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SEMIANNUAL REPORT

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SUBMITTED TO THE U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT

TRANSGENIC SEEDLESSNESS IN GRAPES USING GENETIC ABLATION OF SEEDS

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PROJECT NUMBER: C14 177

GRANT NUMBER: TA-MOU-98-C14-177

PROJECT DURATION

1/4/2000 - 31/3/2004

BODY OF REPORT

Scientific summary

During the last 6 months of research we focussed mainly on the following aspects:

Molecular part: Additional constructs were developed for seedlessness. The contain a new ovary specific promoter and a new suicide gene. These additional two new constructs will be transferred to the South African team during the International Grape Genetic and Breeding Symposium. Both PI's will meet during this event of further discussion and reports.

Tissue culture part: Grape transformation were performed in Israel using the above mentioned constructs. Grape plants are under evaluation for their growth and performance under greenhouse and field conditions.

The Research Accomplishments During the Last 6 Months:

The general strategy

To produce transgenic grapes that express a lethal gene under the control of a seed specific promoter. The toxic gene product will interfere the normal development of the seed, leading consequently to seed abortion and seedlessness.

Engineering of additional expression vectors for *Agrobacterium* - mediated transformation of grape

Promoters:

Our genes were placed under the control of two different promoters, assumed to confer seed specific expression,

1. A seed coat specific promoter, obtained from Dr. Brain Miki and used in the first year of the project.
2. An ovary specific promoter. We have further used the ovary specific promoter DefH9.

Suicide genes

1. The vector containing the *RNase* gene, as a lethal gene, under the control of a seed coat specific promoter was constructed last year.

We have further cloned this gene also under the control of an ovary specific promoter DefH9.

2. We have used the avidin gene as a new potential lethal gene. Avidin is known to bind biotin, an important vitamin required for normal cell metabolism. When trying to overexpress avidin using organ specific promoter, tissue ablation was observed. As an alternative approach for seedlessness, we assumed that expression of avidin under the previously mentioned seed specific promoter may lead to seed ablation and seedlessness.

The engineered cassettes were then removed from the modified pUC derived vector and cloned into a PZP binary vector. The latter vector was then mobilized into the EHA 105 *Agrobacterium* strain.

Construct will be transferred to the South African group for transformation experiments as we did for the previous ones.

Transformation of tomato

Since the evaluation of grape berries will take place behond the scope of this project, we tested the feasibility of our hypothesis as well as our recently prepared construct - in tomatoes. Tomatoes were transformed using the Seed-Coat - RNase construct. Transgenic plants were transferred to the greenhouse and set fruits. Mature fruits were cut and in order to study seed ablation. Out of 12 independent transgenic plants, 9 were totally seedless while 3 had few seeds within the fruits. These promising data supported our hypothesis that a functional *RNase* gene under the control of a seed specific promoter may induce transgenic seedlessness.

Transformation of Grape

Embryogenic cultures of this seeded cultivar were produced in our laboratory. These embryogenic culture were transfer to the South African group for transformation experiments.

Working protocols for transformation were also transferred to the South African group for experiments. Transformation experiments were carried out In Israel using the important grape cultivar "Red Globe. Plants were established and were transfer to the greenhouse for further evaluation and fruit set.

Collaboration:

Travel: Both PI's met twice this year, once in South Africa and once in Israel. An additional meeting will take place during August 2002 in Hungary as both PI's will attend the International Symposium for Grape Breeding and Genetics.

Transfer of know-how: Construct will be transferred to the South African group for transformation experiments as we did for the previous ones. Working protocols for transformation were transferred to the South African group for experiments. Embryogenic culture were transfer to the South African group for transformation experiments.

Publication:

Ginzberg, I., Perl, A., Wininger, S., Badani, H., Ovdat, N. and Kapulnik, Y. (2001). Expression of strepavidin gene impedes growth and development of transgenic tomato plants. Confidential reviewed.

Wang, Q., Gafny, R., Sahar, N., Mawassi, M., Tanne. E. and Perl, A. (2002) Cryopreservation of grapevine (*Vitis vinifera* L.) embryogenic cell suspensions and subsequent plant regeneration by encapsulation-dehydration. Plant Sci. 162:551-558.

PROJECT GRANT NUMBER CDR TA-MOU-98-C14-177
PROGRESS REPORT
1 October 2001 to 30 April 2002

PROGRAMME & PROJECT LEADER INFORMATION

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PROJECT INFORMATION

Project number	CDR TA-MOU-98-C14-177

Project title	Transgenic seedlessness in grapes by using genetic ablation of seeds

Fruit kind(s)	Grapes

PROGRESS REPORT

1. Problem identification and objectives

Shortly state the problem being addressed and the ultimate aim of the project.
State the objectives for the current year and for the following year.

Currently, at the institute, seedless grapes are developed by conventional breeding and embryo rescue techniques involving seedless parents. As crosses are made progeny characteristics are unpredictable and differ from that of both parents. This implicates that a single characteristic of a certain variety cannot be changed in this way. However, by implementing certain specific biotechnological techniques it is possible to alter the seeded characteristic of a known variety to seedlessness. The objective of this project is the development of genetic transformation techniques for important seeded varieties and the development of seedless variants from these by using a seed ablation gene to alter the seeded characteristic to one of seedlessness.

2. Amended workplan (materials & methods)

Give the proposed workplan for continuation if changes are proposed to the original workplan.

Initiation of embryogenic material

Leaflets and petioles of Dauphine, Regal, Bonheur and La Rochelle were cultivated on various media to initiate somatic embryos. The composition of these media are given in the table.

Media used to initiate somatic embryos from *in vitro* leaflets and petioles of the varieties Dauphine, Regal, Bonheur and La Rochelle

1 SEI (Nitsch medium with 1 µM BA + 5 µM 2,4-D) + 10uM ABA
2 SEI + 3 g/l Gelrite (instead of agar) + 10uM ABA
3 Nitsch + 3 g/l Gelrite + 20 uM 2,4 D + 9 uM BA
4 Nitsch + 3 g/l Gelrite + 10 µM 2,4 D + 4,5 uM BA
5 Half MS + 0,8 g casein + 60g/l sucrose + 0,1 g/l inositol + 5 mg/l IASP + 2 mg/l NOA + 0,2mg/ BA + 2g/l activated charcoal
6 Nitsch + Bouquet en Davis (1989) amino acids and 1 g/l instead of 0,1 g/l casein
7 Nitsch + 266 mg/l $Ca(NO_3)_2$ instead of $CaCl_2$ + 3 g/l Gelrite + 20 µM NOA + 4 µM TDZ
8 Nitsch macro + MS micro + Nitsch vit. + 60 g sucrose + 3 g/l Gelrite + 2,5 uM 2,4 D + 2,5 uM NOA + 5 uM CPPU
9 SEI (Nitsch medium with 1 µM BA + 5 µM) 2,4-D Control

Maintenance of somatic embryos

The growth regulator free medium used regularly in our laboratory for embryo multiplication failed to give satisfactory results with Bonheur and Majestic and embryos ceased multiplication and eventually turned brown. Amendments were made by replacing agar with 2 g/l Gelrite and adding 5 uM NOA. This improved the multiplication of somatic embryos. However, the highest success was obtained when the following medium was used : Nitsch macro, MS micro, Nitsch vitamins, Bouquet & Davis amino acids, 2 g/l Gelrite, 0,2 g/l casein, 60 g/l sucrose, 0,1 g/l inositol, 2 g/l activated charcoal, 10 µM NOA, 1 µM BA and 20 µM IAA. This medium is now being used routinely for embryo maintenance of all our varieties.

Transformations

Initially all steps in the transformation procedure were done on solid medium in petri dishes as was the protocol followed in our laboratory with indicator genes. However, overgrowth of *Agrobacterium* occurred more frequently when the seed ablation genes were transferred as compared to our experience with indicator genes. The liquid culture system of Dr Perl for killing *Agrobacterium* and selection of transformants were used instead as it was reasoned that *Agrobacterium* would be more readily exposed to antibiotics in the liquid system. Claforan at 400 mg/l was used for killing *Agrobacterium* and kanamycin at 100 mg/l for selection of transformants.

Germination of putative transformants

Various approaches were followed for the germination of transformants that were selected on kanamycin medium.

The method of Perrin *et al* (2001) was followed to develop embryos into plants. Embryos were transferred from a long-term culture medium onto a pre-conditioning medium. After 4 weeks on this medium embryos were transferred onto a torpedo initiation and development medium. Torpedo stage embryos were transferred onto a medium for conversion into plants.

Other methods involved plating embryos on a germination medium (B15S, containing no growth regulators and 15 g/l sucrose) that was used previously to germinate embryos transformed with indicator genes and plating embryos on shoot elongation medium with 1 mg/l BA in petri dishes as well as in glass jars.

3. Results and discussion

Referring to the objectives, state results obtained to date and list any current benefits to the industry. Include a short discussion if applicable to your results. Please limit this discussion to essential information.

Initiation of somatic embryogenesis:

Somatic embryos developed on medium 4 from Dauphine petioles. Unfortunately these were contaminated by an endogenous micro-organism. The other varieties were put in culture to recently and results will be given in the following report (October 2002).

Transformations

Initially the method of Dr Perl worked quite well, but with later transformations somatic embryogenic material turned brown soon after it was put into liquid culture. Adaptations to the transformation protocol is currently being made

Germination of putative transformed embryos

The method used by Perrin *et al* (2001) proved to be unsuccessful in our laboratory.

The other methods also proved unsuccessful to develop plants from embryos.

Currently transformed embryos are on a maintenance medium to encourage multiplication before further attempts at germination.

Literature

BOUQUET, A. & DAVIS, H.P., 1989. Culture *in vitro* d'ovules et d'embryons de vigne (*Vitis vinifera* L.) appliquée à la sélection de variétés de raisins de table sans pépins. *Agronomie* 9, 565-574.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15: 473 - 497.

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PERRIN, M., MARTIN, D., JOLY, D., DEMANGEAT, G., THIS, P. & MASSON, J.E. 2001. Medium-dependent response of grapevine somatic embryogenic cells. *Plant Science* 161, 107-116.