PANELLA, LEE, USDA, Agricultural Research Service, 1701 Center Ave., Fort Collins, CO 80526. **Measurement of genetic diversity in two populations of *Beta vulgaris*, L. using RFLP markers.**

An important consideration in measuring genetic diversity within populations of cultivated beets or their wild relatives is how many individuals should be examined to make an accurate estimate of the diversity within the population. A large number of plants from two sugarbeet breeding populations were examined using restriction fragment length polymorphism (RFLP) markers (19 possible fragments observed) to estimate the genetic diversity within these populations. There were 76 plants from population 751080H (FC703, PI 590656) and 83 plants from genetic-cytoplasmic male sterile (CMS) population 931016HO1 (52-305 CMS - one of Deming's inbreds). DNA was extracted from each of these 159 plants. The RFLPs were created using probes obtained from the Institute of Crop Science and Plant Breeding at Christian-Albrechts-University in Kiel, Germany. Fragments were visualized as bands on X-ray film using a chemiluminescent detection system. There were two possible bands with an *Eco*R V digest of probe pKP510, three possible bands from an *Eco*R V digest of probe pKP1159 and 14 possible bands from a *Hind* III digest of probe pKP1011. The CMS population was much more diverse when examined with these RFLP markers. This was clearly seen with probe pKP1159 which, when used with an *Eco*R V digest, produced a pattern of eight possible bands through the presence or absence of three RFLPs. The CMS population had all eight possible banding patterns represented. In population 751080H, only five of the eight possible patterns were present, and two patterns were found in 93 percent of the individual plants in that population. The *Hind* III digest and probe pKP1011 produced a possible 14 bands. The 751080H population contained 54 patterns among the 76 plants, while almost each plant in the CMS population had a unique banding pattern (81 patterns in the 83 plants).

Materials and Methods

Fresh leaf material from two sugarbeet germplasm lines was extracted on an individual plant basis. The first line was 931016HO1, which is genetic-cytoplasmic male sterile(CMS) equivalent of 52-305. This is one of "Deming's inbreds" and the population is *rr, MM* and S_{12}. 931016HO1 is bulked seed from 23 CMS plants that had a potential 34 pollen donors. A total of 83 plants from 931016HO1 were examined. The second germplasm line was 751080H (FC 703), which is a bulk of 118 open-pollinated plants arising from a population that had been strongly selected for resistance to Rhizoctonia root rot. Seventy-six plants of this germplasm line were examined.

Five g of fresh or frozen tissue plant tissue was extracted using a modified Saghai-Maroof procedure. Repeated extractions with chloroform-isoamyl alcohol were performed, the DNA was precipitated with ethyl alcohol and suspended in TE. For southern blotting, 8 µg DNA from each plant was digested, run overnight in a 1.0% agarose gel and transferred to a Roche[1] positively charged nylon membrane.

[1] Mention of a trademark or manufacturer by the USDA does not imply its approval to the exclusion of other products or manufacturers.

The RFLPs were created using probes obtained from the Institute of Crop Science and Plant Breeding at Christian-Albrechts-University in Kiel, Germany. For this study, three probes were labeled with digoxigenin, using a Roche[1] PCR DIG probe labeling kit (primers T3& T7). Using a hybridization oven, the membrane was exposed to the probe solution overnight. The following morning, using a Roche[1] "DIG wash and block buffer set", the hybridized membrane was detected with CDP-Star™ as a chemiluminescent substrate. The treated membrane was exposed to X-ray film for about 30 minutes and developed.

The distance bands had migrated from origin was measured and compared to the Roche[1] "DNA marker III, digoxigenin labeled". Because base pair measurement was non-standard, individual linear calculations were done for the largest sized bands (between 21,226 and 5,148 base pairs). Linear regression analysis was done for the remaining portion of the curve (5148 thru 564 bp.) Most individuals were examined on at least two separate X-ray films.

Results and Discussion

There were two possible bands with an EcoR V digest using probe pKP510, three possible bands from an EcoR V digest using probe pKP1159 and 14 possible bands from a Hind III digest using probe pKP1011 (See Table 1). The CMS germplasm was much more diverse when examined with these RFLP markers. This was clearly seen with probe pKP1159 which, when used with an *EcoR* V digest, produced eight possible banding patterns through the presence or absence of three RFLPs. The CMS population had all eight possible banding patterns represented. In population 751080H, only five of the eight possible patterns were present, and two patterns were found in 93 percent of the individual plants in that population. The *Hind* III digest and probe pKP1011 produced a possible 14 bands. The 751080H germplasm contained 54 patterns among the 76 plants, while almost each plant in the CMS germplasm had a unique banding pattern (81 patterns in the 83 plants).

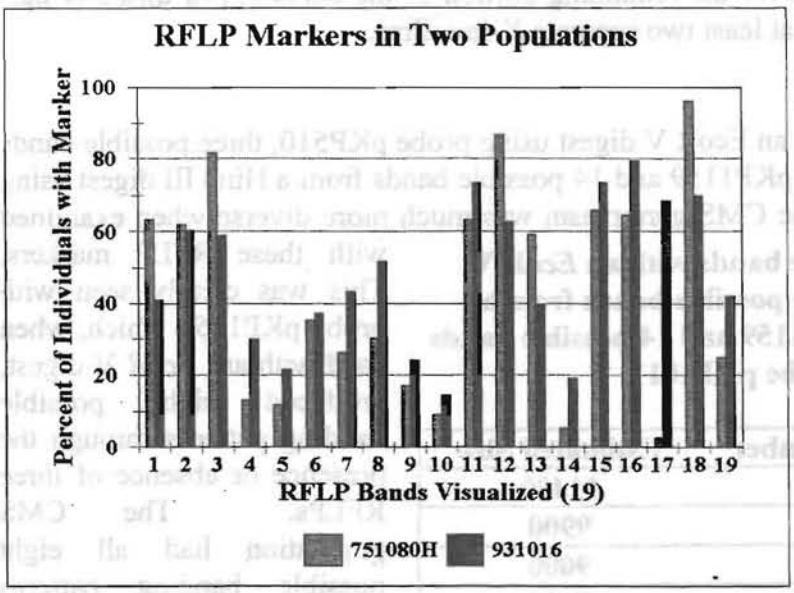
Table 1. There were two possible bands with an *Eco*R V digest using probe pKP510, three possible bands from an *Eco*R V digest using probe pKP1159 and 14 possible bands from a *Hind* III digest using probe pKP1011.

Enzyme	Probe	Band Number	Estimated Size
Hind III	1011	1	11400
	1011	2	9900
	1011	3	9000
	1011	4	7600
	1011	5	6700
	1011	6	6100
	1011	7	4800
	1011	8	4200
	1011	9	3800
	1011	10	3000
	1011	11	1900
	1011	12	1400
	1011	13	1100
	1011	14	900
EcoR V	510	15	7500
	510	16	6900
	1159	17	8800
	1159	18	5700
	1159	19	4400

If we just look at presence or

absence of bands in a each germplasm line (Figure 1), the number that are rare (present in less than 10% of the individuals) or approaching fixation (present in greater than 80% of the individuals) are more common in the germplasm 751080H. In general, the percentage of individuals with the presence or absence of bands in 751080H is more extreme than in 931016HO1. There is a greater amount of diversity present in the population 931016HO1 than in the population 751080H.

Figure 1. The graph displays the percent of individuals in each germplasm line that show a presence of each of the 19 bands examined.



Although the pedigree of 931016HO1 is that of an inbred line, the fact that a CMS (obligate outcrossing) population was sampled indicates that much of the diversity still present in the population was captured. This diversity seems to be even greater than in a self-incompatible population derived from a larger number of parents. Two of the bands in the population showed up in only 5% or less of the individuals in 751080H and, likely, would not have been recovered in a small (5 - 20) sample population.

In collaboration with Irwin Goldman at the University of Wisconsin, these plants and those from two populations from the Garden Beet Group were compared using RAPD markers. These data will be combined to see if the same trends hold true.

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References

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