TRANSCONTAINER

TRANSCONTAINER STAKEHOLDER WORKSHOP:

BACKGROUND DOCUMENT

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CONTENT

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1. INTRODUCTION

1.1 The objectives of Transcontainer

Transcontainer is a European Commission funded research project in the 6th Framework Programme with the following objectives:

- To promote coexistence of GM and non-GM crops in European agriculture by using biological containment strategies in GM crops by improving and simplifying rules for coexistence.

- To develop biologically contained GM crops that are environmentally safe and commercially viable; a series of different strategies are implemented and tested with a view to biological containment of transgene flow from GM plants, including trees, varying from chloroplast transformation to several (combined) techniques that aim at controllable fertility or controllable flowering.

- To assess environmental, human health and socio-economic impacts of the use of biologically contained GM crops in European agriculture.

- To enhance understanding and acceptance of coexistence through biologically contained GM crops by invoking dialogue with and between stakeholders and the general public.

1.2 The objectives of this background document

Transcontainer partners began their work in May 2006 and finalised it by October 2009. The results of this work are presented in this document, in order to provide background information to the participants at the final stakeholder workshop. The overall aim of the stakeholder workshop is to offer different stakeholders the opportunity to express and share their perspectives and comments on Transcontainer with other stakeholders and Transcontainer partners.

The second section of this background document summarises the technical and scientific achievements by Transcontainer. The third section presents the findings from interviews with various stakeholder parties, such as biotechnology, seed and food industries, grain traders, retailers, farmers organisations, environmental and other public interest organisations as well as national coexistence policy makers and advisors. This section further summarises the results from a workshop held with national coexistence policy makers and advisors in Vienna, Austria, on 18 April 2007 and a workshop held with interested parties from Central and Eastern European countries in Plovdiv, Bulgaria, on 7 May 2008.

2. RESEARCH RESULTS FROM TRANSCONTAINER

2.1 Transcontainer's overall objective

Pollination and seed-set are generally required for crops that are harvested for their seed or fruit, and for breeding vegetatively propagated crops. For some crops, prevention of pollen flow between cultivars and non-target plants, like non-GM cultivars or wild plant relatives, can be physically impossible, as it is mediated by environmental factors, such as wind, insects, and the proximity of sexually compatible weedy relatives, all of which are beyond the control of the breeder and farmer. Likewise, dispersal of seeds from crop plants during harvest also creates a possibility for gene transfer to sexually compatible non-target species through outcrossing in subsequent generations. The objective is to implement and test the effectiveness of new and established technologies for the prevention of transgenic pollen and seed spread. The technologies are being developed and tested in both model plants, such as *Arabidopsis thaliana* (arabidopsis), and crop species, such as sugar beet, oilseed rape, rye grass, red fescue, tomato and eggplant, as well as in poplar and birch.

2.2 Chloroplast transformation

2.2.1 Overall aim

Chloroplast transformation aims at preventing the flow of the transgene through pollen of flowering GM plants by incorporating the transgene into the plant chloroplast (or plastid) instead of the nuclear genome. Since pollen usually does not contain chloroplasts, the transgene will be contained within the pollinating mother GM plant. However, the methodology for chloroplast transformation is still problematic for most major crops. The aim was therefore to develop plastid transformation procedures for two major outbreeding crops in the EU, sugar beet and oilseed rape, to enable the containment potential of plastid transformation to be demonstrated.

2.2.2 Plastid transformation in sugar beet

A transformation and selection protocol has been established for sugar beet line Z025. A direct shoot regeneration method using petiole explants was employed. Plastid transformation was carried out by bombarding sugar beet petioles with gold particles coated with either of two sugar beet plastids transformation vectors, pSB1 or pSB2, and selection for spectinomycin–resistannce on a shoot regeneration medium. The transplastomic origin of these calli was confirmed by PCR amplification and by microscopy in which GFP fluorescence was evident within chloroplasts. Homoplasmy of transplastomic calli was determined by PCR using two primers external to the flanking sequences and subsequently confirmed by southern analysis. The homoplasmic plants were transferred to the greenhouse and their phenotype, as well as inheritance of the transgene, is under evaluation. Expression of the herbicide resistance *bar* gene is also under investigation in later transformants obtained with the pSB-*bar* construct. Subsequent efforts have been directed at refining the techniques to develop a more efficient and reproducible protocol. The use of kanamycin resistance as an alternative selectable marker was explored and found to be effective. This work was the first successful chloroplast transformation of sugar beet, and has been published: Marchis *et al,* Transgenic Research 18, 17-30 (2008).

2.2.3 Plastid transformation in oilseed rape

The progress towards obtaining transplastomic oilseed rape plants has been disappointing. No transplastomic plants have been obtained although in the late stages of the project transplastomic callus lines have been obtained from which we are trying to regenerate shoots. Two strategies for plastid transformation have been employed. Firstly the more conventional approach utilising dominant bacterial antibiotic resistance markers, and a biolistic delivery system. A total of four different vectors were constructed for this, utilising either the *aad*A gene (spectinomycin resistance) or the *aph*A gene (kanamycin resistance), some including the herbicide resistance gene, *bar*, and loxP sites to facilitate subsequent marker excision from plastid transformants. Optimal varieties and tissue culture regeneration conditions were also determined. A total of more than 2,000 shots were performed, with the gene gun, using these vectors, but despite some promising shoots developing under selection, none of these proved to be plastid transformants. In the latter stages a greater emphasis was placed on the alternative strategy: utilisation of PEG to deliver to protoplasts vectors containing mutations in the plastome conferring antibiotic resistance mutations for selection (two such vectors were constructed, containing the *bar* gene). This approach has been successfully employed in tobacco, tomato and cauliflower. Despite difficulties in developing a sufficiently efficient protoplast culture system, largely due to contamination problems, we due now have an efficient system which has yielded a number of colonies, now confirmed by PCR analysis, to be plastid transformants. Unfortunately these are slow-growing and recalcitrant to regeneration. Current efforts are directed at this regeneration block through manipulation of the culture conditions. Some improvement is being detected, particularly in the greening of the colonies.

In conclusion, chloroplast transformation has been achieved in sugar beet but not yet in oilseed rape.

2.3 Controllable flowering

2.3.1 Overall aim

The overall aim of controllable flowering is to contain the transgene as long as possible within vegetatively grown crops or trees. Controllable flowering is based on a combination of floral repression systems and floral restoration systems. Containment strategies relying on floral repression are suitable for plants, which are grown for their vegetative parts. Such plants include sugar beet, which is grown for the root part, grasses, which are grown for biomass production, and hardwood trees, which are grown for timber production. While the latter is propagated vegetatively, sugar beet and grasses are propagated through seeds, and therefore ultimately require a floral restoration mechanism that can overrule the floral repression mechanism for seed production. The aim was therefore to develop controllable flowering technologies for commercially important European crops, like sugar beet, red fescue, rye grass, and trees, like poplar and birch.

2.3.2 Floral repression

Three main alleys for floral repression have been pursued:

- Overexpression of floral repressor *TFL1*-like homologs: Overexpression of a *TFL1* homolog seems to be partially effective in sugar beet as two out of 8 lines show complete non-flowering and 3 lines show various degrees of delayed flowering after vernalization. In tall fescue 35 lines out of 39 remained non-flowering over two seasons. The five flowering lines had either no or low transgene expression and the remaining lines had various levels of *TFL1*-like expression. Poplar and birch trees transformed with *TFL1*-like homologs show delayed bud break. But it is too soon to draw conclusions about whether *TFL1*-like homologs are effective floral repressors, as the control plants are not yet at the flowering stage and are expected to flower only next year.

- Downregulation of floral inducer *FUL/AP1*- or overexpression of floral repressor *FLC*-homologs (both MADS-box genes) in dicot species: Downregulation of *FUL/AP1* homologs in sugar beet has efficiently inhibited flowering in six out of seven lines. Poplar and birch trees overexpressing a *FLC* homolog are still in juvenile phase and downregulation of flowering time genes has been observed. Overexpression of a certain miRNA, which influences the regulation of *AP1* in poplars, extends expression of juvenile traits into the second year of growth. Furthermore, expression analysis shows a dramatic downregulation of flowering time genes, indicating that these trees will very likely be late flowering.

- Overexpression or downregulation of putative floral regulators derived from a poplar functional genomics program in perennial species: Thirteen putative floral regulators from poplar has been overexpressed or targeted for RNAi-mediated silencing in poplar. Several of these genes cause downregulation of other flowering genes when expressed in poplar. Expression of a poplar *EBS*-like gene in perennial ryegrass led to a general delay in flowering time, although no linear correlation between expression level and flowering time could be established. Expression of a poplar MADS box gene in tall fescue led to floral inhibition in 3 lines and flowering was only observed in control plants and PCR negative transformants.

2.3.3 Floral restoration

The main component in the floral restoration system is the ethanol inducible *Alc* system (from *Aspergillus nidulans*). This system is convenient for seed propagated species because it allows for flowering only upon ethanol induction in an otherwise floral repressed plant. Flowering induction was thus attempted in the target species either by inducing RNAi-mediated silencing of TFL1 or by inducing AP1-homolog overexpression. An additional task was also to tailor the system for crops, both in terms of on/off expression in the presence/absence of ethanol, and in terms of strength and durability of expression

The entire system was tested in arabidopsis. Very late-flowering TFL1-like expressing plants were transformed with an ethanol inducible *TFl1*-RNAi construct. F2 generation offspring were induced with ethanol vapour and PCR positive plants responded with a significant reduction in flowering time, while null-segregants flowered simultaneously with the late flowering TFL1 background. The same system was also introduced and tested in tall fescue. Almost all lines (45) remained non-flowering independently of the initial *TFL1*-like expression and independently of ethanol treatment. One line displayed initial signs of stem production in the presence of ethanol vapour but further flower development was arrested at this stage. Restoration of flowering in non-flowering *AP1*-RNAi sugar beet plants by ethanol inducible *AP1* expression awaits characterization and thus the conclusion on system performance in grasses and sugar beets still remains quite elusive.

Optimization of the *Alc* system for grasses included transformation of tall fescue and perennial ryegrass with Alc inducible GUS expression constructs containing different combinations of AlcA promoters, which were modified around the AlcR binding sites, and AlcR gene sequences, which were codon-optimized for rice and rye grass. Transgenic plants were subject to five different treatments. 1) 1% ethanol drainage, 2) 1% ethanol leaf spray, 3) ethanol vapour, 4) 1% acetaldehyde drainage, 5) 5% acetaldehyde leaf spray. However, GUS staining revealed that neither of the different constructs nor the different induction stimuli did result in a strong induced staining pattern comparable to the positive control, a maize ubiquitin promoter driven GUS line. Thus the optimization of cis operational elements in the alcA or the condon optimization of the alcR protein did not result in an convincing improvement of the switch system. Samples are now being analyzed by real time PCR for confirmation of the results. This analysis will probably show the same outcome as the visible GUS staining investigation and demonstrate that the switch system is operable, but that the induction is not strong and stable enough.

In summary, floral repression has been achieved in sugar beet, tall fescue, rye grass, poplar and birch with varying levels of success, while floral restoration has been achieved in arabidopsis but not yet in tall fescue and sugar beet. Since technical developments in controllable flowering are still at an early stage, it is too early to draw firm conclusions about their suitability for application under field conditions.

2.4 Controllable fertility

2.4.1 Overall aim

The overall aim of controllable fertility is to prevent and/or delay transgene flow for crops that are harvested for their seed or fruit, and for breeding of vegetatively propagated crops. For the prevention of transgenic pollen and seed spread, several technologies are developed and tested in both model plants, like arabidopsis, and crop species, like oilseed rape, tomato, eggplant and grasses.

2.4.2 Reversible male sterility

Reversible male sterility systems are suitable candidates for transgene containment technologies, providing transgenic male fertile plants are eliminated, female parent lines can be efficiently propagated, and any chemical inducers or restorers that are used to reverse the male sterility meet effectiveness, cost and environmental safety demands. These approaches are being pursued in tomato, eggplant and grasses, and for new technologies, as initial proof of concept, in the model plant arabidopsis. Three main approaches are being followed to engineer reversible male sterility:

- <u>Metabolic starvation through alteration of glutamine synthase activity in the anther:</u> Metabolic starvation technology makes use of dominant negative mutations in the glutamine synthase enzyme to locally inhibit of glutamine biosynthesis in the immature pollen grain or tapetum of the anther. Male fertility can be recovered by spraying the affected plants with glutamine or by *in vitro* pollen maturation. Proof of concept for this technology was obtained previously in tobacco and the technology is now being evaluated in tomato and parthenocarpic (seedless) tomato. A large number of transgenic lines have been developed and are now being evaluated for heritable and reversible male sterility.

- Downregulation of basal transcription factor expression in the anther: Tissue-specific microRNA-mediated gene silencing is being used to downregulate a class of endogenous essential transcription factors. Male sterility is reversed using an inducible system. The technology is still under development in the model species arabidopsis.

- A two component system in which the two halves of the cytotoxic compound barnase are brought together in the anther after crossing: A two-component promoter-toxin system is being developed for grasses, specifically rye grass and tall fescue. The system relies on the generation of two individual breeding lines, each expressing one half of the cell inhibitor (Barnase; Bn5 and Bn3) from two different promoters with overlapping expression regions. The parent lines are fully fertile and can be maintained by selfing, but when they are crossed the offspring will produce each half part of the Barnase in overlapping regions of the plant (i.e. pollen cells). Co-expression of the two parts will allow the protein fragments to combine into a functional enzyme, which will degrade RNA and lead to pollen cell death. Initial experiments using promoters that drive the Bn5 and Bn3 fragments in vegetative tissues suggest that the system functions in grasses. A large number of lines carrying pollen-expressed Bn3 and Bn5 fragments have been generated, crossed and vernalized to induce flowering. Unfortunately, it has not been possible to induce flowering properly, and only a few crosses have been established. However, additional experiments in which Bn3 under control of a constitutive promoter was co-transformed in tall fescue with Bn5 under various tissue-specific promoters or the same constitutive promoter showed strong indications that the system is functional. Whereas on average 45 lines could be retrieved from co-transformation with constructs of non-overlapping activities, only 10 lines could be retrieved from co-transformation where both Bn3 and Bn5 was expressed from the same constitutive promoter. Expression analysis of the transformants further revealed that all lines except for one showed only expression of either Bn3 or Bn5.

2.3.4 Reversible seed lethality

An additional method to obtain gene containment in transgenic plants that are harvested for their fruit or seed is to link the novel transgenic trait to seed lethal genes. The AGL23 MADS-box transcription factor controls chloroplast biogenesis in the model plant arabidopsis: seeds in which the *AGL23* gene is down regulated are morphologically normal, but are albino due to the lack carotenoids and chlorophyll. The mutant seeds germinate, but die soon after as they sensitive to photo-oxidative stress and are unable to photosynthesize. Stage-specific microRNA-mediated gene silencing using temporally-regulated seed promoters has successfully been used to downregulate *AGL23* at different points during seed development. Transgenic arabidopsis lines have been developed that carry an ethanol inducible microRNA-insensitive version of the *AGL23* gene. These lines are now being tested for their ability to rescue seed viability imposed by the *AGL23* microRNA construct.

Since technical developments in controllable fertility are still at an early stage, it is too early to draw firm conclusions about their suitability for application under field conditions.

2.5 Risk assessment and contribution to coexistence

2.5.1 Overall aim

The risk assessment of biologically contained GM crops under development by Transcontainer has been performed in conformity with the principles of the environmental and health assessment as published by the European Food Safety Authority. The EU authorisation of GM plants is based on an event- and case-specific tiered approach which requires detailed assessment of molecular, food/feed safety, and environmental datasets. The economic assessment was done following standard practices for benefit-cost-analysis.

2.5.2 Results and recommendations

Transcontainer carried out the corresponding impact and benefit assessment for four containment strategies based on six molecular technologies. Concerning environmental impact assessment, the most promising containment technologies are (1) chloroplast transformation (see 2.2) and (2) male sterility by metabolic starvation (see 2.4.2). For both the level of containment depends on the rate of seed flow from the GM mother plant and the potential leakage of the technology (assumed to be: <5%). Based on these assumptions gene flow at the pollen level is likely to be reduced by more than 90%.

The other four technologies - (3) male sterility by expression of a toxic protein (Barnase) (see 2.4.2), (4) flowering repression by over-expression of endogenous flower-mediating genes (see 2.3.1), (5) flowering repression by RNAi mediated gene regulation (2.3.2), (6) seed sterility by a miRNA mediated gene regulation (see 2.3.4) are still in early stages of development so that the suitability for application under field conditions has not been proven yet.

The benefit of any containment strategy depends on:

I. The containment goal, which should in case of

I.i. coexistence support separation between GM and non-GM plant cultivation. The benefits are primarily economic since applicable GM plants will have passed a safety approval. The benefits will already start at low containment levels and will in particular provide access to transgenic traits for small farmers facing compliance problems with distance requirements.

I.ii. biosafety standards support high level separation e.g. in case of GM plants for non-food/non feed use like pharmaceutical plants. However, as no system is likely to be 100% tight, a combination of more than one Transcontainer strategy is advisable.

II. The plant species, which inherently determines the characteristics of biological containment regarding pollen, seed, and flower development. Also depending on the intended harvest product as well as the pollination strategy (e.g. wind- or insect-mediated), several Transcontainer strategies are suitable for containment.

III. The practicability in the field, which is dependent on technical and environmental characteristics. Although flower repression offers the highest level of containment, it might be difficult to manage re-induction of flowering (e.g. with ethanol) if seed production is obligatory at a later stage of breeding programs or seeds are the harvest final product.

IV. In the case of oilseed rape, sugar beets, egg plants and tomatoes the main economic benefits are reduced separation distances and resulting compliance costs. For grasses and poplar the access to the genetically modified trait as such is the major advantage. In general, the combination of single Transcontainer containment strategies should be considered for all crops regarding environmental benefits.

3. VIEWS AND PERSPECTIVES FROM STAKEHOLDERS

3.1 National coexistence policy developments

In April 2009, the European Commission reported that 15 Member States have adopted specific legislation on coexistence, while 3 Member States have notified draft legislation to the European Commission.[1] No Member State indicated that it has addressed coexistence by means of non-legislative instruments. In some Member States the development of a regulatory framework is not envisaged in the near future, as the cultivation of GM crops on their territory would be unlikely to take place.

Findings from interviews with national coexistence policy makers and advisors suggest that in most Member States stakeholders, such as biotechnology and seed industries, conventional and organic farming organisations have been given the opportunity to express their views on national coexistence policy making. A few Member States have also consulted environmental and consumer organisations for the development of coexistence measures. In one Member State the organic farming organisation left the consultation process. In several Member States organic farming organisations were satisfied with the outcome, whereas in some other Member States they were not. In one Member State consumer and environmental organisations declined to be part of the consultation process.

Interviews with representatives of biotechnology, seed and food industries, the grain trade, the retail sector and farmers and consumer organisations suggest that these stakeholders endorse the principle of coexistence. Nonetheless, stakeholders hold divergent opinions on: 1) whether or not (national) coexistence measures should be legally binding, and/or; 2) whether or not coexistence measures should be determined at national level.
By contrast, environmental organisations believe that coexistence of GM and non-GM agriculture would not be feasible in Europe. Moreover, they are opposed against the deployment of GM crops in European agriculture *per se*, as their risks to the environment and human health are deemed to be not acceptable.

The majority of Member States has meanwhile designed the coexistence measures in such a way that they prevent the labelling threshold of 0.9 % for GMOs in food and feed from being exceeded. However, a few Member States indicated that they aim at GMO admixture levels to be as low as possible. This approach is generally supported by organic farming organisations. The interviewee from a organic farming organisation further argued that the threshold value of 0.9% should not be viewed as a matter of legislation or governmental policy, simply because the organic market expects a threshold value of 0.1%.

Twelve Member States have adopted coexistence measures for at least one crop, i.e. maize, and in some Member States also for potato, sugar beet, fodder beet, wheat and oilseed rape. Technical coexistence measures are mostly based on spatial segregation, machinery cleansing and crop-rotation. Spatial segregation is generally based on isolation distances between GM crop fields and neighbouring non-GM crop fields. The isolation distances can sometimes be partially or fully replaced by buffer zones between GM and non-GM fields in which sexually

[1] Commission of the European Commission, Report from the Commission to the Council and the European Parliament on the coexistence of genetically modified crops with conventional and organic farming, Brussels, 2.4.2009, COM(2009)153 final.

compatible non-GM crops are grown that are harvested and treated as GM plants. In other Member States, buffer zones are mandatory supplements to isolation distances. One Member States requires GM crop growers to observe an isolation distance between the GM crop fields and sites of established bee keeping. In six Member States segregation measures between GM crop fields and organic fields are more stringent than those for GM crop fields and conventional fields. In addition, different segregation measures are defined by some Member States for fields used for the production of seeds. In all Member States the responsibility for compliance with coexistence measures lies with the GM crop grower and operators dealing with GM seeds or harvests. (See Annex I: Overview of isolation distances as element of national coexistence measures).

The interviewees from the biotechnology and seed industries complained that several Member States are implementing coexistence measures that would be disproportionate and discriminatory against the deployment of GM crops. They further pointed at the variance of segregation measures among Member States. A few interviewees argued explicitly that there would hardly be a need for isolation distances in the case of potato or sugar beet. One interviewee maintained that coexistence in the case of oilseed rape would be rather easily achievable, provided that the threshold for adventitious presence of GMOs in non-GM products would remain at 0.9 %. This view was however not shared by the interviewee from a farmers organisation, who argued that in the case of oilseed rape coexistence would be impossible.

The interviewees from the food industry, the grain trade and the retail sector expressed their concerns that national coexistence policies are not harmonised at the EU-level. Yet, they did not regard the issue of coexistence of direct relevance to their activities. For them there were two other aspects of the GMO debate that really matter 1) a change of regulations allowing the food and feed use of GMOs that have no EU-authorisation but have been authorised elsewhere, and; 2) the adoption of thresholds for the adventitious presence of GM seeds in lots of non-GM seeds.
The interviewees from the biotechnology and seed industries and the farmers organisation also pledged to adopt reasonable and workable thresholds for the adventitious presence of GM seeds in lots of non-GM seeds. According to some interviewees, the absence of such thresholds had already resulted in problems and increasing costs for conventional seed production.

Finally, one of the most contentious issues in public and political debates on national coexistence policies mentioned by almost every interviewee was how to redress liability in case of GM admixture with a non-GM crop, as this may result in economic damage to their producers. Particular when it concerns organic products, as European regulations on organic production and labelling foresee that products requiring labelling according to European Regulation 1829/2003 due to admixture of GMOs can no longer be marketed with an organic label.
At present, insurance products covering risks of GMO admixture do not seem to be available on the European market. Some Member States have set up compensation funds for economic damage resulting from GMO admixture. These funds are usually financed by a levy for GM crop cultivation. A few national coexistence policy makers or advisors indicated that calculations in their country have shown that the finances involved for liability redress would be rather modest. But they also noted that very few liability claims would be made in practice, as they considered the (proposed) technical coexistence measures in their country workable.

The interviewee from the seed industry indicated to favour the approach chosen by the Netherlands and Denmark, where compensation funds are (being) established.

3.2 Biologically contained GM crops for coexistence

The interviews with national coexistence policy makers and advisors suggest that many of them consider the development of biological contained (bc) GM crops a good idea, provided they are reliable and safe from an environmental and human health perspective. In their view, an assessment of the reliability or fail-safety of bc-technologies is of crucial importance as well as an assessment of the consequences of a breakdown of the bc-technologies. Moreover, some interviewees indicated that the deployment of bc-GM crops might affect national coexistence measures, particularly isolation distances, but they stressed that an official viewpoint would have to be developed with great care.
In addition, a few interviewees welcomed the deployment of bc-GM crops to limit the spread of transgenes from GM crops to wild flora. A couple of other interviewees felt that biological containment strategies for GM trees could be very useful. They pointed out that the environmental risk assessment of GM trees is far more complicated than that of GM crops because trees generally spread genes more extensively than crops and forests are not very domesticated areas and often constitute the basis of ecosystems. Biological containment could therefore help to reduce uncertainties in the environmental risk assessment of GM trees.
However, several interviewees expressed serious doubts about developing bc-GM crops for coexistence purposes, also because the coexistence measures in their country are pragmatic and workable. They further warned that the public should not start assuming that biological containment is a necessary requirement for coexistence.
On the other hand, there was wide agreement among most interviewees that biological containment might be very useful for GM crops that produce industrial or pharmaceutical compounds.

The interviewees from the biotechnology and seed industries did not consider bc-GM crops necessary for achieving coexistence in Europe. Though, they usually found the basic science interesting and valuable. A few interviewees suggested that each of the bc-technologies deserves to be evaluated on its merits, because they could help farmers worrying less about volunteers and spread of transgenes from GM crops to non-GM crops.
Notably, almost every interviewee felt that bc-technologies could be helpful for GM crops producing industrial or pharmaceutical compounds. Though, one interviewee expected that such GM crops would be grown in greenhouses.

The interviewees from the organic farmers organisation and the consumer organisation indicated to endorse the deployment of bc-GM crops for facilitating coexistence, provided that they are very reliable. One interviewee further argued that their deployment should be independent of management, like for instance applying of chemicals to induce biological containment, because most incidents with GM admixture so far had been due to management failures.

By contrast, the interviewees from the food industry, grain trade, the retail sector and the farmers organisation pointed out that they had no (formal) position on whether deployment of bc-GM crops might be worthwhile to facilitate coexistence. Though, the interviewees from the grain trade and the farmers organisations felt that if biological containment could help to achieve coexistence of GM and non-GM crops more easily, this might be a useful strategy.

Finally, the interviewees from the environmental organisations did not regard bc-GM crops as a useful tool for achieving coexistence, mainly because their reliability would be doubtful. One interviewee pointed at potential problems with ‘leaky’ promoters, also because selective pressures would push the bc-GM plant to circumvent its biological containment. Another problem might emerge when bc-GM plants with an ethanol-switch are flooded with an excess of water. This could lead the bc-GM plant into a state of so-called ‘anoxia’ that could induce an internal production of ethanol, which might eventually trigger the ethanol-switch at an undesirable point in time.
Another interviewee argued that bc-GM crops are a false solution, as there are many non-GM alternatives available for sustainable agriculture.

3.3 Biologically contained GM crops for hybrid breeding

Most interviewees from the biotechnology and seed industries recognised that several bc-strategies could be deployed for hybrid breeding. However, since the bc-strategies were not expected to be cheap, their deployment would only make sense in case of seeds with high-value traits. One interviewee did not expect that the bc-strategies developed by Transcontainer would be commercially worthwhile for hybrid breeding because of their complex technical nature; otherwise they would have already been developed by the private sector. However, another interviewee argued that particularly in the case of oilseed rape bc-technologies could be interesting to develop hybrid varieties.

One interviewee from an environmental organisation questioned the use of bc-technologies for hybrid breeding. To the interviewee’s opinion, hybrid breeding is mainly profitable for seed industries, whereas breeding of open-pollinated crop varieties would be more beneficial to farmers because such varieties are better adaptable to local circumstances and enable farmers to save seed.

3.4 Public acceptance of biologically contained GM crops

Almost none of the national coexistence policy makers and advisors believed that deployment of bc-GM crops and interviewees would improve public acceptance of GM crops in Europe. This view was shared by almost every interviewee from the biotechnology, seed and food industries, the grain trade and the retail sector.

Only a few national coexistence policy makers and advisors argued that bc-GM crops might contribute to enhance public acceptance of GM crops. Bc-GM crops may address public concerns played out in campaigns by anti-GMO groups, such as the risk of transgene flow to wild flora and the potential emergence of ‘superweeds’. Other interviewees warned that deployment of bc-GM crops might reinforce the public perception that any ‘outcrossing’ from GM crops is risky.
In addition, most of these interviewees expected that some of the bc-technologies under development by Transcontainer would be perceived by environmental organisations and anti-

GMO groups as Genetic Use Restriction Technologies (GURTs) that would jeopardise the practice of farm saved seed.

Almost every interviewee from the biotechnology, seed and food industries, the grain trade and the retail sector also expected that critical groups and environmental organisations would frame one of the bc-technologies under development by Transcontainer as Terminator-technology. Several of these interviewees believed that it would be difficult to communicate to the public that bc-technologies are not deployed because the GM traits would not be safe but only to simplify coexistence, which is an economic issue. As a consequence, there is a risk that the public would perceive such communication as contradictory. One interviewee foresaw that the deployment of bc-GM crops would affect neither the current level of consumer acceptance of GM food in Europe, nor would it change current opposition from environmental organisations, although only a minor part of the consumers would be sensitive for their arguments.

The interviewees from the organic farming organisation and the environmental organisations did neither believe that the deployment of bc-GM crops would increase public acceptance of GM products in Europe. Moreover, one interviewee viewed improvement of public acceptance, one of Transcontainer's communication objectives, rather dubious; it is like pushing consumers in Europe into acceptance of GM crops. Another interviewee maintained that the market in Europe has already decided not to accept GM crops.

5. European Commission support to Transcontainer

Findings from the interviews with national policy makers and advisors indicated that a large majority endorses the European Commission's decision to support to Transcontainer. Some interviewees welcomed every promising strategy to facilitate coexistence. Others argued that the pursuit of scientific knowledge and capabilities as such is important or because the development of biotechnology should be continued in the EU. Some interviewees felt that the research by Transcontainer should have been focussed on main crops, like oilseed rape, sugar beet and grasses.
Notably, a few interviewees had mixed feelings about Transcontainer, because they viewed the coexistence measures in their country as workable and considered feasible by farmers.

Almost every interviewee from the biotechnology and seed industry fully supported the European Commission's decision to fund Transcontainer, albeit for different reasons. For example, one interviewee hoped that the availability of bc-technologies might help to open internal discussions to develop GM crops with pharmaceutical or industrial compounds. Another interviewee indicated to be interested in the basic science, but not from a coexistence point of view.

The interviewees from the farmers and consumers organisations, the food industry, the grain trade and the retail sector had no strong views on the decision by the European Commission to fund Transcontainer. Most of these interviewees felt that any research that could help farmers to grow GM crops in Europe should be supported.

By contrast, the interviewees from the organic farming organisation and the environmental organisations argued that the research by Transcontainer was focused on a problem caused by the biotechnology industry. This research should therefore have been paid by this industry, or, as one interviewee phrased, why should public funds be spent on a techno-fix for the problem of GM contamination of conventional and organic crops caused by big biotechnology companies. Though, on the other hand, this interviewee suggested that public money should be spent to gain better understanding of the molecular mechanisms of bc-technologies, with a view to build an independent and public knowledge base for their risk evaluation. Another interviewee argued that the decision of the European Commission should be considered a waste of tax payers' money, as many public surveys have indicated that consumers in Europe do not accept GM crops and GM food.

ANNEX I:

OVERVIEW OF ISOLATION DISTANCES AS ELEMENT OF COEXISTENCE MEASURES AT NATIONAL LEVEL[2]

AUSTRIA
Not yet defined.

BELGIUM
Flanders and Wallonia: Not yet defined.

CZECH REPUBLIC
Isolation distances and/or buffer zones are requested. Other parameters (as different flowering times) can be voluntary used by farmers. Isolation distances for maize are 70 m towards non-GM conventional and 200 m towards organic maize fields. Buffer strips of one row of conventional maize (around Bt maize) replace 2 m of isolation distance. The maize in the buffer strip must be handled as a GM crop. For potatoes, isolation distances are between three m and 10 m (depending on the orientation of the cultivation lines) towards conventional and 20 m towards organic potato fields.

GERMANY
For GM maize a minimum isolation distance of 150 m towards non-GM conventional maize fields and of 300 m towards organic maize fields. The grower shall take appropriate measures to prevent the significant impairment of areas on which maize is cultivated that is not GM and is intended to be used for seed. No environmental or other parameters are taken into account.

DENMARK
Segregation is based on isolation distances. No environmental or other parameters are taken into account. Segregation measures to segregate fields with GM crops from fields with conventional and organic crops are identical. However, measures for GM crop production and for GM seed production are different. Isolation distances to crop fields are for maize: 150 m; for potato: 10 m; and for beet 20 m.

ESTONIA
(Draft measures) Segregation will be based on isolation distances and buffer zones.

SPAIN
Not yet defined

FINLAND
(Draft measures) Spatial segregation is principally based on isolation distance. If further technical measures prove useful for certain species, more detailed requirements can be given species-specifically. Possible adaptations to local conditions can be introduced by ministerial decree for any given species.

[2] Commission of the European Commission, Commission Staff Working Document accompanying Report from the Commission to the Council and the European Parliament on the coexistence of genetically modified crops with conventional and organic farming, Implementation of national measures on the coexistence of GM crops with conventional and organic crops, Brussels, 2.4.2009, SEC(2009) 408 final

FRANCE
(Draft measures) The cultivation of GMOs is subject to technical conditions, including isolation distances. The technical conditions will be established in a Ministerial Order.

HUNGARY
The competent authority defines the isolation distance as well as other cultivation conditions if necessary, including separate field margin harvesting or sowing of conventional border rows. The minimum isolation distance for maize is 400 meters. There are no separate isolation distances for conventional or organic farming or for non-GM seed production. The minimum isolation distance is legally defined. However, the competent authority may – based on local natural, geographical and other particularities influencing the cultivation activity – at its sole discretion, establish an isolation distance exceeding the minimum.

IRELAND
(Draft measures) Segregation is based on isolation distances only. Proposed isolation distances: Oilseed rape: not yet specified. Maize: 50 m towards conventional and 75 m towards organic maize fields. Potatoes: 20-40 m depending on the category. No environmental or other parameters are taken into account. Isolation distances to organic fields are increased by 50 % compared to conventional production and those to seed production fields are doubled.

ITALY
(Draft measures) Spatial segregation measures consist in: compulsory border rows with non-GM crop, isolation distances, and possible additional border rows with a consequent reduction of the isolation distance. For insect resistant GM crops 20 % of the cultivated area has to be planted with non-GM as refuge-areas. Regions may consider areas with particular climatic conditions (windy or extreme and cyclical weather conditions), where the distances can be tripled, or only doubled if there are suitable wind-breaks or other measures to reduce the wind speed and the dispersion of GM material.

LITHUANIA
A buffer zone of three meters must be established around GM crops where conventional plants of the same species as the GM crops are cultivated and maintained in the same way as the GM crops. Isolation distances are set for sugar beet (50 m), fodder beet (50 m), wheat (50 m), maize (200 m), potatoes (20 m), rapeseed (4000 m), and cross-fertilizing grain (500 m). For fodder beet the isolation distance towards non-GM seed fields is 1000 m.

LUXEMBOURG
Segregation is based on isolation distances only. The following isolation distances apply: maize: 600 m; potato: 50 m; beet: 100 m towards non-GM crop fields and 2000 m towards non-GM seed production fields.

LATVIA
Segregation measures consist of isolation distances and buffer zones. Isolation distances are the following: maize: 200 m; beet: 200 m to non-GM crop fields and 1000 m to non-GM seed production fields; potatoes: 50 m; oilseed rape: 4000 m. In addition, the following buffer zones apply: maize: 1.8 m; beet: 2 m; potatoes: 1.3 m; oilseed rape: 3 m. Furthermore, it must be ensured that the seeds of GM crops do not mix with the seeds for organic and conventional

farming in the process of preparation and packing of seeds. No environmental or other parameters are taken into account.

NETHERLANDS
The following isolation distances apply towards non-GM conventional fields: potato: 3 m; sugar beet: 1.5 m; maize: 25 m. The following isolation distances apply towards GMO-free fields (i.e. organic fields and production under contract for GMO absence): potato: 10 m; sugar beet: 3 m; maize: 250 m.

POLAND
Measures not yet determined.

PORTUGAL
Segregation measures include minimum isolation distances (maize: 200 m towards conventional maize fields or 300 metres towards organic maize fields or where products must comply with certain specific and contractually established conditions with regard to the thresholds of unintended presence of GMOs). These distances may be replaced completely by border rows of at least 24 rows (towards conventional fields) or, to a minimum of 50 metres by border rows of at least 28 rows (towards organic fields). For insect tolerant GM variety refuge areas must be established that are sown with conventional varieties and cover at least 20 % of the total area sown with the GM variety. This band may be also used as a buffer zone. The product obtained from the borders must be included in the product from the GM variety and be labelled as such. The variety to be used in the border must have the same plant cycle as the GM variety. Use of different plant cycles and/or staggered sowing: Staggered sowing or varieties of different FAO classes may be used: If the varieties of maize of the same FAO class are sown at a minimum interval of 20 days; and/or if the varieties of maize are sown simultaneously, the difference between the respective plant cycles must be at least two FAO classes. Measures to minimise the unintended presence of mechanical mixtures: Seed packaging: When preparing for sowing and when sowing, the seed packaging of different varieties must be clearly separated and located in different areas of the store, particularly for GM varieties; at the end of the season, unused seed packaging which has been opened must be sealed and identified. Use of seed drill, combine harvester, dryer and other equipment: All equipment must preferably be used by farmers dedicated to the same production system; in order to avoid the dispersal and mixture of grain from the previous operation, where different production systems are used, seed drills, combine harvesters, dryers and other equipment used must be carefully cleaned after being used in fields cultivated with GM varieties. Combine harvesters, when shared with other farmers dedicated to other production systems or when used by the same farmer to harvest conventional varieties, must, after harvesting a field cultivated with a GM variety, harvest at least an area of 2 000 m^2 of a conventional variety whose production will be labelled as GM. Storage, transport and identification of products produced: The farmer must guarantee the physical separation of maize lots produced using different production systems, from their harvesting to their storage or delivery to the marketing or processing installations; Maize lots of GM varieties must refer to the variety and unique identifier. Farmers may be exempted from having to apply the measures to minimise the unintended presence of pollen or mechanical mixtures if they voluntarily join together to create production areas exclusively dedicated to the cultivation of GM varieties deriving from the same GMO; or when agricultural products produced on a particular farm or in a particular region, both from GM varieties, whether or not deriving from the same GMO, and from

conventional varieties, are intended to be mixed in lots to be labelled as GM. In areas bordering a production area, farmers cultivating GM varieties must comply with the technical measures established in the legislation.

ROMANIA

Minimum isolation distances have to be respected (for maize: 200 m). Further measures include the creation of a buffer area and staggered sowing time. During crop harvest, transport, storage and conditioning of GM production, operators shall take all necessary measures to prevent the physical mixture of the GM products with non-GM production: separate storage, cleaning of sowing and conditioning machines, means of transport, according to standards for seed production.

SWEDEN

The isolation distances and buffer zones are foreseen by the legislation in place. Details will be defined in secondary legislation. Farmers may also choose to use other measures in cultivating GM crops. There are no different segregation measures planned to segregate fields with GM crops from fields with non-GM crops and non-GM seeds. The possibility to have different isolation distances and buffer zones towards organic and conventional farming is still a matter of debate.

SLOVAKIA

Isolation distances have to be respected to non-GM fields of the same species, which are for maize 200 m towards conventional crops and 300 m towards organic crops. Isolation distances can be replaced by buffer strips. For maize, 1 line of buffer strips (non-GM maize) replace 2 m of isolation distance. Buffer strips must consist of 6 lines as a minimum. The buffers trip is harvested together with GM production and marked as GM. For maize, it is also possible to use different maturity classes in agreement with the neighbour.

SLOVENIA

Detailed segregation measures have only been set for maize and potatoes. Isolation distance to non-GM fields of the same species have to be respected (Potatoes: 3 m, maize: 50 m). The SBA can grant exceptions from the distances if there would be e.g. geographical or other aspects that could be found reasonable. For seed production, the general seed production rules apply.