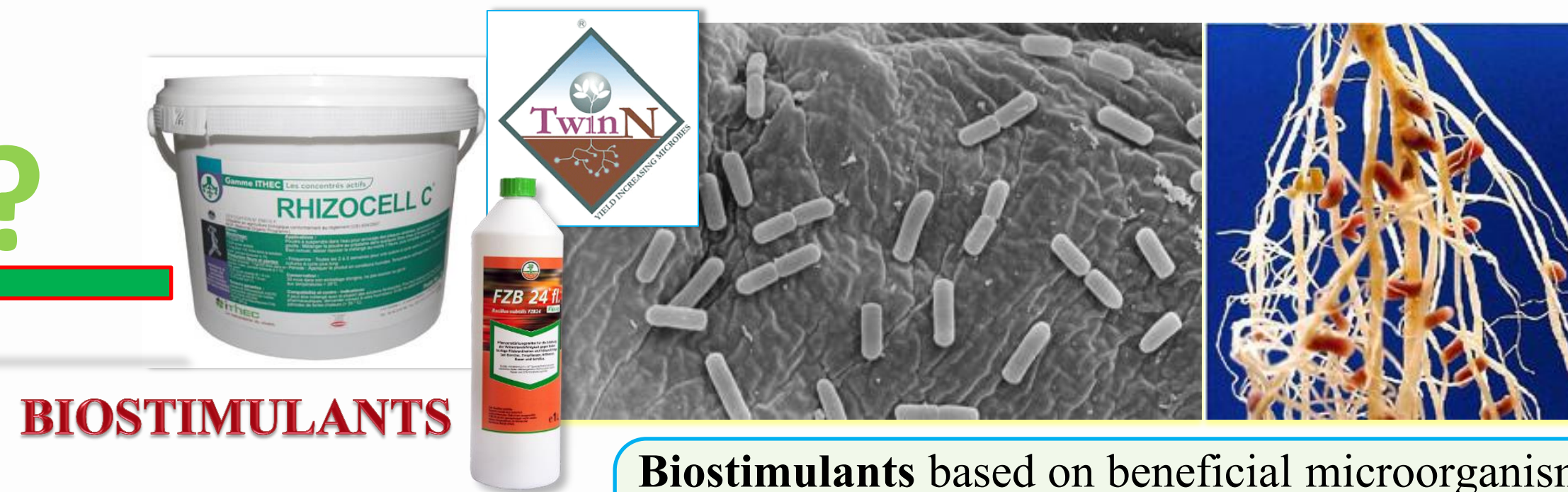
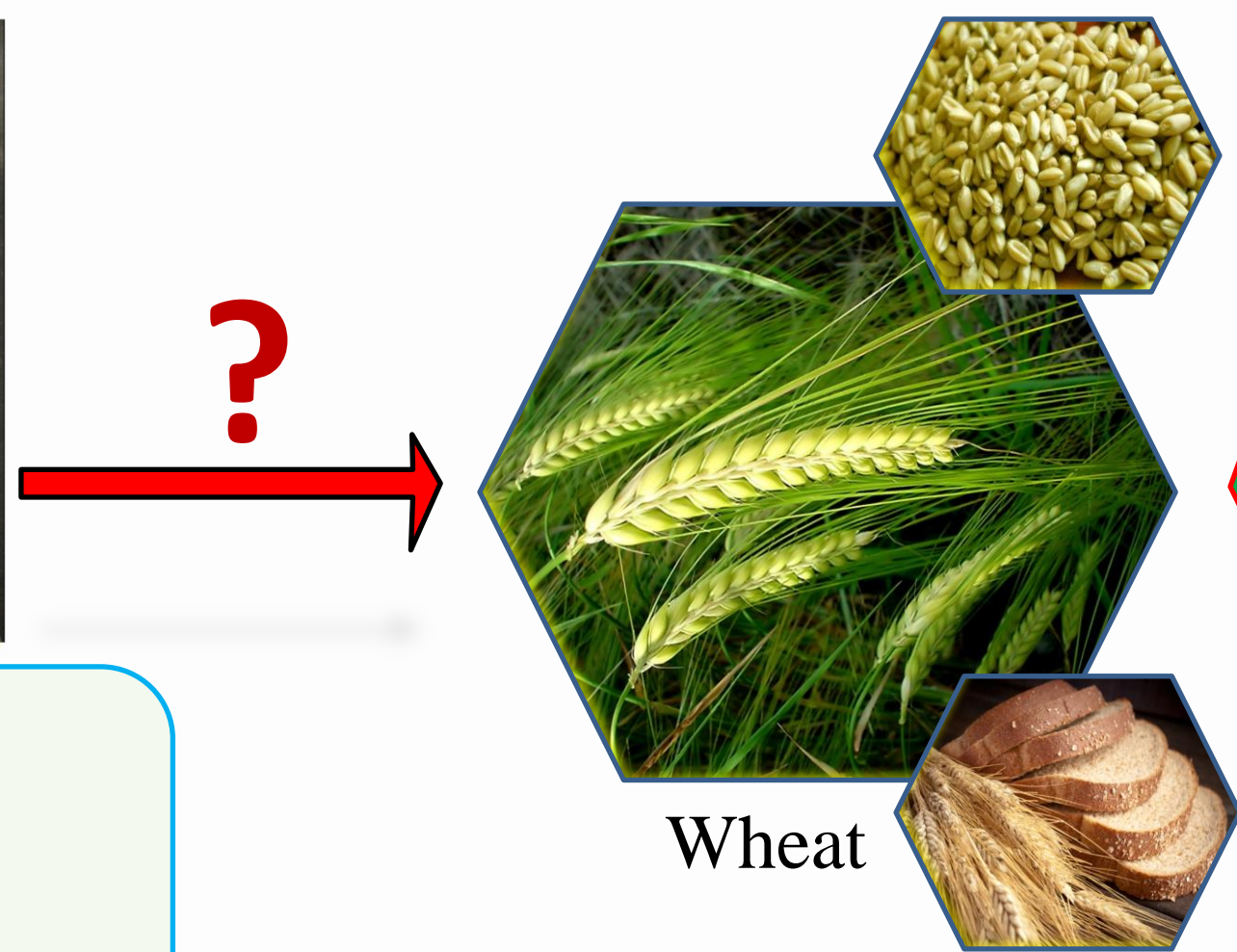
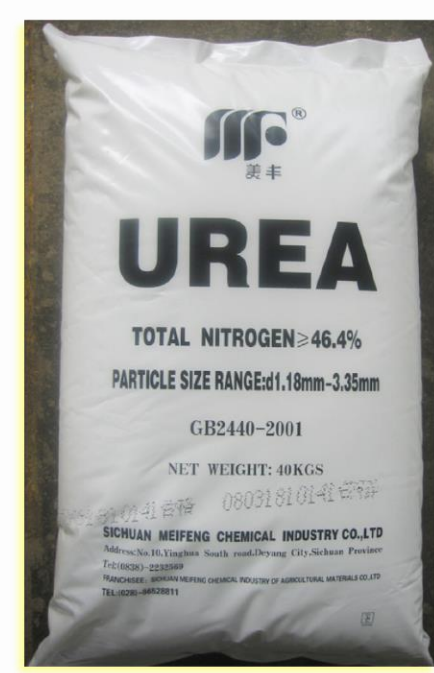


Minh Luan Nguyen<sup>1</sup>, Bernard Bodson<sup>2</sup>, Gilles Colinet<sup>3</sup>, Haïssam Jijakli<sup>4</sup>, Marc Ongena<sup>5</sup>, Micheline Vandenberg<sup>6</sup>, Patrick du Jardin<sup>1</sup>, Stijn Spaepen<sup>7</sup>, & Pierre Delaplace<sup>1</sup>  
 University of Liège, Gembloux Agro-Bio Tech: <sup>1</sup>Plant Biology Unit, <sup>2</sup>Crop Science and Experimental Farm, <sup>3</sup>Soil Science, <sup>4</sup>Phytopathology, <sup>5</sup>Bio-Industries, <sup>6</sup>Animal and Microbial Biology, <sup>7</sup>Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research

## Introduction

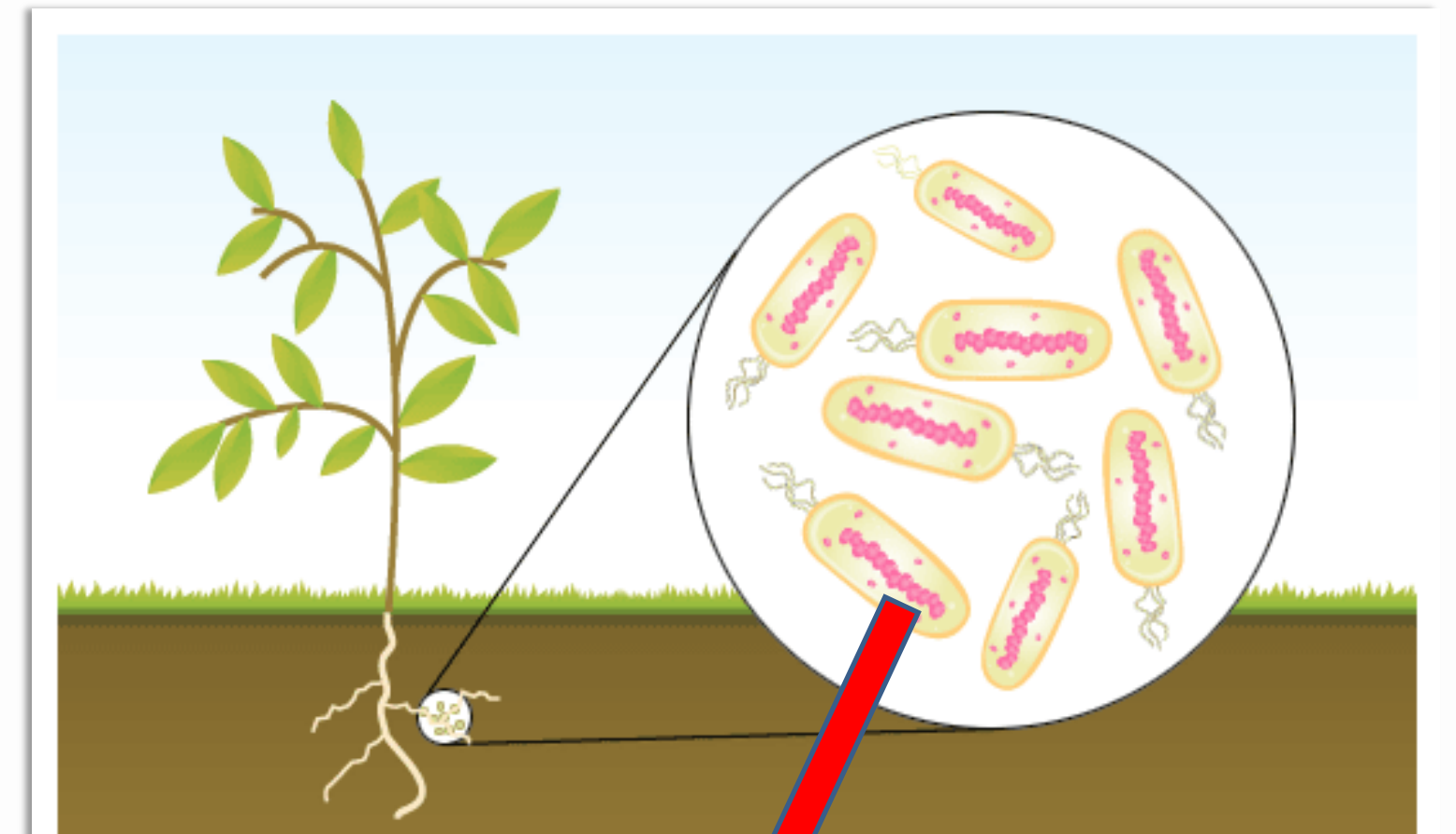


**BIOSTIMULANTS**

Biostimulants based on beneficial microorganism (PGPR) are able to reduce the use of chemical fertilizers and increase the nutrient use efficiency by the plant

- Today's chemical fertilizers:**
- Source of soil & water pollution
  - Greenhouse-gas generators
  - Expensive
  - Reduction of fossil resources (e.g. phosphate)

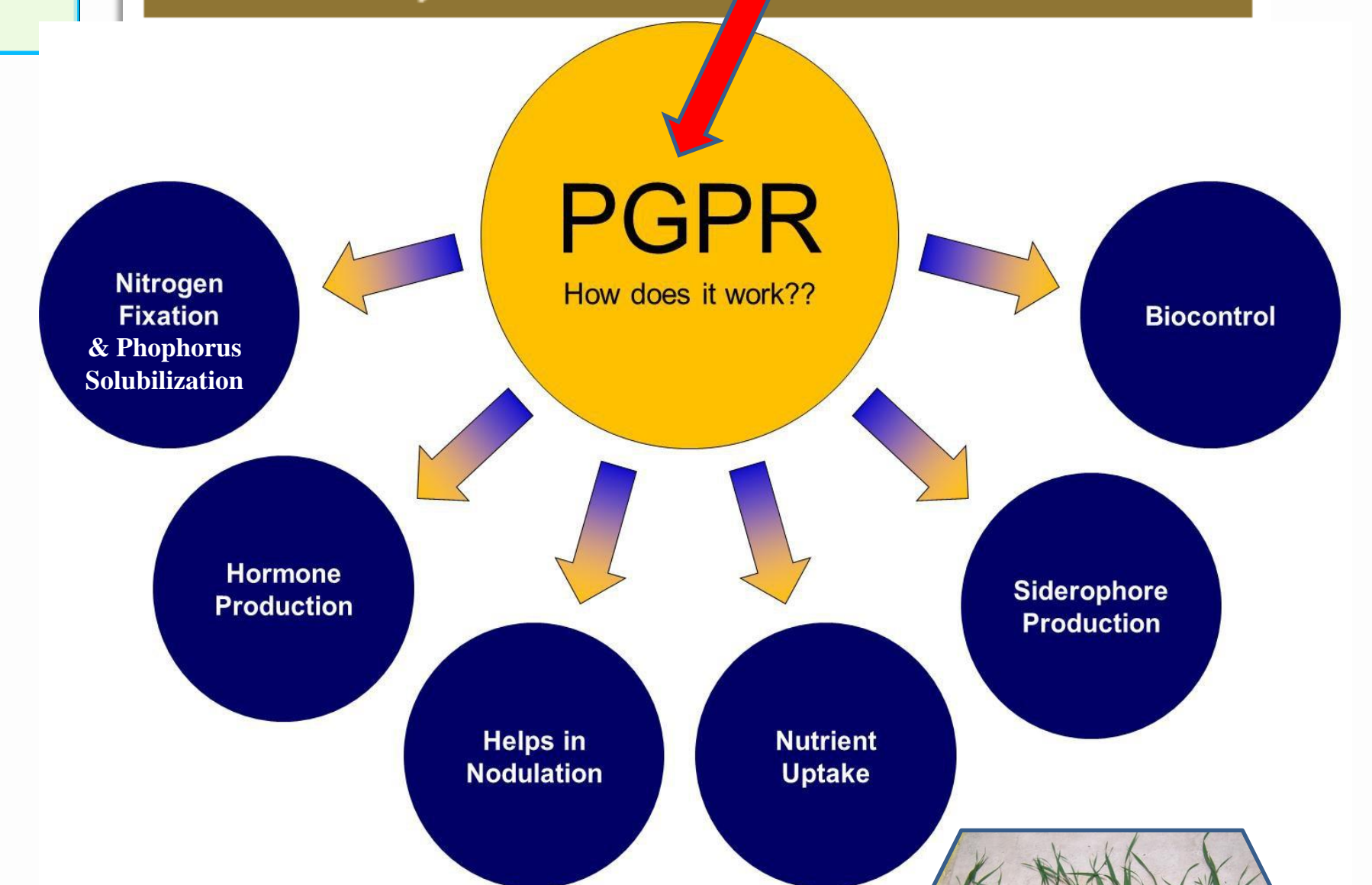
**Biostimulants** are compounds, substances and microorganisms that are applied to plants or soils to regulate and enhance the crop's physiological processes in order to make them more productive. At least 8 classes of compound-based biostimulants exist, including humic substances, complex organic materials, beneficial chemical elements, inorganic salts, seaweed extract, chitin and chitosan, antitranspirants, free amino acids & other N-containing substances<sup>(3, 4)</sup>. In parallel, biostimulants may also include **living microorganisms** like plant growth-promoting fungi (PGPF) and **rhizobacteria (PGPR)**<sup>(1, 2)</sup>. Our present study will initially focus on the last class of products.



## Objective

Development of relevant research tools:

- To assess the impacts of such changes on plant growth, yield, tolerance to abiotic stresses & soil fertility
- To stimulate the increase of beneficial microorganism communities and the decrease of pathogenic ones in the wheat rhizosphere
- To figure out the best agronomical practices to stimulate the beneficial microbial communities under different productions systems



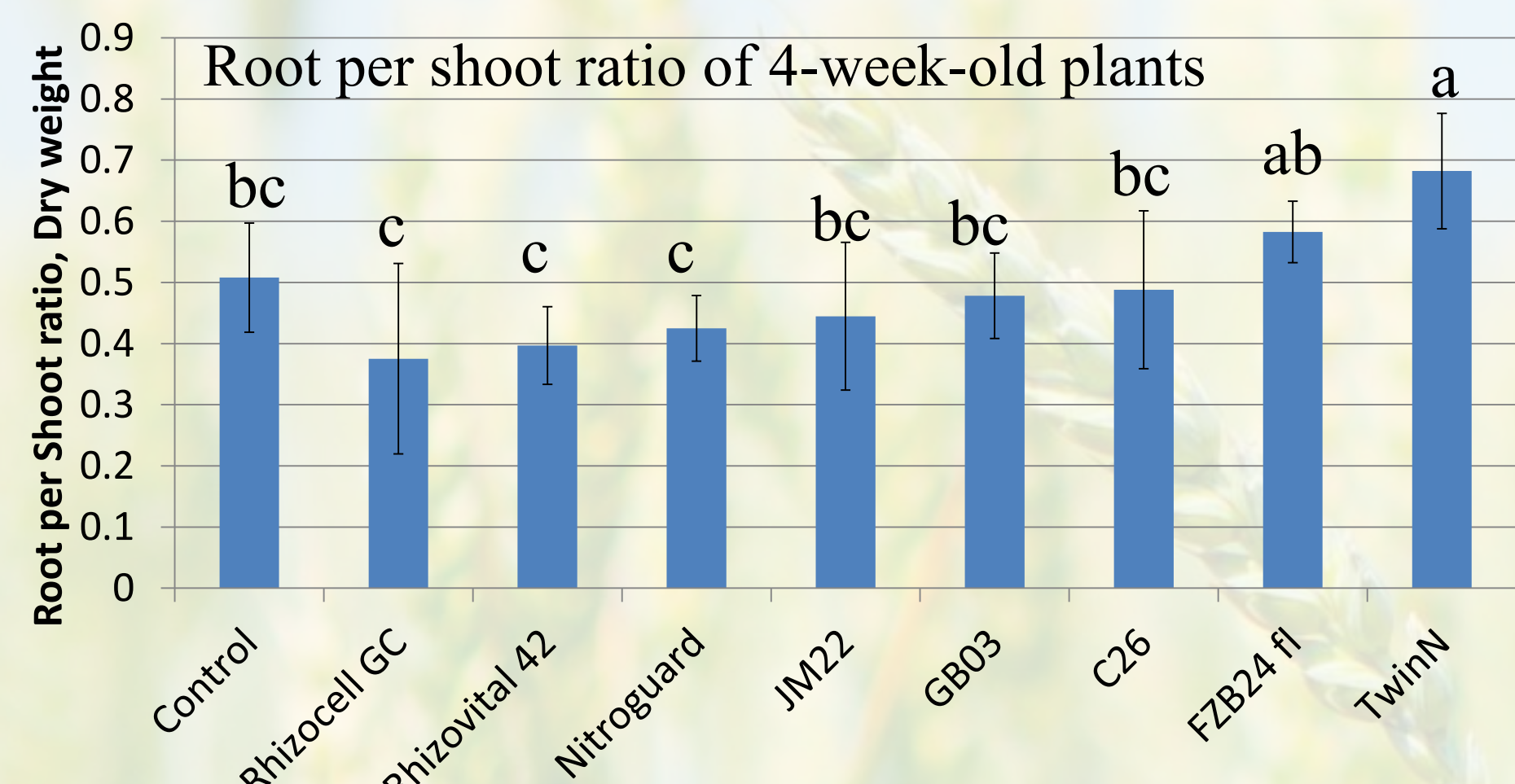
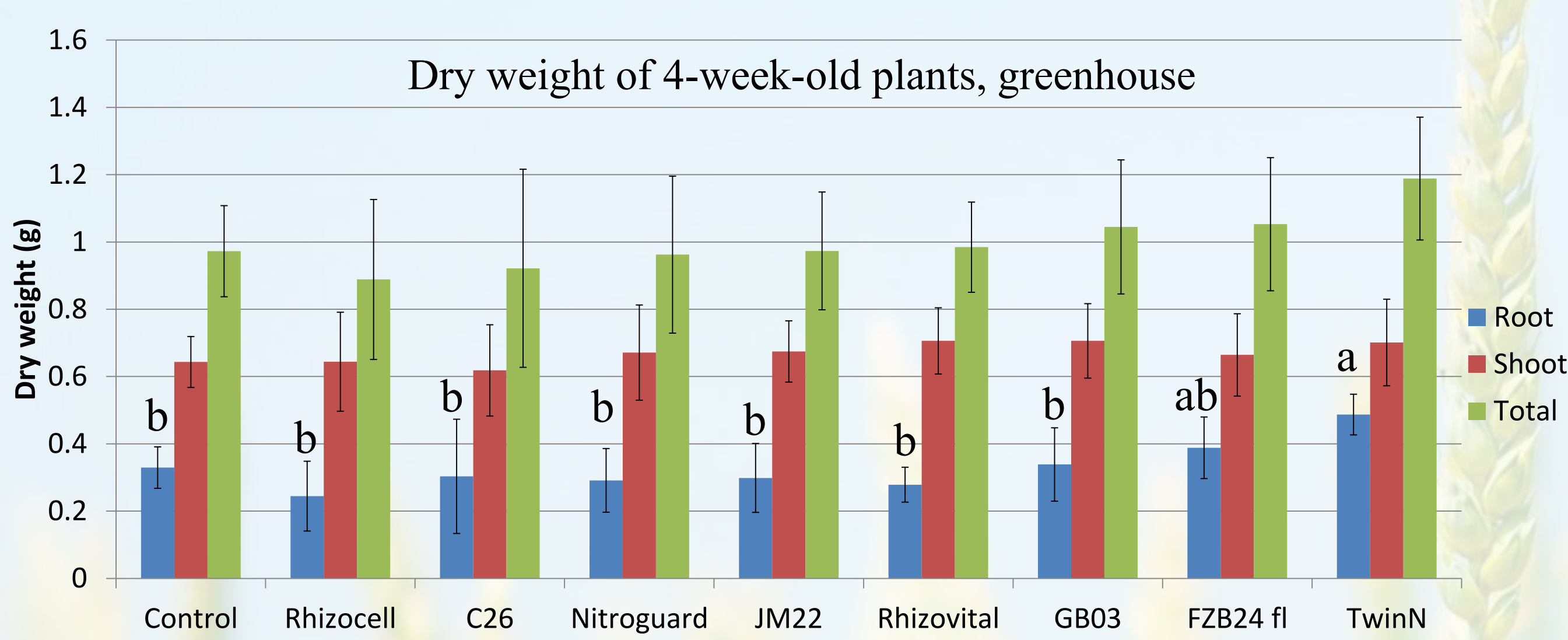
## Materials & method

- **PGPR strains** include in-house strains (*Bacillus pumilus* C26, *B. subtilis* AP-305-GB03, *Enterobacter cloacae* AP-12-JM22) and commercial biostimulant products [TwinN (diazotrophic bacteria); NitroGuard (TwinN + 2 *Bacillus* sp. strains); FZB24 fl (*B. subtilis*); Rhizocell GC (*Bacillus* sp. IT45); RhizoVital 42 (*B. amyloliquefaciens*)]
- **PGPR screening under controlled condition (greenhouse):** Seeds of a spring wheat, *Triticum aestivum* (variety Tibalt) were planted in 30-cm depth PVC tubes filled with field soil (maintained at 16% humidity, no fertilizer) and inoculated with 10<sup>8</sup> cells/plant under LED lighting (flux: 150 W/m<sup>2</sup>). After 4 weeks, plant biomass and tiller number were measured.
- **PGPR screening under field condition in combination with N fertilizer:** Seeds of a winter wheat, *T. aestivum* (variety Forum) were sowed on 2<sup>nd</sup> Dec. 2013 in a criss-cross design. Two fixed factors were used: the PGPR strain (five biostimulant products above and control) and N fertilizer (0, 50, 75 and 100%). The shoot weight, spike number and grain yield will be measured at Zadoks' stage 39, 69 & 100, respectively.

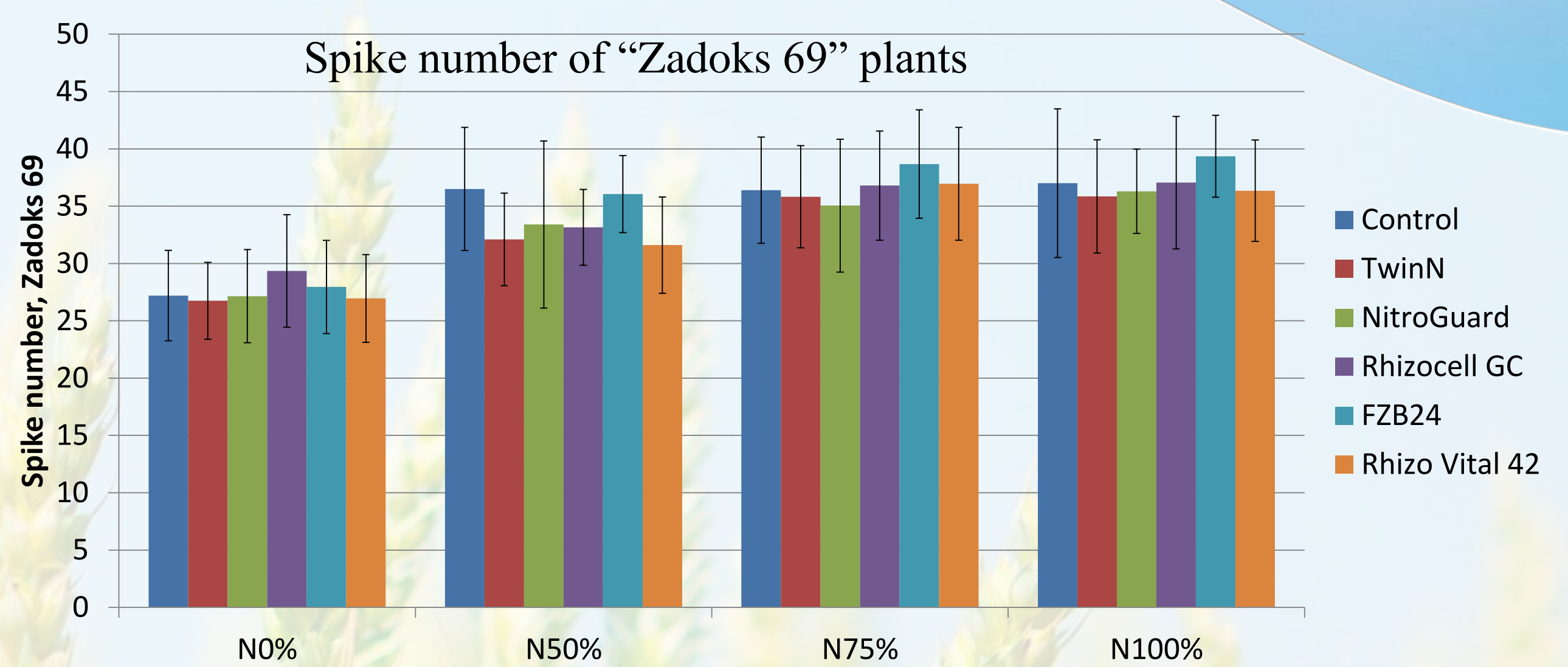


## Result and Discussion

### Spring wheat GREENHOUSE TRIALS



### Winter wheat FIELD TRIALS



#### Results:

- **Under greenhouse condition:** TwinN results in the highest root biomass and root per shoot ratio (one-way ANOVA,  $p=0.000$ ).
- **Under field condition:** (1) A significant increase in spike number was observed in the Control and FZB in combination with N50%, and FZB in N75% ( $p=0.005$  &  $0.031$ , respectively). (2) N75% and N100% have similar spike number and significant higher than that of N0% and N50% ( $p=0.00$ ). (3) FZB24 results in the highest spike number compared to other products and PGPR ( $p=0.009$ ).

#### Perspectives:

- Continue to optimise the growth condition (e.g. fertilizer level) and select the proper plant stage to inoculate PGPR efficiently in greenhouse as well as in field.
- Assess the grain yield of field experiments.
- Identify the potentially stressing condition that could allow PGPR products to express their growth promotion capacity.
- Metagenomic approaches (based on shotgun sequencing of rDNA) will be developed to assess the impacts of biostimulants to soil microbial community.

#### References

- (1) Ahmad, Pichtel, Hayat (2008). Plant-bacteria interactions, strategies and techniques to promote plant growth. Weinheim, Germany: Wiley VCH.
- (2) Bhattacharyya, Jha (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28: 1327–1350.
- (3) Pinton, Varanini, Nannipieri (2007). The Rhizosphere: Biochemistry and organic substances at the soil-plant interface. Boca Raton, Florida : CRC Press.
- (4) du Jardin, P. (2012). The Science of plant biostimulants- A bibliographic analysis. Report to the European Commission, Contract 30-CE0455515/00-96

Contact: ml.nguyen@ulg.ac.be