycling program was optimized for use with the AmpliTaq® DNA Polymerase-Stoffel Fragment in 15 μl reactions carried out in 96-well v-bottomed polycarbonate plates on an MJ Research PTC-lOO™ Programmable Thermal Controller with 10-mer random primers. The program, described in *Materials and Methods*, gave reproducible results with amplification fragments of 480 bp to 1900 bp (data not shown).

Forty random primers were used in the amplification of the population-bulked DNA analysis. Of these, 24 showed bands in all populations (*see* Table 2). The other 16 primers failed to amplify with one population or another. Interestingly, populations PR and SC were consistently the populations that showed no bands. Why this is so is unclear. Table 2 lists the primers that produced bands. Included in the table are the primer sequence, the number of monomorphic bands, the number of polymorphic bands, the number of bands unique to one particular population, and the total number of bands for each primer. A total of 330 bands were scored. Of these, 121 (36.7%) were monomorphic (all populations had the band), 209 (63.3%) of the bands were polymorphic (bands were absent from at least one population), and 60 (18.2%) of the total bands were unique to a single population. The total number of bands per primer varied from 8 to 23. Primer number 40, with only one band, was an exception. These values indicate that a very large number of RAPDs can be generated from a small number of reactions, reducing the cost of using RAPD in marker assisted selection in a breeding program.

Population Grouping and Geographical Distribution

Table 3 lists the coefficients of similarity in pairwise comparisons of populations, calculated using Jaccard's coefficient. The results from the simple matching coefficient and genetic distance calculations were very similar to the values obtained from Jaccard's coefficient. Figure 2 contains the phenogram produced from cluster analysis of the data matrix in Table 3. The phenograms from the simple matching coefficients and the genetic distances produced phenograms (not shown) with almost identical topography as the phenogram produced from Jaccard' s coefficient. The only differences between the three phenograms were the exact values of similarity (or distance) listed at the branch points. Identical branching/grouping patterns were generated by the cluster analysis of all three coefficients of similarity and/or genetic distance. This indicates that the relationships between populations indicated in Figure 2 must be very close to the actual relationships between the populations.

Table 2. Primers used for RAPD amplifications of *Chrysothamnus nauseosus* ssp. *hololeucus* DNA; the primer sequence; the number of monomorphic, polymorphic, and unique bands obtained from each primer; the percentage of total number of bands that were monomorphic, polymorphic, or unique; the average number (per primer) of bands that were monomorphic, polymorphic, or unique (24 out of 40 primers amplified with all populations).

Primer #	Primer sequence (5'-3')	No. of bands monomorphic	No. of bands polymorphic	No. of bands unique	Total no. of bands
2	AGCAGCGAGG	3	8	0	11
4	ACGCTGCGAC	2	9	4	11
5	GGCAAAGCTG	7	9	1	16
6	CAGCGTTGCC	4	19	3	23
7	CAGTTCCCGT	4	10	2	14
9	AAGCGTCCTC	6	6	2	12
10	GGAGTGGACT	5	4	2	9
12	CACGGCACAA	8	7	2	15
13	TGGTTGCGGA	6	10	1	16
14	TGGTCCAGCC	9	10	1	19
16	CCGTCGGTAG	2	10	3	12
19	CCCGAAGCGA	4	9	4	13
20	GTGGCTTGGA	8	3	0	11
21	GTTACGGACC	3	15	6	18
23	GTGCGCAATG	5	16	4	21
27	TCGCATCCAG	5	3	1	8
30	CTTCTCGGAC	5	8	3	13
32	AGAGCGTACC	5	12	4	17
33	TGCCGTGAGA	3	9	4	12
35	AGTCCGCCTG	5	8	4	13
36	ACGGAAGTGG	8	3	2	11
37	CTGAACCGCT	8	9	3	17
39	ACCGCATGGG	6	11	4	17
40	AAGTCGACGG	0	1	0	1
Total		121	209	60	330
%		36.7	63.3	18.2	100
Average		5.0	8.7	2.5	13.8
Std. error		0.5	0.3	0.3	0.9

If the genetic grouping illustrated in Figure 2 is compared to the geographical distribution of the populations shown in Figure 1, an important trend is noticed: genetic grouping resulting from cluster analysis of rubber rabbitbrush RAPD markers produces a phenogram that matches the geographical distribution of the populations. Populations PR and SC, NO and NE, and GO and SQ pair in groups as the most closely related populations. The fact that NE pairs with NO seems to be an exception to the geographical matching observed in the ten rubber rabbitbrush populations. This might be explained by the fact that NO is located just off of a major street in the city of Orem, Utah, while NE is located just off of a freeway exit of the major north/south highway running through Utah. Of all of the populations observed, NE appeared to be the youngest, growing on an embankment between the road and a new golf course. NE is also located on the most disturbed of all of the sites. It is possible that seeds came from the population upstream from Salt Creek Canyon. The rest of the groupings shown in Figure 2 fit the geography nicely. PR, SC, NO, and PC are all located in heavily populated Utah Valley; NO and PC being somewhat isolated from PR and SC by being located in the mouths of canyons adjoining the valley floor. GO and SQ are isolated

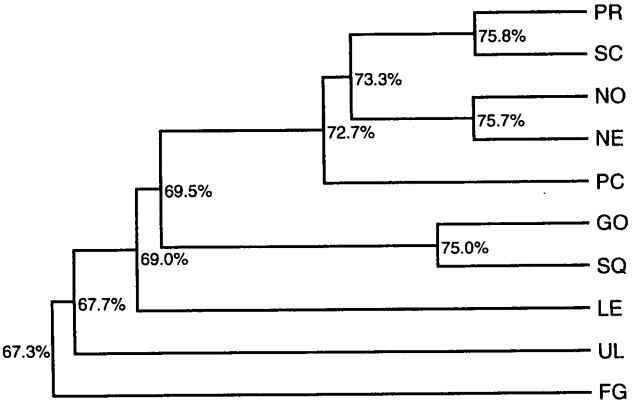

Figure 2. *Chrysothamnus nauseosus* ssp. *hololeucus* populations grouped by UPGMA Cluster Analysis. Values indicate percentage similarity between populations or groups according to the Jaccard coefficient of similarity (×100).

Table 3. Percent similarity of RAPD banding patterns among *Chrysothamnus nauseosus* ssp. *hololeucus* populations, according to the Jaccard coefficient of similarity (×100).

Population	PR	PC	NO	LE	UL	GO	SQ	NE	FG	SC
PR	100.0									
PC	73.5	100.0								
NO	71.6	71.8	100.0							
LE	64.7	70.8	69.7	100.0						
UL	70.4	66.5	66.9	65.8	100.0					
GO	69.9	65.8	62.8	65.8	65.1	100.0				
SQ	72.1	70.9	67.6	70.1	68.6	75.0	100.0			
NE	72.2	72.5	75.7	72.5	66.8	72.8	74.1	100.0		
FG	65.0	66.1	67.9	68.9	64.8	62.0	67.5	72.6	100.0	
SC	75.8	73.0	73.9	69.3	71.4	66.5	72.3	75.4	70.9	100.0

from the rest of the populations by Utah Lake and West Mountain in the north and the Mt. Nebo range to the south and east. LE is isolated from the rest of the populations by the Traverse Mountains to the south. UL is isolated from the rest of the populations by Utah Lake to the north, east and south. FG is isolated from the rest of the populations, being situated east of the San Pitch Mountains. FG, UL, and LE, being in the most isolated areas, are the least similar to any other population. These data indicate that the markers obtained from rubber rabbitbrush RAPDs, do indeed show the genetic relationships of these ten populations.

Interpopulation Genetic Variation Estimated

The degree of similarity between any two of these populations can be obtained from Table 3. If these percent similarities are subtracted from 100% the resulting values indicate the level of genetic variability between the populations. These values range from a low of 24.2% (PR and SC) to a high of 38.0% (FG and GO) polymorphism between individual populations. A total of 63.3% polymorphism (*see* Table 2) when looking at local subsets of the subspecies as a whole indicates that this species has a considerable amount of genetic variation, which could be used as heritable traits in breeding programs.

Anderson (1966) and McArthur et al. (1978, 1979) concluded that strong local differentiation may be the result of predominant inbreeding in *Chrysothamnus*, so that each local population is different from its neighbors.

Identification of population-specific RAPD markers

A high percentage of population-specific markers (18.2% of the total bands) were generated by the 24 random primers used. The number of population-specific markers are listed by population in Table 4. The average number of unique markers was 6.0 per population; the low was 2 (NE), the high was 13 (FG). This evidence supports the idea that NE originated as a transplant from other populations, most likely NO. It also supports the observed trend that geographical distribution is linked to genetic relatedness. The fact that this many population-specific markers can be generated from such a small number of primers indicates that RAPDs would be valuable in a breeding program involving *Chrysothamnus*, and it would be useful in delimiting the phylogenetic relationships within the species and genes on a larger scale.

Table 4. Number of population-specific markers for each *Chrysothamnus nauseosus* ssp. *hololeucus* population.

Population no.	Unique markers
PR	5
PC	6
NO	9
LE	6
UL	8
GO	4
SQ	3
NE	2
FG	13
SC	4
Total	60
Average	6.0
Std. error	1.1

Conclusions

The results demonstrate that *Chrysothamnus nauseosus* is a species that can easily be used in RAPD analyses. The DNA is easy to extract using the procedure outlined herein. RAPDs can be used to differentiate between populations of rubber rabbitbrush, a species with potential as a chemical-producing crop. These markers can be used for phylogenetic analysis of this species, because they clearly mirrored the geographical distribution of the populations—except for one population, which is an explainable exception. The high level of variation found among these populations is most likely indicative of the variation found within the subspecies and species as a whole. This is backed up by the fact that this plant is able to grow in such a variety of conditions over such a broad range. This variation could be utilized in breeding programs to increase the value of this shrub as a crop species. RAPD markers are easily identifiable and could be just as easily linked to important traits to be used in a marker-assisted selection program.

Acknowledgments. The technical advice and expertise of Dr. W. Ralph Andersen on DNA extraction and RAPD techniques is greatly appreciated, as is the aid in selection of populations by Dr. E. Durant McArthur. This research was funded by a grant from Honors and General Education and the Department of Botany and Range Science at Brigham Young University, Provo, Utah.

Literature Cited

Adams, R. P. and T. Demeke. 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). Taxon **42:** 553–571.

Anderson, L. C. 1966. Cytotaxonomic studies in *Chrysothamnus* (Asterae, Compositae). Amer. J. Bot. **53:** 204–212.

Anderson, L. C. 1984. Sympatric subspecies in *Chrysothamnus nauseosus*. *In* E. D. McArthur and B. L. Welch (compilers), Proceedings—Symposium on the Biology of *Artemisia* and *Chrysothamnus*. USDA Forest Service General Technical Report INT-200. Ogden, Utah, pp. 98–103.

Bhat, R. B., D. J. Weber, D. F. Hegerhorst, and E. D. McArthur. 1990. Rubber and resin content in natural and uniform garden populations of *Chrysothamnus nauseosus* subspecies. Phyton **52:** 35–42.

Buchannon, R. A., I. M. Cull, F. H. Otey, and C. R. Russell. 1978. Hydrocarbon and rubber producing crops. Econ. Bot. **32:** 131–45.

Demeke, T., R. P. Adams, and R. Chibbar. 1992. Potential taxonomic use of random amplifies polymorphic DNA (RAPDs): a case study in *Brassica*. Theor. Appl. Genet. **84:** 990–994.

Fu, K.Y. and K. P. Pauls. 1993. Rapid estimation of genetic relatedness among heterogenous populations of alfalfa by random amplification of bulked genomic DNA samples. Theor. Appl. Genet. **86:** 788–794.

Hegerhorst, D. F., D. J. Weber, R. B. Bhat, T. D. Davis, S. C. Sanderson, and E. D. McArthur. 1988. Seasonal changes in rubber and resin contents in *Chrysothamnus nauseosus* ssp. *hololeucus* and ssp. *turbinatus*. Biomass **15:** 133–142.

Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. Annu. Rev. Ecol. Syst. **18:** 23–42.

Kazan, K., J. M. Manners, and D. F. Cameron. 1993. Genetic variation in agronomically important species of *Stylosanthes* determined using random amplified polymorphic DNA markers. Theor. Appl. Genet. **85:** 882–888.

McArthur, E. D., D. L. Hanks, A. Plummer, and A. C. Blauer. 1978. Contributions to the taxonomy of *Chrysothamnus viscidiflorus* (Asterae, Compositae) and other *Chrysothamnus* species using paper chromatography. J. Range Manage. **31:** 216–223.

McArthur, E. D., C. F. Tierman, and B. L. Welch. 1979. Subspecies specificity of gall forms. Great Basin Nat. **39:** 81–87.

McKell, C. M. and G. Van Epps. 1980. Biomass energy production from large growing rangeland shrubs. Logan, UT: Institute for Land Rehabilitation, Utah State University, 78 pp.

Rogers, S. O. and A. J. Bendich. 1985. Extraction of DNA from milligram amounts fresh, herbarium and mummified plant tissues. Plant Mol. Biol. **5:** 69–76.

Stiles, J. I., C. Lemme, S. Sondur, M. B. Morshidi, and R. Manshardt. 1993. Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars. Theor. Appl. Genet. **85:** 697–701.

Tingey, S. V. and J. P. Deltufo. 1993. Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol. **101:** 349–352.

Weber, D. J., T. D. Davis, E. D. McArthur, and N. Sankhla. 1985. *Chrysothamnus nauseosus* (rubber rabbitbrush): multiple-use shrub of the desert. Desert Plants **7:** 172–180, 208–210.

Williams, C. E. and D. A. St. Clair. 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome **36:** 619–630.

Wilkie, S. E., P. G. Isaac, and R. J. Slater. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor. Appl. Genet. **86**: 497–504.

利用基因組核酸混合樣品之逢機擴增多形性片段分析進行十個 *Chrysothamnus nauseosus* 族群之遺傳變異及族群關係之探討

David R. Gang and Darrell J. Weber

Department of Botany and Range Science
Brigham Young University, Provo, Utah 84602, USA

Chrysothamnus nauseosus 係具有經濟實用潛力之作物。此植物屬大型灌木可生產相當量之二次代謝物且可栽培於廣泛之環境條件。本試驗以 10 個地理分野族群，利用其基因組核酸混合樣品進行其逢機擴增多形性核酸 (RAPD) 類型分析， 建立了適合 *Chrysothamnus nauseosus* RAPD 類型分析之 DNA 抽取步驟外，並利用 40 條 10 個鹼基長之逢機引子進行分析。結果顯示，24 條引子可在所有分析族群中有 RAPD 條帶出現，總計有 330 條帶被記錄。這些條帶反應了橡樹地理性之分野可利用於種系發生學之研究。在此 10 族群中，遺傳變異度極高，在所觀測之條帶中， 計 63.3% 之條帶具多形性，而總條帶數之 18.2% 具族群特異性，此種高度變異可利用於育種計畫來增進其價值。此類 RAPD 標誌因子鑑識容易且可用於與重要性狀之連鎖分析。

關鍵詞：*Chrysothamnus nauseosus*；逢機擴增多形性核酸片段；混合基因組核酸；種間變異；Rabbitbrush。