Hot Tomato: Complementation of the Capsaicin Biosynthetic Pathway to Engineer Spicy Tomatoes

Greg Reeves
gr360@cam.ac.uk

The Idea

Chili pepper (Capsicum spp., also chilli pepper, chile pepper or hot pepper) is the most cultivated spice crop in the world with an annual value over $20B (USD). The spicy flavour of chili peppers is due to the accumulation of capsaicinoids, a group of alkaloids that are unique to the Capsicum genus, which are produced exclusively in the placental tissue of chili pepper fruit (Nelson and Dawson, 1923). Capsaicinoids are of major economic importance. They are the active ingredients in many pharmaceuticals, such as analgesic liniments that can treat chronic arthritis, joint and other pain. They are widely used as the active chemical agents in personal defence aerosols (i.e., pepper spray). When ingested, capsaicin has been found to promote weight loss by increasing metabolism and can even inhibit the growth of certain pathogenic microorganisms and several forms of human cancer.

The primary function of capsaicin is to dissuade mammalian predation and promote avian seed dispersal. Mammalian digestive tracts render chili pepper seeds nonviable, whereas the avian gastrointestinal tract chemically and physically softens chili pepper seeds which then non-destructively boosts germination (Bosland and Votava, 2012). Most Solanaceous species possess sufficient alkaloids to cause toxicity to many mammals, thus partially circumventing predation, but chili peppers possess none in their tissues. Without toxic alkaloids, chili peppers evolved capsaicin as a different strategy to deter frugivory from mammals by causing a ferocious burning sensation when eaten. It seems that humans are the only mammal to find this sensation tasty.

All capsaicinoids contain an aromatic vanillyl ring derived from the amino acid,phenylalanine, connected to a branched-chain fatty acid moiety derived from the non-polar amino acids, valine, Leucine or Isoleucine. Thus, it is thought that connection of two pathways in plants: phenylpropanoid and fatty acid biosynthesis, give rise to capsaicinoids. Despite the importance of capsaicin, most of the enzymes in the pathway are poorly characterised; the evolution and the regulation of the pathway is unknown. Chili peppers are the only organisms capable of producing capsaicin, yet all orthologous genes for all of the candidate and confirmed genes of the capsaicin pathway are present in tomato (Solanum lycopersicum) and other Solanaceous plants. Interestingly, these orthologous genes are even expressed to similar levels in tomato placental tissue, save for three enzymes (BCAT, Branched-chain amino acid transferase; KAS, Ketoacyl ACP Synthase; and CS, Capsaicin Synthase), which are not expressed in tomato fruit (Kim et al., 2014). This proposal hypothesises that these genes are the missing components in the ‘tomato capsaicin pathway’.

This proposal seeks to utilise synthetic biology approaches to overexpress these enzymes to complement endogenous genes—forming the capsaicin pathway to yield spicy tomatoes. There are essentially two approaches to achieve this goal --- stable transformation and transient transformation. Stable transformation has advantages in terms of stability and reproducibility; however it is time-consuming and is unsuitable for the rapid screening of wide variety of different genes and constructs for proof-of-principle (Kusnadi et al., 1997). In this project, we will apply transient expression in tomato fruit and leaves for fast screening and validation of the key genes mentioned above. This transient fruit expression approach (Sainsbury et al., 2008) provides a rapid and reliable method to study capsaicin pathway gene function in tomato fruit.

The project would utilise the current models for the capsaicin pathway as a blueprint and would provide a clearer picture of capsaicinoid evolution in Solanaceae. This would demonstrate that the path to capsaicin production is relatively straightforward and that other members of Solanaceae may be evolving capsaicin production. This proposed experiment offers a tool to building synthetic

pathways in plants through complementation of existing components and furthers understanding the evolution of secondary metabolites in plants.

Who We Are

Gregory Reeves, PhD Student (University of Cambridge), gr360@cam.ac.uk
Genetics, B.Sc.; Horticulture, M.Sc.

I formerly worked for Prof. Paul Bosland, director of the Chile Pepper Institute at New Mexico State University (USA) and Prof. Doil Choi, director of the Plant Breeding and Genomics Institute at Seoul National University (South Korea) where I helped investigate the genetic basis for spicy flavour in chili peppers and even broke the world record for hottest pepper. I am currently a PhD student in Prof. Julian Hibberd's Lab at the University of Cambridge where I am studying the genetic basis for the C4 photosynthesis pathway.

Chris Boursnell, Research Assistant (University of Cambridge), cmb211@cam.ac.uk
Computer Science and Artificial Intelligence, B.Sc;
Computational Biology, M.Sc;

I am a bioinformatician working on rice and transcriptomics. I perform sequence analysis and differential gene expression using RNA-Seq data in rice (Oryza sativa) and many species that use C4 photosynthesis in Prof. Julian Hibberd's Lab at Cambridge.

Jie Li, PhD Student (John Innes Centre), jie.li@jic.ac.uk
Agronomy, B.Sc.

I am working with Prof. Cathie Martin to study transcriptional regulation of carotenoid biosynthetic pathway in tomato. During my first year, I modified cow pea mosaic virus hyper-trans (CPMV-HT) systems for over-expression of target genes transiently in tomato fruit, which provides a rapid and reliable approach to study gene function in tomato fruit.

Implementation

Describe what you are planning to do with the funding, including aims, methods, outcomes and who will be involved.

a. Project Aims

1. Synthesise several key enzymes in the capsaicin pathway from chili pepper that are not expressed in tomato.
2. Overexpress these genes in tomato fruit and leaves.
3. Evaluate the presence of capsaicin in transformed tomato tissue.

b. Methods

1. Cloning genes from the capsaicin pathway (G Reeves and C Boursnell, Hibberd Lab, Cambridge)
Three chili pepper genes, BCAT, Branched-chain amino acid transferase (GenBank accession AY034379); KAS, Ketoacyl ACP Synthase (EU616570); and CS, Capsaicin Synthase (AY819029) will be cloned into the pDONR207 gateway vector.

In order to speed the cloning process, the cDNAs for these genes will be commercially synthesised. Once in the gateway vector, they will be ready to be transformed into tomato leaf and fruit tissue.

2. Transient expression of capsaicin genes in tomato fruit and leaves (J Li, Martin Lab, JIC)
Transient expression of the chili pepper capsaicin pathway genes will be used in this experiment over stable transformation, as it will facilitate completion of testing multiple capsaicin pathway genes within the six month timeframe of the project. The expression binary vector containing the three chili pepper genes will be transformed into Agrobacterium tumefaciens and infiltrated into tomato leaf and fruit tissue. The cow pea mosaic virus hyper-trans (CPMV-HT) protein expression systems has been established for transient protein expression in plants (Sainsbury et al., 2008; Sainsbury et al., 2009). This system was developed and subsequently refined to produce very strong expression of proteins of interest at levels up to 20–25% of the extractable protein (Sainsbury et al., 2008) and works in tomato fruit and leaves. Additionally it has been demonstrated that multiple genes can be expressed simultaneously by simply mixing Agrobacterium strains. CPMV Hyper-Trans system was initially established in leaves of N. benthamiana. This will allow for rapid screening of these genes to indicate whether they are sufficient to complement the endogenous orthologues for the capsaicin pathway in tomato.

3. Capsaicinoid quantification by liquid or gas chromatography (G Reeves, Hibberd Lab, Cambridge, and J Li, Martin Lab, JIC)
Infiltrated leaves and fruit will be harvested, dried and ground to a uniform powder. Extraction and estimation of capsaicinoid content will follow the liquid chromatography methods provided by Collins et al. (1995). This will estimate the amount of capsaicinoids in the tomato fruit very accurately and has high-resolution to detect even small quantities (<1ppm) of various capsaicinoids. This method is able to detect glycosylated, oxidised or hydroxylated capsaicinoids, which have reduced or no pungency.

c. Outcomes

1. Initiate an understanding of the genetic components required for capsaicin production in tomatoes.
2. Potential development of ‘spicy tomatoes’.

Benefits and outcomes

Through this OpenPlant grant, we will build upon the knowledge of plant secondary metabolite evolution and on how synthetic biology can produce novel compounds in specific tissues of target organisms (i.e., tomato fruit). We hope to be the first researchers to ever autonomously produce capsaicin, outside of the Capsicum genus. This will have large implications in the tomato industry. As tomato production is largely mechanised and chili peppers are routinely harvested by hand, capsaicin production for extraction purposes (i.e., pepper spray, pharmaceuticals) may be cheaper if done in tomato. In keeping with the ideals of OpenPlant, we intend that the results of all this work to be open source. This ensures that the results are to the benefit of the chili pepper community, tomato community and the greater realm of plant science.

The results of this work will help to improve the understanding of capsaicin evolution in Solanaceae, by demonstrating that phenylpropanoid metabolic pathway and fatty acid biosynthesis (the two halves to the capsaicin pathway) can be simply connected to form a novel compound. This would serve as a good model to produce secondary metabolites through interweaving disconnected pathways in plants.

The broader impact of this project between the University of Cambridge and the John Innes Centre will hopefully lead to a collaborative relationship, student exchanges and shared research projects/grants in the future between both research programmes.

Budget

a. Gene synthesis of capsaicin pathway genes -- £1600
b. Transient expression of chili pepper capsaicin pathway genes in tomato fruit -- £1150
c. Capsaicin quantification costs via High-performance liquid chromatography (HPLC), or Gas Chromatography-Mass Spectroscopy (GC-MS) -- £1000.
d. Travel to/from Cambridge/Norwich -- £250
No additional funding is available for this project.
Total = £4000