ENHANCED EARLY NODULATION OF *MEDICAGO TRUNCATULA* CO-INOCULATED WITH *SINORHIZOBIUM MEDICAE* AND *ACHROMOBACTER XYLOSOXIDANS*

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Efforts to increase the productivity and sustainability of agro-ecosystems have resulted in increased research focused on generating improved microbiological inoculant technologies. Plant growth-promoting rhizobacteria (PGPR) are one area receiving increased attention due to their potential for use as biofertilizers, plant-growth promoters, and biocontrol agents for weed and disease control in farming systems (Siddiqui, 2006).

Although most PGPR research has focused on improving the yields of cereals and other broad acre crops, more recently, some research has indicated that there may be a role for using PGPR as co-inoculants with root-nodule bacteria to increase nodulation and, hence, the yields of pasture and grain legumes (Bai et al., 2003; Camacho et al., 2001; Marek-Kozaczuk and Skorupska, 2001; Zhang et al., 1996). Currently, the mechanisms of PGPR-mediated enhanced nodulation are largely speculative and there is a need for mechanistic studies to be completed in order to understand how these organisms influence nodulation. Understanding these interactions should allow us to improve the consistency and reproducibility of these responses and may allow the development of PGPR inoculants either for specific soil conditions or for specific stress responses.

Research was undertaken to characterize the mechanisms of enhanced nodulation and yields of *Medicago truncatula* when co-inoculated with *Sinorhizobium medicae* WSM419 and a PGPR. Initial glasshouse trials, using four PGPRs from the Centre for Rhizobium Studies (CRS) collection, resulted in the selection of *Achromobacter xylosoxidans* WSM3457 for further study. Subsequent glasshouse trials confirmed that this isolate increased nodule scores, plant nodule mass, and enhanced yields by 25–50% when *M. truncatula* was challenged with a low inoculum dose of *S. medicae* WSM419 (3×10^3 cfu/mL).

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Nodule initiation studies were conducted to determine if co-inoculation of *M. truncatula* with WSM3457 and WSM419 affects the rate of nodule initiation and development. Plants grown in soil under glasshouse conditions were harvested at 5, 7, 9, 11, 14, 17 and 21 days after inoculation. Roots were stained with brilliant green and examined under a dissecting microscope for nodule initials. The data revealed that early nodule development was enhanced with the co-inoculation treatment. Nodule initials were first observed on day 5 and day 7 in the co-inoculation and rhizobium treatments, respectively. Nodules were first evident on the co-inoculated plants on day 7, whereas nodules were only evident on day 9 in plants inoculated with WSM419 alone. This trend continued throughout the sampling period with significantly higher nodule numbers on the co-inoculation treatment by day 17.

A further experiment was aimed at investigating if the enhanced nodulation on co-inoculated plants was a result of PGPR stimulation of *M. truncatula* root development. Four treatments were investigated: (i) inoculation with WSM419 alone; (ii) inoculation with WSM419 and WSM3457; (iii) inoculation with WSM3457 alone; and (iv) an un-inoculated control. There was no significant difference in root length or lateral root formation between *M. truncatula* co-inoculated with WSM419 and *A. xylosoxidans* WSM3457 or inoculated with WSM419 alone. There was a transient increase in root-hair density between days 7 and 9 on all inoculated treatments when compared to the un-inoculated treatment. Although this suggested that bacterial inoculation increased root-hair densities during this time, there was no difference between co-inoculated roots and those inoculated with rhizobium alone. The data suggest that WSM3457 does not increase root development in such a way as to explain the enhanced early nodulation of co-inoculated *M. truncatula*.

These results indicate that *A. xylosoxidans* WSM3457 is exerting its effect over the *M. truncatula/S. medicae* WSM419 symbiosis during the period of the early signalling processes between this legume and its micro-symbiont, resulting in enhanced early root infection by *S. medicae* WSM419. Future work will focus on investigating the mechanisms of this novel interaction.

References

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