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Organisation of the chloroplast genome of kiwifruit (*Actinidia deliciosa*)

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Summary. A restriction map of the chloroplast genome has been determined for kiwifruit, *Actinidia deliciosa.* Single and multiple enzyme digests of kiwifruit chloroplast DNA were hybridised to a set of *Brassica* chloroplast probes, and the kiwifruit bands aligned with the known *Brassica* map. The chloroplast DNA of kiwifruit is typical of the majority of angiosperm chloroplast genomes; it is 160 kb in size, contains a 15–34 kb inverted repeat, and its gene content and gene order are similar to those of the *Brassica* chloroplast genome.

Key words: *Actinidia deliciosa* – *Brassica juncea* – Chloroplast genome – Kiwifruit

The kiwifruit industry is a monoculture, based almost entirely on a single vegetatively-propagated cultivar, "Hayward", that was developed from a small population of seeds introduced into New Zealand from China in 1904 (Ferguson and Lay Yee 1983). There is considerable interest in breeding new commercial varieties of kiwifruit for horticulture, partly to overcome the potential danger posed by the existence of a monoculture, and partly because of the potential advantages for genetic improvement offered by this almost completely unexploited genus.

Kiwifruit, formerly known as a variety of *Actinidia chinensis,* was recently reclassified as a separate species *A. deliciosa* (Liang and Ferguson 1986). Relationships between the 55 recognised *Actinidia* species have been established on the basis of taxonomic characteristics (Liang 1982). We are interested in applying molecular analysis to investigate the relationships between *Actinidia* species. Comparison of restriction fragment length polymorphism between the chloroplast genomes of related plants has proved to be a valuable technique in molecular taxonomy (see Palmer et al. 1983; Palmer et al. 1985; Palmer 1985). In this paper we present the physical characterisation by restriction mapping of the chloroplast genome of *Actinidia deliciosa,* as a first step in a phylogenetic comparison of chloroplast genomes within the genus *Actinidiaceae.*

Materials and methods

Seeds were obtained from controlled pollinations of *Actinidia deliciosa* "Hayward" with known male varieties (obtained from A. Seal, DSIR, Mt Albert). Seed was stored at 4 °C and germinated in potting mix in the glasshouse (natural lighting, 20–25 °C day temperature, 12–16 °C night temperature). Seedlings were watered regularly, and a solution of 0.5 x MS salts (Murashige and Skoog 1962) applied twice weekly. After 5–10 weeks, seedlings were transferred into the dark for 4–5 days, and the leaves were harvested. Chloroplasts were isolated following the procedures described by Palmer (1982, 1985). Leaves were ground using a 15:1 ratio of buffer: tissue, and chloroplasts purified by two cycles of sucrose gradient centrifugation. The incorporation of polyethylene glycol and polyvinyl pyrrolidone in the grinding buffer, and the inclusion of two EDTA washes (see Palmer 1985) are important steps in the isolation procedure for kiwifruit. Chloroplasts were lysed with sodium dodecylsulphate, sarkosyl, and proteinase K, and the DNA released was purified on a CsCl gradient.

Plasmid DNA purification, restriction enzyme digestion, gel electrophoresis, and hybridisation procedures all followed Maniatis et al. (1982). Southern transfer utilised the bidirectional blotting technique (Smith and Summers 1980), but with the solvent and nylon membrane treatments of Reed and Mann (1985). The probes were a series of *Pst*I and/or *Sac*I fragments of the *Brassica juncea* chloroplast genome cloned into pUC vectors (J. Palmer, personal communication). In addition, three gene-specific probes were used: an 1,167 bp *Pst*I-XbaI fragment from the pea *rbc*L gene (J. Palmer, personal communication), a 532 bp fragment from the pea *psb*A gene (Oishi et al. 1984), and a 3.5 kb *Sac*I fragment containing the mung bean 23S

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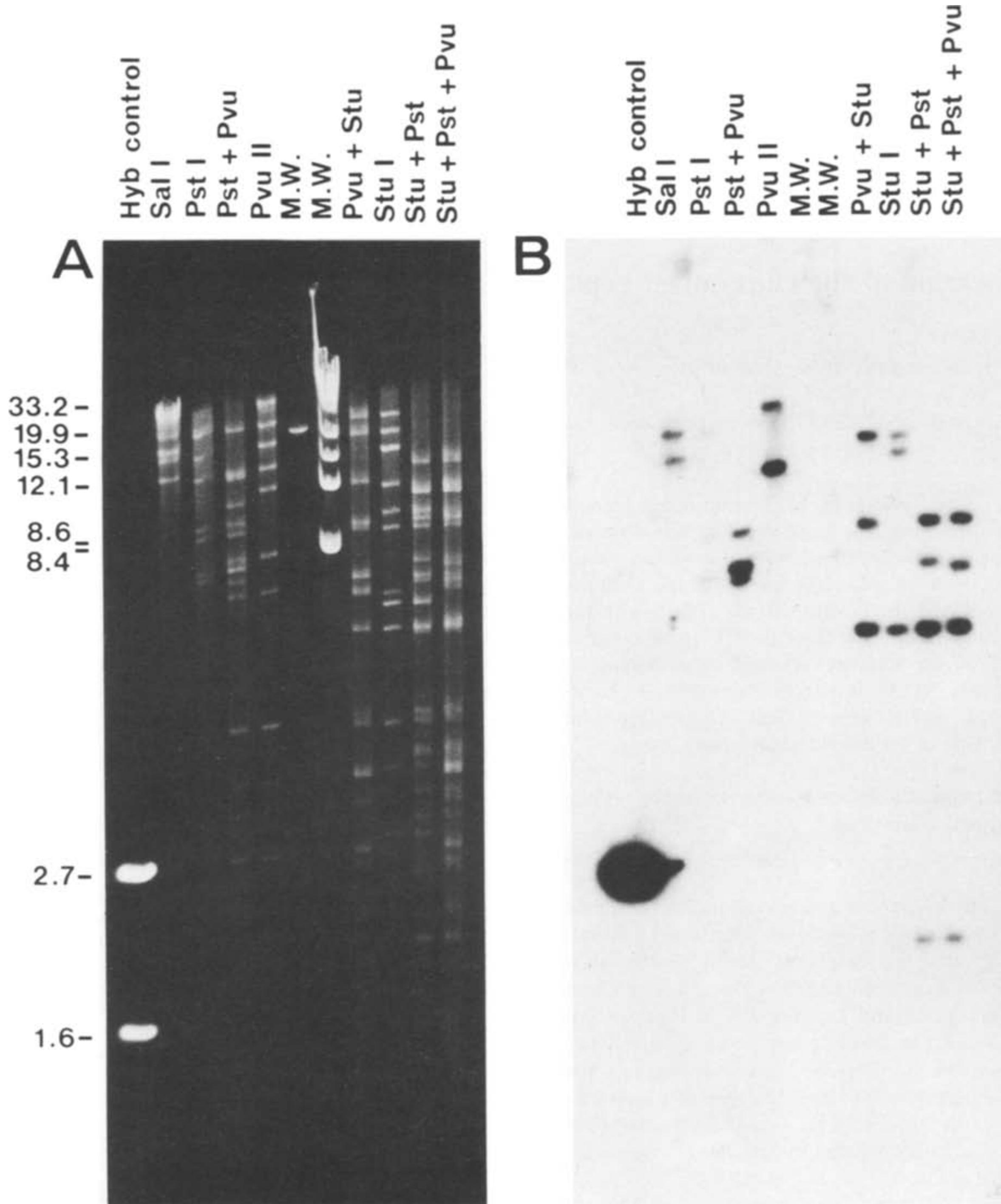

Fig. 1. Overlapping filter hybridisation. *Part A* shows a 0.6% agarose gel containing restriction digests of kiwifruit chloroplast DNA with the enzymes shown. The hybridisation control consisted of a *Pst*I digest of Brassica probe 22. All of the probes used hybridised to the pUC vector (2.7 kb fragment); only probe 22 hybridised to the insert (1.6 kb). The molecular weight markers (MW) were the 23.7 kb *Hind*III fragment of lambda, and a mixture of a *Sal*I and a *Sma*I digest of lambda DNA (sizes indicated on the *left* in kb). *Part B* shows the autoradiograph that resulted from hybridising *Brassica* probe 7 (see Fig. 2) to a nylon membrane after transfer of DNA from the gel

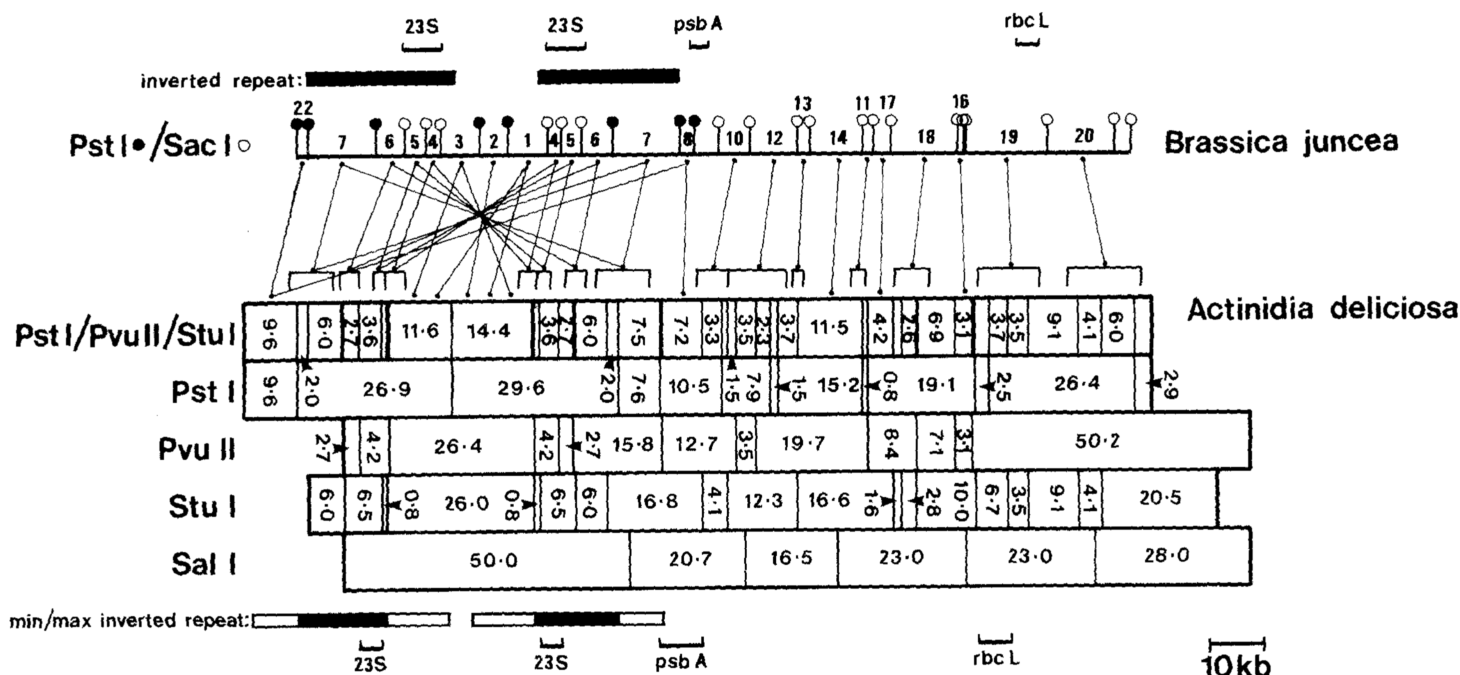

Fig. 2. Hybridisation summary and restriction map of the kiwifruit chloroplast genome. The *top line* of the figure depicts the location of the probes used on the chloroplast genome of *Brassica juncea.* The inverted repeats and the location of the three genes used as gene-specific probes are shown above the map. The *bottom part* of the figure shows the map derived for the kiwifruit chloroplast genome. Hybridisation of the *Brassica* probes to particular kiwifruit fragments is indicated by *lines.* The sizes of the restriction fragments for each of the four enzymes used are shown in kb. A combined *Pst*I + *Stu*I + *Pvu*II digest is also shown, with the sizes of the larger fragments given to allow the individual maps to be aligned. The minimum (*filled bar*) and maximum (*open bar*) size of the inverted repeat in kiwifruit is indicated. Multiple digests with *Sal*I were not performed, so that the *Sal*I restriction map is not precisely aligned with the other three

rDNA gene (Palmer and Thompson 1981). Hybridisation probes were prepared by nick translation of undigested plasmid DNA using a BRL kit. Hybridisation was at 42 °C in 25% formamide, and washes were at 60 °C in 2 x SSC, 0.5% SDS. These conditions would allow hybridisation between sequences with approximately 25% mismatch (Maniatis et al. 1982).

The restriction map was established by sequentially hybridising 19 *Brassica* probes to the digests of the chloroplast DNA shown in Fig. 1. The order of fragments could be established in many cases by observing instances where adjacent *Brassica* probes hybridised to common bands in the kiwifruit digests. In some cases a second enzyme was required to establish the order, and in others the double digests were required. In several cases, small fragments (< 0.7 kb) that would have run off the gel were hypothesised to have been present in the double and multiple digests, in order to obtain a total fragment number that was consistent with those in the single digests. The final map was consistent with all the hybridisation results.

Results and discussion

Chloroplast DNA was isolated from the leaves of young seedlings of "Hayward" using standard sucrose gradient procedures. Initial attempts to isolate DNA from young leaves of cuttings or orchard-grown material were unsuccessful, although parallel experiments with petunias worked well. The difficulties with kiwifruit were attributed to the high polysaccharide content of the material. However, leaves from dark-treated seedlings produced some kiwifruit chloroplast DNA, albeit at moderately low yield (0.3 μg/g fresh weight of leaf) and containing 20–40% contaminating nuclear DNA, as judged by gel electrophoresis (see Fig. 1). The use of seedling material as a source of chloroplast DNA introduced the possibility that the material would not be uniform. However, we observed no heterogeneity in the restriction patterns or Southern hybridisation results. Using seedling material as a source of DNA also raised the question of whether the DNA obtained is representative of the variety "Hayward", since maternal inheritance has not been established for *Actinidia.* However, we feel that this is unlikely, since maternal inheritance is the predominant pattern for angiosperms, and since the cultivars selected in New Zealand have an extremely narrow genetic base and are all likely to have identical chloroplast genomes.

Digests of the DNA with a number of restriction enzymes gave estimates of 155–165 kb for the size of the genome. We used the overlap filter hybridisation strategy (Palmer 1985) to obtain a detailed restriction map for four enzymes (*Pst*I, *Stu*I, *Pvu*II, and *Sal*I). A series

of nineteen probes covering the *Brassica juncea* chloroplast genome (kindly provided by J. Palmer) was used to probe single and multiple digests of kiwifruit chloroplast DNA. Three gene-specific probes (*rbc*L, *psb*A, and the 23S rDNA genes, see Materials and Methods) were also used. An example of one of the gels and a resulting blot is shown in Fig. 1.

A summary of the hybridisation results and the restriction map derived for kiwifruit chloroplast DNA is shown in Fig. 2. The relationship between the *Brassica* and kiwifruit genomes is linear, given the presence of inverted repeats in both molecules. Thus there is no suggestion of large rearrangements between the genomes, although small changes located within individual restriction fragments would not have been detected. In addition, the three genes localised are all in similar positions on the kiwifruit molecule to their homologues on the *Brassica* genome. The kiwifruit chloroplast may have additional genes within its inverted repeat compared to *Brassica juncea,* since there are differences in the hybridisation patterns shown in Fig. 2 for probes 8 and 22, which would be predicted to have identical patterns if the repeats have identical content. The kiwifruit molecule overall is 9 kb larger than the *Brassica* genome (160 vs 151 kb). Because of a lack of restriction sites in the region, our estimate for the size of the inverted repeat in kiwifruit ranges from 15–34 kb. All of the size difference between kiwifruit and *Brassica juncea* would be accounted for if the upper estimate is correct.

In conclusion, we found no evidence for a change in gene arrangement or gene content between the chloroplast genomes of kiwifruit and *Brassica,* two species which belong to the angiosperm subclass Dillenidae. Kiwifruit therefore possesses the canonical type of angiosperm chloroplast genome, similar to spinach (Whitfeld and Bottomley 1983) or tobacco (Shinozaki et al. 1986). Knowledge of the genetic organisation and the restriction map of the kiwifruit chloroplast genome will assist us to compare species relationships within the genus *Actinidia.*

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References

Ferguson A, Lay Yee M (1983) Kiwifruit. In: Wratt G, Smith H (eds) Plant breeding in New Zealand. Butterworths, Wellington

Liang C (1982) Guihaia NZ 4:181–182

Liang C, Ferguson A (1986) N Z J Bot 24:183–184

Maniatis T, Fritsch E, Sambrook J (1982) Molecular cloning. A laboratory manual. Cold Spring Harbour Laboratory, New York

Murashige T, Skoog F (1962) Physiol Plant 15:473–497

Oishi K, Shapiro D, Tewari K (1984) Mol Cell Biol 4:2556–2563

Palmer J (1982) Nucleic Acids Res 10:1593–1605

Palmer J (1985) In: MacIntyre R (ed) Monographs in evolutionary biology: molecular evolutionary genetics. Plenum Press, New York

Palmer J, Thompson W (1981) Proc Natl Acad Sci USA 78: 5533–5537

Palmer J, Shields C, Cohen D, Orton T (1983) Theor Appl Genet 65:181–189

Palmer J, Jorgenson R, Thompson W (1985) Genetics 109: 195–213

Reed K, Mann D (1985) Nucleic Acids Res 13:7207–7221

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng B, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada M, Sugiura M (1986) EMBO J 5:2043–2049

Smith G Summers M (1980) Anal Biochem 109:129–129

Whitfeld P, Bottomley W (1983) Annu Rev Plant Physiol 34: 279–310

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