

Genetically modified grapevine: state of research, possible risks and future scenario.

Francesco Pazzi

Fondazione Diritti Genetici

Via Garigliano 61/a – 00198 – Roma – Italia

Phone: 0039-0645438276

Fax: 0039-0686391315

E-mail: pazzi@fondazionedirittigenetici.org

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Summary

Up the early 1990s grapevine was considered a recalcitrant plant for in vitro regeneration and genetic transformation. Thanks to the development and the optimization of embryogenic cell cultures and the protocols for genetic transformation, the number of publications regarding the development of genetically modified grapevine has become more numerous. If such publications before the early 1990s were concerned with the optimization of protocols for in vitro regeneration and genetic transformation, today focus has turned to the increase in open field experimentation of genetically modified grapevine aiming at investigating its risks and efficacy.

The scope of this review is to evaluate the state of the art regarding research on transgenic grapevine and to delineate its possible future scenarios. To this end, this paper has considered current research in open field trials which can also be considered as those studies which are at the most advanced stage with regard to marketing authorization.

Results have evidenced that at the moment research is not at the level to think about imminent commercialization of genetically modified grapevine. Moreover, the open field experimentation of genetically modified grapevine has raised numerous concerns regarding possible environmental risks and above all possible repercussions on the image of the national wines by wine producers and consumers.

The reputation of genetic engineering offering innovative products for the viniculture sector and its consequent public acceptance will represent, in the future, the main factors on which the commercialization of genetically modified grapevine will depend.

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Introduction

Until the early 1990s grapevine was considered a recalcitrant culture for in vitro regeneration and genetic transformation (Fuchs, 2003). Thanks to the improvement of these techniques though, publications relating to the genetic transformation of grapevine for agronomic and qualitative characters have become increasingly numerous and, likewise, has experimental field trials currently underway to test the efficacy and possible risks prior to commercialization.

If, on the one hand, the application of genetic engineering can offer new perspectives for the wine sector, on the other hand, there is, in many state, a strong opposition in the use of genetically modified organisms (GMOs) derived products yet, due to the possible risks on health and by environmental and socio-economic impacts (Eurobarometer 2008).

Considering that some avenues of research currently in progress are obtaining good results, consumer acceptance of genetically modified (GM) grapevine will represent the principal barrier to the development of such technology.

The aim of this review is to evaluate the state of the art regarding research on GM grapevine and to delineate its possible future scenario. To this end, this paper has considered current research in open field trials which can also be considered as those studies which are at the most advanced stage with regard to marketing authorization. Previous review articles (Fuchs, 2003; Thomas et al., 2000; Perl and Eshdat, 1998; Reisch and Pratt, 1996; Torregrosa, 1995; Torregrosa and Bouquet, 1993; Gray and Meredith, 1992), indeed, have discussed grapevine tissue culture and genetic engineering and at the state of knowledge there are not study reporting the situation of GM grapevine tested in open field trials. For this purpose the research underpinning this

study has been conducted by means of the evaluation of the notifications for the experimental release of GM grapevine in open field trials submitted by the applicant to the specific Competent Authorities and the relating scientific bibliography.

The state of research on transgenic grape

Research on GM grapevine currently under investigation in open field trials concerns the principal agronomic and qualitative traits of the grape as well as its resistance to fungal, viral and bacterial disease and modification of berry pigmentation, sugar composition, flower and fruit development (Table 1). With the exception of only a few cases, the *Agrobacterium tumefaciens* method was utilized for the transformation. In addition to the gene target, the *nptII* gene for kanamycin resistance was used as a marker.

GM grapevine for qualitative traits

To date only Italy and Australia have commenced open field trials of GM grapevine for qualitative traits. The Italian research has demonstrated that the *iaaM* gene derived from the bacterium *Pseudomonas savastanoi* is able to increase the yield of the plants (Constantini et al., 2007), as demonstrated with other crops (Mezzetti et al., 2004). Authorization for these field trials expired in September 2006 and even if the research had given positive results it hasn't been renewed.

The Australian open field trials focussed on studying genetically modified grapevines for berry pigmentation, sugar composition, flower and fruit development. The first studies in open field trials of GM grapevine modified for these traits commenced in 1999 and to date no evidence has been produced to demonstrate the effectiveness of the utilized genes in conferring such phenotypes, unlike results for other vegetables such as

the potato (*Solanum tuberosum*) and carnations (*Dianthus caryophyllus*) (CSIRO, 2003).

Genetically modified grapevine for agronomic traits

The main thrust of research has been at the production of grapevine resistant to the main fungal, bacterial and viral diseases such as powdery mildew (*Oidium tuckeri*), grey mould (*Botrytis cinerea*), Pierce disease and Grapevine Fanleaf Virus (GFLV). The only experimental field trials currently underway to test GM grapevine for abiotic stress are taking place in Canada. These trials concern particularly plants overexpressing the Mn-superoxide dismutase gene. Tests previously undertaken on alfalfa (*Medicago sativa*) have demonstrated plant resistance to low temperatures (McKersie et al., 1999; McKersie et al., 1993). To date there is no evidence to support similar findings for the grapevine.

Among the differing avenues of research currently being pursued with regard to the modification of the agronomic traits of the grapevine, it seems that those being carried out by the National Institute for Agricultural Research (INRA) in France, are at the most advanced stage. This research has shown that some lines of rootstock 41B (*V. vinifera* x *V. berlandier*), once transformed with the coat protein (CP) gene of the GFLV did not exhibit visible GFLV-type symptoms (Vigne et al., 2004). The resistant lines were subsequently tested both in Rumania and in France within the TRANSVIR European project (the Environmental impact assessment of transgenic grapevines and plums on the diversity and dynamics of virus populations), to assess the emergence of viable GFLV recombinants in transgenic grapevines expressing the GFLV CP gene. Results have shown that transgenic grapevines do not assist the emergence of viable GFLV recombinants to detectable levels nor do they affect the molecular diversity of

indigenous GFLV populations (Fuchs et al., 2007), results which differ from the findings of laboratory studies (Latham, 2004).

With regard to studies attempting to obtain fungal resistant grapevine, research has been ongoing in the United States of America (USA) since 1994 in open field trials using GM grapevines with genes that have been shown to confer resistance to powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*) and grey mould (*Botrytis cinerea*) (Grison et al., 1996; Jach et al., 1995). Those genes which appear to have given the best results are the *chi26* gene from barley (*Hordeum vulgare*) and coding for the chitinase enzyme. Line of GM grapevines transformed with *chi26* were also tested in Germany. This experimentation in Germany which was planned to have continued until 2009 has been prematurely curtailed as transgenic plants have been shown to have the same susceptibility as non transformed plants (Bornhoff et al., 2005; GMO-Safety, 2005). These German findings have to date not been confirmed or otherwise by the studies in the USA. Other genes, such as the *Drr206* gene deriving from *Pisum sativum* which is involved in the biosynthesis of lignin and the *Fshp* gene, isolated from *Fusarium* Sp. coding for a DNase have, however, provided more interesting results in defending plants from fungal attacks (Chang, 2005).

Further research in California, Florida and New York State has been studying in open field trials GM grapevine transformed by antibacterial genes. Particular attention has been given to testing GM grapevines resistant to Pierce disease although to date results have been inconclusive.

The competent authorities in South Africa are currently evaluating an application to release into the environment, for experimental purposes, a GM grapevine transformed by the marker gene *uidA* which has been isolated from the *Escherichia coli*. The scope of

this study is to test the long term stability and the expression of the gene. The proposed experimental field trials will be the first of a series to be put into place to test different lines of GM grapevine obtained from the Institute for Wine Biotechnology of Stellenbosch University within the “Grapevine Program”. Experimentation at the moment is taking place only in greenhouse conditions.

Risk assessment of GM grapevine

The main concerns relative to the utilization of GMOs in food sector regard the possible environment, health and socio-economic impacts. An accurate risk evaluation of GM grapevine result therefore of strong importance both for the safety assessment of derived product both for the estimation of the cost/benefit balance derived from its utilization, needed also for a future social acceptance of the technology.

Crossing the modality of grapevine propagation with the possible target of the genetic modification it is possible to identify four different case, each needing a specific evaluation:

- Own-root grapevine;
- Gm scion grafted on GM rootstock;
- GM scion grafted on non GM rootstock;
- Non GM scion grafted on GM rootstock.

Considering that every single transformation event should be value with a case by case approach and due to limited documentation available relative the risk assessment on GM grapevine produced until now, it is not possible to conduct a detailed description on the possible risk derived from GM grapevine. With refer data presents in the risk assessment report in the applications for the experimentation in open field of GM grapevine, submitted by the centre of research involved in the experimentation at the

competent authority and the relative bibliography, it is however possible to make some considerations.

Considering possible environmental impacts, the principal problems could derive from the risk of dissemination of the gene through sexual and asexual propagation of GM grapevine, both through pollen, seeds or vegetative parts of the plant. Such eventuality could cause the growth of volunteers plants that might cross with the possible compatible commercial and wild plants, carrying both problems for the biodiversity as well for the possible coexistence among the different cultural typologies.

Cultivated *Vitis vinifera* sp. is considered self-pollinating and the flower are characterized by a strong cleistogamy and lacking of an efficient vessilar system. For this reason, the possible risks derived from pollen dissemination both for anemophily, both for entomophily impollination should be limited. Considering however that low rate of cross pollination can also occur and there is limited and discordant published information on pollination distance as well influence of insect in fertilizing flower (McGregor, 1976), it will be necessary to define in accurate way pollen flow before commercialization of GM grapevine. It will be an essential condition for risk prevention and to set up coexistence plans among GM, conventional and biological culture. Until now only German and Australian experimentation have foreseen the analysis of vertical gene flow, even if the results have not been published yet.

The dormancy of the seeds and their ability to be transported to long distances by birds and other abiotic and biotic agents, and the ability to originate volunteer plants are factors that may contribute to the dissemination of the gene in the environment. For the mitigation of such risks, until now, experimentations have foresee the bagging of the inflorescences before the flowering, the use of animal proof net and surrounded GM

grapevines by conventional grapevines. To date, published data do not allow to estimate possible impacts derived by dissemination of the gene in a reality cultural context and such measures will be necessary for its mitigation. The use of GM rootstock, where the target allows it, it would contribute to reduce such risks.

Greater part of GM grapevines tested in open field trials were transformed with the gene marker *nptII* conferring kanamycin resistance. Principal worries related to the use of GM plants transformed with antibiotic resistance gene, concerns horizontal gene transfer of such gene toward the microbial flora of the soil. Furthermore, the European legislation, Directive 18/2001/CE for the release in the environment of GMO, foresee before to commercialize GM plants or derived products, it is necessary to evaluate the potential impacts on the soil derived from such technology. Such risks have not been considered in none of the experimentations conducted until now.

Substitution of antibiotic markers with other gene, as also foreseen by the cited directive, it would help to limit such problems that have besides contributed to increase diffidence toward the use of the GMOs today commercialized.

One of the principals aspects considered in the different studies, concerns unintended effects of genetic modification on peculiar characteristics of the product, as for instance total polyphenols and sugar content, acidity, etc. Data reported in the different experimentation seem to show the substantial equivalence among GM grapevines and near isogenics one. Only data from Italian experimentation shown significant differences in total polyphenols content and in some values of acidity (Costantini et al., 2007). In the Australian and Sudafrican experimentation, it is also foreseen to test wine produced from GM grapevine on a group of people to which wines will be administered.

Considering that grapevine productive cycle has a varying duration among the 20-30 years, another very important factor to verify before commercialization of GM grapevine, concerns the genetic stability of the insert, that otherwise could provide the alteration or the loss of function of the inserted gene, altering in this way the characteristics for which the plant has been transformed, besides conducting to potential unintended effects. One of the experimental limits in the evaluation of such parameter, concern duration of the experimentations conducted until now, that has not allowed to fully estimated in a representative way the stability of the specific transformation event in the time. The only available data concern those obtained in French (Vigne et al., 2004;) and Italian (Costantini et al., 2007) experimentation, where stability of the traits were evaluated for 3 consecutive years without show any alteration.

It results instead very difficult make considerations relative possible future implications of social-economic character, even if commercialization of GM grapevine derived products will be destined to open new sceneries and implications, how traceability, labelling and the coexistence among the different cultural typologies. Open field trials conducted until now it underlined however a greater social sensibility toward such applications. The case of the French experimentation is a clear example. In 1999, following the worries related to the possible risks for health, environment and image of the French wine, raised by different institutions and organizations about experimentation in open field trials of GM rootstock, has been interrupted the experimentations in course and denied the applications of new authorizations, with the purpose to allow pilots experience for the construction of a research program on the public participation in the process for GMO authorization. The program has foresee the

experimentation was followed in all of its phases by a group formed by the representatives of the social parts involved to all the levels (INRA, 2001).

The French project represents in its genre the first case in the world of public participations in the process of authorization and management of an experimental field for a GM crop. Such approach, directs to increase the transparency and the visibility in the GMO authorization, could need in the future to increase the confidence of the consumers forward the possible acceptance of GMO derived products.

Discussion and conclusion

Despite of the fact that in the last decade significant progress has been achieved on the development and evaluation of GM grapevine, it is very difficult to foresee its future. On one hand the severe damages caused by grapevine diseases, the strong demand for a reduction in the reliance on toxic agrochemicals for diseases control and the changes of the climatic conditions, open opportunity for practical use of GM grapevine, on the other hand, the success of biotechnologies at offering alternatives to current control strategies and, above all, the consumer's acceptance to the use of GM grapevine derived products could limit its application.

One of the main reasons of consumers' reluctance to consume GMO derived products is due to the fact that these are considered today as a possible cause of health risk. The lack of transparency and visibility in the authorization process and the recent alimentary scandals, are factors that have contributed to increase the distrust toward such products. It will result therefore essential draw up the relationship of trust between science and society, favouring more transparency, visibility and social participation in the GMOs authorization process.

Separation among the different production chains, GM and not GM, is another factor

that will have to be provided so that the GM grapevine could represent a resource for everybody and not a problem. It will need therefore to define what the procedures and the behaviours that will allow coexistence between the different production chains. The validation of detection protocols of the specific grapevine transformation events will result then a fundamental requisite for the control of the efficiency of such strategies.

It will need then to clarify the formalities of GM grapevine registration in the variety register, the labelling of GM grapevine derived products and the questions relative to the patent.

The United States could probably be the first to cultivate GM grapevine for marketing purpose. Indeed, the numerous GM grapevine open field trials conducted to date, have not aroused particular attention from the public opinion and the legislative conditions are more favourable, how for example the unforeseen separation of the different production chain. Instead, in the greater part of the other countries, the applications has aroused a great debate among the different actors of the production chain, many of which have affirmed not to be available to its use (Jonker, 2007; African Centre for Biosafety and Earthlife, 2006; Biowatch South Africa, 2006a; Biowatch South Africa, 2006b; Chicago Tribune press releases, 2006; Fresh Plaza press releases, 2006; Goering, 2006; Winemakers' Federation of Australia, 2003; GMO-Safety, 2002).

Considering however that among the GM cultivars object of the American researches, there are varieties cultivated to a global level, among which Chardonnay, Cabernet Sauvignon, Shiraz, Riesling, Merlot, Pinot and rootstock 41B, the possible commercial authorization of GM grapevine will unequivocally lift a great debate to a world level.

Considering, however, that studies on possible environmental and health impacts are still very limited, as well as prediction of the future social and economical implication

and the efficacy of some GM grapevines reported in the cited study, how for example GM rootstock 41B, has to be confirmed in further study, it result very improbable to suppose an imminent commercialisation of GM grapevine.

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Tab. 1: Summary of the principal GM grapevine field trials in course, recently expired or pending.

Permission							Expected phenotype
Country	date (From/to)	Cultivar	Target Gene ^a	Promoter	Terminator	Source of gene	
Qualitative traits							
Italy ¹	05/1999 09/2006	Thompson seedless; Silcora.	<i>iaaM</i>	<i>defH9</i>	<i>nos</i>	<i>P. savastanoi</i>	Parthenocarpic
Australia ²	06/2003 06/2008	Shiraz; Sultana; Cabernet; Sauvignon; Chardonnay.	<i>ppo</i>	<i>CaMV35S</i> ; <i>sc4</i> ; <i>sc7</i> ; <i>sc4</i> .	<i>ocs</i> ; <i>sc4</i> ; <i>sc5</i> .	<i>V. vinifera</i>	Altered browning
			<i>Ufgt</i>	<i>CaMV35S</i>	<i>ocs</i>	<i>V. vinifera</i>	Altered berry skin colour
			<i>dfr</i>	<i>CaMV35S</i>	<i>ocs</i> ; <i>Sc4</i> .	<i>V. vinifera</i>	Altered anthocyanin/tanni

							n synthesis
			<i>inv</i>	<i>CaMV35S</i>	<i>ocs</i>	<i>V. vinifera</i>	Altered sucrose content of fruit
			<i>sh4</i>	<i>CaMV35S</i>	<i>ocs</i>	<i>V. vinifera</i>	Altered flowers and fruit
Agronomics traits							
Canada ³	NR	Cabernet Franc	<i>Mn-SOD</i>	<i>CaMV35S</i>	<i>NR</i>	<i>A. thaliana.</i>	Cold tolerance
France ⁴	06/2005 10/2009	Roostok 41 B	<i>CP genes from GFLV.</i>	<i>CaMV35S</i>	<i>nos</i>	<i>GFLV</i>	Virus resistant
Romania ⁵	01/2003 30/2006	Russalka; Roostok 41B.	<i>CP genes from: GFLV, GVA and GVB.</i>	<i>CaMV35S</i>	<i>nos</i>	<i>GFLV; GVA; GVB.</i>	Virus resistant
Germany ⁶	07/1999 10/2009 (Interrupted)	Dornfelder; Riesling; Seyval Blanc.	<i>chi26; bgl32; rip-30.</i>	<i>CaMV35S</i>	<i>NR</i>	<i>H. vulgare</i>	Fungal resistant

United State ⁷	03/2006	NR	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	Bacteria resistant	
	12/2011							
	04/2000	Chardonnay;	<i>chi</i>	<i>NR</i>	<i>NR</i>	<i>T. harzianum</i>	Fungal resistant	
	04/2010	Merlot.						
	05/1998	NR	<i>chi</i>	<i>NR</i>	<i>NR</i>	<i>T. harzianum</i>	Fungal resistant	
	11/2008							
				<i>En42; MSI-99;</i>				
				<i>MagII; PGL;</i>			<i>T. harzianum; X.</i>	Fungal resistant;
	04/2007	NR	<i>Synthetic</i>	<i>NR</i>	<i>NR</i>	<i>leavis; A.</i>	Bacteria resistant	
	04/2008		<i>antimicrobial</i>			<i>caudatus.</i>		
				<i>peptide.</i>				
	04/2006	Chardonnay;	<i>PGL; MagII.</i>	<i>NR</i>	<i>NR</i>	<i>A. caudatus; X.</i>	Bacteria resistant	
04/2007	Merlot.				<i>leavis.</i>			
07/2007	NR Rootstock.	<i>CP gene.</i>	<i>NR</i>	<i>NR</i>	<i>GLRaV-2;</i>	Virus resistance		
07/2008					<i>GLRaV-3; GFLV.</i>			

	05/2005	Niagara;					
	05/2009	Concord.	<i>Drr206</i>	<i>NR</i>	<i>NR</i>	<i>P. sativum</i>	Fungal resistant
		Chardonnay;					
	06/2004	Thompson	<i>Polygalacturon</i>				
	06/2014	Seedless; Rootstock Saint George.	<i>ase inhibitor</i> <i>protein.</i>	<i>NR</i>	<i>NR</i>	<i>P. communis</i>	Fungal resistant
	11/2007						Fungal resistant;
	09/2008	NR	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>V. vinifera</i> ; NR.	Bacteria resistant
China ⁸	NR	Thompson seedless.	<i>STS</i>	<i>CaMV35S</i>	<i>nos</i>	<i>V.</i> <i>pseudoreticulata</i>	Fungal resistant
South Africa ⁹	Pending	Sultana; Chardonnay.	<i>uidA</i>	<i>sc4</i>	<i>ocs</i>	<i>E. coli</i>	Visual Marker

Source:

- 1- <http://biotech.jrc.it/deliberate/IT.asp>
- 2- <http://www.ogtr.gov.au/ir/dir031.htm>
- 3- <http://www.inspection.gc.ca/english/plaveg/bio/confine.shtml#sum>
- 4- <http://biotech.jrc.it/deliberate/FR.asp>
- 5- http://ec.europa.eu/research/quality-of-life/cell-factory/volume2/projects/qlk3-2002-02140_en.html
- 6- <http://biotech.jrc.it/deliberate/DE.asp>
- 7- <http://www.isb.vt.edu/cfdocs/fieldtests1.cfm>
- 8- Fan et al., 2008.
- 9- http://academic.sun.ac.za/wine_biotechnology/

^a Gene marker are not considered

NR: not reported