PPP-final report

PPP projects which are under supervision of the "Topsectoren" must file a final report concerning the total project period. This form is used to report the content of the project. There is a separate form for the financial reporting.
The final report will be published on the TKI / topsector website. Therefore, please ensure that there is no confidential information in the final report.
The PPP-final report must be sent, at the latest, by the 1st of March 2020 to the "TKI's": info@tkitu.nl or info@tki-agrifood.nl. For Wageningen Research, the report has to be sent to the "Topsector secretary" of your respective institute.

General information	
PPP-number	KV 1409-029
Title	Genetics and mechanism of Aphid resistance in *Capsicum*
Theme	Topsector Tuinbouw & Uitgangsmaterialen, Meer met Minder
Implementing institute	Plant Breeding Wageningen University and Research
Project leader research (name + e-mail address)	Dr. Ben Vosman (ben.vosman@wur.nl)
Coordinator (on behalf of private partners)	Dhr. R. Linders (rico.linders@syngenta.com)
Project-website address	https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/Genetics-and-mechanism-of-Aphid-resistance-in-Capsicum.htm
Start date	June 1, 2015
Final date	December 31, 2019

Approval by the coordinator of the consortium The final report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the final report.	
Assessment of the report by the coordinator on behalf of the consortium:	x Approved □ Not approved
Additional comments concerning the final report:	

Consortium	
Mention any changes in the composition of the project partners:	-

Summary of the project	
Problem definition	Several aphids can cause problems in pepper cultivation, and produce all kinds of damage, such as chlorosis, necrosis, wilting, defoliation and fruit loss, but the most important damage is caused indirectly by the viruses that are transmitted. Biological control is used, but is quite expensive and not effective in all cases, which is largely caused by the high reproduction rate of aphids on pepper. Chemical control is applied widely. However, increasing resistance of the aphids towards

	the insecticides and environmental concerns related to their use demand that alternatives are sought. Pepper varieties (partially) resistant to aphids would be a welcome tool in the battle and provide an environmentally friendly solution to the problem. In this project we aimed to create the basis for the development of such varieties. During the years 2012-2015 Wageningen UR Plant Breeding together with leading Dutch pepper breeding companies, have evaluated wild relatives of the cultivated pepper for resistance against the aphid *Myzus persicae*. This has resulted in the identification of a resistance source on which *M. persicae* shows a strongly reduced reproduction rate.
Project goals	In this project we aim to analyze the genetics of the resistance that was identified and study the resistance mechanism to facilitate its use in breeding aphid resistant pepper varieties. To reach this goal we will use a molecular genetic approach including: 1. Phenotypic and genotypic characterization of an F2 population derived from a cross between a resistant and susceptible plant. QTL mapping of the resistance. 2. Fine mapping of resistance using F3 lines obtained from F2 plants with suitable QTL genotypes. 3. Production of aphid resistant near isogenic lines (NILs) . 4. Elucidation of the resistance mechanism based on candidate gene(s) from fine mapping, metabolomics and transcriptomic analysis.

Results	
Planned results in the original project plan	The main planned results products of this project are knowledge and plant material. • QTL mapping data • Fine mapping data • Identification of candidate gene(s) for resistance • NILs for the resistance gene(s) • Data on the resistance mechanism (including metabolomics and transcriptomics data)
Achieved results	During the years 2012-2015 Wageningen UR Plant Breeding together with leading Dutch pepper breeding companies, have evaluated wild relatives of the cultivated pepper for resistance against the aphid *Myzus persicae*. This has resulted in the identification of a number of resistance sources, all *C. baccatum*, on which a *M. persicae* population originating from the Netherlands (NL) showed a strongly reduced reproduction rate (Sun et al., 2018). An F2 population derived from a intraspecific cross between an aphid resistant (PB2013071) and susceptible (PB2013046) *C. baccatum* plant was used to study genetics of the resistance. QTL mapping was carried out, resulting in the identification of one major QTL explaining 42% of the variation and a minor QTL explaining 6% in aphid reproduction. The major QTL was validated using F3 lines. Fine mapping of the major resistance QTL resulted in a region of about 96 kb predicted to encode four analogues of resistance genes of the receptor-like kinase family (Sun et al., 2020a). Later on we identified a second aphid population originating from Switzerland (SW) that was (partially) virulent on all tested *Capsicum* accessions. The performance of the two *M. persicae* populations (NL and SW) on the parental lines of the F2 population was analysed with respect to the production of reactive oxygen species (ROS), callose deposition and electrical penetration graph (EPG) analysis. EPG recordings showed a similar feeding behaviour for both aphid populations on PB2013046. On accession PB2013071 population SW was able to feed considerably longer than the NL population. Plants of PB2013046 could not induce ROS accumulation and callose formation

	after infestation with either aphid population, whereas plants of PB2013071 could. The SW population seems to have, at least partly, overcome the resistance of PB2013071 that prevented feeding of aphids from NL population (Sun et al., 2020b). We used transcriptomics to study gene expression in response to aphid feeding by either (NL or SW) population. Differentially expressed genes are probably involved in the compatible and incompatible *M. persicae*-pepper interactions. More genes were significantly up or down regulated in response to the SW than to the NL aphid population. Only a small portion of the differentially expressed genes was regulated by both aphid populations. The NL population induced ROS production genes like peroxidases, while the SW population induced ROS scavenging genes, like catalases and repressed ROS production genes. The SW population was able to induce removal of ROS which accumulated in response to pre-infestion with the NL population, and that pre-infestation with the SW population significantly improved the performance of the NL population on PB2013071 (Sun el al., 2020c). We also collected a number of new *M. persicae* populations from commercial (organic) paprika growers. The response of these populations towards the resistance source varied. Some populations were avirulent to PB2013071, others were virulent on the source. A further characterization of the aphid populations will be needed to shed some light on these differences and the possible involvement of specific effectors. Furthermore the companies involved in the project introduced the major resistance QTL from *C. baccatum* into their commercial *C. annuum* backgrounds, which is not a trivial task as the species are poorly crossable. From one of the companies BC2S1 seeds were available for testing the effectiveness of the resistance in the *C. annuum* background. Unexpectedly, introgression of this QTL into *C. annuum* did not result in a resistant phenotype. A plausible explanation is that another yet unknown factor from *C. baccatum* is needed to get the resistance QTL to work in *C. annuum.* This factor is subject of a follow-up project.
Explanation of changes relative to the project plan	We did not use an untargeted metabolomics analysis as it was already clear very soon that specific components were involved in the resistance. For this reason callose and ROS production were measured in a targeted way.

What was the added value created by the project for:	
Participating "Knowledge Institutes" (scientific, new technologies, collaboration)	For WUR Plant Breeding the project was a very successful one, resulting in the implementation of new methods for the characterization of aphid resistance as well as new knowledge developed that ultimately helped us to acquire a follow-up project. It resulted in four papers published in scientific journals, a PhD thesis that was defended publicly in 2019, as well as several oral presentations, posters, and abstracts.
Participating private partners (practical application of the results, within which period of time?)	The participating breeding companies obtained a lot of knowledge on aphid resistance in *Capsicum.* In addition they have gained experience in making the difficult crosses between *C. baccatum* and *C. annuum*. They have developed advanced plant material from the crosses.
Society (social, environment, economy)	Unfortunately the introgressed resistance gene did not confer resistance in *C. annuum* plants yet. The reasons for this is subject of a follow-up project.

Possibly other stakeholders (spin-offs)	In the follow-up project also a new company will participate.

Follow-up	
Did the PPP result in one or more patents (first filings)?	no
Are there any follow-up projects planned? If yes, explain. (Contract research resulting from this project, additional funding, or new PPP projects)	Yes, a project proposal to continue on the results achieved in the current project was submitted to TKI-TU in 2019 and selected for funding.

Deliverables/products during the entire course of the PPP (provide the titles and/or a brief description of the products/deliverables or a link to a website.

Scientific articles:

1. Sun, M., R.E. Voorrips, W. van 't Westende, M. van Kaauwen, R.G.F. Visser, & B. Vosman (2020a) Aphid resistance in Capsicum maps to a locus containing LRR-RLK gene analogues. Theoretical and Applied Genetics 133:227-237. https://doi.org/10.1007/s00122-019-03453-7
2. Sun, M., R.E. Voorrips & B. Vosman (2020b) Aphid populations showing differential levels of virulence on *Capsicum* accessions. Insect Science, in press https://doi.org/10.1111/1744-7917.12648
3. Sun, M., R.E. Voorrips, M. van Kaauwen, R.G.F. Visser, & B. Vosman (2020c) The ability to manipulate ROS metabolism in pepper may affect aphid virulence. Horticulture Research 7:6. https://doi.org/10.1038/s41438-019-0231-6
4. Sun, M., R.E. Voorrips, G. Steenhuis-Broers, W. van 't Westende & B. Vosman (2018) Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper accession. BMC Plant Biology 18:138. https://doi.org/10.1186/s12870-018-1340-3
5. Sun, M. (2019) The battle between pepper and aphid: genetics and mechanism of host plant resistance. Ph.D. thesis, 125 pp, Wageningen University, Wageningen, the Netherlands.

External reports:

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Articles in professional journals/magazines:

Bladluisresistentie in Paprika, https://www.groeneveredeling.nl/upload_mm/1/f/1/110a39ec-e8cc-44af-a480-6e4f669e65dd_2017%20Nieuwsbrief%20Paprika.pdf

(Poster) presentations at workshops, seminars or symposia.

Oral presentations:

1. Sun, M (2019) Aphid populations showing differential levels of virulence on *Capsicum* accessions, EPS Lunteren meeting. 9 April 2019, Lunteren, Nl
2. Sun, M (2019) Aphid populations showing differential levels of virulence on *Capsicum* accessions. 17th EUCARPIA International Meeting on Genetics and Breeding of *Capsicum* and Eggplant, 12 September 2019, Avignon, Fr.
3. Vosman, B. (2019) Insectenresistentie onderzoek. Vakopleiding voor plantenveredeling. 8 Maart 2019, Wageningen, Nl.
4. Vosman, B. (2019) Breeding insect resistant crops. 10th Brazilian congress of plant breeding. 30 july 2019, Aguas de Lindoia/SP.
5. Vosman, B. (2019) Breeding insect resistant crops. Universidade federal de Lavras. 1 August 2019, Lavras/MG.

6. Vosman, B. (2018) Enrichment of Crop Genepools with Wild Relative and Landrace Diversity. 4th International Conference "Plant Genetics & Breeding Technologies" July 12-13, 2018, Vienna.
7. Vosman, B. (2018) Different Mechanisms of Insect Resistance in tomato and pepper. 15th Solanaceae Conference, Sept 30th - Oct 4th 2018, Chiang Mai, Thailand.
8. Vosman, B. (2018) Bladluis resistentie in paprika. Onderzoekersdag groene veredeling. 13 december 2018, Wageningen.
9. Vosman, B. (2017) Host plant resistance against insect pests in pepper and tomato. Asian Solanaceous Round Table 2017(ASRT-2). February 23-25, 2017, Bangkok, Thailand
10. Vosman, B. (2017) Insectenresistentie onderzoek. Vakopleiding voor plantenveredeling, Wageningen 10 maart 2017.
11. Vosman, B. (2017) Breeding for Host Plant Resistance against insects in the post genomics era. International Symposium on Marine and Agricultural Genomics (ISMAG)". April 19-21, 2017, organized by the Marine Genome 100+ Korea and National Agricultural Genome Program (NAGP) of South Korea. Seoul, South Korea.
12. Vosman, B. (2016) Host plant resistance towards insects. ICE 2016 XXV International congress of Entomology. September 25-30, 2016. Orlando Florida, USA.

Posters:

1. Sun, M (2019) Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper (*Capsicum baccatum*) accession. 17th EUCARPIA International Meeting on Genetics and Breeding of Capsicum and Eggplant, 12 September 2019, Avignon, Fr.
2. Sun, M. (2018) Phloem-based resistance to aphids in pepper leaves', International CRC 973 Symposium, April 8-11, Berlin.
3. Sun, M. (2018) Aphid populations show differential levels of virulence on Capsicum', EPS retreat, July 3-6, Utrecht.
4. Vosman, B., M. Sun, W. van 't Westende, R. Voorrips, (2017) Resistentie tegen bladluizen in paprika, poster Biobeurs, 18 & 19 januari, Zwolle.
5. Sun, M. R.E. Voorrips, W. van 't Westende and B. Vosman (2017) Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper accession. CEPLAS summer school 2017. 5-9 June 2017, Cologne, Germany.

Abstracts:

1. Sun, M., R.E. Voorrips & B. Vosman (2019) Aphid populations showing differential levels of virulence on Capsicum accessions. In: Innovations in Genetics and Breeding of Capsicum and Eggplant (eds V. Lefebvre & M-C Daunay) INRA, Avignon, Fr. PP 56.
2. Sun, M., G. Steenhuis-Broers, W. van 't Westende, B. Vosman & R.E. Voorrips (2019) Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper (*Capsicum baccatum*) accession. In: Innovations in Genetics and Breeding of Capsicum and Eggplant (eds V. Lefebvre & M-C Daunay) INRA, Avignon, Fr. PP 124.
3. Voorrips, R.E., G. Steenhuis-Broers, W. van 't Westende & B. Vosman. (2016) Aphid resistance in a Capsicum collection. In: Proceedings of XVIth EUCARPIA Capsicum and Eggplant working group meeting (eds K. Ertsey-Peregi, Z. Füstos, G Palotás and G. Csilléry) Diamond congress Ltd, Budapest, Hungary. Page 66-68.

Symposium:

1. A symposium on host plant resistance against insects was organized as part of the International Conference on Entomology (ICE2016, Orlando, Fl.). https://esa.confex.com/esa/ice2016/meetingapp.cgi/Session/25614

TV/ radio / social media / newspaper:

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Remaining deliverables (techniques, devices, methods, etc.):

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