Genetic Variation in an Inbred Plant: Variation in Tissue Cultures of Soybean [Glycine max (L.) Merrill]

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ABSTRACT

Although soybean [Glycine max (L.) Merrill] grows as an inbreeding, generally homozygous, plant, the germplasm of the species contains large amounts of genetic variation. Analysis of soybean DNA has indicated that variation of RFLP (restriction fragment length polymorphism) markers within the species usually entails only two alleles at any one locus and that mixtures of such dimorphic loci account for virtually all of the restriction fragment variation seen in soybean (G. max), and in its ancestors, G. soja and G. gracilis. We report here that tissue cultures prepared from root tissue of individual soybean plants develop RFLP allelic differences at various loci. However, these newly generated alleles are almost always the same as ones previously found and characterized in other varieties of cultivated soybean (cultivars). This repeated generation of particular alleles suggests that much of the genetic variation seen in soybean could be the consequence of specific, relatively frequently employed, recombinational events. Such a mechanism would allow inbred cultivars to generate genetic variation (in the form of alternative alleles) in a controlled manner, perhaps in response to stress.

When organisms are constrained to inbreed, they lack access to the genetic variation which is present in the population. Thus, it is difficult to rapidly generate allele combinations which are selectively advantageous. On the basis of data presented here, we propose that such organisms may have evolved internal generators of genetic variation which, by responding to environmental stress, alter the allelic composition of the genome.

Soybean [Glycine max (L.) Merrill] is an inbreeding plant in which the flower opens after pollination. It is usually homozygous for all loci examined. Despite this, soybean has an extensive and varied germ plasm (Hymowitz, Newell and Carrer 1977; Hymowitz and Newell 1980). Cultivars are available which have been maintained as inbred lines derived from original germ plasm collected in many different locations (plant introductions). These inbred plant introductions present a variety of phenotypic and genotypic variants which have been used by breeders to construct new varieties (Hartwig 1973).

Soybean (G. max) and its progenitor G. soja have 40 chromosomes (n:20). It has been proposed that these species originated as auto-tetraploid or allo-tetraploids (Hadley and Hymowitz 1973). Cytological studies of a haploid line show some pairing between chromosomes indicating that these chromosomes could share similar sequences (Crane, Beaversdorf and Bingham 1982). Molecular evidence is also consistent with this idea. For instance, several different leghemoglobin genes can be distinguished (Sullivan et al., 1981) and two genes of the small unit of ribulose 1,5-bisphosphate carboxylase are known (Grandbastien et al. 1986). However, Hadley and Hymowitz (1973) concluded that for all practical purposes, soybean could be considered as diploid and recent genetic evidence (Apuya et al. 1988, see below) has confirmed the view that the chromosomes have diverged sufficiently to allow genetic markers to segregate as if alleles were present on a diploid set of chromosomes.

In preparing a genetic map of soybean, we have used restriction fragment length polymorphisms (RFLP) as genetic markers. RFLP markers have been used in comparative studies of intact organisms (Hellentjarios et al. 1986; White et al. 1985; Feder et al. 1985; Bernatzky and Tanksley 1986; Landry et al. 1987; Burke and Bruford 1987) and of animal cells in tissue culture (Feder et al. 1985; White et al. 1986; Lander and Botstein 1986). Since measurements of the lengths of DNA restriction fragments can be measured directly, RFLP markers are not dependent on growth conditions leading to the expression of a particular phenotype.

We have isolated more than 80, randomly cloned, fragments of DNA (probes) which identify RFLP markers. Of these, more than 50 now have been analyzed for segregation in F2 progeny and the alleles shown to segregate in a typical Mendelian manner (Apuya et al. 1988). The cultivars used were 'Minsoy' (PI 27890, obtained from France in 1910) and 'Noir 1' (PI 290136, obtained from Hungary in 1963). Examination of other cultivars (Apuya et al. 1988; P.
KEIM, R. SHOEMAKER and R. PALMER, personal communication) as well as a survey of several varieties of the wild soybean species *G. soja* and *G. gracilis* [P. KEIM, R. SHOEMAKER and R. PALMER (cited in APUYA *et al.* 1988)], have indicated that, in general, only two alleles characterize a given RFLP locus in soybean (i.e., most loci are dimorphic). Moreover, most of the RFLP allele differences are due to rearrangements of DNA (APUYA *et al.* 1988). [P. KEIM, R. SHOEMAKER and R. PALMER (personal communication) surveyed 48 accessions of soybean (*G. max*), 8 of *G. soja* and 2 of *G. gracilis* using 17 RFLP markers to detect polymorphisms. Only 2 alleles could be found with 15 of these markers. Results for a 16th marker were consistent with a three-allele system. A third allele of the 17th marker was found, but only in 1 of the 58 lines.]

The dimorphism in these inbred lines could be due to a founder effect during the early cultivation of soybean, or it may represent repeated variation of a particular type. The data to be presented below suggest that this second explanation is correct and that soybean, an obligate inbreeder, may have a mechanism for generating genetic diversity perhaps in response to environmental stress.

Plants regenerated from tissue culture are frequently genetically altered (MEINS 1983; EVANS, SHARP and MENDINA-FILHO 1984; ORTON 1984; SCOWCROFT 1985; SCOWCROFT *et al.* 1985). This somaclonal variation has been used to produce plants with new traits (SCOWCROFT 1985) which are genetically stable and inherited in a Mendelian manner as either dominant or recessive traits (MCCOY and PHILLIPS 1982; EVANS and SHARP 1983; LARKIN *et al.* 1984). Many of these changes have been shown to involve molecular changes in nuclear DNA (DEPAEPE, PRAT and HUGET 1983; DIHILLON, WERNSMAN and MIKSCHE 1983; LANDSMANN and UHRIG 1985; BRETTELL *et al.* 1986a, b; BREIMAN, FELSENBURG and GALUN 1987; BREIMAN *et al.* 1987; ZHENG *et al.* 1987) some of which are similar to differences established between plants (CULLIS and CLEARY 1986a, b).

Whereas somaclonal variation has been used to produce new variants of plants of commercial value (LARKIN and SCOWCROFT 1983; RYAN, LARKIN and ELLISON 1987), it has been used infrequently as a tool to infer a process of genetic change in intact plants. In this paper we use RFLP markers to identify somatic genetic change in soybean tissue culture. These changes in turn have provided insight into the processes of genetic variation which give rise to differences between inbred soybean cultivars and have demonstrated that somaclonal variation can provide a powerful tool to study the process of genetic change in plants.

**MATERIALS AND METHODS**

**Plant material.** The soybean cultivars, 'Minsoy' (PI 27890) and 'Noir 1' (PI 290136), were used in these studies (DELANNEY and PALMER 1982). Seed of 'Minsoy,' 'Noir 1,' and an F1 sexual hybrid between 'Minsoy' and 'Noir 1' were obtained from REID PALMER, Iowa State University, Ames, Iowa. Plants were grown in a greenhouse in a standard potting soil mixture at temperatures of 21–30°C during the day and 10–15°C at night. Day lengths were 14 h of light, 10 h of darkness.

Tissue cultures were prepared from leaves, cotyledons, stem or root tissues taken from a single 'Minsoy' or a single 'Noir 1' plant. Soybean seeds were sterilized in 10% Clorox for 20 min, washed five times with sterile water, and germinated at 25°C in the dark, on sterile filter paper saturated in water. After germination occurred (3–4 days), the seedlings were placed under a grow light. Two weeks after germination, the primary leaves, cotyledons, stems and lateral roots were cut into sections and placed in Petri dishes on filter papers saturated with B5 medium (GAMBORG, MILLER and OJIMA 1968) containing 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4D), 3.0 mg/liter naphthaleneacetic acid (NAA), 0.2 mg/liter kinetin and 0.2% (w/v) casein hydrolysate (IGN Pharmaceuticals Inc., Cleveland, Ohio) and incubated at 28°C in the dark. Callus from cotyledons, stems and primary leaves formed after 2 weeks, and callus from roots formed after 4 weeks. The callus was then put into suspension in B5 medium containing 2 mg/ml 2,4D and 0.2% casein hydrolysate. Suspension cultures were grown continuously in B5 medium containing 2 mg/ml 2,4D and 0.2% casein hydrolysate. Suspension cultures were grown continuously in B5 medium containing 2 mg/ml 2,4D and 0.2% casein hydrolysate. Suspension cultures were grown continuously in B5 medium containing 2 mg/ml 2,4D and 0.2% casein hydrolysate.

**DNA isolation:** DNA from plant leaves and tissue culture lines was isolated by a modification of the procedure of DELAPORTA, WOOD and HICKS (1983). The plant or tissue culture material was ground with a mortar and pestle to a fine powder in liquid nitrogen which was quickly added to extraction buffer [50 mM Tris-HCl (pH 8.0), 100 mM EDTA, 150 mM NaCl, 1% SDS, 5 mM 1,10-phenanthroline, and 50 μg/ml proteinase K] at 65°C. Approximately 10 ml of extraction buffer were used per gram of tissue. The mixture was maintained at 65°C for 30–60 min after which one-fifth volume of 5 M potassium acetate was added and the mixture was placed on ice for 30 min. This was centrifuged at 3000 × g for 20 min. DNA was precipitated from the supernatant with 0.54 volume of isopropanol and purified by phenol extractions and equilibrium centrifugation in cesium chloride. The amount of DNA for copy number experiments was measured using a diaminobenzic acid assay (GURNEY and GURNEY 1984). A Perkin-Elmer MPF-66 Fluorescence Spectrophotometer was used to measure fluorescence.

**Plasmid and M13 clones as probes:** The plasmid and M13 clones of soybean DNA which were used as probes in these studies were isolated and characterized previously.
Genetic Variation in Soybean

(APUYA et al. 1988). The methods used in preparing these probes were those already described (QUEMADA, ROTH and LARK 1987; APUYA et al. 1988). Radioactive probes were prepared as already described (QUEMADA, ROTH and LARK 1987; APUYA et al. 1988) using methods of nick translation or primer extension (RIGBY et al. 1977; FEINBERG and VOGELSTEIN 1983).

**Analysis of restriction fragments:** Plant DNA was digested with restriction enzymes according to the manufacturer’s specifications. Digestion (12–16 h) used 25 units of enzyme per 5 μg of DNA. DNA fragments were resolved by electrophoresis through 1% agarose gels, 5 μg of DNA per lane. TPE (80 mM Tris-phosphate pH 7.8, 8 mM EDTA) was used as the running buffer.

The gels were treated with 0.25 N HCl for 20 min and 0.4 N NaOH for 5 min and then transferred to nylon membranes (Biotrace RP, Gelman Science, Inc.) using 0.4 N NaOH (REED and MANN 1985). Membranes were rinsed in 2X SSC (MANIATIS, FRITSCH and SAMBROOK 1982) and dried at room temperature. The membranes were prewashed for 1 h in 1 M NaCl, 0.5% SDS, 50 mM Tris (pH 8.0), 1 mM EDTA and then prehybridized 12–16 h at 42° in 10–20 ml of 50% formamide, 5X SSC, 100 μg/ml denatured salmon sperm DNA, 50 mM phosphate buffer pH 6.5, 5X Denhardt’s buffer, 1% SDS, 2.5% dextran sulfate. These membranes were hybridized 12–16 h at 42° in 5 ml of 50% formamide, 5X SSC, 100 μg/ml denatured salmon sperm DNA, 20 mM phosphate buffer (pH 6.5), 1X Denhardt’s buffer, 1% SDS, 5% dextran sulfate and labeled probe. After this they were washed 1 h in 2X SSC, 0.5% SDS at 42°, 1 h in 2X SSC, 0.5% SDS at 65°, 1 h in 0.2X SSC, 0.2% SDS at 65°, 1 h in 0.2X SSC, 0.1% SDS at 65°, and 1 h in 0.2X SSC, 0.1% SDS at 65°. Autoradiographs were prepared by exposing the nylon membranes to Kodak SB5 X-ray film with intensifying screens (Dupont Cronex Lightning-Plus) at −70°. The nylon membranes were repeatedly stripped and reprobed. To strip the probe, the membranes were soaked in 0.4 N NaOH for 30 min at 42°, and then neutralized for 15–20 min at room temperature in 0.2 M Tris-HCl (pH 7.5), 0.1X SSC, 0.5% SDS.

**RESULTS**

In the course of previous studies using soybean tissue cultures, we had prepared cell suspension cultures from single explants taken from leaves, stems or roots of the same, single, ‘Minsoy’ or ‘Noir 1’ plants. We also had prepared cell cultures from roots of F₁ hybrid plants produced by crossing ‘Minsoy’ and ‘Noir 1’. All of these cultures had been grown continuously for more than a thousand generations. We now have examined these cell cultures for variation of RFLP markers previously characterized by hybridization against digests of DNA extracted from leaves of inbred ‘Minsoy’ or ‘Noir 1’ plants. In the current study, digests of DNA extracted from inbred plants were compared with digests of DNA extracted from the tissue cultures.

Figure 1 compares examples of restriction fragments found in digests of DNA extracted from plants and from tissue culture. The Southern transfers shown were hybridized with the radioactive DNA probes G-8-15 and G-17-1, or 121 and NP-8. These were previously demonstrated by APUYA et al. (1988) to reveal RFLP markers in intact plants. All of these markers have been shown to segregate independently.

The most striking characteristic of the data is that variations in fragment sizes are seen in tissue cultures prepared from roots of homozygous, inbred ‘Minsoy’ or ‘Noir 1’ plants and that these changes result in fragments whose size indicates the presence of another, previously characterized, RFLP allele. In Fig-
cannot be the case since the Southern transfers in polymorphism in which the 4.2-kb allele is found in ‘Minsoy’ leaf DNA and the 8.4-kb fragment occurs in ‘Minsoy’ leaf DNA. Both fragments are present in the DNA from leaves of heterozygous plants. Probe G-17-1 also identifies several EcoRI fragments of which two are polymorphic—the 6.6-kb fragment found in ‘Minsoy’ and the 9.6-kb fragment which replaces this in ‘Noir 1.’ DNA extracted from tissue cultures prepared from cotyledons or stems of ‘Minsoy’ or ‘Noir 1’ plants contain the same fragments as the plant leaf DNA. However, the DNA from tissue cultures prepared from root tissues have changes: probe G-8-15 identifies an additional fragment in the ‘Minsoy’ root culture which is identical in length (4.2 kb) with the ‘Noir 1’ allele. Probe G-17-1 identifies a 6.6-kb fragment (normally characteristic of ‘Minsoy’) present in the ‘Noir 1’ root culture and, in addition, a 9.6-kb fragment (normally characteristic of ‘Noir 1’) present in the ‘Minsoy’ root culture. Finally, the tissue culture prepared from the root of a hybrid plant also shows change, having lost the 8.6-kb fragment corresponding to the ‘Minsoy’ allele of the G-8-15 marker. Similarly, changes are observed with the probes 121 and NP-8. In this case, the BglI digest digests fragments with ‘Minsoy’ root cell culture DNA in which the ‘Minsoy’ RFLP allele corresponding to the 121 probe is missing altogether, but is replaced by a ‘Noir 1’ allele. The ‘Noir 1’ root culture DNA has acquired an additional ‘Minsoy’ allele. The hybrid cell culture DNA is heterozygous as expected. When tested with NP-8 both ‘Minsoy’ and ‘Noir 1’ root cultures appear to have acquired a new ‘Noir 1’ or ‘Minsoy’ allele respectively. Again the hybrid DNA is unchanged.

The simplest, artifactual, explanation of these results might be that the root derived tissue cultures of ‘Noir 1’ or ‘Minsoy’ have become contaminated with ‘Minsoy’ or ‘Noir 1’ cells, respectively. However, this cannot be the case, since the ‘Noir 1’ culture has gained a ‘Minsoy’ allele of the G-17-1 marker but not of the G-8-15 marker. Similarly, the ‘Minsoy’ culture has acquired a ‘Noir 1’ allele for the 121 RFLP marker. Alternatively, one could argue that the new RFLP markers are artifacts resulting from the contamination of particular DNA preparations (‘Minsoy’ DNA by ‘Noir 1’ DNA or vice versa). However, this cannot be the case since the Southern transfers in Figure 1 were reused after stripping radioactive probes from the nylon membranes, and new fragments were observed with some probes, whereas with other probes no new fragments were evident (e.g., in Figure 1, probe G-17-1 ‘Noir 1’ root tissue culture DNA and probe G-8-15 vs the same ‘Noir 1’ root tissue culture DNA; or the ‘Minsoy’ root tissue culture vs the 121 and NP-8 probes). Also, in many instances we have observed the same results with different DNA preparations from these cultures.

The appearance of the new DNA fragments, seen in Figure 1, cannot be explained by the amplification of DNA present in copy numbers too low to be detected. Figure 2 compares the genomic copy number of the fragments detected in root tissue cultures by probes for lectin and G-8-15 with a standard DNA

![Copy number determination for the lectin and G-8-15 sequences.](image-url)
Tissue culture line | No. of RFLP markers examined | Genotype observed (RFLP alleles) |
---|---|---|
'Noir 1' (a) | | |
Root | 22 | 7 1 13 1# |
Stem | 13 | 13 0 0 0 |
Cotyledon | 9 | 9 0 0 0 |
Leaf | 9 | 9 0 0 0 |
'Minsoy' (b) | | |
Root | 16 | 1 9 6 0 |
Stem | 25 | 0 25 0 2## |
Cotyledon | 24 | 0 24 0 0 |

Tissue culture lines were prepared from root, stem, cotyledon or leaf tissue, taken from plants with (a) 'Noir 1' or (b) 'Minsoy' genotypes. The RFLP genotypes observed were: (a) 'Noir 1': Heterozygous 'Noir 1'—'Noir 1'; (b) 'Minsoy': Heterozygous 'Minsoy'—'Minsoy'; or New. Changes in fragments leading to restriction fragment patterns not observed in any plant DNA.

* Additional restriction digest fragments with sizes not observed in the genotypes of plants from which cell suspensions were prepared.

# Absence of a nonpolymorphic restriction fragment, normally present in both 'Noir 1' and 'Minsoy' genotypes.

from plasmid clones corresponding to each probe. The lectin gene, L1, has been established to be present in the soybean genome as a single copy (GOLDBERG, HOSCHEK and VODKIN 1983). As can be seen in Figure 2A, the amounts of this lectin L1 DNA present in 'Noir 1' leaves and in the cell culture prepared from root tissue are about the same and correspond to the amount of plasmid calculated to be present if the clone was present as a single copy (1X) in 5 μg of genomic DNA. Details of the experimental procedure are given in the legend to Figure 2. When the same experimental procedure was used to measure the copy number of the G-8-15 allele, similar results were obtained. The same results were obtained with probe G-17-1 (data not shown). These results demonstrate that the allelic fragments observed in Southern transfers in Figures 1 and 2 are present as single (or at most two) copy sequences in the soybean genome and that this was true also for the new allelic fragments that had appeared in the tissue cultures derived from roots.

Thus, the data in Figures 1 and 2 show that new single copy fragments of DNA appear upon culturing root tissue from homozygous inbred plants. The change corresponds to the acquisition of an RFLP allele, found in other cultivars of soybean as one partner of a pair of alleles previously shown to segregate in a mendelian manner (APUYA et al. 1988).

The data in Figure 1 are not isolated cases. Table 1 presents all of our results in summary form, comparing 25 RFLP markers as they occur in DNA preparations extracted from one set of cell cultures, each prepared from a different tissue of 'Noir 1' or 'Minsoy.' Not all of the markers have been tested against each cell culture. For example, only 22 probes were used to examine the 'Noir 1' root culture and only 16 to examine the 'Minsoy' root culture. Among the 25 probes tested, 17 provided examples of such changes (either from 'Minsoy' to 'Noir 1' or from 'Noir 1' to 'Minsoy' alleles). With 8 of the 25 probes, no changes were observed in any of the cultures. The alleles are indicated according to the genotype of the intact plant in which they are normally found. Two aspects of these data are striking: (1) Almost all of the variation which we have observed in tissue culture is found in the cultures derived from roots, and (2) these changes invariably involved the observation of an additional fragment whose size corresponds to the allele found in the other cultivar—i.e., in 'Minsoy' cultures the appearance of a 'Noir 1' allele, in 'Noir 1' cultures the appearance of a 'Minsoy' allele.

Table 2 presents detailed data for the changes which were observed. The changes do not appear to involve methylation or demethylation of cytosines within the restriction sites, since several of the enzymes involved (DraI, HindIII and TaqI) would not be affected by such changes.

At two loci (probes 121 and 373, Table 2), changes occurring involved the loss of the normal allele found in the homozygous inbred cultivar; an example of such a change was shown in Figure 1 (e.g., for probe 121, a change from a 'Minsoy' to a 'Noir 1' genotype rather than to a heterozygote genotype). These probably result from two events: an allele alteration followed by either a loss of the other chromosome, a mitotic recombination, or perhaps a repetition of the same allele alteration but on the other chromosome.

Several observations support the conclusion that the allelic changes in tissue culture are due to rearrangements of the DNA: (1) In each of five cases which we tested (Table 2 (#)), changes in the alleles were observed using other enzymes and in each such case, the changes observed were the same that already had been observed (APUYA et al. 1988) when the two alleles were characterized using the same set of different enzymes to digest DNA extracted from leaves of 'Minsoy' or 'Noir 1' plants. (2) A change within two adjacent segments of DNA (Table 2, G-17-1&2) was identical with the allelic differences which previously had been characterized in plants as a rearrangement (APUYA et al. 1988). (3) Five changes in tissue culture involved an acquisition of a 'Minsoy' allele by a 'Noir 1' culture and of a 'Noir 1' allele by a 'Minsoy' culture (Table 2(**)). This is consistent with rearrangement, which may occur in both directions with similar frequencies.

One other characteristic of the data should be noted. In Figure 1, an almost equal amount of radioactive G-8-15 probe hybridizes to the 8.6- and 4.2-kb
### TABLE 2

RFLP found in DNA extracted from ‘Minsoy’ and ‘Noir 1’ plants and from tissue cultures prepared from these plants

<table>
<thead>
<tr>
<th>Probe (enzyme) fragment size (kb)</th>
<th>Min. ‘Noir 1’ Hyb. (leaf)</th>
<th>Plant DNA</th>
<th>‘Noir 1’ Tissue Culture DNA</th>
<th>Min. DNA</th>
<th>Root</th>
<th>Leaf</th>
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<th>Root</th>
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Of all of the loci surveyed, only examples showing allele changes are shown. Consequently, examples included in Table 1 in which no changes occurred are not included in Table 2. DNA was extracted from ‘Minsoy’ (Min.), and ‘Noir 1’ plants or from tissue cultures prepared from cotyledon (Cot.), leaf, stem or root tissues of these plants. These DNA preparations were analyzed for changes in RFLP markers (see legend of Figure 1 and MATERIALS AND METHODS: Probes prepared by cloning plant DNA in bacteriophage M13: NN-8, NP-8, NC-24, NN-21, 17/15, 234, 294, 262, 69 and 121. pS-5H1.3 is a plasmid clone of TCM obtained from L. VOKRIN. All other probes were plasmid subclones of plant DNA which had been originally cloned into phage lambda (APUYA et al., 1988).
fragments found in the DNA of cultures prepared from 'Minsoy' root tissue. This pattern of radioactive intensities is similar to the heterozygotic pattern of hybridization of radioactive probes to DNA extracted from leaves of hybrid plants. Similar radioactive patterns can be observed in the other examples in Figure 1. In almost every one of the cases in Table 2, in which a new "allelic" fragment appeared, the intensity of the radioactivity in the hybridization pattern resembled the heterozygous pattern found in DNA extracted from leaves of a hybrid plant.

**DISCUSSION**

We have already explained why the results described in Figure 1 and Tables 1 and 2 cannot be due to contamination of our cell cultures or of a particular DNA preparation. Since our cell cultures were prepared from roots, stems, cotyledons or leaves taken from the same individual plant, we also are certain that our root cultures come from a plant of the correct genotype. We have already noted that in soybean (APUYA et al. 1988) as in maize (HELENTJARIS et al. 1985), most of the differences between RFLP alleles appear to be due to rearrangement. In those cases which we have examined, this also appears to be true of the changes we observe in tissue culture. The finding that the changes encountered in DNA from cultured cells are precisely those fragments found as RFLP alleles in the other cultivar, suggests that the changes which take place are specific and reproducible. This would mean that the rearrangements which are responsible for the change in fragment length are due to some form of specific recombinational (or gene conversion) process.

The precise structures of these rearrangements have not been analyzed. However, in two examples (APUYA et al. 1988) in which genomic DNA fragments previously had been compared to the restriction map of the lambda clone from which the probes were derived (one of these involved an analysis of clone G-17), the comparison indicated that the rearrangements could involve the replacement of one segment of DNA by another, e.g., by gene conversion (for a discussion of this type of mechanism, see BORST and GREAVES (1987)). Other forms of rearrangement which involve specific recombinational mechanisms could be inversion [e.g., as seen in bacteria (SILVERMAN and SIMON 1983; BORST and GREAVES 1987)] or site specific insertion/deletions. Examples of the last mechanism have been reported by VODKIN, RHOADES and GOLDBERG (1983) who have described in soybean the insertion of a transposon-like element in the lectin gene resulting in loss of gene activity, and by GROOSE and BINGHAM (1986a, b) who have described the reversion of an unstable mutation (assumed to be associated with transposition) upon regeneration of alfalfa plants from cultured callus tissue. Distinguishing between the mechanisms of rearrangement responsible for the changes described in Figure 1 and Table 2 must await the comparison of separate clones containing each of the two allelic forms of DNA involved in the polymorphism.

We do not know why cultured root tissue undergoes genetic change whereas cotyledon, stem or leaf derived materials do not. Although suspension cultures derived from these tissues appear identical, callus formation from root is much slower (4 weeks) than formation of callus from cotyledon, stem or leaf (2 weeks). This could indicate that some form of additional stress occurs during the culturing of root tissue which might serve to induce the variation (see below). It is also possible that cell types may differ in their genetic stability and that the generation of diversity in cell culture could be confined to a particular type of cell. All of our root cultures were obtained from lateral roots, which derive from the single cell type, L3 (SUN 1955). Cultures from stems or leaves derive from a mixture of cell types [i.e., L1, L2 and L3 (SUN 1957)] of which cells from a particular tissue (e.g., L2) may selectively outgrow others during callus formation. Again, resolution of this question must await further experiments.

The cultures we have examined have been growing in suspension for more than a thousand generations. Once in suspension, root, stem and leaf cultures all grow at about the same rate (dividing once every 24-30 hr) and we have no evidence indicating that these undifferentiated cells differ in any other characteristic. Therefore, we believe that the variations we observed either preexisted, or occurred when cells were placed in culture. This is supported by the work of others on somaclonal variation (BARBIER and DULIEU
which were selected. Such cells must be rare, since we
are interested in culturing of root tissue. The digestion of root tissue occurs in the intact plant when
root tissue is digested with restriction enzymes. Al-
though we cannot rule out selection, we prefer to
hypothesize that the variation which we observe in
making cell cultures from roots of hybrid F1 plants. So far, we have only observed four examples of the
loss of an RFLP fragment. Whatever the mechanism,
this frequency is low compared to the frequency of change observed in cells from homozygous plants.
This low frequency suggests that heterozygosity may inhibit the process of variation. Such an inhibition could be due to indirect physiological effects (e.g., differences in the interaction of hybrid and parental
tissue with the hormones used for promoting callus
formation) or it could be a more basic effect similar
to the regulatory effect of heterozygosity on the
switching of yeast mating types (Haber 1983; Jensen
and Herskowitz 1984). If we assume that the variation which we observe in making cell cultures from roots is an example of a natural process which occurs in plants (perhaps induced by stress), an inhibitory effect of heterozygosity (i.e., slowing change) would be beneficial. Thus, the amount of variation which we see in inbred, homozygous, cell cultures could lead to
large numbers of loci changing, in which repetition of any change would lead to homozygosity of the rearranged state. Homozygosity for a particular allele might be deleterious in some genetic backgrounds. It could prevent a cell from developing into germline tissue (i.e., the cell would be genetically dead). On the other hand, if heterozygosity at a particular locus inhibited further change at that locus, the effect of allele change would be less drastic, and changes might be transmitted to the germline. In this self-fertilizing plant, this would allow the expression of a wide variety
of homozygous types, and the selection of new combina-
tions of alleles which would be most advantageous.

As seen in Figure 1A, changes have also been ob-
served in DNA extracted from cultured cells derived
from root tissue of hybrid plants. In the absence of change, such DNA preparations should display a heter-
zygous pattern of 2 RFLP fragments. Loss of one
allele, characterized by the loss of an RFLP fragment (as in Figure 1A) is not very informative since it can be caused by any one of several mechanisms: loss of a
chromosome, mitotic crossing over, or to the alteration
of one specific allele to convert it to the other form
(as discussed above). In all, we have examined 25
elements of RFLP markers in one or the other of
different cell cultures from roots of 2 hybrid F1 plants.

In the process of cell culture, do we select rare
changes already present in the plant, or is change
induced by cell culture? It is very difficult to rule out
selection of preexistent change. However, two obser-
vations make it unlikely: (1) We have observed a
massive number of specific changes (about 50% of
the markers (loci) which we have examined) and these
changes appear to be spread throughout the genome.
(Thus, the four examples in Figure 1 are unlinked
RFLP markers. Moreover, examples of the allelic
changes described in Table 2 are found in each of six
RFLP linkage groups established in our laboratory.)
(2) In almost all of these cases, as in the examples
shown in Figure 1, the frequency with which each
change is represented in the cell population is high
(the radioactivity hybridizing to the new fragment is
comparable to that seen hybridizing to the fragment
normally found in this genotype). This leads to the
conclusion that most, if not all, of the changes are
present in all of the cells in the population. Thus, if
changes preexisted in the plant root, almost 50% of
the loci must have been altered in the one or two cells
which were selected. Such cells must be rare, since we
have not seen a population of weakly radioactive
fragments, corresponding to these RFLP alleles on
Southern transfers, when plant DNA extracted from
root tissue is digested with restriction enzymes.
Although we cannot rule out selection, we prefer to
explore the hypothesis that allelic change occurs
during culturing of root tissue.

Following the proposal of McClintock (1984), we
hypothesize that the variation which we observe in
cultures of root tissue occurs in the intact plant when
it encounters stress. (This hypothesis has precedent in
Flax (Cullis and Cleary 1986a, b) where variation in
dNA copy number has been observed in plants
following stress or after preparation of tissue cultures.)
We further speculate that this variation can enter the
germline—i.e., is not confined to root tissue. (For
example, such variation could occur in meristematic
tissues which give rise to flowers and gametes.) This
would explain the type of allelic variation observed
between plant introductions (ApuyA et al. 1988) and
that has recently been observed in varieties of Glycine
soja (P. Keim, R. Shoemaker and R. Palmer, personal
communication), the progenitor of Glycine max
(Hymowitz and Newell 1981).

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LITERATURE CITED


LANDSMANN, J., and H. UHRIG, 1985 Somaclonal variation in


