The Use of RAPD for the Identification of Sugar Beet Varieties

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ABSTRACT

RAPD data have been analysed using multivatiate techniques in order to discriminate between sugar beet varieties. While it was not possible to do so using small numbers of plants per variety, with a larger number discrimination was possible.

Additional Key Words: random amplified polymorphic DNA, DNA fingerprinting, multivariate analysis, cluster analysis, *Beta vulgaris*

Random amplified polymorphic DNA (RAPD) was first used to detect polymorphism in man, soybean, *Neurospora,* maize, and strains of bacteria (Williams et al., 1990). In terms of DNA fingerprinting, where there is a need to identify cultivars of a crop species or to determine parentage within breeding material (Newbury and Ford-Lloyd, 1992), RAPD has been applied to analyse polymorphism within barley cultivars (Weining and Langridge, 1991), and to distinguish between groups of cocoa genotypes (Wilde et al., 1992), and cultivars of soybean (Caetano-Anolles et al., 1991), broccoli and cauliflower (Hu and Quiros, 1991), and rice (Welsh and McClelland, 1990).

Three general criteria have been proposed for varietal identification (Bailey, 1983): distinguishable intervarietal variation, minimal intravarietal variation, and environmental stability and experimental reproducibility. The third criterion has now been shown to apply to characters derived from RAPD for a range of plant material (Munthali et al., 1992). Both distinguishable intervarietal, and lack of intravarietal variation are reported for broccoli and cauliflower (Hu and Quiros, 1991). Sugar beet *(Beta vulgaris* L.) is an outbreeding crop, and despite most modern cultivars being F1 hybrids produced using cytoplasmic male sterility, sugar beet cultivars are far from genetically uniform. Considerable genetic variation within cultivars can be detected using both isozymes and RFLPs (Nagamine et al., 1989a; 1989b), and indeed this variation has been used to identify double-haploid breeding lines (Schmidt et al., 1993). While there is clearly distinguishable intervarietal variation, there is also intravarietal variation to contend with (Nagamine et al., 1989a; 1989b), when attempting to identify sugar beet cultivars using molecular methods.

We have attempted to use RAPD for 'fingerprinting' sugar beet cultivars, and to overcome the problems associated with intravarietal variation by employing numerical multivariate analyses (Francisco-Ortega et al., in press) to distinguish between cultivars. These techniques aim to discriminate through use of a combination of characters which may be large in number, rather than just by one or a few characters used conventionally.

MATERIALS AND METHODS

The plant material which was subjected to RAPD and subsequent numerical analysis comprised two sets:

A. Two/three separate plants of each of seven cultivars: Amethyst, Bingo, Matador, Sandra, Saxon, Ovatio and Rex.

B. Ten separate plants grown from seed of each of two sugar beet

cultivars, Rex and Matador (only seven and eight, respectively, gave scorable results).

DNA was extracted from leaves of glasshouse grown plants, PCR was carried out, and amplification products visualised broadly as described by Munthali et al. (1992).

In a preliminary study, ten random 10-base primers were assessed for their abilities to detect polymorphism. Five of these were then selected on the basis that they revealed polymorphism and that the bands were repeatably produced.

Numerical analyses

After the electrophoretic separation of the PCR products, individual bands were numbered sequentially for each primer and the presence or absence of each band in each plant was scored. A data matrix based on DNA fragments detected per plant was constructed. Hierarchical classifications were obtained after using both Two-way Indicator Species Analysis (TWINSPAN) (Hill, 1979a) and Cluster Analysis (CA) with the unweighted pair group average method (UPGMA) and Jaccard's coefficient of similarity. Detrended Correspondence Analysis (DECORANA) (Hill, 1979b) was used for ordination. These multivariate analyses were carried out using the Clustan 3 package (Wishart, 1987) for CA, and the Cep-pc package (Mohler, 1987) for TWINSPAN and DECORANA.

RESULTS

An initial survey had previously been carried out using 10 primers on 17 plants from five widely differing accessions of beet. These preliminary studies yielded 141 bands, 95 of which were found to be polymorphic. The number of bands given by each primer ranged from 7 to 21, and the number which were polymorphic from 3 to 19. The five primers which gave the greatest number of polymorphic bands were then used for the analyses of the sets of material in A and B.

Analysis of seven cultivars (A)

For the 16 plants for which data could be obtained, a total of 68 bands were scored using the five primers. Out of these, 22 were present in all the plants. Out of the 46 bands which were polymorphic, only 5 were monomorphic within and polymorphic between cultivars. This means that 41 bands varied both between and within the 7 cultivars used. As a result of this, there was no means of separating cultivars by using single marker bands.

With CA, while replicate plants of several cultivars did cluster together (Matador, Bingo, Ovatio), in only one case (Ovatio) did all three replicates fall into the same cluster. In two extreme cases, replicates of a cultivar (Sandra, Rex) fell into different clusters of the major dendrogram division.

The hierarchical classification generated by TWINSPAN yielded 6 "End Groups." The results were generally similar to those from CA. Replicates of Ovatio all fell within the same End Group, as did the only two replicates of Matador. Two out of the three replicates of Bingo occupied the same End Group, but in contrast, each of the three replicates of Sandra fell in different End Groups.

Only the first two axes from DECORANA were used as the remainder had eigenvalues less than 0.1. Results similar to those of the first two analyses were obtained. Again, Ovatio formed a tight cluster within one of the sectors of the scatter diagram, and this contrasted with replicates of Sandra which occupied three different sectors.

Analysis of two cultivars (B)

The same five primers were used to study variation in 20 plants of the cultivars Rex and Matador. Only 15 tracks were actually scored. Most bands were found to be polymorphic between and within the two cultivars (Figure 1), and once again no obvious systematic pattern of bands discriminated between them. One band came close to being discriminatory in that it was present in all plants of Rex, and absent from all except one plant of Matador.

The dendrogram obtained after CA (Figure 2) identified two major groups which represented the two cultivars being studied. One plant of Matador however, stood out as being distinctly different, and was not grouped with either of the two major clusters.

From TWINSPAN seven End Groups were formed, three of these being entirely composed of Matador plants, two entirely of Rex, and two more which each represented single plants of Rex and Matador. The major division in the hierarchical classification completely separated the material into Matador and Rex plants, with the exception of the one Matador plant which did not group in the previous analysis. From this analysis it also was possible to determine that certain DNA amplification products predominated in Rex plants and other amplification products were predominant in Matador. Four amplification products were monomorphic across both cultivars.

The scatter diagram produced from the DECORANA ordination showed considerable variation among the plants of both cultivars. Despite this, there was no overlap between the scatters of each cultivar.

One plant of Matador and one of Rex were close to each other, a situation reflected in the other two analyses, and related probably to the anomalous position of the one Matador plant.

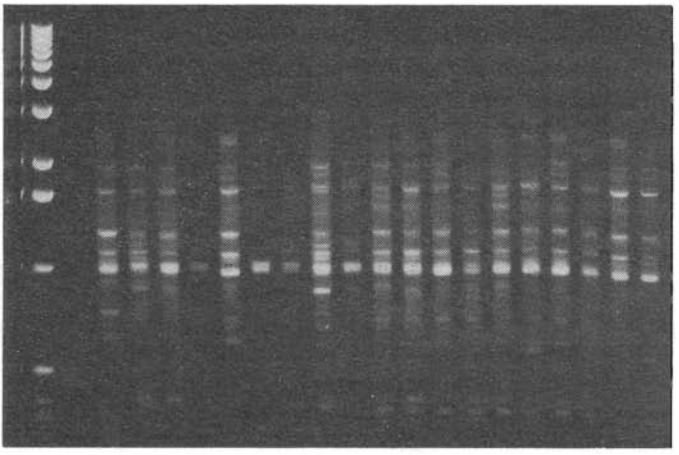

Figure 1. DNA amplification products obtained from plants of two sugar beet cultivars showing intravarietal variation. Tracks 1-10 = Rex; tracks 11-20 = Matador.

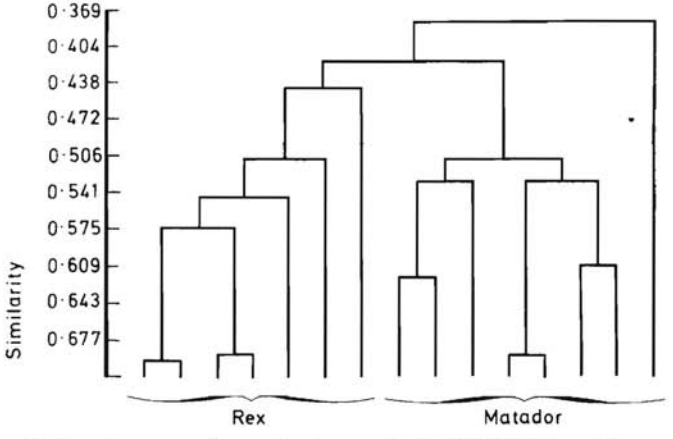

Figure 2. Dendrogram from cluster analysis (UPGMA and Jaccard's coefficient) using RAPD banding information from 5 primers, of plants of two sugar beet cultivars, Rex and Matador.

DISCUSSION

As with both isozymes and RFLPs (Nagamine et al., 1989a; 1989b) intravarietal variation in sugarbeet is easily demonstrated with RAPD. This occurs to such an extent that only a small number of amplified products were found in all replicate plants from any one cultivar, and no products could be identified which were unique to a particular cultivar. This would appear to differ from all published results for other crop species where molecular markers can be identified which are monomorphic within cultivars (Hu and Quiros, 1991; Liu et al., 1992). Superficially then, for sugar beet it appears that the first two of the three criteria set by Bailey (1983) for varietal identification cannot be met using RAPD (as indeed is also the case with isozymes and RFLPs).

The multivariate analyses which have been used, however, do appear to be potentially useful. They have given similar results in terms of classification of plants of sugar beet cultivars. None of the analyses was able to group together the small numbers of replicate plants sampled from all of the seven sugar beet cultivars. However, with TWINSPAN and CA, plants of Ovatio did cluster together, as did two of the three plants of Bingo. It might be inferred from this that these two cultivars show a higher level of genetic uniformity than the others, in particular Sandra, where the three plants fell within three completely separate clusters.

In the remaining analyses, where only two cultivars were used, but with larger numbers of replicates, a much greater prospect for discriminating between cultivars was demonstrated. Based on analysis using (2/3) numbers of replicate plants, Rex and Matador were chosen as contrasts in terms of the way replicates had clustered. The two Matador plants had grouped together, whereas the two Rex plants were completely separate from each other. Using both CA and TWINSPAN, separate groupings of plants from each of the two cultivars was achieved. This was with the exception of one of the Matador plants which proved to have a lower level of similarity to the other Matador plants than all of the plants (Rex and Matador) taken together. Although DECORANA was not able to produce a scatter that would in any way allow the easy visual separation of replicates of all seven cultivars in the earlier analyses, it did so when only the two cultivars were analysed, but again with the exception of one Matador plant.

We have been able to separate two cultivars using only seven or eight samples from those cultivars. It would appear likely that with not much larger numbers of plants accurate discrimination could be

accomplished. Furthermore, larger scale testing of primers could identify those that are more effective in separating beet varieties. The existence of contaminating material within a cultivar would serve to confuse any efforts of discrimination, and in our results, it may be that we have identified such a contaminant within the cultivar Matador. However, the ability to detect contamination may add further strength to the broad applicability of this combination of molecular and analytical techniques.

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