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N_2 FIXATION IN 25 COWPEA GENOTYPES MEASURED AT WA, MANGA (GHANA) AND TAUNG (SOUTH AFRICA) USING ^{15}N NATURAL ABUNDANCE: GENOTYPE X ENVIRONMENT INTERACTION

Alphonsus K. Belane[1] & Felix D. Dakora[2*]

[1]Department of Crop Science, [2]Department of Chemistry, Tshwane University of Technology, Pretoria, Private Bag X680, 0001; phonses08@gmail.com

Field experiments were conducted at Wa and Manga in Ghana, and at Taung in South Africa in 2005, using a randomized complete block design with four replicates to assess the effects of genotype x environment (G x E) interaction on plant growth and symbiotic N_2 fixation as location-specific differences have been indicated elsewhere (Belane and Dakora, 2009). Plants were sampled at 46 DAP (early pod-filling) and assessed for G x E interactions on dry matter yield, $\delta^{15}N$ (‰), %Ndfa and N-fixed. The amount of dry matter produced differed markedly among the three locations, with greater DM yield being obtained at Manga. Dry matter yield ranged from 8.1 g at Taung to 66.1 g. genotype^{-1} at Manga except for the genotypes Apagbaala, Bensogla, Omondaw and Vuli-1 which yielded greater dry matter at Wa. The $\delta^{15}N$ values at Taung were lower (-0.6 to 0.8 ‰) than those at Wa (0.1 to 1.5‰) and Manga (1.3 to 4.8 ‰). As a result, the %Ndfa values also differed ($p \leq 0.01$) among locations with Manga showing a low 19 %, and Taung a high 84 %. The amount of N-fixed by cowpea genotypes also varied with locations. Generally, 16 cowpea genotypes revealed increased N_2-fixation in all three environments. Dry matter was found to be greatly linked to amount of N-fixed.

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TRACE ELEMENT DENSITY IN EDIBLE GRAIN AND LEAVES OF TWENTY ONE NODULATED COWPEA GENOTYPES FROM WA AND MANGA (IN GHANA) AND TAUNG (IN SOUTH AFRICA), MEASURED USING ICP-MASS SPECTROMETRY

Alphonsus K. Belane[1] & Felix D Dakora[2*]

[1]Department of Crop Science, Department of Chemistry[2], Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa; phonses08@gmail.com

Micronutrient deficiency is widespread across the world and represents a major problem to human health, especially in developing countries. Because micronutrients are naturally deficient in many African soils, locally grown cereal crops tend to exhibit high levels of trace element deficiency. Yet many of these trace elements, such as Fe, Zn, Cu, Mn, B and Se, are important not only for the biosynthesis of important enzymes involved in plant and animal metabolism, but also critical for growth and brain development in humans, especially children. In South Africa, overcoming trace element deficiency and meeting the dietary requirements of micronutrients is done by direct supplementation of staple foods such maize flour with these minerals. However, we believe that selecting new genotypes of food crops with greater trace element density is a better approach and a more sustainable alternative to exogenous supplementation. In this study, 21 cowpea genotypes were screened at Wa and Manga in Ghana, and at Taung in South Africa, for their trace element densities in edible grain and leaves. The field experiment was conducted in 2005 using a randomized complete block design with four replications. At early flowering, young edible leaves were harvested from each replicate plot, oven-dried at 40°C, and ground for analysis of trace elements using inductively coupled plasma mass spectrometry (ICP-MS). At physiological maturity, cowpea grain was also harvested and similarly processed for the determination of trace element density. The data showed significant differences in trace element density among the genotypes at the three locations. Edible cowpea leaves showed markedly higher micronutrient densities relative to cowpea grain. There was also a significant genotype x environment (G x E) interaction. For example, Fe accumulation in cowpea leaves was 324.2 $\mu g.g^{-1}$ at Wa, 321.6 $\mu g.g^{-1}$ at Manga, and 551.9 $\mu g.g^{-1}$ at Taung relative to 75.8, 59.8 and 59.0 $\mu g.g^{-1}$ Fe in the grain. The accumulation of Cu, Zn, Mn and B in leaves and grain showed a similar pattern as found for Fe.

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MINING THE SEQUENCE DATA OF RHIZOBIUM LEGUMINOSARUM BV TRIFOLII WSM1325 AND WSM2304.

Lambert Bräu[1], Lynn Goodwin[2], Graham O'Hara[1], Ravi Tiwari[1], John Howieson[1], Ron Yates[3] & Wayne Reeve[1]

[1]Centre for *Rhizobium* Studies, Murdoch University, Murdoch, 6150, Western Australia.[2] DOE Joint Genome Institute, California, USA.
[3] Department of Agriculture Western Australia, Baron Hay Court, South Perth, 6151, Western Australia.

Most clover rhizobial inoculants form effective nitrogen-fixing symbioses with either annual or perennial species (and very few with both). This

constraint provides a considerable barrier to agricultural productivity since background populations of *R. l. trifolii* may nodulate with an incompatible host but ineffectively fix nitrogen (Yates *et al* 2008). Knowledge at the genetic level is essential to develop an understanding of this incompatibility and progress in this pursuit will be greatly enhanced by complete genome sequence information. Thus, the genomes of two *R. l. trifolii* strains were sequenced by the US Joint Genome Institute Community Sequencing Program; the Mediterranean *Trifolium* spp. isolate WSM1325 and the South American *Trifolium polymorphum* isolate WSM2304. Strain WSM1325 is compatible with Mediterranean perennial clovers (ie with American or African perennial clovers whilst the reverse is true for strain WSM2304. To sequence the genomes, a shotgun assembly approach was adopted to assemble a draft genome of each organism. Three libraries were constructed for each strain including one library for each strain in the functional genomics vector pTH1522 (Cowie *et al*, 2006). Assembly of the sequence data is in the draft stage and reveals that the genome of WSM2304 is 6.8 Mb in size with a chromosome of 4.53 Mb and 4 plasmids of 1266, 501, 308 and 258 Kb. The genome has a G+C content of 61.1% and encodes 6590 candidate protein-encoding genes. In comparison, the WSM1325 genome is 7.8 Mb in size (529 contigs of 20 reads or greater), has a G+C content of 60.7% and encodes 7528 candidate protein-encoding genes. We will discuss comparative studies performed on the genome sequences of these two strains.

Yates RJ, Howieson JG, Reeve WG, Bräu L, Speijers J, Nandasena K, Real D, Sezmis E & O'Hara GW (2008). Host-strain-mediated selection for an effective nitrogen-fixing symbiosis between *Trifolium* spp. and *Rhizobium leguminosarum* biovar *trifolii*. *Soil Biol Biochem* 40: 822-833.

Cowie A, Cheng J, Sibley CD, Fong Y, Zaheer R, Patten CL, Morton RM, Golding GB & Finan TM (2006). An Integrated Approach to Functional Genomics: Construction of a Novel Reporter Gene Fusion Library for *Sinorhizobium meliloti*. *Applied Environ Microbiol* 72:7156-7167.

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PROTEOMIC AND METABOLOMIC ANALYSIS OF SOYBEAN ROOT HAIRS COLONIZED BY *BRADYRHIZOBIUM JAPONICUM*

Laurent Brechenmacher[1], Zhen Tian Lei[2], Kim Hixson[3], Marc Libault[1], Seth Findley[1], Roy Lowery[4], Brian Mooney[4], Ljiljana Pasa Tolic[3], Lloyd W. Sumner[2] and Gary Stacey[1,5*]

[1]National Center for Soybean Biotechnology, Division of Plant Sciences, University of Missouri, Columbia, MO 65211, gstacey@missouri.edu [2]Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401, lwsumner@noble.org [3]Mass Spectrometry Facility, Environmental Molecular Sciences Laboratory, Richland, WA 99352, ljiljana.pasatolic@pnl.gov [4]Charles W Gehrke Proteomic Center, University of Missouri, Columbia, MO 65211, mooneyb@missouri.edu [5]Division of Biochemistry and Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65211

Root hairs are single tubular cells formed from the differentiation of epidermal cells, called trichoblasts, on primary and secondary roots. They are involved in water and nutrient uptake, anchorage of the plant into the soil and represent the preferred infection site on leguminous roots by rhizobia. The interaction between soybean (*Glycine max*) and the bacterium *Bradyrhizobium japonicum* leads to the establishment of a nitrogen fixing symbiosis. The bacteria induce the formation of a specific new organ in the plant, the root nodule, in which they reduce atmospheric nitrogen to ammonia, which provides the plant host a consistent nitrogen source. Soybean was selected for this study due to its agronomic importance and its root size, which permits isolation of root hairs in sufficient amount for proteomic studies. In order to investigate the cellular changes that occur in the root hair upon rhizobial infection, we profiled both the root hair proteome and metabolome during the first 48 hours after *B. japonicum* inoculation. Proteins were extracted from root hairs 0, 12 h, 18 h, 24 h, 36 h and 48 h after inoculation and compared to mock inoculated roots. Differential In Gel Electrophoresis (DIGE) was performed and identified 178 spots significantly regulated after *B. japonicum* inoculation ($p<0.05$). As a complementary approach, the proteome and phosphoproteome of *B. japonicum*-infected root hairs are also being analyzed using a non-gel based approach (LC MS/MS). The metabolome was also analyzed over the same time course. Primary metabolites from polar and lipophilic fractions were analyzed by GC-MS. Secondary metabolites were analyzed by UPLC- QtofMS. A total of 1688 metabolites were identified in root hairs by combining both approaches. Statistical analysis identified 121 compounds significantly regulated in root hairs colonized by *B. japonicum* ($p<0.01$). These included trehalose, maltose, lactic acid, apigenin, or afrormosin. The proteomic and metabolomic data, including transcriptomic data also available, are being integrated to provide a systems level view of the root hair response to *B. japonicum* infection.

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PHYLOGENETIC DIVERSITY OF RHIZOBIAL STRAINS NODULATING *CYTISUS TRIFLORUS*

Rajaa Chahboune[1], A. J. Sánchez-Raya[2], E. J. Bedmar[2] & Said Barrijal[1]

[1]Department of Biology, Faculty of Sciences and Techniques, University Abdelmalek Essaadi, Tangier, Morocco, and [2]Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, Granada, Spain.

The genetic diversity of 76 endophytic bacterial strains isolated from root nodules of *Cytisus triflorus* grown in the Aoudal and Fifi regions of the Moroquian Rif was analysed by Repetititve Extragenic Palindromic