ScienceFoundry (2004) 2, 1-7.

DNA Fingerprinting of Potato Varieties

W. Brenna

Grade 8, Clavet Composite School, Saskatoon, SK Canada
Mentors: Dr. T. S. Grewal, Dr. K. Bett (Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK)

The objective of this project was to learn how to extract potato DNA and examine the differences between potato varieties at the DNA level. Potato plants were used as a source of DNA for a study of the comparison of DNA from different potato varieties. Two test potatoes (P11-1 and P23-1) were used to standardize the fingerprinting procedure that gave the best results. Ten other potatoes were then planted; the DNA was extracted using the Cetyltrimethylammonium Bromide (CTAB) method, and individual potatoes were compared by Random Amplified Polymorphic DNA (RAPD) technique. The tested potato varieties were found to vary a lot. The RAPD results were clear and easy to compare. It was concluded that DNA fingerprinting is a powerful technique to study the genetic variation between plant varieties and can be used for plant improvement.

Introduction

DNA fingerprinting uses a couple of different procedures to get a large 'copy' of what the DNA looks like. It is often used in forensic sciences, because it can allow a person to compare the differences in DNA between two samples and you can easily tell if there is a match or if the DNA varies. The main purpose of DNA fingerprinting in plants is to find out if they are genetically related or not. If the DNA between the two plants varies a lot, that means that one of the plants may have a trait in it that may be useful.

One of the main steps of DNA fingerprinting is PCR (Polymerase Chain Reaction). What PCR does is it multiplies the strands of DNA between two points, so that we can run the amplified fragment on agarose gel. The RAPD (Random Amplified Polymorphic DNA) technique of PCR was used to for this experiment on potato varieties.

Methods

DNA Extraction

CTAB method of DNA extraction was used as given by Procunier et al. 1991 (1). The steps are as follow:

1) 2-3 grams of fresh leaf tissue was harvested. Then leaves were cut into small pieces and placed in a pre-chilled mortar. Liquid nitrogen was immediately added and the tissue was ground into a fine powder.
2) The powder was transferred into a pre-chilled 50ml polyallomer extraction tube placed on ice.
3) One volume (15ml) of warm (65°C) 2x CTAB solution (this is 2% CTAB, 100mM Tris-Hcl pH 8, 20mM EDTA pH 8, 1% PVP MW 44 000, and 2.8 M NaCI [autoclave]) was added. Then the tubes were mixed well so that all the powder tissue was suspended.
4) The tubes were incubated for 10 minutes in a 65°C waterbath and shaken once or twice

during this time: gas can build up and cause the lids to pop off.

5) One volume (15ml) of 24:1 chloroform: isoamyl alcohol was added. The tubes were thoroughly mixed by inversion (tipping back and forth slowly) until the phases were mixed.
6) The tubes were spun in a centrifuge for ten minutes at 1500 to 2000 rpm.
7) Ten ml of the supernatant (the top aqueous phase) was transferred to a clean polyallomer tube. Chloroform waste was disposed of in the special waste bottle.
8) 1/10 of a volume of warm (65°C) 10x CTAB solution was added and the tubes were mixed well.
9) Steps 4 to 7 were repeated. This was done to further purify the DNA.
10) Two volumes of ice cold 95% ethanol were added and mixed gently by inverting several times. The DNA precipitated out of the solution at this step.
11) The DNA was placed on ice for about half an hour. (The extractions can be left over night at this point.)
12) The tubes were placed in the centrifuge for 5 minutes at 1500 to 2000 rpm.
13) Ethanol was poured off while making sure the DNA pellet stayed in the tube.
14) Ten ml of ice cold 70% ethanol was added.
15) The DNA was placed on ice for about half an hour. This was to remove the excess salts from the solution. The DNA could be left overnight at this point.
16) The solution was centrifuged for 5 minutes at 1500 to 2000 rpm.
17) The ethanol was poured off. The tubes were inverted at a 45° angle to allow the ethanol to evaporate (1-2 hours).
18) The DNA was re-hydrated by adding TE Buffer.

DNA Quantification

DNA extraction was followed by DNA quantification using a DNA Quantifier (GeneQuant, Pharmacia Inc.). First the DNA was diluted 100 times by adding 99 parts water to 1 part DNA. The DNA was then put inside a cuvette with two very sensitive sides and placed inside the Quantifier. The amount of DNA was measured in micrograms per millilitre as determined by the machine. Then, working concentrations of DNA samples, i.e. 50 ng/ml, were made by diluting the stock solutions.
A test DNA extraction and quantification was done first on two potatoes lines (P11-1 and P23-1) to determine what concentration of DNA was good to use and what primers were best for fingerprinting.

Polymerase Chain Reaction (PCR)

This procedure will multiply the strands of DNA many times over so that when run on a gel it can be seen. Here are the steps for PCR:

PCR reaction conditions

A PCR solution was prepared. It is a combination of a primer (this was chosen at random); *Taq*, an enzyme (DNA polymerase), dNTPs (deoxynucelotide triphosphates), magnesium chloride, the template (the DNA), and water. This was where the best primers and concentrations were determined. At first, the template concentration was set at 25 ng/µL (25 nanograms per microlitre) of template. There was 15.8 µL water, 2.5µL 1 X PCR buffer (Gibco BRL, Bethesda, USA), 1µL of dNTP (100 mm each of dATP, dTTP, dCTP and dGTP - Gibco BRL), 3µL of primer, 0.5µL of template, and 0.2µL of *Taq*. They all had to add up to 25µL. Another cocktail was made with the template concentration doubled: 50ng/µL. Everything remained unchanged except the template went up to 1µL and the water went down to 15.3µL to keep the cocktail at 25µL. Two more DNA concentrations 100ng/µL and 200ng/µL were also tried. Two potato lines were used, there were four concentrations, and there were two primers. The primers used were OPA 15 and OPA 02 (Operon Technologies, California, USA). The tubes were shaken by hand and placed in the PCR machine (Amplitron® Thermocycler, Barnstead/Thermolyne, USA).

Finally we used 100ng/µL template conc. and for this we used 14. 3 ml of water, 2.5ml of 10 X buffer, 1ml of magnesium chloride ($MgCl_2$), 2ml of dNTP, 3ml of the primer, 0.2ml of Taq (*Thermus*

aquaticus), and 2ml of the template. All of this adds up to a 25ml cocktail.

PCR cycling conditions

These were the temperatures the PCR machine set at for the samples. PCR machine started at 94°C for 3 minutes, and then repeated these steps 35 times: 94°C for 1 minute, 37°C for 1 minute, and 72°C for one and a half minutes. Repeating these steps took about three hours. Then, it was programmed to stay at 72°C for ten minutes and then held at 4°C until the samples were taken out.

Gel Electrophoresis

After the PCR amplification step, the samples were run out on an agarose gel. First, 1.5 g of agarose was mixed with 150 mL of 0.5 X TBE buffer to make a buffer solution. Then, that solution was poured into a flask and boiled for three minutes, shaking at half time. Next, 1.5µL of ethidium bromide (it binds to DNA in the solution and glows under UV light) was added and the gel was left to cool for 20 minutes. A 15 cm gel plate was picked and taped over the sides so the liquid wouldn't leak. A comb with the required number of wells and the right thickness was selected and inserted it into the gel plate, so it would make indents when the gel hardens. After the comb was placed correctly, the gel was poured, and allowed to cool for 30 minutes. After the gel was ready, 2 litres of 0.5X TBE buffer was poured into the Horizontal Gel Electrophoresis Apparatus (Gibco BRL, Bethesda, USA), and the gel was placed under the buffer. 5µL of loading dye was added to the amplified DNA samples and each sample (15-20µL) was loaded into its own well. A Marker (DNA ladder) was placed on either side. This acted as a control because the marker moves at a defined rate, so the DNA can be measured up against it. The voltage was chosen for a medium-sized gel apparatus such as the one used here (116 volts). The gel was run for about 2 hours and then turned off. The gel was taken to a photography room with a gel documentation system. UV light shone through the gel and the ethidium bromide in the gel that had attached to the DNA glowed. Pictures were taken of the gel and saved to disk.

The same procedure was used on the ten other varieties of potatoes. The other varieties were P1 and P2 (common grocery store varieties), Shepody, Goldrush, Russet Norkotah, RBBLR, Snowden, Purple Viking, Russet Burbank, and Carib E (known varieties). The random primers used to compare these varieties were OPA-02, OPA-15,OPA-04, OPA-05, OPA-12, OPA-14, OPA-16, OPA-18, OPB-14, OPD-04, and OPM-12. Two University of British Columbia ISSR primers were also used, UBC-824 and UBC-828.

Data Scoring
Dendrogram

To study the relationship between different potato varieties, a dendrogram was constructed. A dendrogram is a pairwise relationship among all the sequences used to determine cluster alignment order. Alignment begins with the two most similar sequences and end with the most distant sequences.

All data was inputted into a Microsoft Excel file. The symbols 1 and 0 were used to represent the bands a DNA fingerprint has (1 means a band is present and 0 means the same band is absent). To do this, we figured out how many total bands there were obtained from a primer. Each column represented a variety of potato and each row represented a band e.g. OPA12-1, OPA12-2, and so on. A '1' was added to the matrix if there was a band in that variety and a '0' if there wasn't one. This data was entered into a computer program called DNAMAN which gave a chart of the percentage relationships between the different potato varieties and a dendrogram showing the relationship.

Results

The results of the DNA extraction were good. This was determined by the PCR. If the PCR hadn't turn out at all for one of the potatoes, this would have shown that the extraction method hadn't worked properly. The PCR results showed how well the primers turned out with the potato DNA (Figures 1and 2).

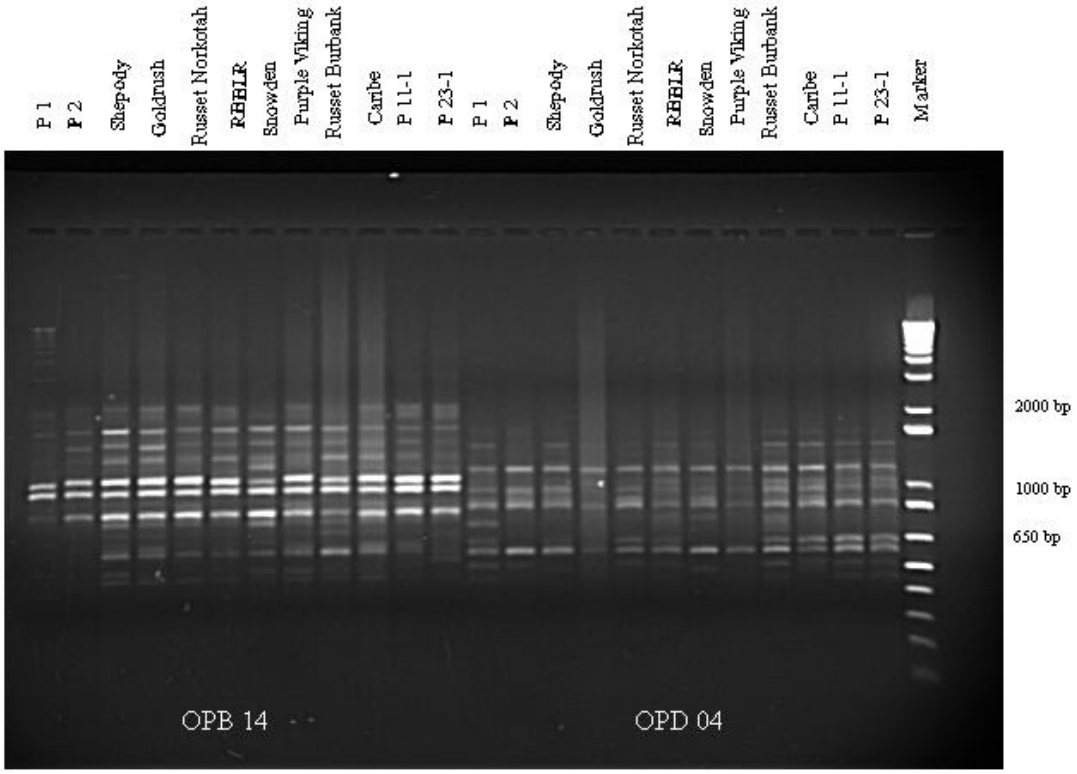

Figure 1. DNA amplification of 12 potato varieties with random primers OPB 14 and OPD 04. It shows many monomorphic bands (band present in all varieties) indicating genetic similarity in these potato varieties. On the extreme right is a marker (DNA ladder) with bands of known size and with this we can figure out the size of other bands.

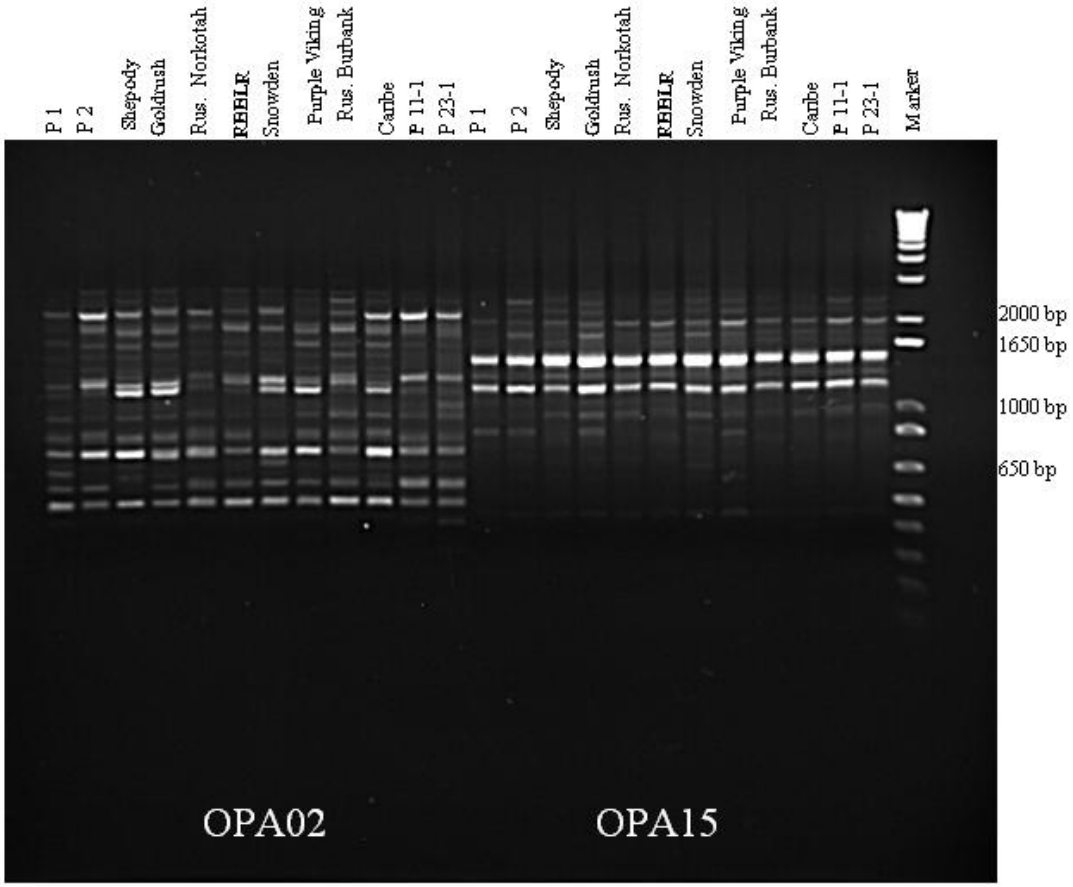

Figure 2. DNA amplification of 12 potato varieties with random primers OPA 02 and OPA 15. It shows many polymorphic bands (band present in one variety but absent in others) indicating genetic diversity in these potato varieties. On the extreme right is a marker (DNA ladder) with bands of known size and with this we can figure out the size of other bands.

Genetic relationships of potato varieties as revealed by RAPD fingerprints

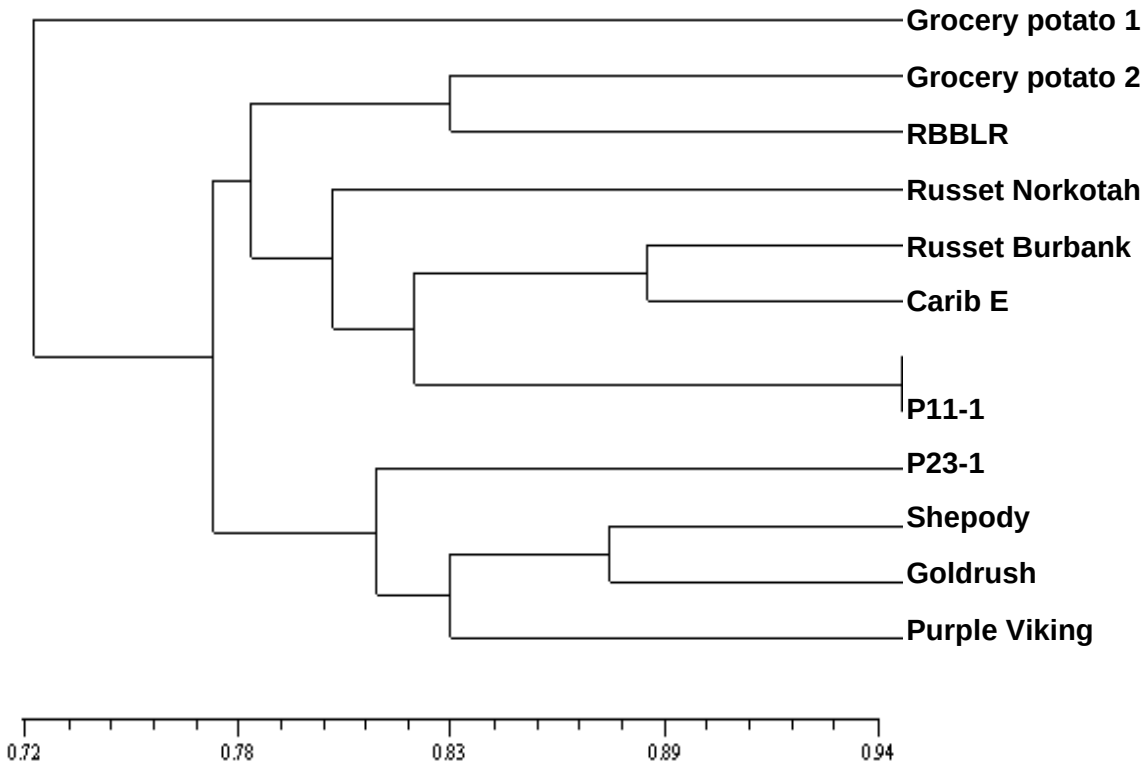

Figure 3. Dendrogram showing the genetic relationship between potato varieties e.g. Test lines P11-1 and P23-1 are genetically similar and Grocery Potato is different from all other known varieties tested.

Initially, on the test potatoes, different template concentrations viz. 25ng, 50ng, 100ng, and 200ng/ml were tried. It was discovered that 100ng/ml was the best, although 50ng/ml was not bad either. In further experiments, 100ng/ml template concentration was used.

Ten random primers from Operon Inc. were tried. It was found that the one with the least number of bands was the primer OPA18, which only gave two monomorphic bands (when a band is present in all tested lines) in all the potato varieties. Whereas, primers OPA02 and OPA14 each produced 14 bands. The Primer OPA02 gave the most polymorphic bands (when a band is present in one line and absent in others) among the potato varieties tested. The data was analyzed and the dendrogram showed the differences between all the varieties (Figure 3). It showed that P1 had no close relation to any other potato variety tested and Russet Burbank was related to Carib E. P2 was closely related (83 %) to the known variety RBBLR. The potatoes with the closest similarity were P 11-1 and P 23-1.

Discussion

The experiment turned out very well. Our question was “How much do potato varieties vary from one another?” The DNA fingerprinting technique (RAPD) we used to demonstrate the variation between potato varieties worked well. The tested potato varieties vary a lot between one another and the PCR results were clear to demonstrate this variation. Variation is desirable because it shows that there is diversity between varieties/line and promotes genetic advances, which can be made within the species. More the diversity, more the chances to improve a particular plant species. For example we could transfer the good characters like disease resistance in one potato variety. The dendrogram showed that no potato tested was more than 88% similar. With DNA fingerprinting, it is possible to tell whether a unknown line (like P1 and P2 from a grocery store) is related to a known variety or not. Our results showed that P1wais not related to any other line tested and P2 was closely related to the known variety RBBLR. The two test lines P11-1 and P23-1 are genetic modification of one variety and our results showed that they are very closely related.

References

Procunier, J. D., Jie, X., and Kasha, K. J. Barley Genetics Newsletter. 1991, 20, 74-75.

Acknowledgements

I would like to acknowledge my parents (D and B Brenna) for entering me in this competition, my high school science teacher, Mr. Johnson, for helping me with anything I needed. I sincerely thank my mentors, Dr T Grewal and Dr K Bett, Department of Plant Science, University of Saskatchewan for all that they taught me about this project. I would also like to thank Aventis Biotech. Inc., Ag-West Biotech. Inc., and their sponsors for holding this competition and supplying money for the experiments. I would also like to thank Dr. G Scoles and research personnel in the Crop Molecular Genetics Lab for allowing me this great work-space. I am thankful to Dr. D Waterer, Department of Plant Science, University of Saskatchewan for providing me with the potato varieties.