

# Stress release for soil

## Mitigating abiotic stresses in legumes using biological nitrogen fixation (BNF)

**B**iological N<sub>2</sub> fixation (BNF) is the process whereby certain soil bacteria (e.g. *Rhizobium*, *Azospirillum*) use their nitrogenase enzyme and convert atmospheric N<sub>2</sub> into ammonia (NH<sub>3</sub>), a usable form of nitrogen (N) that plants and bacteria incorporate into proteins and nucleic acids (Unkovich et al., 2008). Within the legumes, this important process of BNF takes place in highly specialised structures called nodules, which contain the bacteria and are formed on the roots or stems of the plants (Figure 1).

The acquisition of essential nutrients from the soil by plants is one of the crucial steps that not only ensures enhanced growth, but also helps maintain the proper functioning of all parts of the metabolism that ultimately secure plant health. Despite the increasing amounts of fertilizer-N being used in the production of crops, the process of biological N<sub>2</sub> fixation by bacteria is the primary source

of N for agriculture that provides one of the most limiting and very essential nutrient (N) to more than 19000 species of leguminous plants globally.

Leguminous crops such as beans, peas, soybean, lucerne and chickpea, to mention a few, are very susceptible to various abiotic stresses caused by drought, soil acidity, salinity and metal toxicity that limit their growth. Coupled with the increasing threats of climate change effects, these abiotic stresses have huge negative impacts on the overall plant health and productivity. Drought stress is one of the most common types of abiotic stresses caused by low moisture content in the soil. Such low moisture content poses various challenges in the legume-rhizobium symbiosis, one of which being a decrease in the nitrogen (N) derived from biological nitrogen fixation (BNF). Drought stress therefore affects both the legume plants and their symbiotic partners, the rhizobia. While the legume plants show reduced level of nodule initiation, growth and normal activity, the rhizobia respond

to drought stress by having irregular morphology of cells that will eventually lead to a reduction in infection of roots and nodule formation (Zahran 1999).

In line with the International Year of Plant Health (IYPH2020), various plant health related activities have been undertaken by the Agricultural Research Council, at the campus of Plant Health and Protection (ARC-PHP) based in Pretoria, South Africa. The Biological N<sub>2</sub> Fixation (BNF) unit at the ARC-PHP provides different services related to biological nitrogen fixation, including research on legume-rhizobium symbiosis. One of the projects involves the screening of rhizobia with the ability to nodulate and fix atmospheric N<sub>2</sub> in soybean under different drought stress (moisture level) conditions.

The major aim of this project is to screen a wide range of rhizobia strains for nodulation of soybean (*Glycine max* L.) under different water stress conditions in a glasshouse trial. In this project, twenty-five different rhizobia strains deposited at the South African Rhizobium Collection (SARCC) were included

(the rhizobia), it is well known that certain strains of rhizobia are capable of survival under drought stress or low water potential of up to 2 – 15% (Zahran et al., 1999). These drought tolerant rhizobia use different mechanisms to alleviate abiotic stress in soils, one of which is the production of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase. Upon inoculation, the ACC deaminase lowers the plant ethylene level, resulting in longer roots and providing relief from abiotic stresses like drought, salinity and metal toxicity (Gopalakrishnan et al., 2014). It is therefore essential to screen rhizobia that have the ability to survive different drought stress levels and fix atmospheric N<sub>2</sub> by forming effective nodules on the target legume host. As it stands, the screening project described above looks very promising in that a large number of the rhizobia included in the experiment have resulted in satisfactory results representative of which is indicated in Figure 1. The most effective strains (i.e. those that resulted in the formation of active pink nodules with better plant vigour) will be further screened in a field trial experiments. The ultimate aim will be the selection of elite drought tolerant rhizobia which will be developed into commercial

inoculants for soybean cultivation under drought stress conditions in South Africa. ■

Dr. Ahmed Idris Hassen (PhD)  
Agricultural Research Council, Plant Health and Protection  
[HassenA@arc.agric.za](mailto:HassenA@arc.agric.za)  
072.274.4380

### REFERENCES

Zahran, HH (1999). *Rhizobium legume symbiosis and nitrogen fixation under severe conditions and in an arid climate*. *Microbiol. Mol. Biol. Rev.* 63: 968-989  
Gopalakrishnan S., Sathya A, Vijayabhathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2014) *Plant growth promoting rhizobia: challenges and opportunities*. *3 Biotech* 5: 355-377.  
Unkovich M., Herridge D., Peoples M., Cadisch G., Boddey R., Giller K., Alves B. and Chalk P. 2008. *Measuring plant-associated nitrogen fixation in agricultural systems*. *ACIAR Monograph No. 136*, 258 pp.  
Hassen AI, Mtsweni PN, Bopape FL, van der Linde EJ, van Vuuren A, van Wyk S, Smit C (2020). *Project on screening of rhizobia strains that nodulate soybean (Glycine max L.) under drought stress conditions*. (Unpublished).



Figure 1. Screening of rhizobia that nodulate and fix atmospheric nitrogen (N<sub>2</sub>) in soybean. Top: Experimental design in the glasshouse for the drought screening trial (left) and laboratory manipulation of the different rhizobia to be screened before inoculation onto the seeds (right). Bottom: Soybean roots with several conspicuous pink nodules after treatment with different rhizobium strains at 50% moisture reduction, compared to an un-inoculated control treated with full moisture capacity, and no nodule formation (far left). Source: Hassen et al. 2020, unpublished.