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Chapter 5

General discussion

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Introduction

*Agrobacterium tumefaciens* is a Gram-negative phytopathogen which is known to be the causal agent of crown gall disease in a diversity of dicotyledonous plants. Crown gall is characterized by the formation of tumors, usually close to the root crown. Virulent *Agrobacterium* strains are always carrier of a tumor-inducing (Ti) plasmid [1,2]. A section of this plasmid, the transfer DNA (T-DNA) is delimited by two specific DNA sequences: the *right border* (RB) and the *left border* (LB) [3]. *Agrobacterium* is able to perceive sugars and phenolic compounds that form indicators for damaged plant cells leading to the production of a range of virulence (Vir) proteins [4,5]. The T-DNA is nicked by the relaxase VirD2, facilitated by VirD1, at both the RB and LB sequence [6]. VirC1 enhances the nicking of the T-DNA by interacting with VirD1, VirD2 and VirC2 [30]. As a result of the expression of Vir proteins, a single stranded copy of the T-DNA (T-strand) is formed. For this, VirC1 binds to *overdrive* (OD), a DNA sequence located upstream the T-DNA RB [28, 29]. The T-strand remains covalently attached to VirD2, and is transferred via a type four secretion system (T4SS) to the recipient cell. After translocation from the *Agrobacterium* cell to the plant cell, the T-strand can integrate into the genomic DNA of the host cell.

Prior to genomic integration, the T-strand must be targeted to the nucleus. Several proteins are involved in the nuclear import of the T-strand. The effector protein VirE2 binds to the T-strand in the recipient cell where it covers the entire length of the T-strand thereby protecting it from degradation by plant nucleases [7-9]. The nucleoprotein complex that is formed in the plant cytosol from the T-strand and VirE2 is termed the T-complex. In *Arabidopsis thaliana* it has been shown that VirE2 also interacts with VIRE2 BINDING PROTEIN 1 (VIP1) [10]. VIP1 is a transcription factor that can be targeted for nuclear import by the mitogen-activated kinase (MAPK) MPK3 that is part of the plant cell defense mechanism against pathogens [11].

Although the natural host range of *Agrobacterium* is restricted to the plant kingdom, a number of research groups have managed to apply AMT to several other organisms ranging from *Streptomyces*[12], yeast[13], filamentous fungi[14] to sea urchin[15]. Because of its short generation time allowing for easy transformant screening and analysis, the AMT of yeast has developed into a highly valued tool to study the role of host factors in the T-DNA integration process [16].

Towards T-DNA processing and integration in *Saccharomyces cerevisiae*

Cocultivation studies using *Saccharomyces cerevisiae* as a model organism resulted in important new insights regarding the involvement of the DNA repair mechanism in T-DNA integration [16,17]. Adding to this, we started the development of an all-in-yeast system, to allow for a more detailed study of T-DNA processing, T-strand formation, and T-DNA integration. Such a model system would enable a more thorough and reliable screening for host components important for T-DNA integration than is possible when
relying on the AMT of yeast. The protocol for the AMT of yeast introduces variables that can influence the AMT efficiency, like the humidity of the plates or the adhesion of the Agrobacterium cells to the yeast cells that could obscure the variables that are the actual target of the experiment. Therefore an all-in-yeast system that removes many hard-to-control variables from the equation could fully employ the readily available collections of yeast strains harboring individual knockout mutations for annotated genes.

For this purpose, a vector harboring an artificial T-DNA substrate, pArT1, as well as two galactose inducible virulence gene expression cassettes were prepared equipped with ORFs expressing the Agrobacterium Vir proteins VirD2, VirD1, VirC2, VirC1 and VirE2, as well as the Arabidopsis ORF encoding the Arabidopsis transcriptional activator VIP1. The latter protein might increase the nuclear targeting of VirE2 in yeast since for yeast cells it has been shown that VirE2 needs to be co-expressed with VIP1 to induce nuclear uptake of VirE2 [18]. These genes were collected as they all are known to function in T-DNA processing or in the nuclear targeting of processed T-DNA. Initially, a fully plasmid-based system for the processing and integration of T-DNA was assembled. The already available mutant yeast lines could then simply be equipped with the newly developed plasmids.

Initially, a fully plasmid-based system for the processing and integration of T-DNA was assembled. The already available mutant yeast lines could then simply be equipped with the newly developed plasmids. To provide the yeast cells with a vector to serve as an artificial T-DNA-like substrate (pArT1) the ARS/CEN yeast shuttle vector pRS313 [19] was equipped with an RB and LB sequence, where the RB was preceded by an overdrive (OD) sequence. A promoterless KanMX marker gene was placed in between these border sequences. After the VirD2 mediated nicking of both border sequences, the promoterless KanMX ORF could then be released as a T-strand. Integration of this T-strand into a transcriptionally active region of the yeast genome should reconstitute KanMX marker gene expression and thus provide us with a selectable trait for T-DNA integration events. Prior testing for a possible level of kanamycin (G418) resistance in pArt1 containing cells by plating cells on media with different G418 concentrations indicated that cells were killed above a concentration of 50 µg/µl. When performing experiments with larger cell numbers and with two plasmids encoding the Vir proteins it appeared that up to 1-3% of the plated cells were resistant for G418 concentrations of up to 200 µg/µl. Further analysis of the obtained G418 resistant colonies revealed that the G418 resistance was pArT1 plasmid-based and was not due to insertion of T-DNA into the yeast genome. The acquired G418 resistance was independent of the expression of virulence genes but did correlate with the presence of additional ARS/CEN type vectors besides pArT1, even when these plasmids did not carry any inducible ORFs. Since plasmid instability caused by the simultaneous usage of several plasmids apparently frequently resulted into the stochastic expression of the KanMX marker on pArT1 another approach was required. To reduce the occurrence of G418 resistance, two measures were taken.
Firstly, an extra piece of stopcodon-rich DNA was inserted into pArT1, just upstream the RB sequence. This modification resulted in the vector pArT1stop which was supposed to prevent any translational read through into the KanMX ORF from upstream translation starts. Transcription at this area of the plasmid could be enhanced by unforeseeable epigenetic changes. Secondly, the virulence gene expression cassettes were integrated into the yeast genome, thus preventing the presence of similar plasmids that might recombine with pArT1stop. These measures strongly reduced the background levels of G418 resistance to values in principle low enough to detect even very small positive contributions of Vir proteins to T-DNA integration. In that manner, it seemed to be very promising that the conditions allowing for the expression of VirD2 resulted into increased numbers of cells that acquired G418 resistance compared to the empty vector control (Figure 11, Chapter 2). The addition of ORFs encoding other virulence proteins, including the VirD2 assisting protein VirD1 [6], did not further elevate the frequency of G418 resistance.

A Southern blot of DNA derived from G418 resistant yeast colonies revealed additional bands that might be indicative for potential T-DNA integration events (Chapter 2, Figure 11). However, it finally turned out that the G418 resistance was plasmid-borne in all cases; transferring the plasmids isolated from the G418 resistant strains to a fresh WT yeast strain always resulted into new G418 resistance and Southern blot analysis of genomic DNA isolated from cured plasmid-free strains proved that the KanMX ORF had disappeared. Evidence for genomic integration of T-DNA produced in yeast had thus not been obtained.

The molecular explanation regarding the origin of G418 resistance found in the experiments with pArT1stop and integrated expression cassettes has remained unclear. It is tempting to speculate that the association of VirD2 with single stranded border sequences during plasmid replication challenges accurate DNA replication, resulting in the generation of aberrant plasmids which then can express the KanMX gene. Since any further insights regarding the source of these events would not address the research questions we originally started out with, it seemed not prudent to further analyze the plasmids that conferred G418 resistance. Instead, it was concluded that the tools developed thus far did not allow for a sufficiently high level of all-in-yeast T-DNA production to support the intended investigations regarding the role of yeast factors in T-DNA integration.

Not all parameters that are of importance to the development of an all-in-yeast T-DNA production system could be investigated in detail; by lack of antibodies it was for instance not possible to verify the expression of unmodified Vir proteins in yeast. However, GFP fusions demonstrated that all expression cassettes were in principle active. In the cases that there could be a problem due to a clear difference in codon usage between Agrobacterium and yeast, yeast optimized codons were inserted. The minimal border sequences used in Chapter 2 proved to support a high level of DNA transfer, comparable
to the larger border sequences that were used for the cocultivation studies described in Chapter 3. It is conceivable that changes in orientation and position of border sequences in respect to the origin of plasmid replication could affect the possibility for VirD2-mediated border processing. Results from experiments addressing this issue, showed changes in the background frequency of G418 resistance, but no evidence was found indicating that successful T-DNA formation and integration had occurred (data not shown). One other experiment that was performed led to the conclusion that it is unlikely that in yeasto border processing by Agrobacterium Vir proteins takes place while using plasmids equipped with an ARS/CEN replication origin as a substrate. This experiment will be addressed below in the paragraph discussing Chapter 3 “Involvement of Rad52 in T-DNA circle formation during Agrobacterium tumefaciens mediated transformation of Saccharomyces cerevisiae”.

Hence, without clear cues on how to improve upon the construction of an effective and flexible all-in-yeast T-DNA processing and integration system, it was decided to focus on cocultivation based studies instead to get more insights in particular aspects of AMT. During these studies it was possible to use tools and insights that were obtained during the attempts to generate the all-in-yeast system.

### Involvement of Rad52 in T-DNA circle formation during Agrobacterium tumefaciens mediated transformation of Saccharomyces cerevisiae

In a pioneering study performed by our laboratory circular T-DNAs could be recovered after AMT of yeast cells [13]. In a recent publication, similar T-DNA circles were isolated from Agrobacterium infested plant tissue [20]. The recovery of T-DNA circles from yeast cells as well as from plant cells is indicative that T-DNA circle formation might be a general trait of AMT in addition to genomic T-DNA integration. From a biological point of view, the formation of T-DNA circles could benefit Agrobacterium, giving it the opportunity to express genes from the T-DNA circles even before genomic integration of the T-DNA is accomplished. Since the T-DNA circles do not contain free 5’ and 3’-ends, the T-DNA should be insensitive to exonuclease mediated degradation in the recipient host cell. The formation of T-DNA circles could also be of great interest for plant biotechnologists that are searching for new techniques that allow transient expression of genes in plant cells without the requirement to disrupt the genome.

Here, we investigated the impact of the presence as well as the type of T-DNA border sequences on a binary vector upon the occurrence of T-DNA circles. Additionally, we investigated the effects of mutations in the DNA repair mechanisms present in the recipient yeast cells, since previous research demonstrated that genomic integration of T-DNA can be mediated via the different DNA repair mechanisms [16,17]. Involvement of the DNA repair machinery could very well also be relevant to T-DNA circle formation. A new series of binary vectors was constructed, the pOphis series, all provided with a yeast ARS/CEN origin of replication.
Any circular T-DNA that would be formed in yeast could thus be maintained as a chromosomal type of plasmid. The pOphis series consisted, among others, of pOphis RB LB, pOphis RB, pOphis RB RBLB and a borderless vector pOphis.

The results that were obtained using the different yeast genotypes clearly indicated that the homologous repair (HR) DNA repair proteins Rad52 and Rad51 are involved in T-DNA circle formation, but not the nonhomologous end-joining (NHEJ) protein Ku70 (Figure 3 of Chapter 3). At first sight, these results were surprising since most of the pOphis vectors that were used did not contain sufficiently long regions of internal homology to support HR or no internal homology at all. However, these results would make perfect sense when multimeric T-DNA structures are formed, either in Agrobacterium or in yeast, which are consequently resolved into T-DNA circles by the HR pathway of the recipient cell. Current models for conjugative plasmid transfer assume that a rolling circle replication-like mechanism in the donor strain feeds the T4SS with single stranded DNA [21-24]. Experiments were therefore performed to test if such a mechanism could underlie the formation of concatemeric T-DNA structures. The obtained results indicated that following AMT of all plasmids, thus pOphis RB, pOphis RB LB, pOphis RB RB, and pOphis RB RBLB, a similar reduction of 70-75% in transformation efficiency was found when comparing AMT with \textit{rad52Δ} recipient cells to AMT with the congenic WT strain. After analysis of T-DNA circles that were rescued from transformed yeast cells, it appeared as if pOphis RB was always translocated in its entirety while for vectors with two or more borders the transfer was mostly restricted to the region between two border sequences (Figure 6, Chapter 3). Since the transformation efficiencies of yeast cells that received partial vectors and complete vectors were similarly reduced by the \textit{rad52Δ} mutation, therefore both types of entering T-strands were supposedly concatemeric. This observation prompted us to discard the rolling circle replication-like mechanism as a source for T-DNA concatemers since a rolling circle replication-like mechanism cannot be the source of multimers of partial vector sequences.

Sequence analysis of the RB area of T-DNA circles rescued from transformed yeast cells (Figures 7 and 8, Chapter 3) showed that in virtually all cases perfect border fusions were present, as if joined perfectly by VirD2. Interestingly, in addition to the reconstitution of RB sequences, also processed RB and LB sequences were found to be perfectly fused. Since the NHEJ reaction mediated by Ku70 was shown to be of negligible importance to T-DNA circle formation, a model for T-DNA circle formation was constructed wherein T-DNA concatemers are formed by a chaining reaction catalyzed by VirD2 and are subsequently resolved by the HR pathway of yeast into T-DNA circles (Figure 9, Chapter 3). As a follow up to the results of Chapter 2 “Towards T-DNA processing and integration in \textit{Saccharomyces cerevisiae}”, pOphis RB LB was modified to test if in yeasto T-DNA processing mediated by \textit{Agrobacterium} Vir proteins could occur. By the addition of an \textit{URA3} gene to section B of pOphis RB LB, it should be possible to select for a Vir protein mediated processing event.
After recirculation of the T-strand derived from pOphis RB LB a smaller plasmid could be reconstituted lacking the URA3 gene. A positive selection for the formation of these smaller circles is available in the form of 5-FOA that selects against cells that produce uracil. A major benefit of this approach is that for the detection of T-DNA processing it does not depend on the integration of T-DNA in transcriptional active genomic DNA. A proof of principle experiment yielded very few yeast colonies that apparently had inactive uracil synthesis pathways (data not shown). However, a slightly higher amount of Ura auxotrophic colonies was also retrieved from the negative controls that did not express any Agrobacterium virulence genes. The latter observation prompted us to discard the idea that yeast episomes equipped with Agrobacterium border sequences form a suitable substrate for virulence protein mediated T-DNA processing. As was explained previously in the discussion of Chapter 2, differences in the plasmid topology between yeast and prokaryotic cells like Agrobacterium and E. coli [25] could account for the observation that T-DNA present on pOphis RB LB is processed in Agrobacterium but no prove for T-DNA processing was obtained using yeast.

Enhanced gene targeting mediated by translocated I-SceI during the Agrobacterium mediated transformation of yeast

Random integration of the T-DNA is a natural consequence of using AMT for plant transformation [26]. The inherent unpredictability of the future integration site of the translocated transgene is commonly regarded as a major limitation of AMT, as it can lead to disruption of host genes or to the presence of undesirable regulatory elements at the genomic integration site. To avoid random integration of T-DNA, the HR system, that is naturally present in plants, might offer a means to target T-DNA to a predetermined genomic locus that comprises a strong homology to the incoming T-strand. Unfortunately, HR-mediated integration of DNA molecules are usually rare events in plants, estimated to occur once per $10^4$ to $10^5$ integration events [27,28]. Fortunately, the levels of HR can be boosted by the induction of a DNA double strand break (DSB) at the target locus [29-31].

To further characterize possible variables regarding targeted T-DNA integration, the effect of different chromatin states was investigated. Additionally, it was assessed if DSBs can be induced during cocultivation by the co-delivery of fusion proteins containing I-SceI nuclease domains to facilitate HR mediated T-DNA integration during AMT. The latter would be a very useful addition to the techniques that are currently used to induce DSBs to achieve T-DNA targeting via HR in plants since no extra DNA encoding such a nuclease needs to be present in the recipient cell.

The obtained results indicated that a decrease in the nucleosome occupancy of the target locus had no measurable impact on the efficiency of T-DNA integration at this locus.
However, the combined translocation of nuclease proteins and T-strands during AMT greatly facilitated HR-mediated integration of T-DNA (Figure 5, Chapter 4). In cases where *Agrobacterium* mediated translocation of specific nucleases for DSB induction at the target locus occurred to cells with an elevation of the nucleosome occupancy of that target locus, it was found that the presence of nucleosomes severely reduced the positive effect of the nuclease on the transformation efficiency. Apparently the nucleosome occupancy influenced the level of access that was granted to the nuclease to the target locus.

Surprisingly, during the course of performing the experiments mentioned above, it was also found that the agent that was used to induce nucleosome removal from the target locus, i.e. growth on galactose instead of glucose, had a severe impact on the AMT efficiency of the yeast strain YPH250 (Figure 3, Chapter 4). Especially T-DNA integration via the NHEJ pathway was more efficient if the cocultivation was performed on galactose containing agarose plates. The yeast strain RSY12 proved to be largely unaffected by this “galactose effect” and was therefore used to perform the experiments that required manipulation of the nucleosome occupancy of the target locus. Especially because growth on glucose resulted in an improvement on the AMT efficiency of the NHEJ binary vector pSDM8000, which usage usually results in very low transformation efficiencies, a deeper knowledge of the mechanisms behind the “galactose effect” might be beneficial. The T-DNA integration itself seemed not to be of a different nature than AMT of yeast performed on glucose plates: sequencing data of five inverse PCR products showed a random integration pattern typical for T-DNA integration. When comparing growth curves of RSY12 and YPH250 on galactose, YPH250 is clearly disadvantaged, hardly showing any growth on galactose medium. However the growth curves for glucose were similar between the two yeast strains.

Possibly difficulties in the uptake of galactose forms the basis for the observed differences between the AMT of YPH250 and the AMT of RSY12. There are also some indications from literature that the yeast strain S288C, which is ancestral to YPH250, had a defect in the galactose permease *GAL2* [32]. An alignment of the amino acid sequence of the galactose transporter protein *GAL2* from RSY12 and YPH250 showed that two amino acids were varying between the two strains, of which one was predicted to be located at the inside of the Gal2 pore. Possibly, these variations influence the capacity of these yeast strains to gather galactose from their environment. Because of time limitations no experiments have been performed to link the mutations in the YPH250 *GAL2* gene to the ability to grow on galactose medium. Although not supported by solid evidence, the reduced growth we observed for YPH250 on galactose medium could be instrumental for the enhanced levels of NHEJ mediated T-DNA integration we observed. Possibly starvation has a yet unknown impact on some of the mechanisms important for T-DNA-integration like the nuclear uptake of DNA or DNA repair.
Double stranded DNA formation as a limiting factor for transkingdom AMT

Although useful and reliable protocols have been developed for the AMT of yeast [13] and filamentous fungi [14], the transformation efficiencies that could be achieved have always remained limited. This is especially true if the T-DNA is forced to integrate via the NHEJ pathway, thus in cases of absence of sequence homology between the T-DNA and the genome of the recipient cell [16,17].

Several steps of the AMT pathway could be considered to be potentially rate limiting, already starting with the adhesion of Agrobacterium to the intended recipient cells. Observations at the start of AMT of yeast cells on a cocultivation plate, using a confocal microscope show that only a small fraction of the yeast cells is actually bound to the excess of Agrobacterium cells that is usually present (P.A. Sakalis, personal communication). Later on in the AMT process, it can be envisaged that one or more of the different Vir proteins that are transported to recipient cells might not exert the required AMT supporting function, either due to limiting concentration or to lack of a required interaction partner within the recipient cell. Considering the transferred effector proteins VirE2, VirE3, VirD5 and VirF [33,34], an example of the latter limitation is the absence of a yeast homolog of VIP1 from Arabidopsis thaliana [18]. Another example of a deficiency in a recipient cell could be the observation that the absence or presence of the virulence protein VirF bears no impact on the AMT efficiency in yeast [13] although VirF proved to be of importance to the AMT of tomato and a number of other plant species [35-37]. Strikingly, although considered of vital importance to the AMT of plants, the absence of some of the natural interactors of Agrobacterium effector proteins in yeast cells [18,38-40] does apparently still allow for nuclear import of the T-strand or T-complex and the consequent genomic T-DNA integration [13].

Apart from the issues regarding the Vir proteins and potential interactors mentioned above, several thus far unnoticed aspects of AMT might provide ample opportunities to improve upon AMT efficiency. The galactose induced elevation of NHEJ-dependent AMT shown in Figure 3 of Chapter 4, could very well be an example of such a hitherto unknown experimental variable. Furthermore, during the finalizing steps that are required for the T-DNA integration mediated by the DNA repair mechanisms of the recipient cell there could be multiple moments where very important determinants for the efficiency of AMT need to be operational. Support for the latter hypothesis might be found when further exploring the phenomenon that when performing AMT of yeast using the KanMX marker gene for the selection of transformants often many miniscule colonies are visible, besides the larger colonies which clearly represent stable G418 resistant transformants. Although for lack of time these smaller colonies were never thoroughly analyzed, it might very well be that they represent cells that only transiently obtained G418 resistance. This would mean that a T-strand, containing the KanMX marker gene, has entered these yeast cells but was degraded before it was incorporated into the genome.
Nevertheless, due to transient expression of double stranded T-DNA in the original recipient cell as well as some of its descendants, growth of a small colony might be supported around relatively few G418 detoxifying cells. In that manner, the observation of minuscule colonies during experiments aimed at T-DNA integration via the NHEJ-pathway, might indicate that in the majority of cases the incoming T-strands are expressed but do not end up in the genome, thereby pointing out that instead of T4SS mediated translocation or the proper functioning of effector proteins, it are the final steps that take place just prior to the genomic incorporation of the T-DNA that are apparently rate limiting.

A comparison of all the transformation efficiencies resulting from the different experimentations described in the different Chapters of this thesis shows that the highest transformation efficiencies were generally obtained with the ARS/CEN typed T-DNA circle forming binary vectors that are described in Chapter 3. AMT with these binary vectors is more efficient than when using binary vectors that can integrate via HR and are even several orders of magnitude more efficient than when using binary vectors that must rely on NHEJ for integration of T-DNA. Strikingly, the smaller colonies mentioned above were generally absent from the transformant selection plates used with T-DNA circle forming vectors. This could indicate that when incoming T-strands can be maintained as episomal structures, AMT frequency would approach the maximal potential as far as it is related to the frequency of successful contact and T4SS mediated transport of macromolecules. For AMT of yeast, this frequency is between 1 and 5 transformants per 1000 yeast cells under the most optimal conditions thus far identified.

For the repair of DSBs via the HR or the NHEJ DNA repair machinery, the synthesis of a complementary DNA strand is required. Since the involvement of the host DNA repair mechanisms in T-DNA integration is well documented [16,17,31] I would like to postulate that the formation of a complementary strand is a prerequisite for T-DNA integration, thereby forming an important rate limiting factor in the AMT of yeast and possibly also other organisms. The ARS/CEN typed binary vectors would then have an advantage since they are equipped with a yeast replication origin and are thereby automatically a target for yeast primases leading to an efficient conversion to dsDNA.
Future perspectives of the application of genetically modified plants

Much has been said and written about the application of AMT of plants in plant biotechnology. Meanwhile, AMT has become the method of choice for the genetic modification of crops like corn and soybeans. In the United States, genetically modified foods have been on the menu for over 16 years. How much more can be expected from this small soil born bacterium, more than a 100 years after *Agrobacterium tumefaciens* was first described in Science by Erwin Smith and Charles Townsend?[41] With further research, AMT could be put to use to meet some of the needs that are expected to become more prevalent in a future with a growing world population and limited resources.

Some of these applications that are worth looking forward to, are the development of drought resistant plants that can be grown at localities now unavailable for agriculture and the optimization of the maximal amount of yieldable biomass to make full use of the available agricultural lands. From an environmental point of view, the development of pest resistant plants would also be beneficial since such plants could effectively reduce the amount of pesticides that are used now. The sympathetic “Golden rice” project, that resulted in genetically engineered rice plants that produce beta-carotene, a precursor of vitamin A, proved that it is possible to perform very challenging research without a commercial drive [42]. However, the strict regulations concerning the application of genetically engineered plants have severely delayed the application of this technology [43].

Looking at the potential of applying genetically modified (GM) plants to solve actual problems, one could wonder why research institutes that could be developing new applications of GM plant lines are mostly focusing on other technologies instead. Possibly, the negative associations that both the general public and policymakers have regarding GM plants play a major part in this. The few GM crops that are put to use were developed purely for commercial reasons, adding little to the general interest. However, the general reluctance to embrace this technology cannot simply be disposed of as a critique on commercialism or as technophobia. There can be a strong willingness to accept new technology or commercialism if the resulting products bear sufficient attraction to the consumer. The wide range of new technologies that led to the development of smartphones had no difficulties at all to become widely accepted and even admired. Adding to this view, an example of a GM organism that did receive considerable popularity is the “GloFish” from Yorktown Technologies, a transgenic fluorescent fish that comes in several bright colors and is available in pet shops throughout the United States. Clearly, apparent usefulness is not a relevant selling point for new technology.

By performing more research on the subject of AMT of plants, with a special focus on potential problem solving applications, it will become apparent that when it comes to the application of GM plant lines, it is prudent to go against popular believes and fears in order to accomplish something very worthwhile.
References


