PN-ADI-141

FINAL REPORT

Covering period:
August 1, 2003 to July 31, 2005

Submitted to the U.S. Agency for International Development; Bureau for Global Programs, Field Support and Research; Center for Economic Growth

TRANSFORMATION OF ORNITHOGALUM BREEDING LINES FOR VIRUS RESISTANCE

Principal Investigator: Avner Cohen
ARO - The Volcani center, Dept. Ornamental Horticulture, Bet Dagan, Israel

Collaborator: Gurling Bothma
ARC – Roodeplaat, Vegetable and Ornamental Plant Institute, Pretoria, South Africa

US-AID CDR Project: C18-006
Grant Number: TA-MOU-99-C18-006

Grant Project Officer: William Crane

Duration of project: August 1, 2000 to July 31, 2004

Table of Contents:

	Page
Executive summary	3
Section I	
A. Research Objectives.	4
B. Research accomplishments	5
B.1. Activities at the ARO.	5
Summary of the main scientific accomplishments of the project:	5
Establishment of in vitro tissues of *Ornithogalum* suitable for transformation	
Construction of transformation vectors	7
Particle Bombardment-Mediated Transformation of *Ornithogalum dubium*	8
Molecular verification for the presence of the genes	9
Molecular verification for the presence of the genes	10
B.2. Activities at ARC-Roodeplaat.	11
Cloning of the OrMV constructs	11
Biolistic transformation for stable transformation of the Israeli constructs	11
Leaf disk transformation of *Agrobacterium* with the Israeli promoters	12
C. Scientific impact of collaboration	15
D. Description of project impact	15
E. Strengthening of developing country institutions	16
F. Future work	16
Section II	
. Managerial issue	17
Budget	17
Special concerns	17
Collaboration, travel, training and publication	17
E. Requests for American Embassy Tel Aviv or AID actions	17

Executive summary

The project aims to develop virus-resistant *Ornithogalum* plants of two varieties, one from South Africa (*O.thyrsoides* x *O.dubium*) and one from Israel (*O.dubium*) using pathogen-derived resistance. Transformation protocols using particle bombardment-mediated method (at the ARO), or *Agrobacterium*-mediated method (at ARC-Roodeplaat) to deliver genes into the plant tissue were developed.

At the ARO, *O. dubium* cell clusters were bombarded with four target gene constructs (the viral coat protein (CP) and replicase (Nib) genes, each under the control of the UBQ3 and ΔSV promoters). Since stable GUS expression was evident in the cultures bombarded with all the four constructs, the cultures were transferred to a selective medium supplemented with 80 mg/l kanamycin. Following a selection period in the dark, the cell clusters were transferred to the light for a regeneration of transgenic plantlets. GUS expression was evident in most of the plants recovered from the cultures. Hundreds of transgenic plants were recovered for each of the target genes and the presence of the selective marker (nptII), the reporter gene (GUS), and target gene was verified by PCR analysis.

The South African team calibrated the system for *Agrobacterium*-mediated transformation of leaf tissue as an alternative to nicroprojectile-driven transformation of callus. Plants recovered following selection on kanamycin were GUS-positive. The optimized *Agrobacterium* method was used to transform leaf disks of the South African *Ornithogalum* clone with the same constructs used by the Israeli team to transform cell clusters of *O. dubium* using particle bombardment. Most of the recovered plantlets showed GUS expression. The presence of the transgenes was confirmed by PCR analysis.

Following the molecular verification for the presence of the transgenes, The transgenic plants developed both in South Africa and in Israel will have to pass an important tests: The transgenic plants will have to be challenged with virus infection using either mechanical means or by virus-carrying aphids, in order to determine whether the selected *Ornithogalum* plants carrying the various transgenes are, infect, resistant to virus infection.

Section I

A. Research Objectives.

The objectives of the project were:

Generating virus-resistant *Ornithogalum* plants of two varieties, one from South Africa (*O.thyrsoides* x *O.dubium*) and one from Israel (*O.dubium*) using pathogen-derived resistance. Establishing transformation protocols using particle bombardment-mediated method (at the ARO), or *Agrobacterium*-mediated method (at ARC-Roodeplaat) to deliver genes into the plant tissue. The steps for achieving theses goals included:

1. Establishment of in vitro tissues of *Ornithogalum* suitable for transformation including the calibration of the types of tissue, culture media and conditions for plantlet regeneration.
2. Determining and optimizing the conditions for successful transformation of etiolated cell clumps by particle bombardment (at the ARO), and *Agrobacterium*-mediated transformation of leaf discs in breeding line A2 (at ARC-Roodeplaat).
3. The cloning of genetic construct that include the viral coat protein(CP) of the Israeli isolate of OrMV and virus replicase (Nib) genes, isolated in South Africa, each under the control of the UBQ3 and ΔSVB promoters.
4. Transformation of *Ornithogalum* breeding lines with the gene constructs and selection of transgenic plants
5. Molecular verification of the presence of the target-genes in the regenerated plantlets.
6. Challenging the transgenic plants with virus infection and estimation of the level of virus resistance conferred by the target genes.

B. Research accomplishments

B.1. Activities at the ARO.

Summary of the main scientific accomplishments of the project:

Establishment of in vitro tissues of *Ornithogalum* suitable for transformation

Tissue culture and plantlet regeneration: Two alternative plant tissues were initially considered as a possible material for transformation. The first being leaf section from either aseptic cultures or greenhouse-grown plants, the second being de-differentiated cell clusters from liquid cultures. We have a well-calibrated system for adventitious plantlet regeneration from *O. dubium* leaf sections. Agar-solidified medium #206 (MS+2 mg/l BA+0.1 mg/l +3% sucrose) is used at the ARO for this purpose. The second approach, using cell clusters grown in liquid culture was initially excluded out of concern for somaclonal variation, vitrification and a loss of morphogenic potential. We have nevertheless, decided to pursue this approach following its successful implementation in transforming *Lilium longiflorum*. *O. dubium* cell cultures were started from callus that developed from basal parts of plantlets of a selected clone from our breeding lines (ATD) propagated in a long-term culture on a solid medium #206 in the light. This firm, compact callus was maintained in a liquid medium on a gyratory shaker (100 rpm) in the dark until ready to be used. Upon transfer to the light (16 h photoperiod at 24^0C), this culture became embryogenic and developed plantlets. The dark-grown cell clusters maintained their regenerative capacity for many months with no apparent decline in efficiency.

Competency of liquid-grown cells for transformation by particle bombardment. We constructed a particle inflow gun (PIG) that was used to propel 1.5-3 mg gold particles coated with the appropriate constructs. The plasmid DNA for the particle bombardment was isolated from *E. coli* strain DH5α, purified using plasmid Midi-Kit (Qiagen Inc., Valencia, CA, USA) and concentrated to 1μg/μl prior to bombardment. The ratio of 1μg DNA to 0.7 mg gold particles in 9 μl ethanol was used. Samples of 2-3g cell clusters were used for each bombardment and every sample was bombarded twice. In order to evaluate the competency of liquid-grown cells for transformation cell clusters were bombarded twice with gold particles coated with the plasmid pUBQ3*uid*A(GUS). The two plates had nearly 7000 blue spots of the transient GUS reaction. Two important findings were evident: first, that callus that was more compact appears to be more competent for particle bombardment than "loose"

clusters and, second, that the transfer of the cultures to a fresh medium one week prior to the bombardment also significantly increased the number of the blue spots.

Kanamycin as a preferred selectable marker: Our attempt to use the herbicide Basta as a plant selectable marker did not yield any transgenic *Ornithogalum* plants. Obvious difficulty in obtaining putative transgenic plants resulted from the drastic response of the explants. The presence of 1 mg/l Basta yielded too many "escapes" while 2 mg/l caused the tissue to bleach and excrete oxidation products into the medium with no obvious shoot regeneration. The use of the herbicide Basta as a selectable marker was discontinued in both ARC and ARO because of a very narrow margin between a concentration that is harmful to *Ornithogalum* and one that allows a regeneration of non-transgenic plants. We had similar difficulty in the transformation of *Lilium longiflorum*, which we overcame after changing the selectable marker to kanamycin. We have used the same approach in the current project and tested kanamycin as an alternative marker also for *Ornithogalum*. It was clear that when Ornithogalum cells were exposed to kanamycin there was a strong reduction in cell proliferation and a total block in regeneration but the medium remained clear with no obvious excretion of oxidation products or cell death even in concentration as high as 150 mg/l. Kanamycin was found to totally block differentiation and development at concentrations as low as 20 mg/l, even when applied at a later stage when some differentiation had already initiated. A two-week exposure of such cultures to kanamycin was sufficient to cause bleaching and death even when the tissue was subcultured onto a kanamycin-free medium. Kanamycin, therefore, was proven to be the appropriate selective marker for *Ornithogalum*.

Construction of transformation vectors:

Four different gene constructs were built for transformation purposes. These included two target genes: the coat protein gene (CP) of the Israeli isolate of OrMV (Zeidan et al., 1998), and the virus replicase gene (*NIb*) isolated in South Africa. These were placed under the control of one of the two constitutive promoters: the *Arabidopsis* polyubiquitin promoter (UBQ3) and the strawberry vein-banding virus deleted promoter (ΔSVB, accession no. AF331666), and cloned into a pCAMBIA2301 vector that has both *npt*II gene, conferring kanamycin resistance, and a GUS reporter gene (both under control of 35S promoter). In the construction of the chimeric gene standard procedures of DNA restriction, subcloning and propagation in *E. coli* were employed. Firstly, a NOS terminator was PCR cloned into pCAMBIA2301 KpnI-EcoRI sites to create pCAMBIA-

2301-NOS. The SVB deleted promoter (ΔSVB) and the *Arabidopsis* UBQ3 promoter were then PCR cloned into the HindIII-BamHI sites of pCAMBIA2301-NOS to create pCAMBIA2301-ΔSVB-NOS and pCAMBIA2301-UBQ3-NOS. The Israeli OrMV-CP and the South African OrMV *NIb* genes were then PCR cloned into the BaMHI site or BamHI-KpnI sites of either pCaMBIA2301-ΔSVB-NOS or pCaMBIA2301-UBQ3-NOS, respectively to create pCaMBIA2301-ΔSVB-CP or *NIb* and pCaMBIA2301-UBQ3-CP or *NIb*. Sense orientation of the CP gene was verified by PCR and restriction analysis. All other constructs were verified by restriction analysis. The constructs were handed over to the South African research team at ARC-Roodeplaat. All four constructs with the two target genes gave very good transient GUS expression after bombardment of cell clusters grown in liquid cultures.

Particle Bombardment-Mediated Transformation of *Ornithogalum dubium*.

Ornithogalum cell clusters with high competency for transformation were bombarded with gold particles carrying the cloned vectors that contained the target gene for their ability first, to cause transient GUS expression in the cultured cells. Two days following bombardment with plasmid DNA the bombarded cells were recultured onto liquid proliferation medium #206 supplemented with 80 mg/l kanamycin for prolonged selection in shake flasks in darkness. The cultured cells were subcultured into a fresh medium at 1-2 week intervals. The *O. dubium* cells were kept on the proliferation medium for 6 months for the development of clusters of transformed cells prior to the transfer to the regeneration medium in the light. Following the prolonged selection in the proliferation medium the dark-grown cell cultures were transferred to shoot regeneration medium in the light (16 hours photoperiod). Plantlet regeneration in *Ornithogalum* was induced on MS medium devoid of any growth substances, with 3% sucrose and 50 mg/l kanamycin. Upon regeneration, plants were transferred to agar-solidified MS medium containing 50 mg/l kanamycin for continued plant growth and development. It is worth noting that the developed plantlets were, initially, highly vitreous. It took several months on an Agar-solidified medium for these plants to recover from the hyperhydricity and develop into normal-looking transgenic plants. This caused an unforeseen delay in the project. The entire pathway for the development particle bombardment mediated transformation of *Ornithogalum* dubium is presented in plate 1.

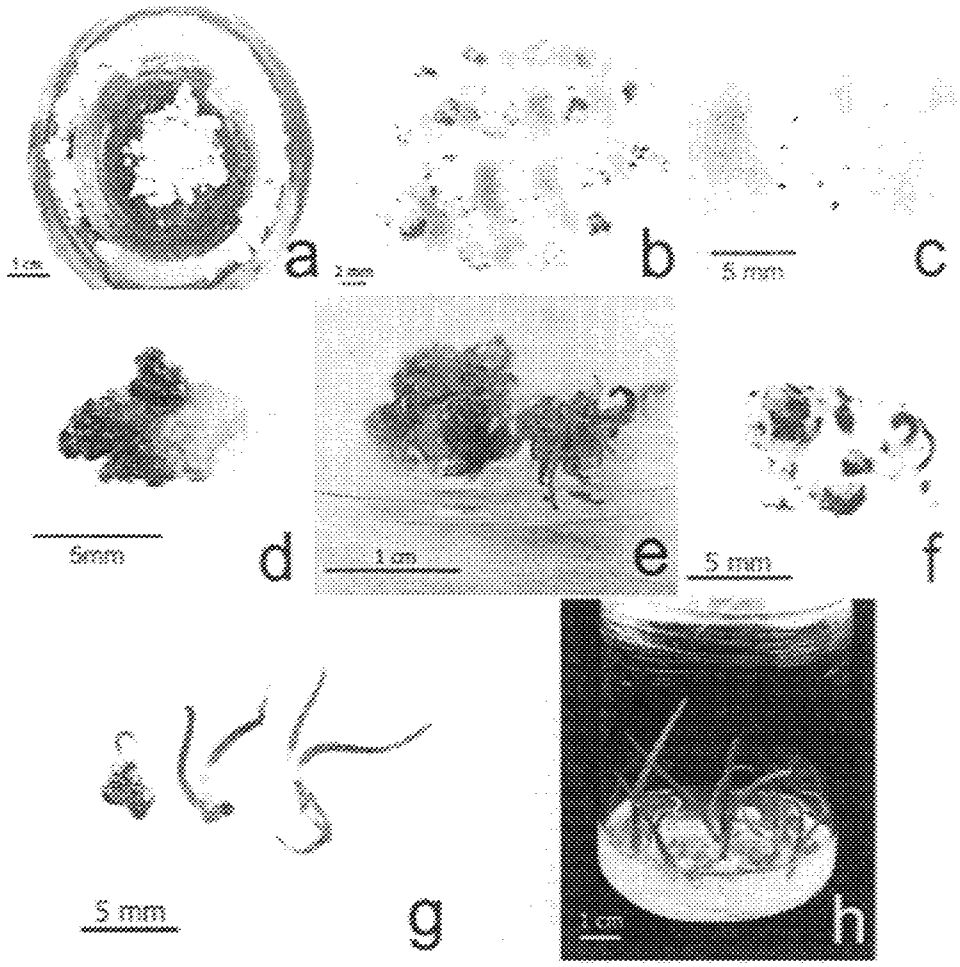

Plate 1. Genetic transformation of *O. dubium*: **a:** Cell clusters of *Ornithogalum* grown in liquid medium 206 in darkness prior to the bombardment. **b:** Transient GUS expression in competent cells bombarded with pUBQ3genGUS. **c:** Stable expression of the reporter gene in cells grown in selection medium containing 80mg/l kanamycin for 54 days. **d:** The development of organized transgenic meristematic centers in the liquid medium after prolonged selection. **e:** Plantlet regeneration in light-grown cultures in liquid MS medium containing 80 mg/l kanamycin. **f:** GUS expression in regenerating cell clumps from the culture in **e**. **g:** GUS expression in transgenic plantlets regenerated in the liquid cultures. The plantlet on the right is a non-transgenic control. **h:** Transgenic *Ornithogalum* plants on solid MS with 50mg/l kanamycin.

Molecular verification for the presence of the genes

Leaves were collected from shoots surviving on medium with 50 mg/l kanamycin and control shoots regenerated from non-bombarded explants. DNA was isolated from the leaves according to the procedure of Fulton et al. (1995). Approximately 500 ng of DNA was used as the template for PCR. Amplification of the *nptII* gene utilized the upstream primer 5' - GCC CCT GAT GCT CTT CGT CCA GAT C - 3' and the downstream primer 5' - TCG GCT ATG ACT GGG CAC AAC AGA C - 3' resulting in amplification of a 434 bp fragment. The PCR reaction was performed in 30 µl reaction volume with 1x buffer (JMR Holding, London), 1.5 mM $MgCl_2$, 0.2 mM dNTP mix plus 7.5% DMSO, 0.25 µM of each

primer and 0.75 units of *Taq* DNA polymerase. The reaction was 2 min at 95^{0}C, followed by 35 cycles of 60 sec at 94^{0}C, 45 sec at 57^{0}C, and 90 sec at 72^{0}C with a final extension at 72^{0}C for 5 min. For amplification of the GUS (*uid*A) gene the forward primer 5' - GAC GGC CTG TGG GCA TTC AGT CTG G - 3' and the reverse 5' – GTG TAG AGC ATT ACG CTG GCA TGG A - 3' were used, resulting in amplification of 487 bp fragment. For the amplification of OrMV-CP gene the forward primer 5' - CG GGA TCC ATG GGC AAA ACG AGA TAC ATT TCA G -3' and the reverse primer 5' -GG GGT ACC TCA CAT ATT AAC ACC AAG TAG TGA ATG - 3' were used, resulting in amplification of 900 bp - the entire length of the cloned gene. For amplification of the replicase (*NIb*) gene the forward primer 5' – GGA AGG GTA CAA CAA CGT GCT CAG -3' and the reverse primer 5' – GCT ATC GAA CTG TGA GCC GTC AGC – 3' were use to amplify a 490 bp fragment. The PCR reaction was performed in 30 µl reaction volume as above. PCR products were separated on 1% agarose gels and visualized with ethidium bromide.

The putative transgenic plants of *O. dubium* were analyzed by PCR for the presence of the transgenes (Figs. 1 and 2).The presence of the *npt*II gene was confirmed in all of the analyzed plants, as was expected since the plants were maintained on high levels of kanamycin through all stages of development. In fact, the majority of the plants showed co-transformation of both the reporter gene (GUS), the selectable marker (*npt*II), and the target gene (CP or *NIb*).In some plants, however, the presence of GUS was not confirmed indicating a possibility for genetic recombination (Fig. 1).

Molecular verification for the presence of the genes

An important issue to be dealt with successfully in order to fulfill the original objectives of this project is that the transgenic plants will have to be challenged with virus infection in order to estimate the level of resistance, if any, conferred by the target genes. This has been done in other plants using either mechanical means or by virus-carrying aphids. Ornithogalum plants grown in commercial fields get infected readily by virus-carrying aphids and the entire propagation stock gets totally infected and become commercially worthless in the third year. Infecting the transgenic plants artificially has been proven to be a challenge. Virus was extracted from virus-infested *Ornithogalum* plants by grinding with mortar and pestle in the presence of phosphate isolation-buffer. Control (non-transgenic) plants were sprayed with abrasive carborundum powder and the virus was applied to the

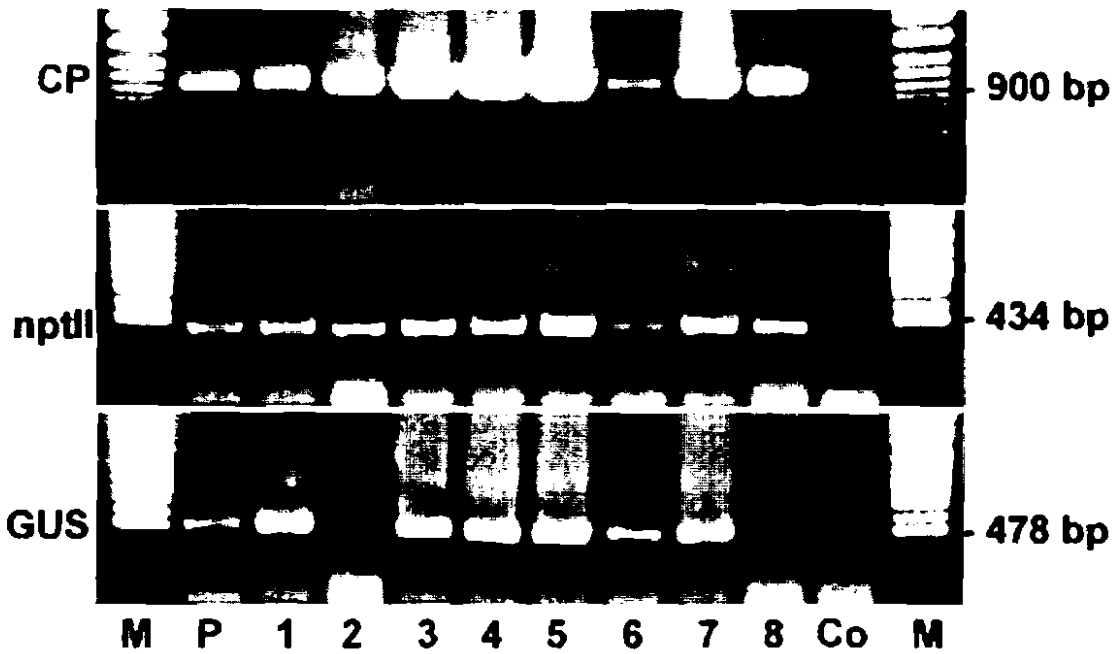

Fig. 1. PCR analysis for the reporter gene (GUS), the selectable marker (*npt*II), and the target CP gene in putative *Ornithogalum dubium* transformants. Lanes marked with M present 1 kb DNA ladder. Lane Co is an untransformed control and lane P is the plasmid vector. The plants in lanes 2 and 8 show the presence of the *npt*II and the CP gene but not that of the GUS reporter gene.

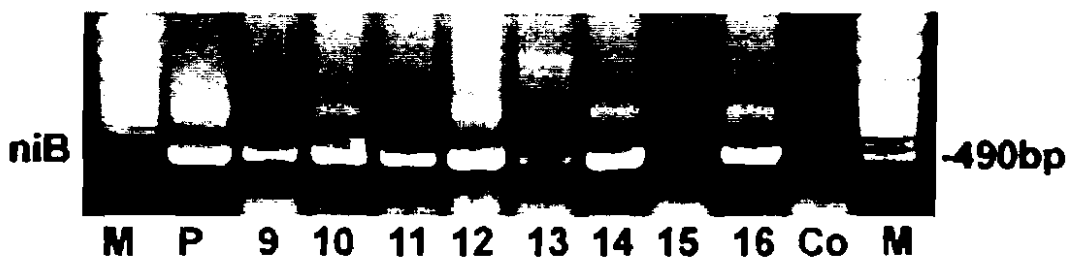

Fig. 2. PCR analysis for the target replicase (*NIb*) gene in putative *Ornithogalum dubium* transformants. Lanes marked with M present 1 kb DNA ladder. Lane Co is an untransformed control and lane P is the plasmid vector. The target gene is missing from the plant in lane 15.

leaves, using a cotton-pad. Alternatively, aphids were allowed to feed on virus-infected plants and than allowed to infect the virus-free (control) *Ornithogalum* plants. The same procedures were used to infect *Chenopodium quinoa* and *Chenopodium amaranticolor* normally used as test plants for virus infection. Symptoms appeared on the test plants within 7-10 days whereas the Ornithogalum plants had virtually no symptoms. The challenged

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plantlets were tested by ELISA. 21 days after the infection the frequency of challenge plants that were tested positive to ELISA was very small. The highest frequency of infection was 50% and many times there was no infection at all. Test plants that were used as control plants had, in the same experiments, infection rates of 100% suggesting that the problem originates in the Ornithogalum plant material rather than the virus-extraction method or the infection method. Using the virus-carrying aphid method did not have any advantage over the mechanical method, they both showed very low rates of infection of the *Ornithogalum* plants.

The results meant that we could not attempt to subject the transgenic plants to these test before further improvement of the infection methods. In addition, we had to clonally propagate each and every transgenic plant in order to minimize future false-negative results. This problem caused a further delay in the culmination of the project.

B.2. Activities at ARC-Roodeplaat

1. Cloning of the OMV constructs

Due to the unsuccessful cloning of the ARC-Roodeplaat constructs into the pCAMBIA vectors, it was decided to continue the experimental work with the constructs designed in Israel. These constructs, IS-OrMV-CP (*Ornithogalum* mosaic virus coat protein gene) and SA-OrMV-NIB (*Ornithogalum* mosaic virus replicase gene) are under control of either the ΔSVB or the UBQ3 promoter. The constructs were transformed to *E.coli* and DNA was isolate with a Qiagen® mega plasmid kit.

2. Biolistic transformation for stable transformation of the Israeli constructs

Ornithogalum callus was bombarded with the different Israeli constructs using the BioRad PDS Helium device® for stable transformation. Two days after bombardment the tissue was transferred to regeneration medium (0.1mg/l NAA and 2mg/l BA) supplemented with 80mg/l kanamycin (for selection). These plates were cultured in the growth room with a 16hr photoperiod at 23°C. The plates were transferred to fresh medium with the selection agent kanamycin every 2 weeks. Although some shoots regenerated soon after bombardment on the selection medium, the kanamycin started to have an effect and the callus which became discolored and died off. No successful transformants of the *Ornithogalum* hybrid A2 were obtained using the BioRad PDS Helium device®.

3. Leaf disk transformation of *Agrobacterium* with the Israeli promoters

Agrobacterium tumefaciens strain AGL1 was transformed using the constructs cloned in Israel, IS-OrMV-CP and SA-OrMV-NIB under control of either the ΔSVB or the UBQ3 promoter. The optimized *Agrobacterium tumefaciens* protocol developed during 2002/3 was used to transformed *Ornithogalum* A2 leaf disks. Leaves of *in vitro* plants were dissected into pieces and incisions were made on the surface to create wounding sites for transformation. The leaf explants were then dipped into the *Agrobacterium* cell suspension and co-cultivated for 3 – 5 days. The leaves were transferred to pre-selection medium with 250 mg/ℓ cefotaxime. After 11 days the leaves were transferred to selection medium containing 80mg/l kanamycin and 250 mg/l cefotaxime.

The shoots developed on medium with 2mg/l BA, 0.1mg/l NAA supplemented with 80mg/l kanamycin were subcultured to MS medium with 80mg/l for rooting. In the previous year five putative transgenic plants were selected on kanamycin (Table 1). Number A2:1 transformed with ΔSV-nib-OMV, Number A2:4 transformed with the UBQ3 IS-OrMV-CP gene, number A2:9 transformed with the ΔSV-SA-OrMV-NIb gene, number A2:24 transformed with the UBQ3 IS-OrMV-CP gene and number A2:32 transformed with the ΔSV SA-OrMV-NIb gene survived on selection medium with 80mg/l kanamycin. All of the leaves stained blue, indicating Gus activity. DNA was isolated using the Genelute plasmid kit®. PCR was performed on these shoots in order to determine if the *GUS* and *nptII* genes were integrated into the genome of these plants. It was found that number A2:1, A2:4, A2:24 and A2:32 contain the *bar*Gus gene and plants A2:1, A2:9, A2:24 and A2:32 contain the *nptII* gene. Therefore, it can be concluded that plants A2:1, A2:24 and A2:32 are stable transformants (PCR results in previous year report).

Fourteen more putative transgenic plants, numbers 41 – 54, all transformed with the ΔSV SA-OrMV-NIb gene construct (containing the South African OMV replicase gene with the strawberry vein-bending virus promoter), were selected on kanamycin (80mg/l). PCR was performed on these shoots in order to determine if the *gus*, *npt*II, OMV replicase or the OMV coat protein genes were integrated into the genome of these plants. The presence of the *gus* gene was confirmed using the following GUS primers: GUS *intron* L and GUS *intron* R. The presence of the *npt*II gene was confirmed using the following *npt*II primers: *npt*II L and the *npt*II R. The GUS gene fragment is approximately 1 000 b.p. long and the *nptII* gene fragment is approximately 700 b.p. long. Fifty ng of DNA was used per reaction.

The $MgCl_2$ concentration was 4mM. Both primers sets anneal at 64 °C. The following cycle profile was used (Table 1.)

Table 1. PCR cycle profile

Temperature	Time (seconds)	Number of cycles
95 °C	120	1
94 °C	60	
64 °C	45	35
72 °C	90	
72 °C	300	1
4 °C	600	1

Figures 3 and 4 show the bands obtained from the PCR reactions on 1% agarose gels. These results showed that the plants have been stably transformed with the constructs and that *Agrobacterium tumefaciens* can be used for transforming *Ornithogalum* breeding line A2.

Table 2 shows the plants that survived on selection medium and which stained positive for GUS activity. They also had the correct PCR bands for the gus and *npt*II genes. They were transformed with the *Ornithogalum* replicase Gene, OrMV-NIb or the *Ornithogalum* Coat protein gene, IS-OrMV-CP.

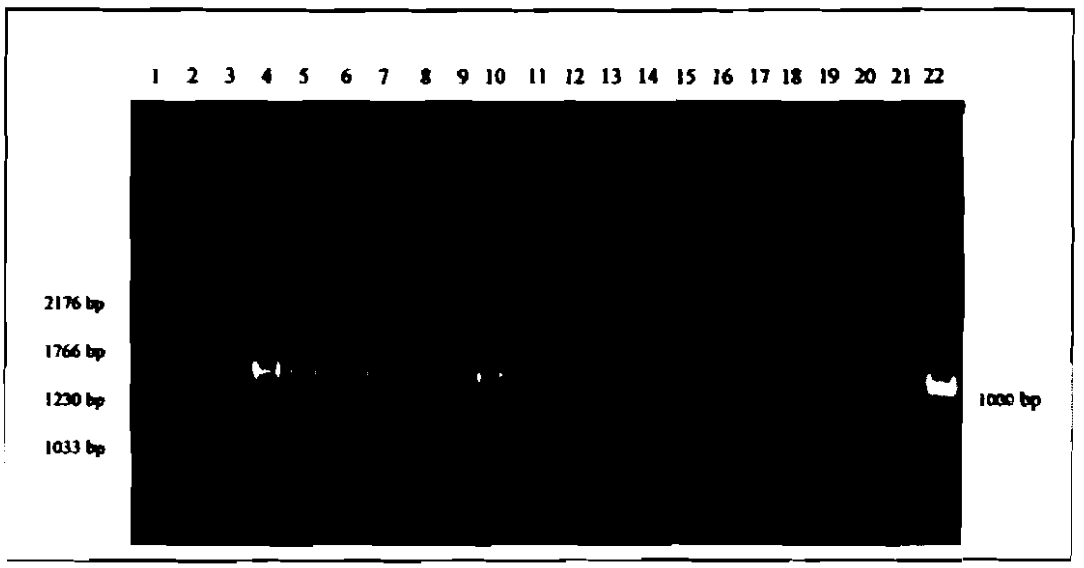

Figure 3: The presence of the *Gus* gene was confirmed using the GUS intronL and GUS intronR primers. (1) MM VI; (2) water control; (3) A2:negative control; (4) A2:1; (5) A2:4 ; (6) A2:9; (7) A2:24; (8) A2:32; (9) A2:41; (10) A2:42; (11) A2:43; (12) A2:44; (13) A2:45; (14) A2:46; (15) A2:47; (16) A2:48; (17) A2:49; (18) A2:51; (19) A2:52; (20) A2:53; (21) A2:54; (22) *Gus* positive control

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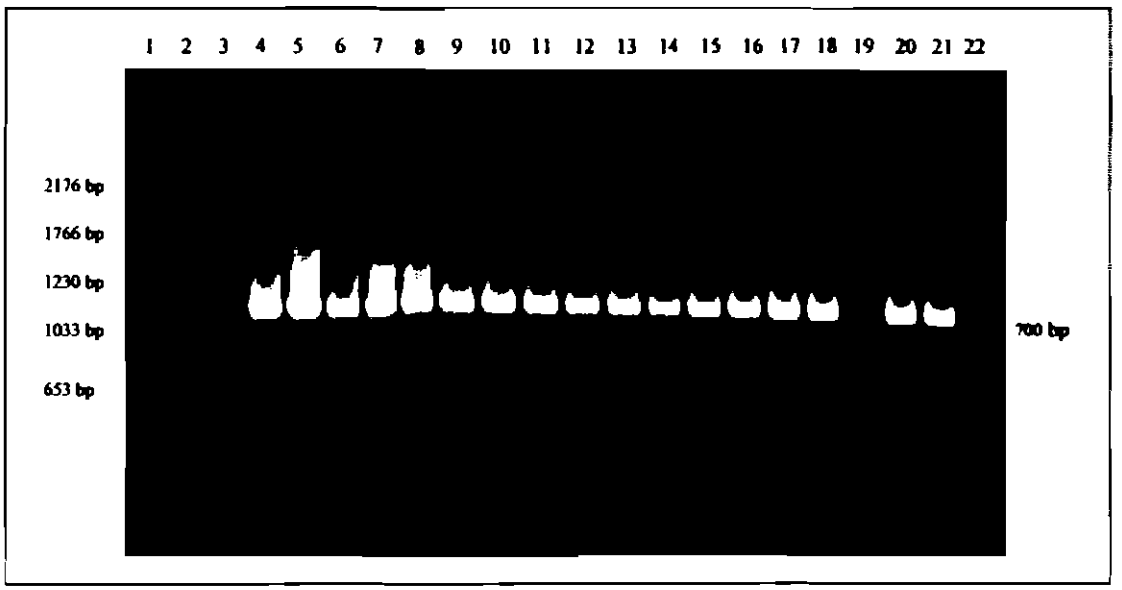

Figure 4: The presence of the *npt*II gene was confirmed using the *npt*II L and the *npt*II R primers. (1) MM VI; (2) water control; (3) A2:negative control; (4) A2:1; (5) A2:4 ; (6) A2:9; (7) A2:24; (8) A2:32; (9) A2:41; (10) A2:42; (11) A2:43; (12) A2:44; (13) A2:45; (14) A2:46; (15) A2:47; (16) A2:48; (17) A2:49; (18) A2:51; (19) A2:52; (20) A2:53; (21) A2:54; (22) *npt*II positive control

Table 2: Table listing the putative transformed plants

Plant #	Promoter	NIb gene	CP gene	Expression of GUS activity	PCR result for the *GUS gene*	PCR result for the *npt*II gene
A2:1	ΔSV	x		None	x	x
A2:4	Ubq3		x	Moderate	x	x
A2:9	ΔSV			None	-	x
A2:24	UBQ3		x	Strong	x	x
A2:32	ΔSV	x		Faint	x	x
A2:41	ΔSV	x		Strong	x	x
A2:42	ΔSV	x		Faint	x	x
A2:43	ΔSV	x		Strong	x	x
A2:44	ΔSV	x		Faint	x Faint band	x
A2:45	ΔSV	x		None	x Faint band	x
A2:46	ΔSV	x		None	-	x
A2:47	ΔSV	x		None	x Faint band	x
A2:48	ΔSV	x		Faint	x Faint band	x
A2:49	ΔSV	x		Faint	-	x
A2:51	ΔSV	x		Strong	x Faint band	x
A2:52	ΔSV	x		Faint	-	-
A2:53	ΔSV	x		None	-	x
A2:54	ΔSV	x		Faint	-	x

From the results shown in Table 2 it can be concluded that the following plants are not stably transformed with the *gus* and *npt*II genes; A2:9, A2:46, A2:49, A2:52, A2:53 and A2:54.

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The putative transformants were multiplied using leaf material on medium containing 2mg/l BA and 0.1mg/l NAA. These plants will be challenged with the virus in the glasshouse.

C. Scientific Impact of Collaboration

In order to find the most efficient promoters for the use in *Ornithogalum* transformation, six different promoters were compared at ARC-Roodeplaat for their efficiency to bring about transient GUS expression in *Ornithogalum* callus by microprojectile bombardment. Three of them were constructed by the Israeli team and the other three were constructed in South Africa. The South African compared the functioning of six promoters in callus of Ornithogalum A2. Since no one promoter performed discernibly better than the others and all of them were functional and gave more or less the same results, it was decided to use UBQ3 and ΔSVB promoters. UBQ3 was used after proving to be very efficient in driving GUS reaction. The ΔSVB was used because it was found as efficient as 35S in other systems as well as being free of patent restrictions. The Israeli team cloned both the Israeli isolate of OrMV coat protein and the NIb (replicase) gene that was isolated by the South African team, under the control of UBQ3 and ΔSVB. These four constructs were used by both collaborating groups in the attempt to transform *Ornithogalum* clones using either *Agrobacterium*-mediated transformation (in South Africa), or the particle acceleration method (in Israel). All the construct use include kanamycin as a selectable marker following the mutual decision made to discontinue the use of the herbicide Basta as a selectable marker for transgenic *Ornithogalum*, due to the narrow margin between concentrations that allow "escape" of non-transgenic plants and the one that caused necrosis and cell death. Calibrating the methods for artificially infecting plants with virus should be another issue for collaboration. The inefficiency of the present infection methods is evident in both South Africa and Israel. We need to improve these methods in order to verify that the transgenic plants are, in fact resistant to virus infection and put them to a test in both countries.

D. Description of Project Impact

The *Agrobacterium* method seemed to be more efficient for the South African *Ornithogalum* than the biolistic transformation method. In contrast, the Israeli self-constructed particle inflow gun (PIG) used for biolistic transformation, was found very efficient in transforming

the Israeli selection of Ornithogalum *dubium*. In fact, the system is so efficient that the number of putative transgenic plants is too large to handle. The BioRad PDS Helium device[8] was found by far less efficient than the self-constructed inflow gun assembled at a fraction of the cost at the ARO. It is possible that this gun is at least partially to blame one of the reasons for the unsuccessful attempts by the South African team to transform callus via particle bombardment. *Agrobacterium*-mediated transformation is usually less effective for the transformation of monocotyledous plants. The research team at ARC-roodeplaat calibrated the system for *Ornithogalum*. The knowledge gained in this project may prove very useful for future transformation of other bulbous crops.

E. Strengthening of Developing Country Institutions

Techniques learned and the knowledge gained from the project using either the microprojectile technique or *Agrobacterium*-mediated transformation could be used for future transformation protocols for other bulbous crops, many of which are indigenous to South Africa. In ARC-Roodeplat, there is an active breeding program of *Lachenalia* an important flower bulb, which is also affected by the same virus. In addition, the system could also be used to transform other *Ornithogalum* breeding lines with other genes of merits, like ones that confers resistance to bacterial diseases such as *Erwinia*, which is a major cause of yield losses in *Ornithogalum*, in *Lachenalia*, Zanthedeschia, as well as in many other bulbous crops. The transgenic plants, if proven to be resistant to the virus, could be distributed as such following rapid commercial propagation. Alternatively it could be used as a source of virus resistance in the active breeding program aiming to offer high quality propagation material for the growers.

F. Future Work

In published reports on attempts to transform various crops for virus resistance, two things are evident. The first, is, that target gene does not have to be expressed in order to prompt resistance to the virus. In fact in most cases, virus resistance is induced by gene silencing rather than expression. Secondly, the presence of the virus-resistance gene in the transgenic plants does not necessarily mean that the transgenic plant is actually resistant. Consequently, transgenic plants have challenged with virus infection in order to confirm their state and level of resistance to virus infection. The rate of the confirmed infection using either mechnical means or by virus-carrying aphids was too low for confirming whether the

transgenic plants are in fact resistant. The method of infection has to be perfected in order to bring the project for its successful conclusion.

Section II

Managerial issues

None

Budget

As per previously submitted invoice

A. Special concerns

None

B. Collaboration, Travel, Training and Publications

Results of this project were reported, in part, at the IX- Symposium on Flower-bulbs held in Niigata, Japan in April 2004 and was published as a paper:

Cohen, A., et al. 2005. Particle Bombardment-Mediated Transformation of *Ornithogalum dubium* for Ornithogalum Mosaic Virus Resistance.

Acta Hort. 673:183-190.

C. Requests for American Embassy Tel Aviv or AID actions

None