Final Publishable summary

Executive summary

Among red fruits, raspberry is a horticultural crop with a high value due to its unique flavours and sensory quality, to its proven “health promotion – disease prevention” (HPDP) properties, and to the high but still increasing competition between production worldwide. While the world market of raspberries has increased by 60% in the last decade, the relative share of the products originating from Europe has increased in a lesser proportion. Challenges to the European market include providing highly productive cultivars rich in bioactive compounds as well as cultivation schemes requiring significantly reduced chemical inputs. In this context, the major achievements of the QualiRedFruits project have been to provide a consortium of European SMEs with the necessary research activities aimed at combining - for the first time - varietal selection of raspberries for greater HPDP properties (antioxidant molecules) with new cultivation systems based on biotisation (inoculation of beneficial microorganisms) and elicitors (stimulators of natural defence), in order to optimize the production of healthy raspberry plantlets and ensure high-quality fruits without resorting to the excessive use of polluting chemicals.

Technical data related to producer and consumer criteria were assembled for about 100 raspberry varieties/cultivars. These indicated over one third with interesting traits. Further analyses for fruit quality, antioxidant HPDP components and/or for resistance/tolerance against major diseases highlighted the genotypic dependence of these characteristics and defined several cultivars with a high exploitation potential. Whilst providing competitive arguments for the market of fresh fruits or derivative products, such information is also important for breeding-based creation of new genotypes with attractive traits. In this context, (i) protocols for pilot hybridization between interesting genotypes and varieties were optimised and procedure guidelines were established, (ii) an approach for genotyping raspberry resources was developed to support certification for registration and to trace material on the market for producer protection, and (iii) a methodology, based on cryopreservation, was elaborated with the aim of guaranteeing long-term conservation and availability of genetically stable, pathogen-free germplasm for selected raspberry material.

An innovative scheme was established for raspberry plant cultivation from in vitro microplants or cuttings to field and greenhouse conditions, based on the combined use of biotisation to improve plant growth/fruit yield and compatible elicitor treatment to effectively reduce root rot damage. Efficient mycorrhizal fungi and beneficial bacteria were selected and procedures were recommended for optimized biotisation of raspberry plants in substrates before outplanting, combined with effective elicitor application in real production systems. PCR-based diagnostic probes were generated to track persistence of introduced beneficial microorganisms and to evaluate the phytosanitary status of plant materials, soils and substrates. Field and greenhouse trials in 7 locations (5 countries) confirmed the clear benefit of biotisation and/or elicitor treatments on fruit yield or plant protection against root rot, but they also highlighted the importance of growing environment and plant genotype in determining raspberry crop responses. Raspberry genotype and geography/location of production were likewise major factors influencing antioxidant characteristics of raspberry fruits although the agronomic treatments had little clear impact.

Project context and objectives

The QualiRedFruits (QRF) project was co-funded by the European Commission and involved seven Small and Medium Enterprises (SMEs) and five Research and Technological Development (RTD) performers altogether representing seven different European Member and Associate States. This project dealt with the improvement of quality and agricultural procedures of raspberries considered as a paradigm of red fruits because of their high value due to:

- a unique panel of flavours and sensory quality,
- a proven "health promotion – disease prevention" (HPDP) property, and
- a high but still increasing competition between production worldwide.

Despite its continuous increase in the World market during the last decade, the European market share has remained stable around 20% and may decrease in the next decade. In this context, the major innovative aspects of the QualiRedFruits project are to provide a consortium of SMEs with the necessary research activities aimed at combining - for the first time - varietal selection of raspberries for greater HPDP properties with new cultivation systems based on biotisation (inoculation of beneficial microorganisms) and elicitors (stimulators of natural defence), in order to optimize the nursing of healthy raspberry plantlets and ensure high-quality of the fruits without resorting to the use of polluting chemicals.

Moreover, the sustainable development of new production strategies requires that molecular tools be developed to:

- monitor beneficial microorganisms introduced into biotised raspberry culture systems,
- assess plant sanitary status,
- enable the tracing of raspberries on the market for producer protection against biopiracy and,
- guarantee long-term conservation and availability of germplasms of high quality raspberry varieties selected during the project.

Raspberry crop

In the global fruit production system, a dominant position belongs to the berry fruits and especially raspberry production. The major production regions are: Russia, Europe and the Pacific Coast of USA and Canada. Many other countries, such as Chile, New Zealand, Australia and China are entering into the market as they supply the fresh market during winter in the northern hemisphere.

In Europe, most of the raspberry production is in the northern and central countries and higher regions of Italy. Greece, Portugal and Spain are new comers. In many production areas, the fruit is grown for the high-value fresh market, but in Eastern Europe (Serbia, Poland, and Hungary) a high proportion of the crop is for further processing, i.e. frozen preparations including individual quickly frozen (IQF) fruits, juice, concentrate, and preserves. These products are then packaged and sold directly to the consumer or further processed into jam, jelly, dessert topping, pie filling, ice cream, yoghurt, etc. The popularity of raspberries continues to grow as shown by the increasing number of raspberry-containing products on supermarket shelves.

Key figures

Statistics about production are controversial at both world and national levels. Roughly, the worldwide production is about one million tonnes per year. A half of it enters into the regular market where an average 20% comes from Europe. Since the market price of raspberries varies from 1 to 7 €/kg paid to the producers and 7 to 10 € paid by the consumers, the economical significance of this crop on the global market is rather high.

Context of the project

The QualiRedFruits project is undertaken at the crossroad between the evolution of the fruit market, especially red fruits, and the progress of plant physiology.

Market trends – In addition to the continuous request for increased productivity and decreased production costs, the new trends of the market are dominated by the increasing consumers' interest in the potential of red fruits in health promotion and disease prevention (HPDP) mainly resulting from their potentially high content in antioxidant molecules (AOM). Additionally, there is an increasing social awareness and requests for agricultural practices globally safer for the environment and free from noxious chemicals in the context of sustainable development and food safety. In the near future, these new features may significantly add to the conventional quality criteria which are: the fruit size and firmness, the colour, the ease of harvest and the taste (in priority order).

Scientific and technical progress in plant physiology – The mechanisms which regulate the interactions between the soil and the plant roots are not fully elucidated yet. Nevertheless, the role of fungi and bacteria in these interactions is better known and laboratories have developed a new knowhow to monitor the plant roots/bacteria/fungi symbiosis in order to protect plants from their major diseases such as fungi and viruses. Plant conditioning is known as "biotisation". Additionally, it has been established that the plant self-defences against several pathological agents can be enhanced by new treatments without pesticides or fungicides and known as "elicitors".

Major objectives

The current market trends and progresses in plant physiology and molecular biology have created a timely opportunity to identify raspberry varieties with higher quality in terms of

AOM content and consistent with innovative cultural practices respectful of the environment. The QualiRedFruits key objectives of the project are to:

- screen raspberry varieties for their AOM content and compare with the varieties currently dominating the market;
- design and deliver on-purpose cultural practices suitable for the selected varieties and making use of beneficial micro-organisms (biotisation) and natural elicitors of plant defence reactions in order to drastically reduce chemical input and increase AOM synthesis in the selected varieties.

Considering the improvement of the competitiveness of the SMEs involved in the project as the global and ultimate prospective, the newly design chain from plant biotisation, plant nursing and farming must be strengthened by an adequate quality assurance scheme for both plant production and further raspberry prodction. The project is thus meant to bring operational advantages to the SMEs participants while contributing, as a pioneer, to the evolution of fruit production in a broader prospective. This includes the development and adaptation of complementary technologies for:

- monitoring of the biotisation system towards large scale industrialisation,
- assessing plants sanitary status,
- tracing raspberries to combat biopiracy, and
- preserving germplasms of the processed varieties on a long term.

Ultimate challenges of the project

While the world market of raspberries has increased by 60% in the last decade, the relative share of the products originating from Europe has increased in a lesser proportion although the production capacity per unit of cultivated area has doubled during the same period of time. **Reasonable business anticipations suggest that the EU dependence in volume and in value may unfortunately decrease by 10% in the 5 years after the new varieties and innovative cultivation procedures resulting from the QualiRedFruits project are actually demonstrated and consolidated at the field level. This reduction of imports to the benefit of European actors is expected to reach 10% per 5-year time slot and should likely continue for 2 decades.** This will require that several challenges be met and namely:

- broadening the offer to customers by introducing on the market new cultivars originating from European technologies;
- underpinning such new European varieties thanks to a standardised assessment of their content in bioactive compounds as the role of these compounds in HPDP is increasingly established by medical research worldwide;
- offering varieties that require significantly reduced chemical treatments as the result of innovative cultivation procedures;
- orienting the relevant regulatory framework towards the establishment of certification procedures based on easy genotyping of the new varieties and easy assessment of the sanitary status of the plants;

- Forging arguments to promote the informative labelling of the marketed fruits and processed derivatives concerning their HPDP properties including measurement of AOM content.

Implementation strategy and expected outcomes

The main directions followed by the project are:

- selection of raspberry varieties with high fruit quality (including AOM) and tolerance/resistance to fungal diseases;
- development of new methods of plant biotisation and elicitor treatment for healthier plant production;
- conservation, genotyping and plant health assessment;
- production field trials.

Since the project approach from laboratories to fields had to deal with seasonal constraints, parts of the work was undertaken in parallel instead of sequential developments.

Objective indicators involved molecular markers for:

- the specific identification of varieties together with a cryo-preservation protocol for germplasms' long-term conservation;
- the identification of beneficial and pathogenic micro-organisms in order to assess the sanitary status of plants;
- the biomarkers-based approach tested in real production trials, from *in vitro* culture to the field for validation.

Finally, appropriate controls and reproducibility tests enabled the partners to formulate recommendations for quality production of raspberries taking into due consideration both the fresh fruits market of higher quality and the processed fruit market.

Main S&T results/foregrounds

Formal delivery of results by RTD performers to SME participants

The results of the QRF project which had to be delivered to the SME participants by the RTD performers according to the transaction of the Grant Agreement and the DoW are reminded in the Table 1 below.

Table 1 – Results and share percentages of these results expected by the SME participants from the RTD performers in the QualiRedFruits project.

		SME Participants					
		SICOLY	PREDICAT	INOPLANT	MYBATEC	DIEFFENBACH	LA PRUNE LORRAINE
		P11	P02	P03	P04	P05	P12
Result ID	**Description**						
R01	Identification of raspberry varieties with enhanced antioxidant molecule content and/or tolerant to pathogenic fungi that could be used as commercial varieties and/or in a breeding programme of new commercial varieties **FROM P06, P08, P10**		**10%**			**30%**	**60%**
R02	Identified beneficial microbes for plant biotisation **FROM P06**			**100%**			
R03	Identified elicitors of natural plant defence mechanisms for improving raspberry resistance against pathogenic fungi **FROM P07**		**10%**			**30%**	**60%**
R04	Defined protocol for the combined use of biotisation and elicitor treatment **FROM P06, P07 P08**		**10%**			**30%**	**60%**

R05	Defined protocol for raspberry cryo-preservation **FROM P06**			**100%**			
R06	Genotyping of interesting raspberry varieties and hybrid lines **FROM P09**	**70%**					**30%**
R07	Defined protocol for plant sanitary status assessment **FROM P06, P10**	**70%**			**30%**		
R08	Innovative scheme for raspberry plant production from *in vitro* to field, requiring low amounts of chemical input and more compatible than conventional with respect to the environment **FROM P06, P07,P08, P09, P10**	**50%**	**10%**	**10%**		**30%**	
R09	Recommendations to SMEs for producing and managing biotised plants, and for elicitor treatment procedures **FROM P08**	**60%**	**10%**			**30%**	

Independently from the Deliverables reporting the achievements in the project Work Packages, **each Result R01 to R09 has been formally delivered in a detailed internal Report available to fix the Joint Exploitation Agreement among the SME participants and to serve as foreground reference for any further work to be carried out after the end of the project.**

The internal reports executive summaries are presented in the Table 2 below.

Table 2 – Summaries of internal reports on Results supporting the transfer of background from RTD performers to SME participants in the QualiRedFruits project.

Result ID	R01
Disclosing Institution	Agroscope Changins-Wädenswil Research Station ACW Centre de recherche Conthey Route des vergers 18 1964 Conthey, Switzerland
Authors	Zo-Norosoa Camps, Christoph CARLEN, P08-ACW Kaloyan Kostov, Slavcho Slavov, Rossitza Batchvarova, P10-ABI Anton Ivancic, Andrej Šušek, P09-UMFKVB Tomasso Sozzi, Vivienne Gianinazzi-Pearson, Silvio Gianinazzi, P06-INRA
Nature	Report

Dissemination level	Confidential
Title	Identification of raspberry varieties with enhanced antioxidant molecule content and/or tolerant to pathogenic fungi that could be used as commercial varieties and/or in a breeding program of new commercial varieties
Date of disclosure	30/11/2012 - Project Month 24

Summary

- The aim of WP1 was to select cultivars riche in AOM and tolerant to diseases with a high exploitation potential with regard to specific cultivation procedures, to breeding and to Intellectual Property Rights.
- A screening of about 100 varieties and genotypes of raspberries made it possible to define 37 available floricane and primocane fruiting raspberries varieties to be tested by entering them into the experimental approach of the project and finally support a future breeding program.
- Concerning AOM synthesis of floricane fruiting cultivars the most interesting red cultivar is Elida. Elida showed high contents of Vitamin C, phenolics, ellagitannins and high antioxidant capacities. Elida may support a future breeding program in combination with a high yielding cultivars producing firm fruits such as Tulameen. Another approach is to breed a high yielding cultivar with big, light red fruits with Bristol or some wild genotypes (SL, F) to get progenies with high contents of health related compound. These progenies may have to be backcrossed with another high yielding cultivar to get a cultivar with big, red fruits and a high yield.
- Concerning AOM synthesis of primocane fruiting cultivars, the most interesting varieties were Joan J and Kweli. It would be an interesting approach to cross these two cultivars, because their positive attributes concerning AOM synthesis are very complementary. On the other hand, Kweli, a high yielding cultivar with big, firm fruits, has an interesting potential for healthy fruits and in combination with specific cultivation procedures (biotisation, elicitor treatments) to produce high value fruits.
- Concerning the tolerance to diseases (such as Botrytis cinerea and Leptosphaeria sp and Phytophthora sp.) of floricane fruiting cultivars, Elida showed good tolerance against Botrytis cinerea and Schopska Alena and Williamette against Phytophthora sp. The other cultivars were not different from each other concerning the tolerance to these diseases. Concerning the primocane fruiting cultivars Sugana, Autumn Bliss, Joan J (Dieffenbach) and Erika showed interesting tolerance to Botrytis cinerea and Leptosphaeria sp.. Erika has a good tolerance to Leptosphaeria sp. and the two Bulgarian cultivars Iskra and Samodiva against Phytophthora sp.
- Data on technical aspects of hybridisation of new cultivars including pollination, seed harvesting, stratification of seeds and plantlets production were given related to experiences developed in this project. The biggest problems were to regenerate plantlets from seeds obtained from crossing of two genotypes.
- In order to capture the greatest value from plant-related intellectual property assets, a number of types of statutory intellectual properties rights for SME involved in breeding new varieties and/or SME involved in biotisation of plants are described and discussed such as plant variety protections (PVPs), plant patents, geographic indications, trademarks and trade secrets. SME participants should be aware of all options for further IP protection and use them to their greatest advantage. The vari-

ous forms of IP protection for plants can be used alone or in combination.

Result ID	R02
Disclosing Institution	Institut National de la Recherche Agronomique 17 Rue Sully, BP 86510, 21065 Dijon Cedex, France
Authors	Vivienne Gianinazzi-Pearson Silvio Gianinazzi Tommaso Sozzi
Nature	Report
Dissemination level	Confidential
Title	Identified beneficial microbes for plant biotisation
Date of disclosure	30/11/2012

Summary

- Three arbuscular mycorrhizal fungi are identified for efficient raspberry plant biotisation: Glomus intraradices isolates LPA8, LPA7 and LPA54.
- Two beneficial soil bacteria are identified for efficient raspberry plant biotisation: Pseudomonas fluorescens sp. I13 and Sinorhizobium meliloti.
- A composite inoculum has been defined based on the compatibility between the bacterium Sinorhizobium meliloti and the arbuscular mycorrhizal fungus Glomus intraradices isolate LPA8.
- A biotisation procedure is provided for the inoculation of Glomus intraradices isolate LPA8 and Sinorhizobium meliloti during the acclimatization of vitroplants or cuttings of different raspberry cultivars.
- The described biotisation procedure is highly reproducible and the methodology has the advantage of rapidity and applicability to a large number of plants.

Result ID	R03
Disclosing Institution	Scottish Agricultural College West Mains Road, Edinburgh EH9 3JG, UK
Authors	Dale Walters
Nature	Report
Dissemination level	Confidential
Title	Identified elicitors of natural plant defence mechanisms for improving raspberry resistance against pathogenic fungi
Date of disclosure	26/06/2012

Summary

- Cultivar differences exist in raspberry responses to foliar or root drench application of the plant defense elicitors Bion® (acibenzolar-S-methyl), BABA® (beta-aminobutyric acid), Yea Foliar (chitosan) and methyl jasmonate.
- In Tulameen cultivar plants, only Bion® reduces root rot damage by P. fragariae var rubi and increases root development.
- In Meeker cultivar plants, all four elicitors significantly decrease root rot damage and increase resistance to P. fragariae var rubi , with best control achieved using methyl jasmonate.

• The elicitor Bion® is identified as effectively increasing resistance to the root rot pathogen in both Tulameen and Meeker raspberry cultivars. • Increased resistance elicited by Bion® in Tulameen plants is associated with enhanced activity of the defence-related enzymes peroxidase, beta-1,3-glucanase and cinnamoyl alcohol dehydrogenase (CAD) in leaves. • BABA® appears to increase 'tolerance' to raspberry root rot in Tulameen and Meeker plants, and enhance CAD activity in Tulameen leaves.

Result ID	R04
Disclosing Institution	INRA - Institut National de Recherche Agronomique, France
Authors	Vivienne GIANINAZZI-PEARSON, Silvio GIANINAZZI,Tommaso SOZZI at INRA - Institut National de Recherche Agronomique, France Dale WALTERS at SAC – Scottish Agricultural College, Scotland Christoph CARLEN, Zo-Norosoa ANDRIANJAKA-CAMPS at ACW - Eidgenoessisches Volkswirtschaftsdepartement, Switzerland
Nature	Report
Dissemination level	Confidential
Title	Defined protocol for the combined use of biotisation and elicitor treatment
Date of disclosure	20/11/2012
Summary	
• Indicators have been defined for monitoring compatibility in raspberry plants between biotisation and plant defence elicitor application: mycorrhizal development, plant growth, defence-related enzyme activities, fruit production and AOM content/antioxidant activities in leaves and fruits. • Protocols are described for the combined use of biotisation and elicitor application in greenhouse and real production systems of raspberry plants and fruits. • Treatment of raspberry plants with the plant defence elicitors BION®, BABA®, methyl jasmonate or chitosan is compatible with biotisation with a composite microbial inoculum (Glomus intraradices isolate LPA8/Sinorhizobium meliloti) under greenhouse production conditions. • The compatibility of BION® application with plant biotisation has been confirmed in a real production system of organic farming for raspberries..	

Result ID	R05 – R06 – R07
Disclosing Institution	University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, 2311 Hoče, Slovenia
Authors	A. Šušek, M. Šiško, A. Ivančič at UMFKBV – Univerza v Mariboru, Fakulteta za kmetijstvo in biosistemske vede (University of Maribor,Faculty of Agriculture and Life Sciences) V. Gianinazzi-Pearson, T. Sozzi at INRA - Institut National de Recherche Agronomique, France

	R. Batchvarova at ABI-Agrobioinstitute A. Gollotte at INOPLANT, France
Nature	Report
Dissemination level	Confidential
Title	Conservation, genotyping and plant sanitary status assessment
Date of disclosure	14/11/2012

Summary

- The cultivars Meeker, Glen Ample, Rose de Core d'Or, JJR, Sicoly S1 and Polka were successfully identified with microsatellites markers.
- Specific molecular primers for 5 plant pathogenic fungi (Phytophthora fragariae, Thielaviopsis basicola, Leptospheria coniothyrium, Aphanomyces euteiches, Pythium ulitmum) were designed.
- Specific molecular primers for 3 beneficial bacteria (Sinorhizobium meliloti, Pseudomonas fluorescens sp. I13, Paenibacillus sp. B2) were designed.
- Specific molecular primers for 2 beneficial fungi (Glomeromycota, Glomus intraradices) were designed.
- Cryopreservation protocol based on encapsulation/vitrification was developed for raspberry varieties Tulameen and Meeker.

Result ID	R08
Disclosing Institution	SRUC (formerly SAC - Scottish Agricultural College) West Mains Road, Edinburgh EH9 3JG, UK
Authors	Dale Walters
Nature	Report
Dissemination level	Confidential
Title	Innovative scheme for raspberry plant production from in vitro to field, requiring low amounts of chemical input and more compatible than conventional with respect to the environment
Date of disclosure	14/12/2012

Summary

- The aim of WP04 was to test raspberry varieties and methods developed in work packages 1, 2 and 3 under real production conditions. In order to achieve this aim, field trials were conducted in Switzerland, France, Slovenia, Bulgaria and Scotland, using the same basic design and layout of the trials and joint protocols for treatment. The overall aim was to test the effect of biotisation and elicitor (Bion®) treatments, alone and in combination, on growth and yield of raspberry varieties, and on control of diseases, particularly raspberry root rot. The sanitary status of the plants was examined to ensure the presence of mycorrhiza and the absence of pathogens. The content of antioxidant molecules (AOM) and the antioxidant capacity of the plants receiving the different treatments was also determined.
- The results highlight the importance of geography/location, and genotype, in determining the response of a raspberry crop to agronomic treatments:
- Biotisation and elicitor treatments had no significant effect on factors affecting fruit yield in Switzerland.
- There were significant effects of the treatments on growth of raspberry plants and fruit yield in Bulgaria, although genotype differences were observed in fruit yield.

• Genotype differences were also found in Slovenia, where elicitor, and biotisation plus elicitor treatments, provided increased fruit yields in Polka, but not in Tulameen. • In France, the combination of biotisation and elicitor provided good fruit quality and yield, while the elicitor treatment provided good vegetative growth and root development. Here, the elicitor treatment (Bion®) was associated with some phytotoxicity. • Under Scottish conditions, poor weather in 2011 and extremely poor weather in 2012 affected the trial, again highlighting the role of geography, with its accompanying differences in weather, on crop performance. Nevertheless, the field trial in Scotland confirmed the genotype differences observed in the other field trials, and also showed that in Polka, the elicitor can provide some protection against Phytophthora root rot, while in Tulameen, the combination of biotisation and elicitor provided best protection against root rot, under very heavy inoculum potential. • Analysis of AOM contents and antioxidant activities in the leaves and fruits from plants in the different field trials also highlighted the importance of genotype, followed by geographic location, while agronomic treatments had little effect. • In most of the field trials, elicitors and biotisation were compatible, suggesting that in most production systems for raspberries, the elicitor Bion® and biotisation can be used together.

Result ID	R09
Disclosing Institution	SRUC (formerly SAC - Scottish Agricultural College) West Mains Road, Edinburgh EH9 3JG, UK
Authors	Dale Walters
Nature	Report
Dissemination level	Confidential
Title	Formal set of recommendations to SMEs for producing and using biotised plants, and for elicitor treatment procedures
Date of disclosure	14/12/2012
Summary	
• Based on the experiments carried out in the four work packages comprising this project, recommendations can be offered to SMEs. • A protocol has been defined for the combined use of biotisation and elicitor treatment, based on (i) isolates of mycorrhizal fungi and beneficial bacteria selected by P06 INRA for their efficiency and compatibility, (ii) a procedure optimized by P03 INOPLANT for biotisation of raspberry plants before outplanting, and (iii) an effective elicitor treatment developed by P07 SAC for reducing development of Phytophthora root rot symptoms in plant production systems. • Specifically, the following recommendations can be given to SMEs: • Since the plant defence elicitor BION® is compatible with biotisation when applied under greenhouse conditions, its application can be recommended during the standardized procedure for large scale production of biotised raspberry plants to provide effective control of Phytophthora root rot. • In some environments (e.g. Bulgaria, France and Slovenia), and in certain cultivars (e.g. Polka and perhaps Tulameen), biotisation and the elicitor Bion® can be recommended to protect raspberry plants against Phytophthora root rot and to increase plant growth under field conditions.	

- In Scotland, depending on the raspberry variety, biotisation and / or the elicitor Bion® can be recommended to reduce the damaging effects of root rot under field conditions.

Detailed achievements of the project

Selection of raspberry varieties with high fruit quality and tolerance to fungal diseases

Current interest by consumers, media and medical actors in the health benefits of several components of fruit and vegetable aliments having pharmaceutical properties (often referred to as "nutraceutical" in English and "Alicament" in French) has created an increasing interest in the berry industry. There is a consensus that berries are a nutritional and nutraceutical powerhouse, and that raspberries rank near the top of all fruits for antioxidant strength. Past and current medical research shows likely benefit of regularly consuming raspberries against cardiovascular diseases, diabetes, inflammation, some cancers and age-related degenerative diseases. Such benefit is proven to result from antioxidant molecules (AOM) (Kresty *et al.*, 2001; Puupponen-Pimiä *et al.*, 2005; Coates *et al.*, 2007). The concept of AOM refers mainly to non-nutrient compounds in foods and covers a broad range of molecules including flavonoid or non-flavonoid polyphenolics, phenolic acids, vitamins and other potential organic antioxidants (Beekwilder *et al.*, 2005). Anthocyanins and ellagitannins are the major contributors to the total antioxidant activities of raspberry (Mullen *et al.*, 2002 ; Beekwilder *et al.*, 2005 ; Battino *et al.*, 2009): ellagitannins contribute around 50% and anthocyanins about 25%. AOM synthesis in raspberries varies between plant varieties; most red raspberry varieties presently on the market contain relatively low levels of AOM compared to black raspberry, blackberry and wild raspberry.

Raspberry plants are prone to parasites, fungal diseases and virus infections, which make the use of pesticides a conventional cultivation practice. Chemical treatments generate potentially noxious food residues which counterbalance the positive effect of berry diet upon human health (Łozowicka *et al.*, 2012), but little success has been achieved to find alternatives to fungicides to control fungal diseases (Ançay *et al.*, 2005; Xu *et al.*, 2012). Three fungal pathogens have a high potential to cause serious raspberry yield loss. Root rot due to *Phytophthora fragariae* var. *rubi*, the main cause of raspberry plant death, cannot be effectively controlled by chemicals and only prophylactic measures can be adopted by planting healthy raspberry plants in non-contaminated soils (Ançay *et al.*, 2005). *Leptosphaeria coniothyrium*, which attacks raspberry canes, causes variable disease severity depending on the year, situation, weather and varieties (Williamson *et al.*, 1986). Finally, *Botrytis cinerea* causes the fruit grey mould responsible for significant economic loss (Xu *et al.*, 2012; O'Neill *et al.*, 2012).

Technical data on raspberry varieties/cultivars with a high exploitation potential related to enriched AOM content of fruits and tolerance to disease are therefore an important pre-requisite to an integrated approach including the selection of plant varieties more resistant to main pathogens and breeding-based creation of new genotypes with combined producer and consumer defined traits.

Pre-selection and evaluation of raspberry varieties

About 100 commercially and collection available varieties and genotypes of raspberry were screened on the basis of literature and project partner experiences for their high economical importance and contrasting traits concerning consumer criteria (fruit sensory quality, firmness), farming criteria (yield, fruit size), potentially higher quantities of antioxidant molecules (HPDP properties) and/or tolerance to major fungal diseases in raspberry production systems. A total of 33 available floricane and primocane fruiting raspberries cultivars as well as 4 wild or semi-wild floricane genotypes were defined and grown to fruit for further analyses.

Figure 1: Illustration of colour quality parameters of fruits of some selected raspberry cultivars

- *AOM content and antioxidant capacities*

- Appropriate methodologies for extraction and analysis of health-related compounds in raspberry fruits and leaves were defined and applied to 23 floricane and 11 primocane cultivars or genotypes for the characterization of AOM compound contents (vitamin C, total phenolics, anthocyanins, ellagitannin) and antioxidant capacities (DPPH radical inhibition, Ferric Reducing Antioxidant Power, Oxygen Radical Absorption Capacity).
- Overall, the variation in antioxidant capacities of floricane and primocane cultivars followed that in terms of AOM content, with cultivars rich in the major phytochemicals showing the higher antioxidant activities. Results clearly indicated that leaf antioxidant analyses cannot be used to predict fruit antioxidant capacities. No correlation was found between leaf and fruit antioxidant capacities, or between leaf antioxidant capacities and fruit AOM content.
- In general, floricane fruiting cultivars with interesting agronomical, physical and sensorial quality parameters had relatively poor antioxidant activity and health related compounds. From factorial discriminant analyses, floricane raspberries could be classified in four principal groups depending on their anthocyanin constituents and in four main clusters according to ellagitannin patterns, whilst primocane genotypes were distinguished into three categories for their anthocyanin patterns and two main classes for ellagitannin composition.
- Three primocane cultivars gave a significantly higher yield and fruit size than all other cultivars whilst four other cultivars (two floricane, two primocane) and some of the wild genotypes showed high potential in terms of AOM content and antioxidant capacities. These could be promising for future breeding in combination with high yielding cultivars producing large, firm fruits.

- *Tolerance to fungal diseases and fruit malformation*

- 11 floricane and 9 primocane fruiting cultivars were examined for tolerance to three pathogenic fungi causing major diseases in raspberry production systems. Detached leaf and stem bioassays against infection by *Phytophthora fragariae var. rubi, Leptosphaeria coniothyrum, Botrytis cinerea*) showed that none of the studied floricane fruiting cultivars possess good tolerance to all three fungal diseases. However, one floricane cultivar demonstrated good tolerance towards *B. cinerea,* and two others against *P. fragariae*.

Figure 2: Response of raspberry cultivars to the P. fragariae var rubi in detached leaf (left panel) and stem inoculation (right panel) tests.

- For primocane fruiting cultivars, one showed significant tolerance to all three fungal diseases. An interesting level of tolerance against *B. cinerea* and *L. coniothyrum* was observed for another three cultivars, and against *P. fragariae* for two.
- None of the floricane or primocane fruiting cultivars grown in a project field site showed more than 2 % of fruit malformation. No virus or MLO was detected and canes deformed fruits in one year did not necessarily have them in the following year. Conditions during flowering, especially extreme temperatures, may impact on fruit malformation

Pilot hybridizations

- In order to set the pace for breeding innovative raspberry genotypes with high exploitation potential related to increase the AOM content of raspberry fruits tolerance to diseases, pilot hybridizations were performed and successfully achieved between some interesting genotypes and varieties. Technical procedures (emasculation, pollination, seed harvesting, seed stratification, plant regeneration) were optimised and guidelines were provided to SMEs. Intellectual property rights relative to novel plant-related assets have also been communicated.

Figure 3: *Choice of suitable developmental stages of raspberry flowers for emasculation and pollen samples*

- Higher leaf antioxidant activities and total phenolics content were identified in some hybrids between the cultivated and wild genotypes. However, plants were too young to be transferred to the field for fruit evaluation and this will be performed in the 2013 season.

In conclusion, the expectancies of the project SMEs have been met with regards to obtaining reliable information and defining several raspberry cultivars or genotypes which are enriched with healthy compounds, and which meet farmer and consumer criteria requirements. Also in line with the SME expectancies, new analytical tools have been introduced for the identification of raspberries having a high AOM content and thus enabling new marketing approaches to promote raspberry consumption based on objective quality criteria. In this context, knowledge about the qualitative and quantitative contents in health-promoting compounds of raspberry plants or fruits, as well as of their tolerance to diseases, can provide competitive arguments for SMEs in order to increase their market share of fresh fruits or derivative products from fruits and leaves. Moreover, the information can support a future breeding program by suggesting cultivars with a potential to increase AOM content of the fruits or leaves of newly developed cultivars, or their tolerance to three major fungal diseases in raspberry production systems. In the context of a future breeding program, various types of statutory intellectual properties rights (IPR) for plants such as plant variety protections (PVPs), plant patents, geographic indications, trademarks or trade secrets have been communicated to the project consortium.

Raspberry plant genotyping, conservation and health assessment

Variety/cultivar registration is an important issue of plant genetic resource characterisation and utilisation. Traditional methods to identify raspberry cultivars for the granting of plant breeder's rights are based on phenotypic observations. The incorporation of new methodologies into plant material certification schemes will accelerate and optimise the identification process of genotypes and offer guarantee of plant sanitary status. Genetic fingerprinting (genotyping) can provide exact data on the genetic diversity existing in raspberry collections of available genetic resources for breeding programs, and independently of developmental stage or environmental factors that may influence the phenotype. Genotyping techniques based on microsatellites, also known as Simple Sequence Repeats (SSRs) or short tandem repeats (STRs), are highly informative (show high polymorphism), technically simple (simpler than other techniques), robust and suitable for automated allele detection and sizing (Rafalski & Tingey 1993). In addition, the utilization of fluorescence-based automated DNA detection and fragment sizing offers a potential improvement of the efficiency and affordability of variety testing (Hayden *et al*. 2008). Since raspberry species are vegetatively propagated (not by seed), the identification of genotypes will offer a standardised reference for certification of any cultivar and for controlling its propagation. This approach may prove to be crucial in the context of variety ownership rights in the event of the creation of new genotypes.

Novel techniques for ensuring long term conservation of raspberry materials, like cryopreservation (Reed *et al*. 2001; Wang *et al*. 2005; Keller *et al*. 2006; Reed 2008), can provide producers with easy access to stable genetic resources and a back-up to recover varieties when genetic divergence or sanitary problems occur. In this context, the development of molecular tests to detect beneficial or pathogenic micro-organisms in plant materials, soil and substrates will provide rapid and reliable information that is crucial for raspberry cultivation systems. The basic methods for detection and identification of plant-inhabiting micro-organisms applied in practice include symptom observation, isolation from attacked tissue or soil, and morphological characterization of the isolated organism. Molecular techniques based on PCR provide additional information and certainty of the microbial detection and identification process (Bonants *et al*. 1997, Vandemark *et al*. 2000, Gollotte *et al*. 2004, Farmer *et al*. 2007, Nilsson *et al*. 2008). The combination of both approaches in the routine observation of plant sanitary status would facilitate the accuracy of the diagnostics and lead to an optimized regime of the plant protection activities.

Plant genotyping

- A molecular technique to genotype raspberry material was developed based on the discriminating power of Simple Sequence Repeat (SSR) sequences in 19 microsatellite loci. SSR primer sets designed from the microsatellite loci were used in PCR of DNA extracted from fresh, young leaf tissue to evaluate the genetic diversity in a germplasm collection of 49 raspberry genotypes (semi-wild, wild, floricane, primocane). Capillary electrophoresis of PCR products generated SSR sequences which revealed 167 polymorphic alleles at the 19 microsatellite loci.
- Dendrogram analysis of genetic relationships based on SSR data arranged the 49 investigated raspberry genotypes in five main clusters, with a larger genetic distance between genotypes than between clusters. One cluster included wild and semi-wild raspberry germplasm, whilst one local variety and genotypes resembling cultivated floricane or primocane cultivars were grouped in the other four clusters. Analyses of genetic diversity in the cultivars identified 8 duplicate varieties.
- An SSR-based PCR protocol was optimized for the identification of raspberry varieties considered to be commercially important. A specific fingerprint (electrotrace), which can be used for identification, was obtained for each variety using combinations of SSR primer sets for selected microsatellite loci. The most specific microsatellite loci were selected for each variety from PCR reactions conducted with the 19 optimized SSR primers.

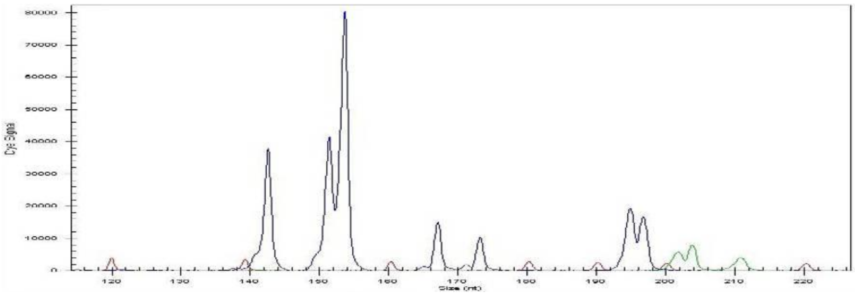


Figure 4. SSR-based electrotraces used for identification of one raspberry cultivar based on a combination of microsatellite loci

- The optimized protocol was successfully applied to six raspberry varieties. Seven of the most specific microsatellite loci were used for one floricane variety, and combinations of six loci were used for 2 other floricane varieties and 3 primocane varieties. To reduce labour and costs for PCR product detection, a multiplex of three primer sets labelled with two different fluorescent dyes were combined. Applicability of the developed protocol for variety identification was verified in a ‘blind’ test on anonymous DNA samples from five raspberry genotypes.

Conservation of raspberry genotypes

- In the context of long-term conservation and stability of high quality raspberry varieties/ genotypes, a cryopreservation technique adapted from published literature was first successfully applied to *Medicago sativa* seeds in pre-experimentation trials. The same protocol, however, gave toxic effects when applied to shoot tips of three raspberry varieties. An alternative procedure was developed for raspberry tissues, in which treatment with low sugar concentrations, together with adapted osmotic loading and plant vitrification solutions, improved tissue survival and enabled some plant regeneration from raspberry shoot tips or root fragments after storage in liquid nitrogen.

Plant sanitary status assessment

- A molecular method, based on PCR detection, was developed to track the presence or absence of beneficial or pathogenic micro-organisms in raspberry cultivation systems. Specific PCR primers were designed on sequences in the DNA region coding for rRNA and used on soil or plant material from raspberry cultures.
- Molecular marker sets were obtained to detect three beneficial bacteria and one mycorrhizal fungus used in biotisation technologies, and for six fungal pathogens, of which three are important pests in raspberry production systems (*Phytophthora fragariae, Leptospheria coniothyrium*, *Botrytis cinerea*).
- Specificity of the PCR detection method was verified under different laboratory conditions and on soil or raspberry plant tissues colonized by the targeted organisms. The approach was successfully applied to check for the absence of pathogenic fungi from substrates or composite inoculum used for biotisation.
- Applicability of the molecular protocols for routine monitoring of the targeted fungi under real production conditions was demonstrated directly on fungal-colonized raspberry plant tissues using DNA from leaves infected by *P. fragariae* var. *rubi* or *L. coniothyrium*, and of roots or soil colonized by *P. fragariae* var. *rubi* or AM fungi, sampled from field trials.

Figure 5: PCR detection of a mycorrhizal fungus in root samples of biotised raspberry plants after plantation into a field site (left panel) and of P. fragariae in Phytophthora-contaminated soil samples (right panel).

In conclusion, the methodology developed for genotyping of raspberry material can be exploited in furthering certification procedures for registration of established or new variety/cultivar cultivars and for their control during propagation or across the market. The protocols defined for detection of beneficial or pathogenic micro-organisms provide rapid and reliable procedures for plant sanitary status assessment, and so promote the production of healthy biotised raspberry plants not contaminated by pathogens. Both these achievements meet the SME expectancies of proven methods for ensuring plant quality in future certification procedures. In addition, optimization of the cryopreservation technique opens the possibility of long-term, genetically stable conservation of pathogen-free raspberry material which represents an added value resource for producers.

Development of new methods of plant biotisation and elicitor treatment for healthier plant production

Biotisation is a biotechnology consisting in the inoculation of young plants with beneficial micro-organisms (bacteria and/or fungi) in order to increase plant vigour (Gianinazzi *et al*., 2003). In the case of raspberry, biotisation of young plants during the acclimatisation phase has proved useful for improving plant survival and growth, and for increasing tolerance to *P. fragariae* var *rubi* (Gollotte *et al*. 2009, Lemoine *et al*. 2000). Arbuscular mycorrhizal (AM) fungi are the most significant of beneficial soil micro-organisms as they establish a symbiosis at the interface between soil and the roots of most high value agricultural and horticultural plants. Although AM fungi are present in most ecosystems, their populations are generally limited in the field under conventional agriculture because of their sensitivity to practices using large quantities of fertilisers and pesticides. Moreover, micro-propagated plants and substrates used in raspberry production are usually devoid of AM fungi so that biotisation can be very useful in such cases to ensure plant vigour and health. Biotisation can also be beneficial to field-grown plants, for example under organic farming conditions or when reduced amounts of fertilisers and pesticides are used.

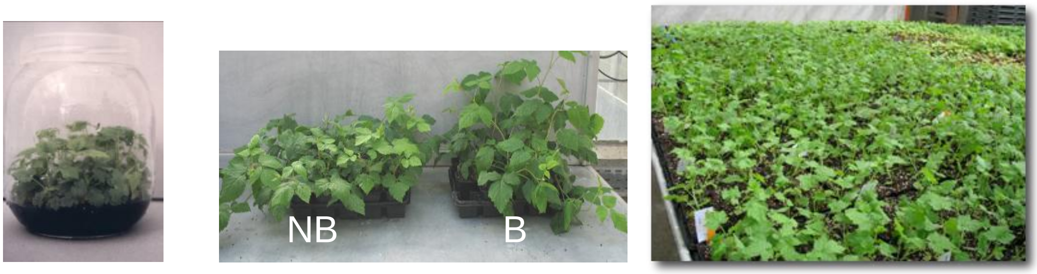
Fighting against cryptogamic diseases is still a problem in raspberry cultivation, although a few raspberry varieties can be identified which show good tolerance to *Phytophthora fragariae* var. *rubi* (see above), and producers have to resort to the use of chemical fungicides which can be pejorative to the environment and human health. Elicitors of natural plant defence mechanisms have been shown to provide useful disease control in many crops against a range of economically important pathogens (Walters, 2011), and various natural agents (e.g. cell wall fragments, plant extracts) can induce plant resistance to subsequent pathogen attacks, both locally and systemically (Walters *et al*. 2005, Walters & Heil 2007). This induced resistance does not lead to complete disease control, but rather to a reduction in pathogen development and disease symptoms. The defence mechanisms involved are those used in other forms of plant resistance to pathogens and include enhanced defence-related enzyme activities as well as synthesis and accumulation of a range of phenolic compounds, many of which possess antioxidant properties. Agents which mimic natural elicitors of resistance have been developed and commercialised for broad-spectrum disease control using the plant's own resistance mechanisms, but these have not been tested on raspberry (Walters & Heil 2007).

AOM synthesis in plants depends on agricultural practices as well as plant varieties and climatic conditions (Anttonen *et al.*, 2005). Mycorrhization has been reported to increase polyphenol content in grapevine and sweet basil, carotenoid level in sweet potato, and antioxidant activity in raspberry (Krishna *et al*. 2005, Farmer *et al*. 2007, Toussaint *et al*. 2007, Gollotte *et al*. 2009). Also, treatment with plant defence elicitors has been reported to increase total phenolic content and antioxidant activity in sweet basil (Kim et al. 2005), and to increase total phenolic, anthocyanin and antioxidant content of raspberries and blackberries when sprayed onto fruits during the ripening phase (Wang & Lin 2000, Wang & Zheng 2005, Wang *et a*l. 2008). However, the impact of combining these innovative agricultural practices on plant content in AOM has yet to be characterised.

Whilst the identification of plant defence elicitors which enhance resistance to major fungal diseases in raspberry represents a new alternative to chemical control, knowledge about their compatibility, or synergy, with biotisation in raspberry plant production systems is so far inexistent, although positive or no effects have been reported in other plants (Murphy et al. 2000, Sonnemann *et al*. 2000, Tosi & Zazzerini 2000, Hause *et al*. 2007). The enhancement and the consequent standardization of raspberry plant biotisation coupled with elicitor treatment are expected to be of competitive advantage for the SMEs in terms of healthier plant production and fruit quality.

Raspberry biotisation

- Suitable mycorrhizal fungi and beneficial bacteria were selected for their compatibility in the biotisation of raspberry microplants, and a multiplication procedure was established for the production of appropriate mycorrhiza and bacteria inocula. A first composite inoculum for biotisation in raspberry production was defined based on the compatibility of one beneficial bacterium with a mycorrhizal fungus, together with its persistence after inoculation and its synergistic effect on mycorrhizal plant growth.
- A highly reproducible procedure for biotisation of raspberry microplants or cuttings with the composite inoculum was optimised during plant acclimatisation using an appropriate substrate and fertilisation régime. This was subsequently up-scaled to provide material on a large scale for tunnel and field production trials of four raspberry varieties.



(i) (ii) (iii)

Figure 6. Large scale production of biotised micropropagated raspberry plants multiplied in vitro (i) and biotised during the acclimatization period (ii) and grown in a greenhouse (iii). The growth response of plants to biotisation (B) compared to non-biotised plants (NB) can be observed (ii).

- The feasibility of using biotisation was tested under the conditions of field soils or inert substrates used by SMEs to grow raspberry plants. When biotised and acclimatized vitroplants of two floricane raspberry varieties were outplanted into an organic-based field production system, mycorrhizal levels were maintained and non-biotised plants quickly developed mycorrhiza after outplanting. Plant-derived liquid manure proved to be compatible with mycorrhiza development and had a slight positive effect on raspberry fruit size of biotised plants.

- Four commercial potting substrates, currently used by SME partners for raspberry plant or fruit production, proved to be poorly compatible with mycorrhiza development in a biotised floricane variety under greenhouse conditions. Nevertheless, residual beneficial effects of initial biotisation on plant behavior were observed (absence of mildew, better growth, no lodging). Also, higher fruit yields were obtained in plants that had previously been biotised in two of the commercial substrates and then outplanted into a small field trial.
- Introduction of better adapted mycorrhizal fungi, modications in the P fertilisation regime and/or amendment of the substrate composition overcame the problems of incompatibility in a commercial substrate and greatly improved mycorrhiza development in biotised raspberry plants.

Elicitor treatments

- Four plant defence elicitors (reported to control fungal diseases in other plants) were tested under greenhouse conditions in order to define the most effective product for control of *P. fragariae* var. *rubi* in raspberry production systems. Plantlets of two floricane raspberry varieties were treated with the elicitors by foliar spraying or root drench and challenged with *P. fragariae* two days later. None of the elicitors significantly affected growth of either raspberry variety in the absence of *Phytophthora*.
- Effectiveness of the tested elicitors on root rot development was genotype-dependent: all four elicitors (E1-E4) clearly reduced development of disease symptoms, indicative of resistance, in one raspberry variety whilst only one of the elicitors (E1) was effective in the other variety. In the case of another elicitor (E2), the growth of the treated plants in both raspberry varieties was less affected by pathogen attack in spite of the development of root rot symptoms, which suggests an increased tolerance to the disease induced by the elicitor.

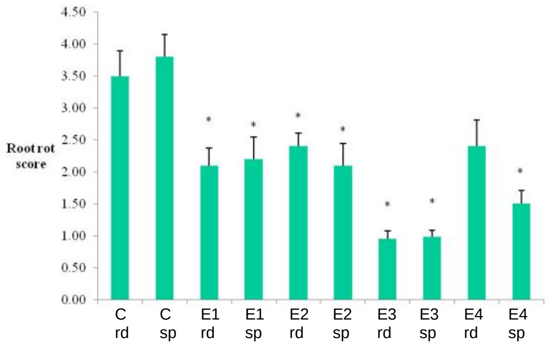


Figure 7: Reduced root rot development by P. fragariae var. rubi induced in a raspberry variety by root drench (rd) or foliar spray (sp) treatment with 4 plant defence elicitors (E1-E4) compared to untreated controls (C).

- Analyses of raspberry root and leaf samples for the response of three enzymes known to be related to defence activation in other plants showed that elicitor treatments differentially affected the early response of enzyme activities to *P. fragariae* var. *rubi* depending on the elicitor, the raspberry variety and the treated plant tissue. The elicitor E1 showed most effect, stimulating all three enzymes in the leaves of one raspberry variety, and one enzyme in the roots of both varieties. The effect of the other three elicitors was limited to one enzyme in roots (E3, E4) or in leaves (E2).

Compatibility and synergy between elicitor treatments and biotisation

Compatibility between biotisation and elicitor treatment was evaluated under greenhouse and organic farming conditions using as indicators: mycorrhizal development, plant growth, defence-related enzyme activities, fruit production and AOM content/antioxidant activities in leaves and fruits.

- Under greenhouse conditions

- The four elicitors which can reduce *Phytophthora* root rot in raspberry plants were compared for effects on mycorrhizal development and plant growth during the large scale greenhouse production of biotised raspberry plants. Elicitors were applied three times as an aerial spray at two week intervals to non-biotised or biotised raspberry plants after acclimatization, and effects monitored up to 8 weeks. All four elicitors were compatible with biotisation. Although mycorrhizal development decreased slightly following a second elicitor treatment, levels subsequently increased to reach those comparable to plants not treated with the elicitors. None of the elicitors altered the beneficial effect of biotisation on raspberry plant growth as compared to non-biotised plants.

Figure 8. Growth of biotised (left panel) and non-biotised (right panel) plants treated with elicitors.

- Root and leaf samples of the raspberry plants were analysed after the last elicitor application for the response of the three defence-related enzymes. Effects were not conclusive and varied between the enzymes, plant parts and biotisation. Whilst two enzyme activities were decreased by the elicitor in the leaves of all plants, two showed increased activities in the leaves or roots of elicitor-treated, biotised plants.
- The elicitor (E1)-treated raspberry plants were transferred into fresh substrate and overwintered in an unheated, unlighted greenhouse; the elicitor was foliar applied again at early and late flowering stages. Mycorrhizal colonization in biotised plants was not significantly affected by the elicitor treatment and was maintained through the overwintering period. Biotised plants developed a higher number of buds and flowers than non-biotised ones in spring. During the first five weeks of harvest, total fruit yield and number were greater in biotised compared to non-biotised plants with values for the yield of biotised plants ranging from +37% to +60% in presence or absence of the elicitor. At the last harvest after 9 weeks, no differences were observed between treatments.
- The influence of biotisation and/or elicitor treatment on AOM content and antioxydant activities in the overwintered raspberry plants was not consistent between leaves and fruits. Leaflets of non-biotised plants treated with the elicitor showed an increase in some AOM and antioxidant activities whilst in biotised plants, the levels of AOM and antioxidant activities decreased as compared to non-biotised plants and this trend was accentuated by elicitor treatment. Different observations were made for fruits where elicitor application tended to decrease AOM and antioxidant activities in non-biotised plants, whereas in biotised plants treated with the elicitor, on the contrary, the levels of AOM, but not antioxidant activities, were higher in fruits as compared to biotised or elicitor-treated, non-biotised plants

- Under organic farming field conditions

- Organic farming conditions enhanced compatibility in elicitor-treated, biotised plants in a pilot field trial. Biotised and non-biotised plants of two floricane raspberry cultivars transplanted into an organic production system were foliar treated or not with a plant defence elicitor (E1) at 4, 8 and 12 weeks after plantation and at bud break, early and full flowering the following year. Treatment with the elicitor had no negative effect on mycorrhizal development, whatever the raspberry cultivar. At 12 weeks after transplantation, there was a synergistic effect of two plant-based liquid manures on mycorrhizal development for the two raspberry cultivars and mycorrhizal levels were higher in the elicitor-treated plants compared to untreated plants.
- No significant variation was observed in the mean fresh weight of fruits from either raspberry cultivar between plants treated or not with the elicitor, whether they were biotised or not before outplanting, probably due to mycorrhization of the non-biotised plants by AM fungi indigenous to the field soil. However, application of both liquid manures increased fruit size in previously biotised plants of one cultivar compared, and this synergistic effect was accentuated in elicitor-treated plants.
- Clear differences in AOM content and antioxidant activities existed between the two raspberry cultivars grown under organic farming field conditions. The impact of biotisation and/or elicitor treatment on AOM contents and antioxidant capacities varied between the two raspberry cultivars, having no effect in one cultivar, across treatments, and decreasing slightly with elicitor treatment alone or combined with biotisation at outplanting.

In conclusion, the objectives of associating biotisation and elicitor treatment in the production of raspberry microplants or cuttings have been attained through the combined use of (i) growth-promoting and compatible AMF isolates and beneficial bacteria, (ii) an optimized procedure for large scale biotisation of raspberry plantlets before outplanting, and (iii) effective elicitor treatment for reducing development of *Phytophthora* root rot in raspberry plants. The successful utilisation of both biotisation and plant defence elicitors resulted in improved plant growth/fruit yield, even though treatments did not consistently influence AOM content or antioxidant activities of raspberry fruits under the experimental conditions. The defined methodology has the advantages of being highly reproducible, rapid and applicable to a large number of plants, without excessive cost, destined to greenhouse or field production systems. Also, the adaptation of commercial substrates to biotisation is important in order to give a competitive advantage to SMEs in soil-less as well as soil-based production systems. Altogether, these results represent a promising step towards the development of innovative plant production procedures respectful of the environment.

Biotisation and elicitor application in real production systems

In order to evaluate the response of raspberry varieties to biotisation and plant defence elicitor treatment under real production conditions, protocols based on a common experimental design and layout were defined for use either in the field or under polythene tunnels, in soil or in soil-less substrates, depending on the trial location. Recommendations were formulated for the application of one elicitor (E1) with a clear potential to reduce development of root rot symptoms in different raspberry varieties whilst being compatible with biotisation. Four raspberry varieties (3 floricane, 1 primocane) were subjected to four treatments (untreated, biotised before outplanting, elicitor, elicitor + biotised) at 7 locations in 5 countries (Switzerland, Slovenia, Bulgaria, Scotland, France). Elicitor treatment was programmed to protect raspberry plants against four major diseases: root rot, cane blight, spur blight and grey mould. Fertilization practices were applied according to the producer.

Productivity in soil-based cultivation systems

Figure 9: Field (left panel) and tunnel (right panel) soil-based raspberry production systems

- No drastic changes were observed in the composition of microorganisms in soils as a result of biotisation and/or elicitor application. Mycorrhizal levels remained high in all biotised plants outplanted to soil, and roots of non-biotised plants became colonised by indigenous mycorrhizal fungi to comparable levels after 2 to 3 months. Elicitor applications had no consistent effect on mycorrhiza development, whatever the trial location. Low phosphate fertigation favoured mycorrhiza development.

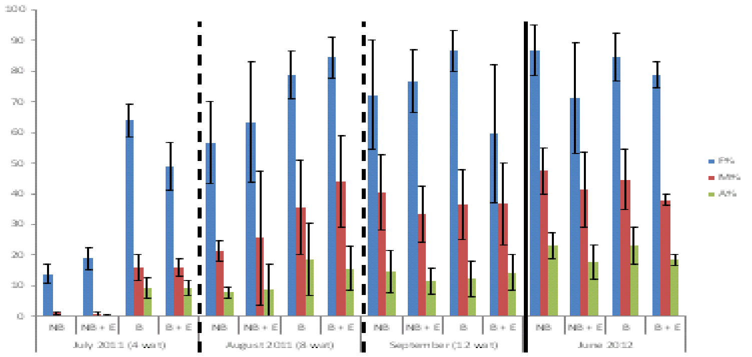


Figure 10: Mycorrhizal colonisation in a floricane raspberry variety grown in soil at a field site in Switzerland

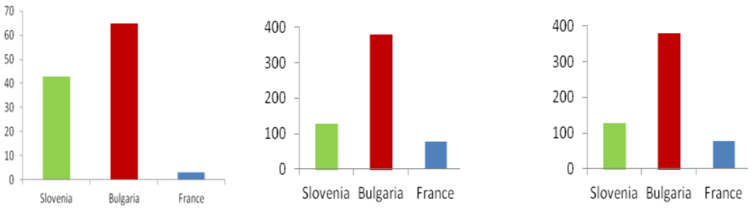
- Biotisation and elicitor treatment had significant positive effects on raspberry fruit yield at a Bulgarian field site, although genotype differences were observed. A primocane raspberry variety responded best to each treatment separately whilst biotisation, with or without elicitor, was most effective for a floricane variety. Genotype differences were also found in soil-based production system under tunnel in Slovenia, where elicitor or biotisation plus elicitor treatments provided increased fruit yields in the primocane variety, but not in a floricane variety, although in this case, the genotype variation may have been related rather to differences in cold tolerance during overwintering.
- Genotype differences were again observed in responses to biotisation and/or elicitor treatments between primocane and floricane varieties outplanted into a *P. fragariae* var. *rubi*-heavily infested field site in Scotland, in this case in sensitivity to root rot. Elicitor treatment of primocane plants provided some protection against *Phytophthora*, while the combination of biotisation and elicitor provided best protection against the fungal pathogen in the floricane variety. In contrast, biotisation and/or elicitor treatments had no significant effect on factors affecting fruit yield of a floricane variety outplanted into another *Phytophthora*-infested field site in Switzerland.

Productivity in soil-less cultivation systems

- Mycorrhization of biotised plants tended to be low after outplanting and then increased to reach good levels in the second year of raspberry plant production. Mycorrhiza development was not adversely affected by elicitor treatment and was enhanced by suppression of P fertilization in a P-rich substrate.
- Biotisation and/or elicitor treatments had no significant effect on factors affecting fruit yield of a floricane variety produced in a soilless greenhouse production system in Switzerland, whilst the combination of biotisation and elicitor provided best fruit yield of primocane plants in a soilless production system under plastic tunnel in France. Here, elicitor treatment provided good vegetative growth and root development, although elicitor treatment induced some early phytotoxicity symptoms.

AOM content and antioxidant capacities

- Growing (geographical) location, including climate and culture conditions, significantly influenced AOM content and antioxidant capacities of raspberry fruits with, in general, fruits from the field site in Bulgaria showing the highest contents of health related compounds. Fruits from production on soilless substrate had higher contents of anthocyanins but lower contents of total phenols and antioxidant capacities compared to fruits from plants grown in soil.
- Plant genotype was also an important factor influencing AOM content and antioxidant activities in raspberry fruits. Taking all the growing locations and cultural treatments together, one floricane variety was significantly richer in total phenolic content, the primocane variety in total anthocyanin content and another floricane variety was intermediate for both AOM.
- Neither biotisation nor elicitor treatment had any clear impact on AOM production and antioxidant activities in fruits or leaves at each location. Nevertheless, leaves or fruits of some raspberry varieties from production systems with higher mycorrhizal colonisation showed overall higher total phenols and antioxidant capacities than elsewhere.



M **AC** **Total phenols**

Figure 11: Mycorrhization (M), antioxidant capacity (AC) and AOM (total phenol content) in leaves of a primocane variety grown at three different locations

In conclusion, the compatibility observed between plant defence elicitor treatment and biotisation in the production trials, conducted over two years in the field, under polythene tunnels, in soil or using soil-less substrates, indicate that these agronomic practices can be used together in most production systems for raspberries. Results highlight the importance of geography/location and plant genotype in determining the response of a raspberry crop to biotisation and/or elicitor application. There is a clear potential to use these agronomic innovations in some environments and in certain raspberry cultivars, to increase fruit yield or to improve plant protection from the damaging effects of root rot by *P. fragariae* var *rubi*., although treatments did not consistently influence AOM content or antioxidant activities of raspberry fruits. Recommendations have been made to the project SMEs for the successful utilisation in raspberry production of both biotisation to improve plant growth/fruit yield and plant defence elicitors which effectively reduce *Phytophthora* root rot, even though treatments did not consistently influence AOM content or antioxidant activities of raspberry fruits under greenhouse or organic farming conditions.

General conclusions

The project results clearly show that in raspberry the most important factor influencing fruit yield, fruit quality, antioxidant HPDP molecules in fruits or leaves, and resistance/tolerance to diseases is the plant genotype (cultivar). Several cultivars have been defined which meet SME producers and consumer criteria concerning in particular enrichment in healthy compounds or interesting tolerance to fungal disease. Growing location also significantly influenced fruit yield and fruit quality such as AOM content and antioxidant capacities of raspberry fruits with, in general, fruits from a field site in Bulgaria showing the highest contents of health related compounds. Also, fruits from production on soilless substrate had higher contents of anthocyanins but lower contents of total phenols and lower antioxidant capacities compared to fruits from plants grown in soil.

The approach of genotyping developed for the genetic characterization of interesting raspberry material can further certification procedures for registration of established or new variety/cultivar cultivars and for their control during propagation or across the market. Also, the rapid and reliable molecular protocols applied to plant sanitary status assessment will promote the production of healthy biotised raspberry plants not contaminated by pathogens. Both these achievements meet SME expectancies of proven methods for ensuring plant quality in future certification procedures and provide a sound base to build a solid net flow of a large number of healthy raspberry plants within the European Community. In addition, the possibility of long-term, genetically stable conservation of pathogen-free raspberry material through techniques like cryopreservation represents an added value resource for producers.

The innovative procedures combining biotisation to improve plant growth/fruit yield with plant defence elicitor treatment to reduce *Phytophthora* root rot symptoms can be successfully applied to raspberry production following a defined protocol. This is based on the use of efficient and compatible isolates of mycorrhizal fungi and beneficial bacteria in a procedure optimized for biotisation of raspberry plants before outplanting, together with an effective elicitor treatment developed for reducing *Phytophthora* root rot in plant production systems. Based on the project results, an innovative scheme can be proposed for raspberry production from in vitro or cuttings multiplication to field or greenhouse conditions, which is more compatible than conventional systems with respect to the environment. In this context, a formal set of recommendations has been set to SMEs for producing and using biotised plants, and for elicitor treatment procedures.

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Potential impacts and main dissemination activities and exploitation of results

A complex legal environment for IPR protection in agriculture

The QualiRedFruits partners have paid considerable attention to the possible strategies in order to take the best advantage of the results while considering the several facets of such strategy including IPR protection, raising awareness about the new generation of Raspberries and deriving exploitation plans grounded on the best possible impact anticipated for the project onto producers, consumers and intermediate bodies.

The QualiRedFruits SMEs faced some difficulties to find out the most appropriate balance between:

- having the maximum impact through e.g. intensive dissemination of the results with the ultimate intention of strengthening their position in the market of new raspberries with advantageous features and,
- sufficiently protecting the intellectual property they have generated (or obtained from RTD performers) in order to strengthen their share of any new business derived from the new Raspberries for as long as possible to gain competitiveness especially against competitors (inside and outside the European Union) benefiting from cheaper workforce and less restrictive regulation regarding the use of chemicals along the fruits production chain.

Moreover, the domination of the big players over the distribution market and the contrasting weaknesses of the consumer organizations do not encourage any innovation which does not increases distributors' profits and decreases the prices for the consumers in a short term whichever the quality of the end product could be. The key question is thus whether a satisfactory IPR protection policy can be designed in order to face the current context with a reasonable guarantee of sufficient return on investments.

IPR protection legal context - According to M. Blakeney, J.I. Cohen, and S. Crespi (Intellectual Property Rights and Agricultural Biotechnology) the decision to adopt an intellectual property rights (IPR) system for agricultural research hinges on several factors affecting the use of genetic materials and germplasms. Research programs in developing and developed countries therefore seek clarification on the rights of and access to research innovations and genetic resources. The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs) and the Convention on Biological Diversity (CBD) are meant to provide such clarification. Countries pursuing commitments under both the TRIPs agreement and the CBD must also address the possible conflict between provisions regarding IPR in the two agreements. In addition, decision makers want to improve their understanding of innovation in farming communities. In some countries, they are examining these alongside the innovations produced by scientists.

Patent laws were originally considered unsuitable for the protection of plant varieties (PVR) developed by traditional breeding methods. Some countries therefore introduced special national laws for PVR in the 1960s, as did the International Union for the Protection of New Varieties of Plants (UPOV), established in 1961 but this is still an evolving legal framework. Originally, the scope of PVR concerned the production for purposes of commercial marketing and offering for sale of the reproductive or vegetative propagating material. Under UPOV, Researchers using biotechnology techniques alongside traditional breeding methods should be able to obtain both types of protection as appropriate. Nevertheless, the status of PVRs in UPOV remains complex, not easy to handle at SMEs' level, and suffers from many exceptions and peculiarities;
According to the **Agriculture and Rural Development European Committee** the most important types of IPR in agriculture are geographical indications and Community Plant Varieties (CPV) rights. Protection granted by geographical indications focuses on preventing misuse of names which could mislead consumers as to the origin of agriculture products and their quality or characteristics. CPV and CPVO are the European counterparts of PVR and UPOV with the same advantages and limitations. The Community Plant Variety Office is a European Union agency which manages a system of plant variety rights covering the 27 Member States. Interestingly, it offers a CPVO online filing system which enables users to apply for a Community plant variety right in English, Dutch, German and French. The CPVO legislation regroups all of the regulations and other legislative texts in force applicable both to the CPVO and to the system of Community plant variety protection. The obvious necessity of such a European legal framework in the context of international competition for strategic crops is poorly efficient when it comes to fruits and vegetables having a limited quantitative impact on the global food market. At this level, the current legal framework appears very complex to be easily taken up by a majority of actors and decision makers in SMEs and production entities (farmers). They are paralysed by the huge amount of texts and amendments regarding each of the chapters on *Basic Regulation*, *Implementation of the Basic Regulation*, *Varieties Denomination*, *Fees Regulation* and *Implementing rules on the agricultural exemption to Community plant variety rights provided for in Article 14 of the Basic Regulation*. Only large professional organizations employing teams of dedicated lawyers can deal with such a complex legal framework. **Obviously, a much simpler interface should be offered to farmers and SMEs to actually convince them to enter into a strong IPR protection strategy as far as the niche markets of fruits and vegetables are concerned.**

Given the poorly incentive and complex legal environment summarized above, the QualiRedFruits partners have decided to act in two steps:

- **Step 1 will last 3 years and is meant to promote and consolidate rather than protect the results obtained by the project.**
- **Step 2 will start by the end of the first step and is meant to concentrate on the protection of the knowledge, knowhow and practices should the promotion of the results be sufficiently encouraging and successful by the end of step 1.**

The implementation of this strategy is enabled and granted by a dedicated and consistent Joint Exploitation Agreement (See Deliverable D06.03) signed by all the SME participants.

First step: PROMOTION AND CONSOLIDATION OF RESULTS

The screening of European raspberry varieties for their AOM content and/or tolerance towards pathogenic fungi actually resulted in the identification of genotypes to be used commercially straight away or as parent lines in a breeding programme. The pilot hybridisation between an AOM rich variety and a commercial variety will be useful for following the inheritance of the traits of interest (AOM content and tolerance against pathogenic fungi). This would require surveillance for a period longer than the duration of the QRF project in order to ascertain the stability of the hybrids characteristics. **During this first 3-year step, the actual inheritance of the traits of interest (AOM content and tolerance against pathogenic fungi) has to be demonstrated to the actors along the chain from breeders, farmers and producers organizations outside the QRF consortium. It is a basic requirement to further enter into a Plant Variety Protection scheme.**

As shown by the QRF project, biotisation of raspberry using selected mycorrhizal fungi and beneficial bacteria, together with optimised growth substrates and fertilisation regime, will enable sustained association between plant roots and the beneficial micro-organisms all along plant production and thus improve the beneficial effect expected from this technology in soil and soil-less systems. Combining biotisation and plant defence elicitor treatments is a new area nearing the market for raspberry production. Nevertheless, this newly developed cultivation technologies must be consolidated **during this first 3-year step,** to actually convince **the actors along the chain from breeders, farmers and producers organizations outside the QRF consortium** about the new and high potential advantage of bioactive compound content (AOM) and reduced use of pesticides when compared to traditional raspberry producers. These new technologies could be exploited in organic farming and very likely adaptable to other red fruit productions systems thus consolidating the position of other SMEs on the European market.
Since the key elements of knowledge and knowhow which underpinned the demonstration and consolidation mentioned above are, and will be kept confidential, all the promoting activities to be undertaken during the 3-year first step after the project are not expected to jeopardize the protection that will be implemented during the second step.

Second step: CERTIFICATION AND GEOGRAPHICAL INDICATION

Two complementary directions will be taken in parallel and concern the certification and the geographical indications respectively.

CERTIFICATION: The tools developed in the project for genotyping, cryopreservation and diagnostics of diseases, are meant to foster, during this second step, the **development of a certification scheme for production of Raspberry with the traits of interest (AOM content and tolerance against pathogenic fungi) according to the detailed protocols and procedures developed during the project.** Genotyping of raspberry varieties is essential for protection against fraud. Optimal cryopreservation is strategic for the long term and safe conservation of such varieties. Monitoring of beneficial and pathogenic micro-organisms in growth substrates, soil and plant tissues, is an important diagnostic tool for assessing and possibly monitoring plant sanitary status and thus enables plant producers to certify plant health and in particular the absence of *P.fragariae* var. *rubi* thus fighting against the spreading of the disease. The QRF certification is compatible with any approach of variety protection. Nevertheless, the QRF partners are aware that protection is going with the financial capacity to fight against fraud. So far and given the fragmented market of most fruits and vegetables, it is not clear whether any business building on license fees for a given variety should generate sufficient profit to support all expenses to defend the protected rights. As already emphasized, the legal framework and business models which apply to the strategic crops at international level do not apply to the niche market of red fruits although the international competition is high and has a real impact on the agriculture and food economy.

GEOGRAPHICAL INDICATION: As shown by the QualiRedFruits project, combining biotisation and plant defence elicitor treatments to enhance the quality of plantlets, plant and fruits is somehow modulated by environmental factors including, but not limited to, soil and climate. Consequently, the recommendations formulated by the project to the SME participants do not overcome the intrinsic properties of geographical locations where the fruits are produced. Consequently, the partners will seriously consider two of the European Union schemes of geographical indications and traditional specialities known as protected designation of origin (PDO), protected geographical indication (PGI) to promote and protect names of quality agricultural products and foodstuffs. They are based on the legal framework provided by the Council Regulation (EC) No 510/2006 of 20 March 2006. This law (enforced within the EU and being gradually expanded internationally via bilateral agreements between the EU and non-EU countries) ensures that only products genuinely originating in a well defined area are allowed to be identified as such in commerce. The legislation first came into force in 1992. The purpose of the law is to protect the reputation of the regional foods, promote rural and agricultural activity, help producers obtain a premium price for their authentic products, and eliminate the unfair competition and misleading of consumers by non-genuine products, which may be of inferior quality or of different flavour. These laws protect the names of wines, cheeses, hams, sausages, seafood, olives, beers, Balsamic vinegar and even regional breads, fruits, raw meats and vegetables.
This system is similar to appellation systems used throughout the world, such as the “appellation d'origine contrôlée (AOC)” used in France, the “denominazione di origine controllata (DOC)” used in Italy, the “denominação de origem controlada (DOC)” used in Portugal, and the “denominación de origen (DO)” system used in Spain. In many cases, the EU PDO/PGI system works parallel with the system used in the specified country, and in some cases is subordinated to the appellation system that was already instituted.

In conclusion, the ultimate goal of the QualiRedFruits project, during the decade after completion, is to create competitive advantages for the European stakeholders based upon enhanced quality and quality assurance, hygiene, food safety, nutrition, labeling, consumer health concerns, environmental-friendliness and geographical indications. The reasonably anticipated gain on the international market for the European producers chain is at least 10% in the next decade compared to current status and in contradistinction with the slow decrease observed during the last decade.

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