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B.T.

FINAL REPORT

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Plastom Manipulation in Plant Protoplast Systems

S. Izhar, M.R. Hanson, R. Frankel, J. Beckmann,
E.M. Aususbel

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Plastom manipulation in Plant protoplast systems

Names of Investigators:

Principal Investigator: S. Izhar

Cooperating Investigator(s): M.R. Hanson

Name and Address of Affiliated Institutions:

Principal Institution: Agr. Research Organization (ARO)

The Volcani Center, Bet-Dagan 50250

Cooperating Institution(s): University of Virginia

Charlottesville, Va. 22091

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Signatures:

Principal Investigator

Institution's Authorizing Official

Hanay Izhar

Yuval Eshdat
Dr. YUVAL ESHDAT
Head, Div. for Research Projects and Budgets
Agricultural Research Organization

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C. Abstract

The main efforts in this projects centered about cytoplasmic male sterility (cms) in Petunia, the manipulation of this trait via protoplast fusion, molecular characterization of the cytoplasmic organelle DNA with special reference to cms, and the inheritance of the cms, a nuclear gene for male sterility and male fertility restoration gene.

In the somatic fusion experiments we showed that direct transfer of sterile and fertile plasmons is possible and that there may be nuclear control on the survival of the hypothetical cytoplasmic sterility (ste) or fertility (fte) elements in the somatic hybrid products (Izhar et al 1984).

In one experiment we describe large population of somatic hybrid plants with tetraploid hybrid nuclei and plasmonic variation. Most of the somatic hybrid plants (3923) were homoplasmic male fertile, 92 plants were homoplasmic male sterile and 32 were heteroplasmic plants containing both ste and fte. Somatic and gametic sorting out occurred only in sterile tissue. Nevertheless, in some cases, the two kinds of elements coexisted in a single heteroplasmic cell, prevailing trough several meiotic cycles (Izhar et al 1983).

Molecular analysis of the mitochondrial (mt)DNA was done by endonuclease restriction and Southern blot hybridization. We identified no clear sterile-specific correlation between the mt DNA restriction patterns of

cytoplasmic male sterile somatic hybrids and those of the male sterile parent. Similarly, we found no clear relationship between the mt DNA restriction patterns of male fertile somatic hybrids and those of the male fertile parent (Boeshore et al 1983).

In further study we found consistent differences between normal fertile derived mt DNA and cms mt DNA with respect to two specific fragments which were identified by endonuclease restriction and southern blot analyses. Restriction maps of the two related mt genome regions were constructed. These maps indicate that sequence arrangements shared by the two genomes adjoin sequences which are not shared at the corresponding locations in the two genomes. We identified and mapped a point where the mt genomes from the cms parent and the fertile parent diverge from each other.

These restriction maps allowed us to test whether a mtDNA arrangement unique to the cms parent segregated with the cms phenotype in a population of cms and fertile somatic hybrids. We found that a mtDNA arrangement unique to the cms parent was present in all 17 stable sterile somatic hybrids tested and in none of the 24 stable fertile somatic hybrids tested (Boeshore et al, 1984).

In further attempts to study the interactions between cms and the nuclear sterility allele in Petunia (e) we described genetic analysis of the interaction between cms, and the fertility restorer allele (Rf). The main findings in this study are: (1) The nuclear sterility allele can coexist in one or two dosages with the ste in somatic cells or female gametes;

(2) the presence of the fertility restorer allele Rf is not required for the coexistence of ste and e (Evenor and Izhar, 1984).

E. Description of results of cooperation

Constant exchange of plant materials and methodologies took place between the two laboratories. F. Ausubel was in Israel and had very useful discussion about the project. Irit Lifshitz spent two months in M.R. Hanson's Laboratory to learn mtDNA techniques. She collaborated with M.R. Hanson's staff which yielded joint publications. S. Izhar visited Charlottesville in November 1981 and for short sabbatical in 1982. Ellen Clark from Charlottesville spent the summer of 1982 in Bet Dagan doing cpDNA work. M.R. Hanson visited Israel in 1980. Both laboratories benefited greatly from this collaboration.

F. Benefit to Agriculture

This project deals with basic research. However, transfer of cms by protoplast may have immediate application in several crops. We found mixture of mtDNA in somatic hybrids with little sorting out. This suggest possible recombination of mtDNA in the somatic hybrid and combining some useful mt markers.

Recently we isolated and mapped mtDNA region which may be the sequence related to cms. if this will prove to be true, attempts to transfer cms by molecular means will be made.

G. Publications

1. Gilboa, Nehama, Dvora Lapushner and R. Frankel (1982). Plasmotypic heterogeneity in temperature among isonuclear substitution lines of Nicotiana (submitted).
2. Lifshitz, Irit, S. Izhar and J. Beckmann (1982) Isolation of circular mitochondrial DNA from suspension culture cells in Petunia. Plant Mol. Biol. Newsletter 3: 6-7.
- ✓ 3. Izhar, S., Miriam Shlicter and Dvora Swartzberg (1983) Sorting out in somatic hybrids of Petunia and the prevalence of the heteroplasmon through several meiotic cycles. Molec. Gen. Genetics 190: 468-474.
- ✓ 4. Boeshore, M.L., Irit Lifshitz, M.R. Hanson and S. Izhar (1983) Novel composition of Mitochondrial Genomes in Petunia somatic hybrids derived from cytoplasmic male sterile and fertile plants. Mol. Gen. Genetics 190: 459-467.
- ✓ 5. Evenor, Dalia and S. Izhar. Coexistence of cytoplasmic and nuclear genes for male sterility in Petunia. Molec. Gen. Genetics (in press).
6. Izhar, S., Yona Tabib and Dvora Swartzberg. Reciprocal transfer of male sterile and normal plasmons in Petunia. Theoret. App. Genetics (in press).
7. Boeshore L.M., MR. Hanson and S. Izhar. A variant mitochondrial DNA arrangement specific to Petunia sterile somatic hybrids. Plant molec. Biology (in press).
8. Izhar S., 1984, Male sterility in Petunia in k.c. sink (ed) The Genus Petunia, Springer Verlag (in Press).

9. Hanson M.R. (1984), Stability, variations and recombination in plant mitochondrial genomes via tissue culture and somatic hybridization. in: 1984 Oxford Survey of Plant Molecular and Cell Biology.
10. Hanson M.R. and M.F. Conde (1984) Functioning and variation of cytoplasmic genomes : Lessons from cytoplasmic - nuclear interaction affecting male fertility in plants. in: Inter Rev. Cytology (in press).