Drought conditions may have affected the uniformity of beetle infestation in the field, thus tests with excised trifoliates were conducted to more critically evaluate the resistant lines. Results of these tests were similar to those of field evaluations, but lines 3 and 4 proved to be more susceptible (Table 1).

Similar results were also obtained in feeding tests with seedlings of the same 13 lines and these results and those of field evaluations were closely correlated (r = .63\*\*, 12 df). Seedling tests did not detect susceptible lines as accurately as did the excised trifoliate test, but ratings of the more resistant lines were similar in both tests. Additionally, results of both tests were also highly correlated (r = .71\*\*, 11 df). The results of these tests indicate the usefulness of each to a breeding program. The utility of excised trifoliate and/or seedling screening tests of F<sub>2</sub> plants in the greenhouse is yet to be determined. These tests would greatly increase the efficiency of the breeding program. Both techniques are now being used to increase the efficiency and precision of insect resistance evaluations.

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## 1) Characterization of cytoplasmic diversity in soybeans.

Soybean cultivars which occupy a majority of the U.S. acreage trace to only a few maternal parents. According to a recent report (1972) by the National Academy of Sciences, the maternal ancestors and their combined frequency of occurrence in the parentage among Northern and Southern varieties are: 'Mandarin' 51%, 'Illini' 23%, 'Tokyo' 11%, 'Dunfield' 8%, 'Mukden' 4%, and 'Roanoke' 4%. Four of these parents are introductions from Manchuria, one from Japan, and one is of unknown origin. The limited geographical origin and the paucity of maternal parents is a reasonable basis for concluding that a high degree of cytoplasmic uniformity exists in currently grown soybean cultivars. The consequences of cytoplasmic uniformity in maize suggest that plant breeders should give consideration to choosing parents diverse in cytoplasmic constitution. We report here preliminary results from a study designed to assess cytoplasmic diversity in soybeans.

Because classical genetic analyses are not applicable to a study of cytoplasmic traits in soybeans, a restriction endonuclease fragment analysis (Nathans and Smith, 1975) was used. This approach is contingent on the presence of specific sequence differences among the organelle DNAs. Levings and Pring (1976) recently described the application of this technique in characterizing mitochondrial DNA in maize. The method consists of an analysis of the DNA fragments generated from digestion of organelle DNA by a site-specific restriction endonuclease. The resulting fragments are separated by gel electrophoresis, producing a "fingerprint" of the original DNA molecule. The fingerprints of organelle DNAs from test genotypes can then be compared. In this study, mitochondrial DNAs (mtDNA) isolated from the hypocotyls were digested with the restriction endonuclease <u>Bam</u> 1 from <u>Bacilis amyloliquifaciens</u> H.

In addition to the six maternal ancestors noted above, 'Arksoy', 'Dorman', <u>Glycine soja</u> and <u>G. gracilis</u> were chosen for study. The variety 'Lincoln', from the cross of Mandarin X Manchu, was substituted for Mandarin due to problems with seed availability. Arksoy, introduced from Korea, was chosen as a representative of an additional geographic location. Dorman, from the cross Dunfield X Arksoy, has been used as a parent in a number of improved varieties. <u>Glycine soja</u> and <u>G. gracilis</u> represent the supposed progenitor, and an intermediate species between the progenitor and <u>G. max</u>, respectively.

Electrophoresis of the restricted mtDNA fragments resulted in approximately 40 bands. Banding patterns of all six maternal ancestors were identical. Figure 1 is a schematic representation summarizing the differences in electrophoretic banding patterns that were observed among the mtDNAs examined. Only the top 5 cm of the gel is shown since no differences were detected in the remainder of the gel. The center banding pattern represents a fingerprint of mtDNA from Dorman and is identical to the fingerprints obtained for all the maternal ancestors as well as for <u>G</u>. <u>gracilis</u>. Arksoy, the variety of Korean origin, differs from Dorman by a single missing band at approximately 3 cm. The wild species, <u>G</u>. <u>soja</u>, differs by six bands, all occurring in the upper 4 cm. In general, there was a high degree of homology among the banding patterns of the mtDNAs examined. The heterogeneity in banding patterns reflects

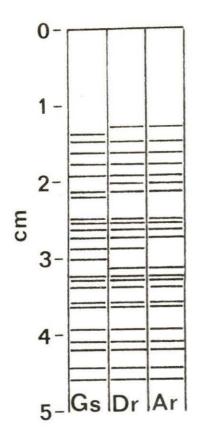


Figure 1. Schematic diagram illustrating the different banding patterns obtained by gel electrophoresis of soybean mtDNA digested with restriction endonuclease <u>Bam</u> 1. Sources of mtDNA were: (Gs) <u>Glycine soja</u>, (Dr) Dorman, and (Ar) Arksoy. Only upper 5 cm of the 10 cm gels is shown.

differences in the number and position of cleavage sites only and are not necessarily related to specific fitness traits. Nevertheless, these results clearly demonstrate a high degree of uniformity among mtDNAs of the maternal ancestors when digested by Bam 1. This conclusion is reinforced by the differences in banding patterns observed for G. soja and Arksoy compared with the maternal ancestors. These results, then, tend to substantiate the premise that cytoplasmic uniformity cannot be overlooked as a potential hazard to production in soybeans. Of further interest is that restriction endonuclease analysis provides a powerful tool for soybean breeders in detecting maternal parents of diverse cytoplasmic constitution for use in varietal development.

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