



Minutes of the 1ST Conference of COST Action CA15223 “Modifying plants to produce interfering RNA”

Rome, Italy

15-17/02/2017

1. Welcome to participants

The participants were welcomed by Prof. Bruno Mezzetti, Chair of the Action, and by Dr. Jeremy Sweet, Vice Chair of the Action. Dr. Francesco Loreto, Director of the Agriculture Department of CNR (National Research Center) welcomed all participants to CNR and expressed his very high appreciation to the program of the conference. The Chair then asked to the WG leaders to move experts of each WG in different rooms and to lead separate meetings for WG1, 2, 3 while WG4 and 5 had a joint meeting.

2. Outcome of the WG meetings

Synthesis of the outcome of the separate WG meetings (15/02/2017 – Afternoon):

WG1 RNAi Technology

A first WG1 workshop was organized at the iPLANTA conference with a total of about 30 WG1 members and experts in RNAi mechanisms. The session started with a brief introduction on our tasks and deliverables for the WG, as described in the project. A total of 11 presented were presented as oral presentation together with a display of 7 posters with newest achievements on RNAi mechanisms. Presenters were from Belgium, Bosnia and Herzegovina, Bulgaria, Estonia, France, Germany, Greece, Israel, Italy, Macedonia, Poland, Slovak Republic, Slovenia, Sweden, Turkey, UK. The WG1 members presented and discussed different aspects on RNAi mechanism and interactions and also the delivery routes and optimizations. Applications in insects, plants, fungi, viruses and weeds were also presented. All in all, there were very good interactions and scientific discussions among members, supporting also the high level of expertise and the needs for advances in technology within the theme of WG1. Hence, the discussion confirmed the needs for more scientific collaboration among members.

The WG1 discussed also on the organization of workshops, alone and/or in collaboration with WG2 and WG3, and the workshops, how to keep the WG active and update each other, and opportunities for STSM applications.

WG2 Application of RNAi technology in GM plants

The tasks and deliverables for this working group, as described in the original application, were discussed. A call for interested individuals to contribute to these was made by the WG leaders who will follow up via

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



email. The WG2 leaders proposed to start writing a scientific review to cover the current and future applications of RNAi technology for horticulture and agriculture. The WG discussed applications of RNAi in terms of the 'target' and saw both common challenges and specific application bottlenecks depending on whether the targets were *in planta* (ie. viral or plant transcripts), or those in fungi, insects, nematodes etc. The WG also appreciated the significant interest in sprayable RNAi applications which are likely to be commercially available in the next 5 to 10 years. These applications are of particular interest to this COST action because regulatory frameworks are not yet in place for this new class of PPP.

The WG meeting also incorporated five talks and five posters presented by scientists from eight different member states. These presentations covered progress and challenges in RNAi for targets in plant allergens, viruses, fungi and insect pests, and stimulated relevant discussion.

Considering the integrated themes of the whole action, it was quite clear that joint activities with the other working groups would be beneficial. There was a clear interest expressed to get some of the WG2 representatives to Ghent meeting (in 2017) and to organise future events in common also with other WGs (3,4 & 5) might be beneficial from the learning point of view as especially pointed out by the younger researchers. Thus, we have to make further planning and scheduling for the coming meetings.

WG 3: Specific biosafety issues associated with RNAi

A brief introduction to the deliverables of WG3, especially regarding the development of specific biosafety protocols and post-market monitoring requirements and methods for RNAi plants as well as regarding possible case studies of current RNAi applications for risk assessment, was given. This also included the risk assessment issues and open questions raised during the 2014 EFSA workshop on RNAi-based GM plants.

Due to the absence of 2 speakers (Stuart J. Smyth and K. Purnhagen) only 3 presentations were given during the WG3 meeting, leaving plenty of time for discussion. K.M. Parker presented data on the environmental fate of dsRNA in terms of mechanistic studies on dsRNA adsorption and degradation in laboratory systems. An integrated pest management program for the RNAi-based control of the pollen beetle *Meligethes aeneus* as well as an environmental risk assessment of using trap crops as a delivery platform was introduced by H. Hokkanen. The issue of RNAi-based pesticides – although initially not included in the iPLANTA COST Action – is considered as an important new task because it is highly related to RNAi-based plants. Finally a presentation on the investigation of functional transfer of plant-derived dsRNA into mammalian cells by *in vitro* and *in vivo* models was given by B. Mateescu.

There was general agreement that the biosafety issues cannot be considered separately from the mechanisms of RNAi action and from the development of new RNAi systems and that therefore joint meetings are desirable.

WG4: RNAi socio-economics

The meeting of WG4 included eight presentations. All presentations addressed socio-economic issues related to the use and introduction of RNAi technologies. The presentations ranged from issues related

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



to the farm level applications, issues related to the food and feeds supply chain to aggregated assessments at national and EU level as well as the legal and political economy of decision making on RNAi technologies at EU and member state level. Important aspects with respect to the economic assessment at different levels of aggregation are related to including uncertainties and irreversibilities. In general, modelling tools are available to address these issues. Applications to assessing RNAi technologies are rare. One presentation addressed the issue with respect to the use in oil seed rape, where the market can be differentiated into food, feed, and fuel. What became clear during the discussions is that the application of RNAi technologies cannot be discussed in isolation. They have to be seen within the context on the debates surrounding the use of new plant breeding technologies. Often, the final product in the case of seeds is produced using a combination of different plant breeding methods and regulations affecting one method has implications for the use other methods as well as the development new products (seeds as well as plant protection). It became also clear that the development of plant protection agents using RNAi technology will not easily pass the regulatory hurdle. They will be assessed as carefully and rigorous as other plant protection strategies and developers may face similar problems (regulatory delay, high approval costs) as reported for the case of seeds. At this point in time empirical evidence is not available and deserves attention. At policy level it became clear that regulations in the EU as well as at international level have a strong science based approach, but that the EU has been moving away from this over the past decade and consumer issues, where fears with respect to new technologies in plant breeding have been raised, have become increasingly important in policy decision making at EU as well as local level.

WG5 RNAi Communication platform on plant RNAi

Due to the nature of WG5 depending on input from the other WGs, in order to decide what to communicate and how from the iPLANTA COST action, this first meeting was focused on communication in general. Because of the low contribution to this first specific WG meeting, our session was combined with WG4. This way we got a substantial insight into this WGs activity during this session, through crosstalk between WG4 and WG5. We ended the joined session with a presentation from K Minol, J Freitag and K Sinemus on Framing a communication strategy for RNAi GM plants based on their experiences from past EU and national research projects. Their experience was that open access web based platforms were hacked and consequently down for substantial periods of time. They also experienced that written statements were abused by cutting and pasting parts of sentences to undermine the meaning of texts and create negative impacts in order to discredit the COST action's work and intentions. Their conclusions where to avoid written text, open internet pages for the public to read and abuse, and to focus the communication to film cuts, live interviews and the like which are not so easily abused. The WG 5 leader claimed that written text still should be used, and that by using a Facebook platform with clear guidance for communication and removal of possible abusers, there would be warning before blocking of the pages. This has been successfully practised on several groups on Facebook on GM over the last few years. It was further suggested that the iPLANTA Facebook platform should open up for page open to all interested stakeholders. This has however not yet been fully discussed or decided upon.

2. Outcome of the IPLANTA Conference (16/02/2017 – Full day)

Synthesis of the outcome of the separate meetings of:

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



WG1 RNAi Technology

The WG1 session oral presentations were very good interactions and scientific discussions on different aspects on RNAi mechanisms and interactions and also the delivery routes and optimizations. Applications in insects, plants, fungi, viruses and weeds were presented. All in all, there were very good interactions and scientific discussions, supporting also the high level of expertise and the needs for advances in technology within the theme of WG1. Hence, the discussion confirmed the needs for more scientific collaboration among members.

WG2 Application of RNAi technology in GM plants

The conference talks were all case studies which in all indicated multiple use (considering different plant species, insects, fungi and viruses) of RNAi technology. Generally, the conference was clearly indicating the need for this kind of coordinated action (COST) to make joint activities potential. Considering the Ghent meeting in 2017 to be joined by WG1 members it might be beneficial if also some of the WG2 members might join (but for that we probably should apply extra funding).

In addition, there were interesting poster presentations but separate time for poster presentations (the authors available by their posters) were not arranged and also the time for those should have been a bit longer. Thus this might be something which we can improve for the next meetings.

WG3 RNAi biosafety

During the Conference (day 2), general overviews related to the safety issues linked to RNAi-expressing plants were given through presentations related to environmental and food safety and risk assessment.

N. Papadopoulou (European Food Safety Authority) explained the applicability and limitations of the European risk assessment framework under which RNAi plants are evaluated. EFSA has promoted studies in the field by launching three procurements on RNAi on: 1. molecular characterization, 2. food/feed safety, 3. environmental risk assessment.

O. Christiaens and S. Arpaia focused on environmental aspects of RNAi plants (possible off target effects, effects along the food chain, environmental persistence, etc.), while H. Kuiper discussed general requirements for food/feed safety and the possibility of using “omics” techniques in support to risk assessment.

The presentations were well received by the audience and animated discussions followed after each lecture. Knowledge gaps identified were particularly debated.

WG4: RNAi socio-economics

Socio-economic issues including cost-benefit analyses are important when assessing the impacts and safe use of RNAi technologies especially in the EU context. The RNAi technology cannot be discussed in isolation and has to be seen from a socio-economic perspective in the wider context about the debate of

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



agriculture development and the role of technical change and the debates around new plant breeding technologies in particular. WG4 will initiate further activities to bring together scientists working on these topics to increase the knowledge base. The COST Action is an excellent forum to communicate these issues to politicians and other decision makers at EU level but also in individual countries.

WG5 RNAi Communication platform on plant RNAi

WG5 depend on contributions from all remaining four WGs, and focused on the need for close communication and contact with the remaining COST action. That was specified by the need to participate in all meetings hold by the action, which was also supported by the other WGs and comments from others during the round table discussion this first day. We also invited volunteers from all countries ideally the MC members to secure good communication, meaning from all parts of the action to all stakeholders, which would reach the most by national further spread within each country. Feedback from the national level back to the action to secure ownership and cost efficient time use would secure optimal success of the action and WG5s activities in particular.

3. Outcome of the Round Table and General Discussion (17/02/2017 – Afternoon)

WG1 RNAi Technology

WG1 members see as an important topic the mechanisms and delivery of ds/siRNAs . Interactions with WG2 and WG3 on applications and biosafety aspects are also important, suggesting for a joint workshop in year 1 or/and 2 of the COST action. One option is to join with WG3 at the IOBC conference on GMOs in integrated plant production (Sept 4-6, 2017) in Ghent. Other options are on miRNA interactions with RNAi mechanisms and applications for crop protection and selective pest control. The WG1 members will send also new proposals to the chair/vice-chair.

WG1 members agreed in the support of international conference participation and then reporting back on the useful aspects for the WG1. Useful can be to organize a WG1-supported session on RNAi mechanisms at the European Congress of Entomology, organized in Naples/Italy in August 2018; this symposium has a high reputation and is reaching a large audience with >500 participants. Other suggestions are welcome, for instance covering aspects on delivery techniques, applications in plants, interactions with miRNAs and viruses.

Thus, WG1 members together with chair/vicechair will make further planning and scheduling for the coming meetings; a list with e-mail addresses is collected by the vicechair to be used for WG1 mailings/updates.

WG2 Application of RNAi technology in GM plants

Round table discussions were lively and they were partly based on skype meeting in the previous week. The round table discussions considering themes of WG2 also supported the ideas of joint meetings. We were also discussing about common efforts to review the present state of art of RNAi technology (could be common effort of several / all WGs to be published e.g. by Springer). This was most welcomed by WG5, which would be happy to participate in such dissemination activities.

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



WG3 RNAi biosafety

It was stressed that despite the selectivity of RNAi there is still the possibility of unintended effects and that there is lack of information on the expected exposure in nature. Knowledge gaps need to be identified, e.g the effect of dsRNA on mammals. With respect to this issue, information from medicinal RNAi applications can be useful to assess unintended effects from RNAi plants in mammals. Some of the questions raised during the discussion concerned the transgenerational and environmental stability of dsRNA expression. When developing protocols for risk assessment, it was seen as important to discriminate “nice-to-know” from “need-to-know” information.

WG4: RNAi socio-economics

In the round table the importance of different stakeholder groups in the debate on RNAi technologies and new plant breeding technologies in general has been stressed. The important contribution of the iPLANTA cost action can be on the economic implications of different policy choices on the development of RNAi technologies in the European Union and the implications this might have for the structure of the seed and plant protection sector in the EU, where current policies favour concentration in the sector and discriminate against smaller companies.

WG5 RNAi Communication platform on plant RNAi

During the presentations we saw how media can be used to reach all possible stakeholders, form opinions and eventually policy making. The final discussion picked up where we left the first day, and elaborated further on the need for active and direct information flow from all parts of the action to WG5, for further spread of results to all stakeholders. Topics raised during the talks the day before were also raised, such as how to use different media to reach out to the general public in addition to specific stakeholder groups. The suggestion of putting together a book through CABI was raised. Some referred to presentations the day before showing how Western Europe increasingly lag behind concerning food security, while South America have greatly increased their food export during the last few years at the same time as they adopted GM varieties. This needs to be communicated in order to possibly reach concerns and raise the discussion of technology adoption for food production, not having technology only revolutionizing the way we live, work, move and communicate. We ended by asking how we should best secure communication in the next year to all stakeholders and how we (WG5) could be present in all iPLANTA meetings to get adequate overall information of the actions activities.

4. Summary of the major outcomes from the iPlanta Conference (15-17/02/2017)

1. RNAi Applications on virus, fungi and insects - to identify the technical challenges that are general to all target pest/pathogens and those challenges that are specific to one or another target.
2. RNAi Applications on plant development and maturation abiotic stress, and fruit shelf Life
3. RNAi Environmental risk assessment
4. RNAi and human health benefits and/or risk

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.



5. RNAI future for molecular plant protection.
6. Approaches for risk assessment and risk management of RNAi plant protection products; both for their use in EU and as residues on imported food crops. This is a very timely topic and we have a real opportunity to influence. CAN EU APPROVE A MOLECULAR CROSSTALK?
7. Stakeholders experiences.
8. Rules or deregulation: what is the better strategy.
9. Calculate and communicate the socio-economic impact of not applying the RNAI technology and products.
10. Communication strategies (set up a group of experts working on it).

Inclusiveness Target Countries: Bosnia-Herzegovina, Bulgaria, Cyprus, Czech Republic, Estonia, Croatia, Hungary, Lithuania, Latvia, Luxembourg, Malta, Montenegro, Poland, Portugal, Romania, Slovenia, Slovakia, the former Yugoslav Republic of Macedonia, Republic of Serbia and Turkey.