TTI-GG PROJECT AGREEMENT

Regarding

"Bridging the genomes in lily: creating an EST mapping framework for introgression breeding"

The undersigned:

1. Van Zanten Flowerbulbs B.V., established in Hillegom, whose registered office is at 1e Loosterweg 1A (2182 BL) Hillegom, hereinafter also referred to as "party 1",lawfully represented by A.F. Veldhuyzen van Zanten;
2. MakBreeding B.V. established in 't Zand, whose registered office is at Wagendwarspad 4 (1771 RN) Wieringerwerf, hereinafter also referred to as "party 2", lawfully represented by N. Mak;
3. Vletter & Den Haan Beheer B.V., established in Rijnsburg, whose registered office is at Oegstgeesterweg 202a (2230 AE) Rijnsburg, hereinafter also referred to as "party 3", lawfully represented by A. Vletter;
4. Testcentrum voor Siergewassen B.V., established in Hillegom, whose registered office is at Hyacintenlaan 10 (2182 DE) Hillegom, hereinafter also referred to as "party 4", lawfully represented by G. van Klaveren;
5. Worldbreeding B.V., established in Breezand, whose registered office is at Burg. Lovinkstraat 105 (1764 GD) Breezand, hereinafter also referred to as "party 5", lawfully represented by P.J. Kos;
6. De Jong Beheer B.V., established in Andijk, whose registered office is at Kerkepad 28 (1619 AE) Andijk, hereinafter also referred to as "party 6",lawfully represented by W. van der Kooij
7. C. Steenvoorden B.V. established in Hillegom whose registered office is at Veenenburgerlaan 86 (2182 DC) Hillegom, hereinafter also referred to as "party 7", lawfully represented by C. Steenvoorden;
8. Van den Bos Breeding B.V. established in Honselersdijk , whose registered office is at Postbus 15, 2675 ZG Honselersdijk, hereinafter also referred to as "party 6",lawfully represented by R. le Clercq

   and

9. the private limited liability company Plant Research International B.V., established in Wageningen, whose registered office is at Droevendaalsesteeg 1 (6708 PB) Wageningen, The Netherlands, hereinafter also referred to as: 'PRI', lawfully represented by the General Director, Prof.Dr. R.J. Bino.

All parties hereinafter together and individually to be referred as "**Parties**" and/or "**Project Partners**"; Parties 1, 2, 3, 4, 5, 6, 7 and 8 hereinafter to be referred to as "**Commercial Project Partners**"; and Party 9 to be referred to as "**Academic Project Partner**".

WHEREAS:

A the foundation Stichting Technologisch Topinstituut Groene Genetica ("**TTI GG**") is a public-private partnership between the Dutch plant breeding and plant propagation industry and the Dutch plant sciences community, providing a platform for joint research on plant genetics, phytopathology and physiology.

B The Parties have made a proposal to TTI-GG for a project in "Bridging the genomes in lily: creating an EST mapping framework for introgression breeding" ("**Project**"). The Project has been granted and has the project number 33601003.

C The Parties would like to lay down in writing the conditions under which the Project will be executed in this Agreement (**"Agreement")**.

HAVE AGREED AS FOLLOWS:

Article 1 – Applicable Rules and Definitions

1.1 The Project and this Agreement should comply with the obligations laid down in the following documents:

- **EZ subsidieregeling IOP-TTI** – module van de experimentele Kaderregeling subsidies innovatieprojecten as published in Staatscourant no. 221 November 14, 2005;
- Articles of Association of TTI- GG executed on 4 May 2007
- **Rules and Regulations** with respect to Management of Projects (**R&R M)** adopted on September 2, 2008
- **Rules and Regulations** with respect to Financial Obligations (**R&R F)** adopted on November 14, 2007
- **Rules and Regulations** with respect to Industrial and Intellectual Property Rights (**R&R IP)** adopted on September 2, 2008
- Any other rule adopted pursuant to article 8.4 of TTI GG's Articles of Association

The Rules and Regulations (**R&R M, R&R F** and **R&R IP)** are enclosed as Annex 1 and are an entire part of this Agreement.

1.2 All the definitions provided for in the Rules and Regulations apply mutatis mutandis to this Agreement. Specially important for this Agreement are the following definitions:

- **Foreground IP Rights**: all Intellectual Property Rights to subject matter developed as a result of the activities undertaken in a Project **(**R&R IP**)**.
- **Foreground Knowhow**: All knowhow developed as a result of the activities undertaken in a Project **(**R&R IP**)**.
- **Intellectual Property Rights**: all intellectual property rights, including but not limited to patent application rights, patent rights, breeder's rights, copy rights and database rights **(**R&R IP**)**.
- **Background IP Rights**: all Intellectual Property Rights other than Foreground IP Rights **(**R&R IP**)**.
- **Background Knowhow**: All knowhow other than Foreground Knowhow **(**R&R IP**)**.
- **Project Budget:** Estimated costs incurred to carry out the Project tasks **(**R&R F**)**.
- **Affiliate:** A person who directly, or indirectly through one or more intermediaries, controls or is controlled by, or is under common Control of another Person **(**R&R F and R&R M**)**.
- **Control**: The possession, direct or indirect, of the power to direct or cause the direction of the management and policies of a Person, whether through the ownership of voting securities, by contact or otherwise **(**R&R M**)**.

- **Person**: Any corporation, limited liability company partnership, trust, joint stock company business trust, unincorporated association, joint venture, governmental authority or other legal entity of any nature whatsoever **(**R&R M**)**.

Article 2 - Project

2.1 Parties shall carry out the Project in accordance with this Agreement and the Project Proposal presented to TTI-GG which sets out the goal, duration and scope of the Project. The Project Proposal is enclosed as Annex 2 and is an integral part of this Agreement.

2.2 Parties shall make the contributions to the Project as provided in the Project Budget enclosed as Annex 3.

2.3 Ronald Snijder of Van Zanten Flowerbulbs B.V. has been appointed as Project Leader.

Article 3 – Confidentiality

3.1 Each Party shall be bound by the obligations of confidentiality as provided in Article 18 of the R&R M.

Article 4 - Publication

4.1 All publications shall comply with the provisions on publications provided by article 14 of the R&R IP and article 12 of R&R M.

4.2 Authorization of a publication as provided in Article 14 of the R&R IP shall be withhold in the following situations:

- a commercial interest is harmed because of the infringement of Parties' Background and/or other Confidential Information of any Party; or
- the information can be used as prior art in a future patent application. In this case, a publication can only be delayed with a maximum period of six months.

Article 5 -Background Knowhow and Background IP-Rights

5.1 The Parties shall also contribute to the Project with the Background Knowhow and Background IP Rights as provided in Annex 4.

5.2 The Background knowhow and Background IP Rights shall remain confidential and the Parties shall only use them for the purpose of the Project.

5.3 The Parties shall remain the owner of their respective Background Knowhow and Background IP Rights brought to the Project.

5.4 Each Party hereby grants to all Parties a royalty free, non-transferable right of use of its Parties' Background Know How and Background IP- Rights, solely for purposes of Research if and to the extent described in the Project Proposal.

Article 6 – Foreground Knowhow and Foreground IP- Rights

6.1 The provisions regarding to the ownership and use of any result of the Project are found in the R&R IP.

6.2 All Foreground Knowhow shall be owned by the Party generating it.

6.3 According to the R&R IP, TTI GG initially acquires the ownership of all Foreground IP-Rights. The processes of identification of protectable material and of filing for protection, including the costs hereby involved, are provided in Article 3 to 6 of the R&R IP.

6.4 The Parties shall be able to acquire the Foreground IP Rights as provided in Article 8 to 10 of the R&R I and IP. The compensation to be paid to acquire such intellectual property rights is equal to the market price -/- the Parties' proportional contribution as specified in Article 12 of the EZ subsidieregeling IOP-TTI.

6.5 Parties shall procure that no third party (including employees of such Parties) can claim rights (including licenses) to the results of a Project. Prior to the start of the Project, Parties must inform each other on any possible prior commitments to third parties which may in any way conflict with this obligation and Parties will consult in good faith on the measures to be taken to resolve such conflict.

6.6 When necessary for the use of Foreground Knowhow and Foreground IP-Rights, each Project Partner will grant a non-exclusive worldwide, non-transferable license to the Background Knowhow and Background IP-Rights upon first request of another Party and under the terms to be agreed by the Parties at such time.

Article 7 – License on Foreground IP Rights and Foreground Knowhow

7.1 Parties shall make available their Foreground Knowhow to other Parties under confidentiality as described in article 3 (Article 18 of the R&R M).

7.2 The Party that acquires Foreground IP Rights as provided in paragraph 6.3 above, shall grant the other Parties a non-exclusive, royalty-free worldwide, non transferable license thereon for research within the Project and without the right to sublicense.

7.3 On request of a non acquiring Party, The Party that acquires Foreground IP Rights as provided in paragraph 6.3 above, shall grant the other Party a non-exclusive, royalty-bearing, non transferable license thereon for commercial purposes, such under market conditions and with respect to the proportional contribution of such Party.

7.4 Each Commercial Project Partner can at all times and in its own discretion obtain a non-exclusive, non-transferable worldwide royalty free license to all Foreground Knowhow for commercial purposes.

7.5 The Academic Project Partner shall have the non-exclusive, non-transferable, royalty-free right to use all Foreground IP-rights and Foreground Knowhow of other Parties for internal academic research purposes. For the sake of clarity, the Academic Project Partner shall not use such Foreground IP-Rights and Foreground Knowhow in research projects, which obligate the Academic Project Partner to assign rights, in particular intellectual property rights or licenses, to Third Parties,

Article 8 – Transfer of material

8.1 In the event that during the course of this Agreement it might be necessary to exchange material, the receiving and/or the providing party can demand the conclusion of a material transfer agreement, prior to the exchange of the material, which agreement has to be in accordance with the Rules and this Agreement.

8.2 For the sake of clarity, material in the context of this Agreement has to be interpreted as, but is not limited to, biological material, particularly plant material and seeds.

Article 9 - Term

9.1 The Agreement shall take effect on 01/01/2009 and shall end on 31/12/2012; Parties shall implement the Project as specified in the Project Proposal within this period of time.

9.2 Six months before the termination of the Agreement, the Parties shall consult whether an extension of the period provided in paragraph 9.1 is necessary.

9.3 Termination of the Agreement shall be without prejudice to the obligations of Parties pursuant to the Rules and Regulations.

Article 10 – Termination

10.1 The Parties shall be bound by the provisions regarding termination of the participation provided in Article 14 and 15 of R&R M.

Article 11 - Miscellaneous

11.1 In case of a conflict between this Agreement and the Rules and Regulations, the latter shall prevail.

11.2 The invalidity or unenforceability of any term of or any right arising pursuant to this Agreement shall not adversely affect the validity or enforceability of the remaining terms and rights.

Article 12 Applicable law and disputes

12.1 This agreement shall be governed by and construed in accordance with Dutch law.

12.2 All claims and disputes arising out or relating to this Agreement or the Project shall be first referred to the competent Court in The Hague (The Netherlands).

Executed in eightfold by the Parties below,

Van Zanten Flowerbulbs B.V.
Name: A.F. Veldhuyzen van Zanten
Function: director
Date:
Place:

MakBreeding B.V.
Name: N. Mak
Function: director
Date:
Place:

Vletter & Den Haan Beheer B.V.
Name: A. Vletter
Function: director
Date:
Place:

Testcentrum voor Siergewassen B.V.
Name: G. van Klaveren
Function: director
Date:
Place:

Worldbreeding B.V.
Name: P.J. Kos
Function: director
Date:
Place:

De Jong Lelies B.V.
Name: W. van der Kooij
Function: director
Date:
Place:

C. Steenvoorden B.V.
Name: C. Steenvoorden
Function: director
Date:
Place:

VandenBos BreedingB.V.
Name: R. le Clerq
Function: director
Date:
Place:

Plant Research International B.V.
Prof. Dr. R. J. Bino
Function: General director
Date:
Place:

_____

ANNEXES:

Annex 1 Rules and Regulations

Annex 2 Proposal

Annex 3 Project Budget

Annex 4 Background Knowhow and Background IP

Annex 2

PROPOSAL FOR TTI GREEN GENETICS third call for cluster projects

The applicant

Company / organisation:	Van Zanten Flowerbulbs B.V.		
Contact person[i]:	Ronald Snijder		
Adress:	1e Loosterweg		
Zip code:	2180AA	City:	Hillegom
Telephone:	0252-535323	GSM:	+31-6-12304211
Fax:			
E-mail:	r.snijder@royalvanzanten.com		

Title of the project

Bridging the genomes in lily: creating an EST mapping framework for introgression breeding

Partners[ii]

The companies involved in this project:	Contact persons:
C1. Van Zanten Flowerbulbs BV , Hillegom	Ronald Snijder
C2. MakBreeding BV, ‘t Zand	Erwin Hoogendijk
C3. Gebr. Vletter & Den Haan Rijnsburg	Arie Vletter
C4. Testcentrum, Hillegom	Arie Peterse
C5. Worldbreeding BV, Breezand	Pieter-Jan Kos
C6. De Jong Lelies BV, Andijk	Moritz Ceulemans
C7. C. Steenvoorden BV, Hillegom	Kees Steenvoorden
C8. VandenBos Breeding BV	Rene le Clerq
The research institutes involved in this project:	Contact persons:
R1. Plant Research International, Plant Breeding - Wageningen UR	Jaap van Tuyl & Paul Arens
R2.	
R3.	
R4.	

Time

Duration of the project in months:	48 months
Is the project substantially dependent on growing seasons?	NO
If yes, in what month of the year does the project need to start?	

Research personnel needed

How many PhD students are involved in the project?	1- 2 fte
(optional) How many post docs / junior researchers are involved?	0 / 0.5 / 1 / 1.5 / … fte
(optional) How much input of technical assistants is foreseen?	……0.5….. (units fte)
(optional) How much input of senior researchers is foreseen?	……0.4….. (units fte)
Does new personnel need to be hired for this project?	NO
If yes, what type of new personnel needs to employed?	

Are candidates for this position / these positions already available?	
[iii](optional) Will any knowledge institute researchers be positioned at participating companies or vice versa? Please indicate yes or no.	

Aim of the project

Describe the aim of the project (max. 200 words).
To anticipate to the tightening of Dutch legislation with respect to the use of pesticides and the commitment of the sector to reduce spraying in general, new requirements are put on cultivar characteristics concerning disease and insect resistance. One of the main problems in lily are viral diseases. Virus infections can not be prevented by sanitary measures apart from aphid control. In the recent past the use of crop protection substances has increased due to the an increased use of mineral oil and pyrethroïdes to control aphids. Therefore virus and aphid resistances are important traits to study, in order to develop new cultivars adapted to future growing conditions with minimal pesticide input.
The aim of the project is to develop a mapping framework based on EST sequences which derived markers can be used in a range of progenies and interspecific breeding material originating from crosses between representatives of the different lily sections without the need for repeated marker production. As such the intention is to set up a molecular toolbox that can be used to trail any trait in future. Framework will initially be used to develop molecular tools that will allow directed introgression breeding on resistance against viruses as well as resistance against aphids.

Project idea

Describe the proposal. Focus on the scientific background and innovative aspects of the proposal, and how the proposal contributes to the aims of TTI-GG as expressed in the Business Plan (min. 500, max. 2.000 words).
Please indicate specifically how and to what extent your research project meets the criteria as presented in the TTI GG business plan and focus document with regard to using at least two out of the three disciplines mentioned (genetics, plant physiology including biochemistry and seed physiology) and phytopathology (including seed pathology, entomology, nematology and virology) in relation to adaptation of crops to new growing conditions, climatic conditions and environmental conditions in open and protected cultivation, namely: closed greenhouses, automation, robotisation, and salination and drought.

Background

Lily growing and breeding

The lily breeding and growing industry in the Netherlands has for decades been an economically healthy sector with an evident positive contribution to our national balance of trade. As a result the Netherlands has the world's largest production area of lilies, covering in the last 5 years more than 4000 ha. More than 2 billion lily bulbs (75% of the world production) are produced in the Netherlands, mainly for cut flowers. The economic value of the lily cut flower production can be estimated worldwide at a level of approximately 2,000 Million Euro. In the Netherlands, lily is ranked as the fourth most important ornamental crop (statistical data from VBN 2007).
The genus Lilium can be classified into seven sections. In general, wild species within each section are relatively easy to cross and the hybrids are fertile. These interspecific hybrids

especially within and between the sections Leucolirion, Archelirion and Sinomartagon represent a large part of the currently registered cultivars. Among these groups there is a wide variety of valuable characters such as flowers size, color, flowering time and resistance to different pathogens. Because intersectional crossing is the only way to combine valuable traits, the introduction of wide interspecific hybrids (ie hybrid combinations from two or more different sections) is increasing.
Breeding schemes up to now almost completely rely on classical breeding and selection methods, therefore it is very difficult to breed for traits that are defined by multiple loci as break up in additive effects between these loci might occur in these inter-specific crosses. Marker assisted breeding can speed up the selection process and because lily has a long generation time these methods can shorten the time to market of new cultivars with desirable combinations of traits considerably. Little is known about the presence of virus- and aphid- resistance within the different sections of lily. Screening of germplasm for these traits will contribute to our knowledge on the possibilities for breeding for sustainability. Increased levels of resistance to virus and aphid infestation will be needed to adapt new growing conditions, identification of sources of resistance and of markers linked to genes involved in resistance are necessary steps to obtain this goal.

Resistance to viruses

Virus diseases affect quality and quantity of lily cut flower production and so they appear to be one of the most important pathological problems for the cultivation of this flower (Bellardi et al. 2002). The symptoms associated with virus presence can affect leaves (vein-clearing, mosaic, necrosis, chlorotic, yellow or brown spots, green stripes between the vein, curling, etc.) and flowers (size reduction, malformation, breaking patterns on the petals, etc.), but all these symptoms are intensified when plants have mixed virus infections. Also environmental conditions (e.g. temperature) can reduce or intensify symptom development considerably (Allen, 1972). Lily symptomless virus (LSV) is frequently found in the sections Leucolirion, Sinomartagon, Archelirion although large differences occur within the Oriental (Archelirion) and Asiatic (Sinomartagon) hybrids. High levels of resistance are likely to be present in Pseudolirium and *L. henryi* (Leucolirion section) as within these genotypes no virus infections have been observed (van Tuyl pers. comm.).
A high level of resistance to Lily mottle virus (LMoV) has been found in some Asiatic (Sinomartagon) hybrids. For cultivar Connecticut King this resistance has been found to be monogenic and its position has been mapped, conversion of linked markers into easily useable PCR markers for marker assisted selection is in progress but hindered by the repetitive nature of the fragment identified by the marker.
Next to this there are also indications for the presence of other resistances (partial or complete) among the different sections (including Sinomartagon) that have not been carefully characterised.

Aphid resistance

Up to now there have been no studies published on aphid resistance in lily but from field observations it is clear that differences between varieties exist. Whether this is based on differences in preference or that it is a true resistance remains to be established. Most viruses in

lily are non-persistently transmitted by aphids. Which aphid species have the largest contribution to virus transmission and what is the variation in this between specific aphid lines within a species is currently unknown. Therefore within this proposal virus resistance and aphid resistance will be separately studied as far as possible. For aphid resistance focus will be on screening of germplasm for factors that influence population development. Two factors contribute to a reduction in population development on a crop ; repellence and antibiosis. For non-persistently transmitted viruses repellence is of crucial importance, as it is reduce the number of contact moments between aphid and plant. Repellence levels will be measured for those genotypes that show a very significant reduction (80-90%) in population development.

Project proposal

Genotyping

The application of resistance markers in breeding programmes for bulbous crops is still in its infancy. The majority of research on molecular markers for bulbous crops has been done with lily by Plant Research International (PRI) that internationally has a leading position in this area. Despite the extreme large (more than 25 x larger than tomato) genome size of the Liliaceae and initial problems related to that, two maps have been produced in which AFLP®, NBS and DArT markers are aligned. Although especially the DArT technology has proved itself useful in marker production the applied markers have limitations in that their use is limited to a specific cross with often a relatively low number of traits and in the limited possibility to detect additive genetic variation due to the dominant nature of the used markers. Conversion of markers has been cumbersome with AFLP® and NBS markers due to the abundant presence of repetitive DNA. Although this has improved by the use of DArT markers the issue still remains for specific marker conversions.

Markers derived from EST sequences have the advantage that they are targeted to genes which generally are low copy and that the markers can be co-dominantly scored and thus provide more information. The main advantage of ESTs is that it can be expected that presence and position on chromosomes will be similar between genotypes because phenotypic variation is mainly caused by variation within the genes and expression levels rather then by presence/absence of genes. Therefore, markers based on EST sequences can be expected to be much wider applicable even among the different genomes that are brought together by the wide inter-specific crosses between members of the different lily sections. By setting up a marker set based on EST sequence information from the different sections within lily, a framework can be created that can be used in a wide range of material.

Currently there are two mapping populations for lily available at PRI (AA: Connecticut King x Orlito and LA: White Fox x Connecticut King) which can be used as reference maps to map the ESTs. After mapping of the ESTs in the reference maps, subsets of markers can then be selected for interval mapping in other crosses and in association studies. Once linkage in such cases has been established, other ESTs from the reference maps that have been mapped within the interval between which a trait of interest has been found, can be used for further fine mapping and identification of markers for Marker Assisted Selection (MAS) and eventually cloning of the gene of interest.

Because the build up of populations suitable for segregation studies takes a long time (~ 8 years)

in lily and from currently available populations it is not known whether they contain traits of interest, an association mapping study will be performed to map traits of interest. Association mapping can be applied to collections and selections of varieties (Malosetti et al 2007). Genotypes will be selected from the most important lily sections to identify associations within each of these sections and the set as a whole (correcting for structure). Next to that hybrids derived from crosses between these different sections will be included if possible (e.g. OA-material supplied by the companies). By including wide interspecific hybrids admixture mapping may enable confirmation of map positions. Initial studies on insect resistance and virus resistance can be extended to other important traits in future projects by phenotyping the whole set of genotypes for those traits.
Starting material for marker development will be mRNAs from different genotypes. The use of mRNAs will provide a complexity reduction for sequencing on a new generation sequencer (454). Before sequencing mRNAs will be tagged per genotype and subjected to a normalisation procedure to prevent sequencing a few highly expressed mRNAs. Between sequences of different genotypes SNPs will be identified and used for an 1536 Illumina SNP array in which offspring of the two populations and the genotypes for the association mapping will be genotyped.

Phenotyping

Virus and aphid resistance

Virus resistance will be assessed in two different trials: An 4 year field experiment in which genotypes will be tested for percentage of virus infection (number of infected plants per genotype) and level of infection. Also included will be a greenhouse test with controlled infections; either mechanical infection or infection through contaminated aphids.

Because there are only descriptive indications of differences in aphid resistance first results obtained from a small scale study on development and mortality in a no choice test on a set of 50 widely chosen genotypes will be awaited. Based on the results of this initial small scale study in the second year a larger study will be set up to study the presence and segregation of insect resistance in the set of genotypes chosen for the virus testing. In no choice tests, aphids will be put in clip on cages on the plant. Adult survival and nymph production will be monitored.
In the third and fourth year results from the initial no choice tests can be verified by choice tests in which aphids behavior towards different genotypes that are available will be monitored. If the initial screening for population reduction (first year) does not identify genotypes with a very significant reduction a no-go decision will be effectuated for the further screening for aphid resistance by the project partners.

In the phenotypic screening a wider set of material will be used compared to the genotyping where only material from the three main cultivated sections (Oriental, Asiatic and Longiflorum) will be used. The reason for this is in the number of genotypes needed in association mapping to obtain sufficient statistical power.

Innovative aspects
Very recent breakthroughs in sequencing technology and SNP detection platforms have made the

proposed EST approach above feasible for all crops including ornamentals. Currently the know how is still confined to a few research groups and the project in this form would be new in ornamental breeding. Marker and sequence data will become available that can be used for future studies for other traits within different backgrounds.

Broad screening of virus resistances in wide set of material, identification of sources of resistance, markers linked to virus resistance can give an important impulse to innovation in lily breeding.

Insect resistance is little studied, identification of sources of resistance is very important in increasing the sustainability of lily breeding.

Contributions to TTI-GG aims

1. The project will elucidate the distribution of important phytopathological traits (virus and aphid resistance) in the three main important sections of lily.
2. Breeding tools will be developed that will enable marker directed breeding in lily a crop with an exceptional large genome that has a life cycle of 2-4 years.
3. Within the bulbous research group at Wageningen UR – Plant breeding, three PhD students are currently active, it is emphasized that when the proposal is honoured, a part of their research will be directed to topics that fall within the tasks mentioned above. Additionally, the project should result in at least one PhD. This will assure that the project will contribute to scientific innovations in the genetics of lily disease resistance, resulting in at least four publications in peer reviewed international journals.

Work plan: Give a detailed plan of the activities, divided into work packages (minimum one, maximum six work packages)

Work package	**Description of the activities (max. 200 words per work package**;
WP1	**Sequencing of ESTs**

	Extraction of mRNA will be performed from different tissues combined in equimolar amounts and used as a template for cDNA synthesis. The obtained cDNAs will be subjected to a normalization process to enrich for low-copy mRNAs. The normalised cDNAs from different genotypes will be tagged and subsequently pooled. Normalised cDNAs will be sequenced by a dedicated sequencing company on a 454 sequencer that has been updated to accomodate the 1,000,000 reads (400 bp) option that recently has become available. The EST sequences will be annotated based on sequence similarity (BLAST analysis), and collected in a searchable database. SNPs and SSRs within and between samples will be identified using in house developed software (Tang et al. 2006; Tang in preparation). Choice of tissues for mRNA isolation and selection of genotypes will be done in close discussion with all partners involved.
WP2	**Phenotyping for virus resistance** Screening for resistance against LSV and LMoV in a wide assortment of species, cultivars (from the different sections) and wide interspecific hybrid material. Sample sizes of about 100 genotypes per lily section (O, A and L) will be selected for testing, next to that this set will be extended by a number of Trumpet hybrids, inter-specific hybrids (OA, LO, LA) and wild species (eg. *L. henryi*) For each genotype a number of repetitions, four rows of six plants (24 plants in total) will be planted alongside with plants infected by both viruses. Next to the field trail there will be a single year greenhouse test for virus resistance in which plants will be either be mechanically infected or by means of infected aphids. All material used will be tested for virus at the onset of the project as well as during the the field trial.
WP3	**Phenotyping for aphid resistance** Screening for aphid resistance will be performed in an initial screen of 50 genotypes (representing a wide set of germplasm) during year 1 with a no-choice set up and based on aphid performance. If this shows clear indications for differences in population performance of aphids between lily genotypes the used screening will be extended to the same set analysed for virus resistance. Choice tests will be performed on a small subset of genotypes to obtain more background knowledge on the mechanism of the observed differences from the no-choice tests.
WP4	**Mapping of EST markers in reference map and genotyping collection** Mapping and genotyping will be performed using a 1536 Illumina SNP array. This will allow the simultaneous mapping of the markers in the reference maps (mainly LA map) as well as genotyping of the representatives of the Oriental, Asiatic and Longiflorum groups and hybrids. Mapping will be performed using the software package Joinmap.

WP5	**Association mapping** The genotypic data and the phenotypic data (WP5) will be combined using developed software and approaches available within Wageningen UR to identify the positions of QTLs for resistance. To make the markers closely linked to traits of interrest fully applicable in a marker assisted breeding program, a protocol will be developed and marker analysis will be further optimised to allow high through put genotyping using a state of the art technology-platform to be chosen at that moment based on costs and ease of use.

Division of the work

Indicate which partner is responsible for which work package, using the symbols P and S (P = primary responsible partner, S = secondary involved partner).

	R1	R2	R3	R4	C1	C2	C3	C4	C5	C6	C7	C8
WP1	P				*							
WP2	P				*							
WP3	P				*							
WP4	P											
WP5	P											

* A breeding commission will be appointed which consists of representatives of the companies and that will supervise activities and take any decisions regarding plant material

Time table

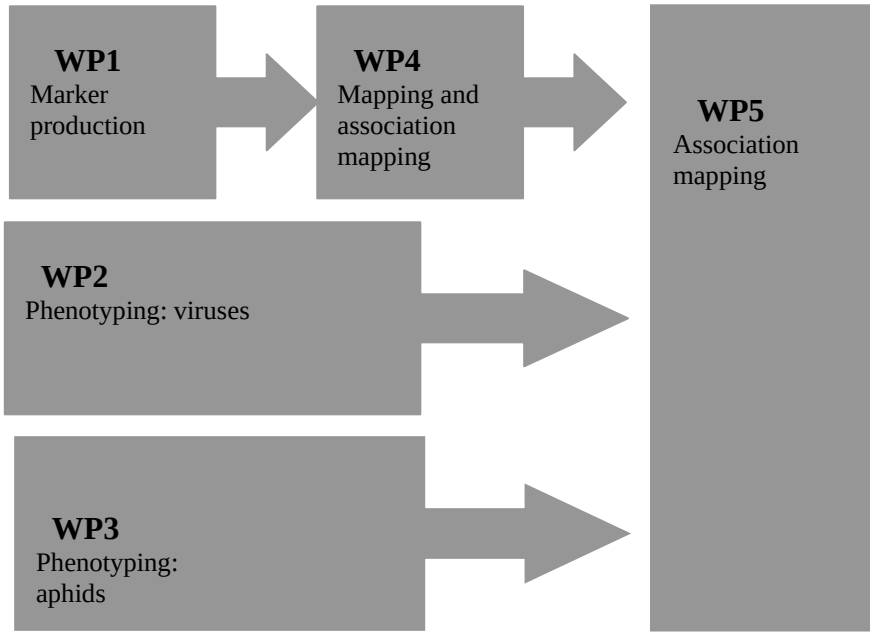
Indicate when the activities will take place using only the symbols X, XX and XXX (X = some activity, XXX = much activity).

Months →	0-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48
WP1	XX	XXX	XXX	XX				
WP2	XX	XX	XX	XX	XX	XX	XX	X
WP3		X	XXX	XX	XXX	XX	X	
WP4			X	XX	XXX	XXX	X	
WP5						X	XXX	XXX

Risks

What might cause possible failure of this project? (max. 50 words) Confounding effects of sub-structuring may lead to false positive markers in the association mapping.
How have you minimized this risk of possible failure (e.g. alternative plan, go/no-go decisions)? (max. 50 words) Material from different sections have to be chosen carefully and potential sub-structuring has to be accessed by analysis of SNP data.
If go/no go decisions are scheduled, when are they foreseen (in terms of time and/or in terms of work packages)?

Are work packages interdependent in the sense that one cannot proceed if results of another are not yet available? If yes, please give a schematic representation of the inter-dependencies.



Finances

Please fill out the attached Excel file (four worksheets). The project budget should conform to the TTI GG Rules and Regulations with respect to Financial Obligations and the Annex 1 connected to it.

Deliverables

How would you rate the potential for this project to result in patentable results? (scale 0 – 100%)	0
Please estimate the number of patents to result from this project	0
How many PhD theses are foreseen at the end of the project?	1
What is your best estimate of the resulting number of scientific publications in scientific journals with an impact factor higher than 3.0 ?	2
Within what period of time after the end of this project do you expect economic benefit of this project?	5-10 / > 10 years Lily life cycle is 2-3 years
Do you expect new plant varieties in the future to contain traits or technologies developed in this project? If yes, how wou you rate the expected added value or the expected market share the time period mentioned above.	YES 5%

Disclosure of results

Do you expect results or know how that can or will not be	NO

protected by intellectual property rights but publication of which may damage or hamper the project or its Partners?	
If yes, describe the type of results as indicated above, and describe any measures you will take to prevent unprotected results to interfere with the publication of PhD theses within this project.	
How do you intend to communicate the results to non-participating parties? In scientific publications.	

Scientific track record:

How many peer reviewed scientific publications in this specific field of research have been written by the research institutes involved (first author only)? (Please attach a list of references).	**> 10**

Submitted elsewhere and/or collaboration with other initiatives?

Has the project proposal previously been submitted elsewhere?		NO
[iv]If yes, where, and what was the result?	Where: ……. Result: …….	
Have you submitted this project proposal elsewhere for simultaneous or additional subsidy?		NO
If yes, where?	Where:	
[v]Do you intend to share facilities that are or may be subsidized by other initiatives?		NO

Confidentiality

All proposals will be treated confidentially by TTI Green Genetics, as outlined in the call for proposals. An external Review Committee will evaluate the proposals. The list of members of the Review Committee will soon be available on www.greengenetics.nl. If you have any suggestions for a potential member of the review committee who is in your opinion competent and integer, please notify TTI GG on the name and affiliation of such a person.
Members of the programme board will be nominated by the TTI GG board and will be obliged to sign a secrecy/non disclosure agreement.

TTI Green Genetics may confidentially forward and recommend relevant proposals to other related initiatives, such as WCFS+ or Food and Nutrion Delta (FND) foundation, solely for the purpose of determining whether the project may be eligible for (co-)subsidy from such sources. If you do not want your project proposal to be forwarded, please tick this box:

☐ I do **not** grant the right to forward this proposal to other parties eligibility purposes.

[i] This person is expected to be responsible for correspondence with TTI Green Genetics and will be used to address the project consortium.
[ii] In case more than eight companies or more than four research institutes are involved please mention them on a separate attachment.

[iii] TTI GG strives for the exchange of researchers between knowledge institutes and industry. To accommodate researchers from participating knowledge institutes to perform a substantial part of their research work on site at a participating company or vice versa, TTI GG will compensate partners for additional expenses, such as travel costs or housing costs, that result from such an exchange.
[iv] Please attach a copy of the end evaluation of your submission.
[v] Please specify which facilities and their subsidy status

Appendix II : References for the scientific track record

Plant Research International harbors many experts in the field of, map construction, marker development, development of resistance tests, software packages and new statistic and genetic analytical approaches and QTL mapping. On bulbous crops a long and outstanding research program has been running for almost 30 years already. Below, some of the many possible references are given.

1. Alvarez AE, Tjallingii WF, Garzo E, Vleeshouwers V, Dicke M, Vosman B 2006. Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to aphid Myzus persica. Entomologia Experimentalis et Applicata 121 (2): 145 - 157.
2. Bink MCAM, Uimari P, Sillanpää MJ, Janss LLJ, Jansen RC (2002) Multiple QTL Mapping In Related Plant Populations Via A Pedigree-analysis Approach. Theor App Genet 104: 751-762
3. Gao ZS, WE van de Weg, JG Schaart, HJ Schouten, DH Tran, LP Kodde, IM van der Meer, AHM van der Geest, J Kodde, H Breiteneder, K Hoffmann-Sommergruber, D Bosch, LJWJ Gilissen (2005) Genomic cloning and linkage mapping of the Mal d 1 (PR-10) gene family in apple (Malus domestica). Theoretical and Applied Genetics 111: 171–183
4. Haymes KM, Van de Weg WE, Arens P, Maas JL, Vosman B, Den Nijs APM (2000) Development of SCAR markers linked to a *Phytophthore fragariae* resistance gene and their assessment in European and North American strawberry genotypes. J Am Soc Hortic Sci 125:330–339
5. Jansen J.(2005) Construction of linkage maps in full-sib families of diploid outbreeding species by minimizing the number of recombinations in hidden inheritance vectors. Genetics 170: 2013-2025
6. Lim, K-B, JD Chung, Bernadette CE Van Kronenburg, MS Ramanna, JH De Jong & JM Van Tuyl, 2000. Introgression of *Lilium rubellum* Baker chromosomes into *L. longiflorum* Thunb.: a genome painting study of the F1 hybrid, BC1 and BC2 progenies. Chromosome Res. 8(2): 119-125.
7. Lim, KB & JM Van Tuyl 2007. Lily , Lilium hybrids. In: Flower breeding & genetics: Issues, challenges and opportunities for the 21st century. Chapter 19 page 512-532 Kluwer Academic Publishers, Dordrecht (Ed. N.O. Anderson),
8. Linden CG van der, DCAE Wouters, V Mihalka, EZ Kochieva, MJM Smulders, B Vosman (2004) Efficient targeting of plant disease resistance loci using NBS profiling. Theor App Genet 109: 384-393.
9. Malosetti Zunin M, Linden CG van der, Vosman B, Eeuwijk FA van (2007). A mixed-model approach to association mapping using pedigree information with an illustration of resistance to Phytophthora infestans in potato. Genetics 175 (2): 879 - 889.
10. Marasek, A, Mizuochi, H and Okazaki, K 2006. The origin of Darwin hybrid tulips analyzed by flow cytometry, karyotype analyses and genomic in situ hybridization. Euphytica 151: 279-290.
11. Snijder, RC & JM Van Tuyl, 2002. Disease test to soft rot caused by *Erwinia carotovora* subsp. Carotovora for tubers and seedlings of *Zantedeschia* (Araceae). Eur. Journal of Plant Pathology 108: 565-571.

12. Straathof, ThP, W Eikelboom, JM van Tuyl & D. Peters, 1996. Screening for TBV-resistance in seedling populations of Tulipa L. Acta Horticulturae 432: 392-399.
13. Tang J, JAM Leunissen, RE Voorrips, CG van der Linden, B Vosman 2008. HaploSNPer: a web-based allele and SNP detection tool. BMC Genetics: 9 (23).
14. Van Heusden, AW, Jongerius MC, JM Van Tuyl, TP Straathof & JJ Mes 2002. Molecular assisted breeding for disease resistance in lily. Acta Hortic 572: 131-138..
15. Van Tuyl, JM. and Creij, MGM 2007. *Tulipa gesneriana* and *T.* hybrids. In: Flower Breeding and Genetics, Chapter 23, Springer Verlag, pp 623-641.
16. Wittenberg AHJ, T van der Lee, Cl Cayla, A Kilian, RGF Visser, HJ Schouten (2005) Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. Molecular Genetics and Genomics 274: 30-39.

Annex 4

Background Knowhow and IP-rights

1. Selected genotypes from PRI-lily-genotype collection (including the molecular mapping populations Connecticut King x Orlito (891338, 101 clones), Longiflorum x Connecticut king (006001, 100 clones).
2. Materials used in the project and made available by the participating companies are owned by these commercial partners. Background is limited to brought in material. Additional information concerning the genetical background may be given on a voluntarily base by the project partners and cannot be regarded as Background Knowhow and IP Rights as described in Article 12 of the R&R I&IP. Any information given by companies on the genetic background of plant material to PRI will be treated as confidentially and will not disclosed by PRI to other involved Partners.