**Flavonoids are plant-derived compounds that regulate positive and negative interactions between chickpea with its natural symbiont Mesorhizobium ciceri and the plant-parasitic nematode Meloidogyne javanica**

Jason Ng[1]
Haniza Zainal Abidin[1] and Ulrike Mathesius[1]

[1] The Australian National University

Legumes are unique due to their ability to form beneficial interactions with rhizobia. Nitrogen fixation during legume-rhizobia symbiosis allows legumes to thrive in nitrogen (N)-poor soil environments, enriches the rhizosphere with N compounds for future crops, and circumvents the application of costly fertilisers. In the field, legumes are also exposed to multiple pathogens, including plant-parasitic nematodes (PPNs). PPNs are soil-borne, microscopic roundworms that collectively account for >USD100 billion (~15%) of global agricultural yield loss. Root-knot nematodes (RKNs; *Meloidogyne spp*.) are the most damaging group of nematodes that parasitise almost every vascular plant, including legumes. RKNs induce the formation of galls in infected roots, which are essentially nutrient sinks where female RKNs grow and reproduce.

Flavonoids are plant-derived compounds that play multiple roles in rhizospheric interactions. During the legume-*Rhizobium* symbiosis, flavonoids are exuded from host roots, which will then be recognised by the compatible *Rhizobium* species and induce *Rhizobium* nodulation (nod) gene expression. Rhizobia produce nodulation factors that are recognised by the compatible legume hosts to initiate nodule formation. Flavonoids have also been shown to affect PPN behaviour, including their motility, chemotaxis (repellent activity) and mortality. However, little is known about which flavonoid compounds are effective nod gene inducers for different legume-*Rhizobium* partners. Much is also unexplored about which are the most potent flavonoid compounds in reducing nematode parasitism.

Our work focusses on the interaction between chickpea (*Cicer arietinum*) with *Mesorhizobium ciceri* and *Meloidogyne javanica*. We hypothesised that distinct sets of flavonoid compounds are optimal for positive and negative interactions with rhizobia and nematodes, respectively. A selection of 20 chickpea varieties common to Australian growers were cultivated in individual sand pots in the glasshouse. We showed that nematode infection reduced chickpea yield by an average of two-fold while rhizobia inoculation increased yield by an average of more than 2.5-fold, compared to control plants. Root flavonoid exudate was extracted from the rhizospheric sand. We analysed flavonoid exudates using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by targeting 40 flavonoid compounds. These compounds were chosen based on their potential to act as nod gene inducers/nematode repellents by assessing their known activities in other legume-*Rhizobium*/nematode combinations. We found a very significant variation in flavonoid exudate concentration across the 20 varieties under control, nematode-infected and rhizobia-inoculated conditions. Two flavonoids - kaempferol and genistein were strongly correlated with gall numbers in nematode-infected plants, whereas 2-hydroxyflavone, chrysin, isoliquiritigenin and taxifolin were strongly correlated with nodule numbers in rhizobia-inoculated plants. We performed motility tests to investigate if certain flavonoids could affect nematode movement and showed that several flavonoids, including kaempferol, genistein, medicarpin, isoliquiritigenin and coumesterol could affect (accelerate or reduce) nematode movement in a concentration-dependent manner. Next, we also performed chemotaxis assays and demonstrated that some of these flavonoids (medicarpin, coumesterol and isoliquiritigenin) could repel nematodes to varying degrees. Some of these flavonoids could also potentially be strong nod gene inducers. Our goal is to establish flavonoid markers in chickpea that is indicative of high nodulation and tolerance/resistance towards PPNs for use in breeding programs and seed priming technologies.