Alfalfa to get back into planetary boundaries

2025 world alfalfa congress
FRANCE REIMS-CHÂLONS

Closing the nitrogen cycle: from alfalfa symbiotic fixation to crop and animal production

Thamialami I. & Palmonari A.

Plenary session 4

FROM 03 > TO 06 nov. 2025

Alfalfa to get back into planetary boundaries

2025 world alfalfa congress
FRANCE REIMS - CHÂLONS

Pre-sowing inoculation of rhizobia and arbuscular mycorrhizal fungi as a way to increase alfalfa (*Medicago sativa* L.) varieties productivity on acid soil

Nedeljko Racić, Vladeta Stevović, Snežana Anđelković, Dejan Soković, Jasmina Radović, Zoran Lugić, and Vladimir Zornić

Institute for forage crops, Kruševac, Serbia

ИНСТИТУТ ЗА КРМНО БИЉЕ КРУШЕВАЦ

FROM 03 › TO 06 nov. 2025

Introduction

- More than just a forage crop
- Builds soil fertility
- Agrotechnical benefits
- Importance

Challenges of Alfalfa on Acidic Soils

⚠️ Growth limited by soil acidity and Al^{3+} toxicity

⬇️ **Low alfalfa nodulation, root development, and phosphorus availability**

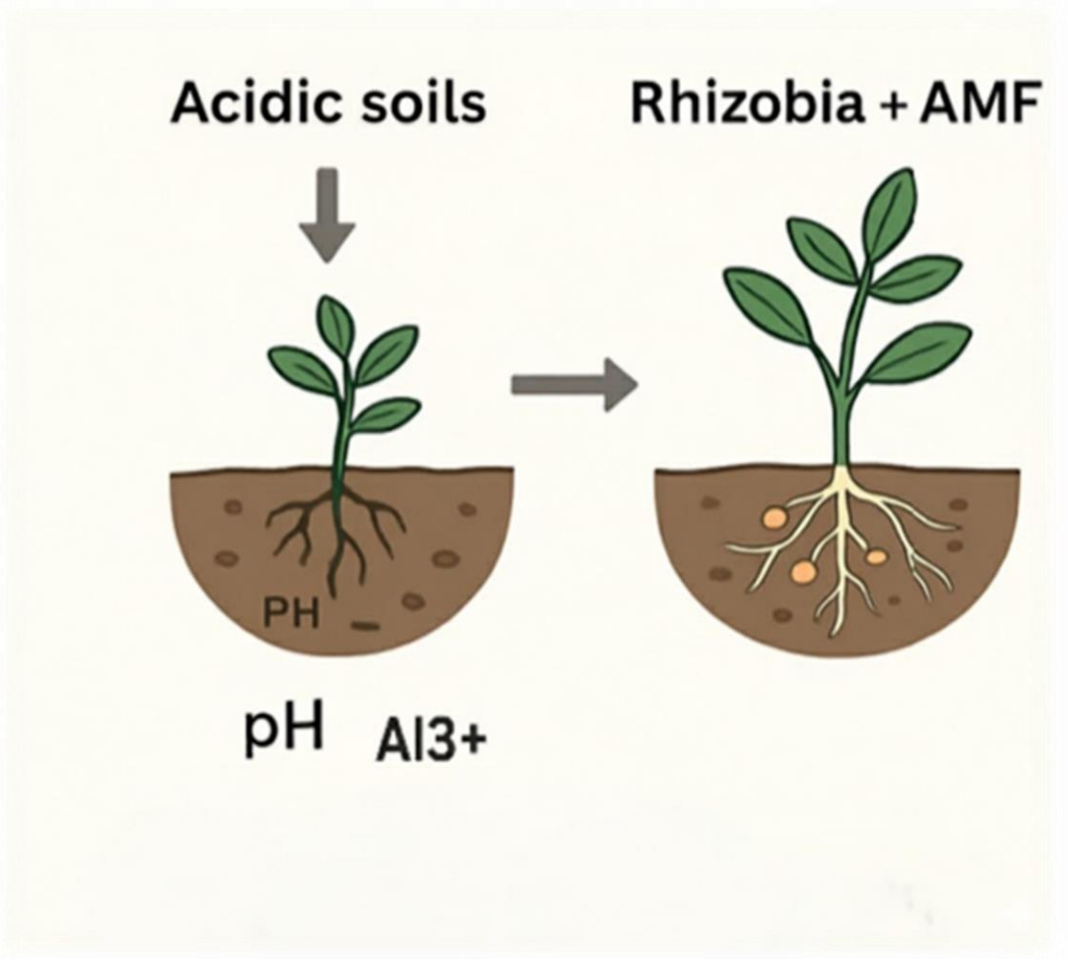
�Biofertilizers = economic and ecological alternative

🌿 Symbiosis: Rhizobia ↑ N fixation

🍄 AMF ↑ P uptake, stress tolerance

📈 Benefits:↑ yield,↓ fertilizer use, ↑plant health

🔬 Co-inoculation (Rhizobia + AMF) → more nodules, higher N fixation, better absorption



Research Objectives

Effect of rhizobia, AMF, and combined inoculation on:

🔬**Nodulation & Colonization** (nodule number & mycorrhizal colonization)

🌱**Biomass Productivity (**Impact on shoot & root dry matter yield)

🥛**Forage Quality** (Influence on protein yield & phosphorus content)

Materials and metods

Experimental Design

1. Inoculation treatments

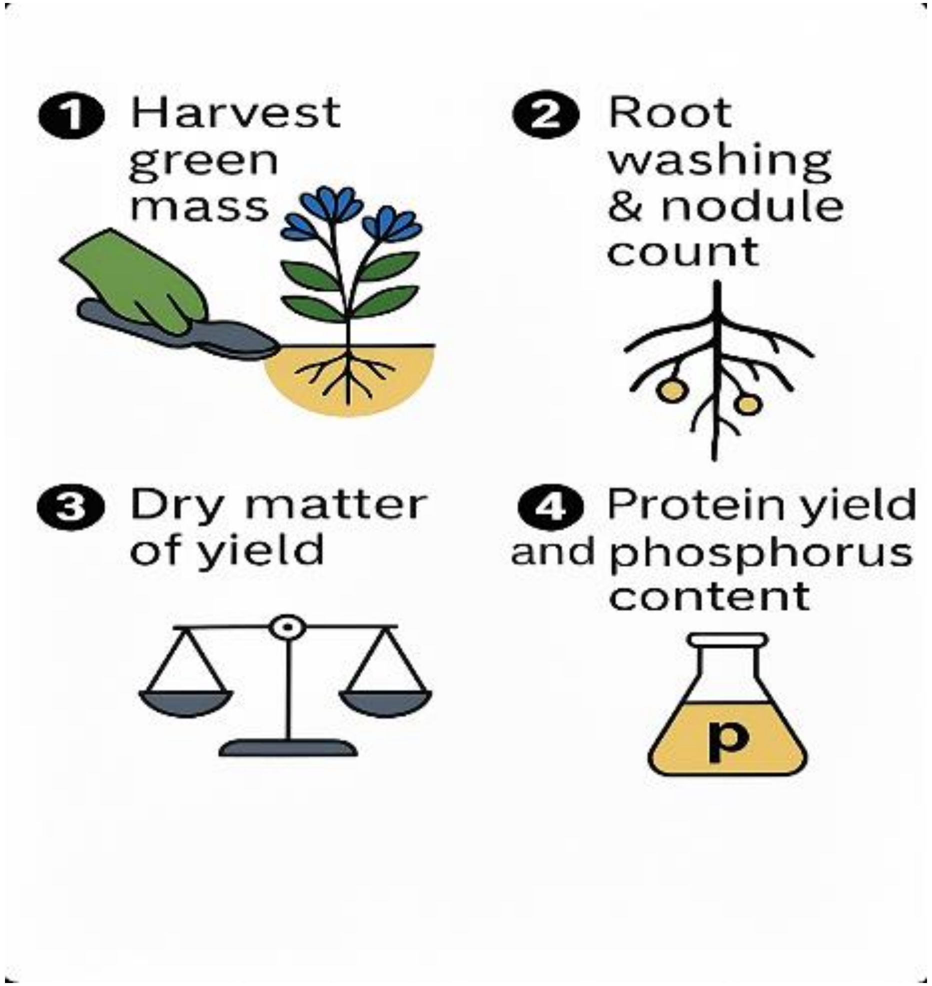
- **C** Control
- **R** Rhizobium (*Sinorhizobium meliloti* 218)
- **MF** Mycorrhizal fungi (*Rhizophagus intraradices and Funneliformis mosseae*)
- **RMF** RMF Rhizobium + Mycorrhizal fungi

2. Alfalfa varieties

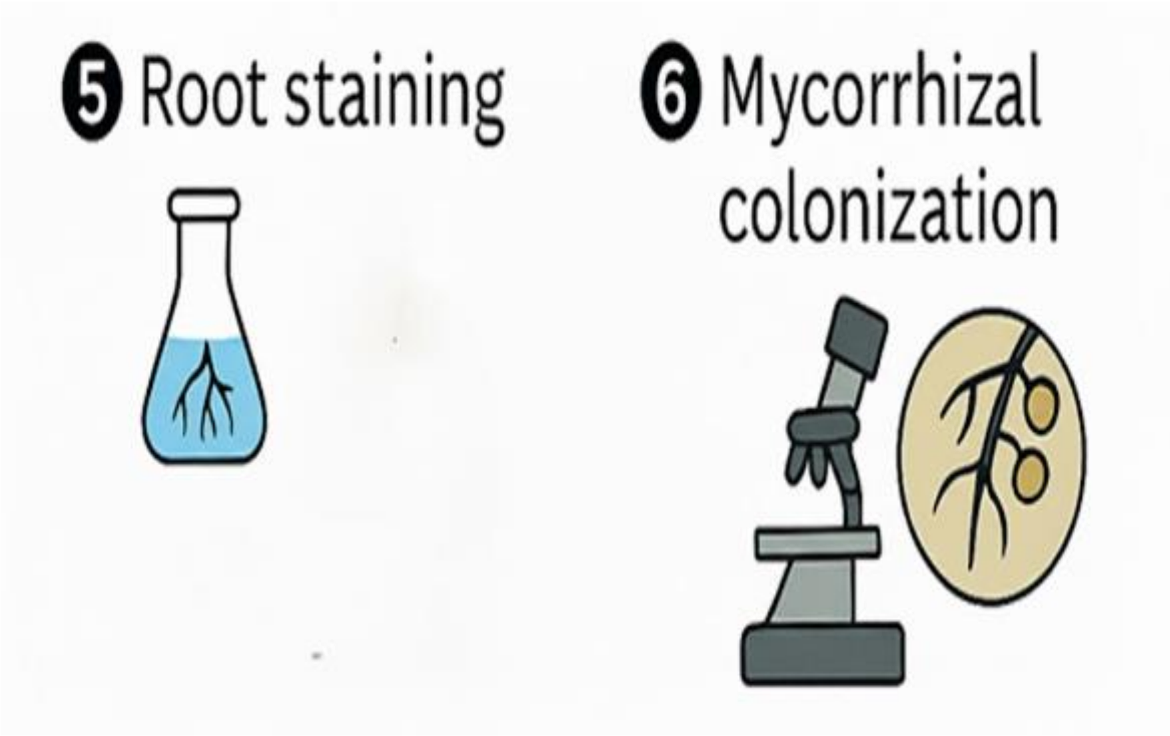
K-28, Zuzana, Nijagara

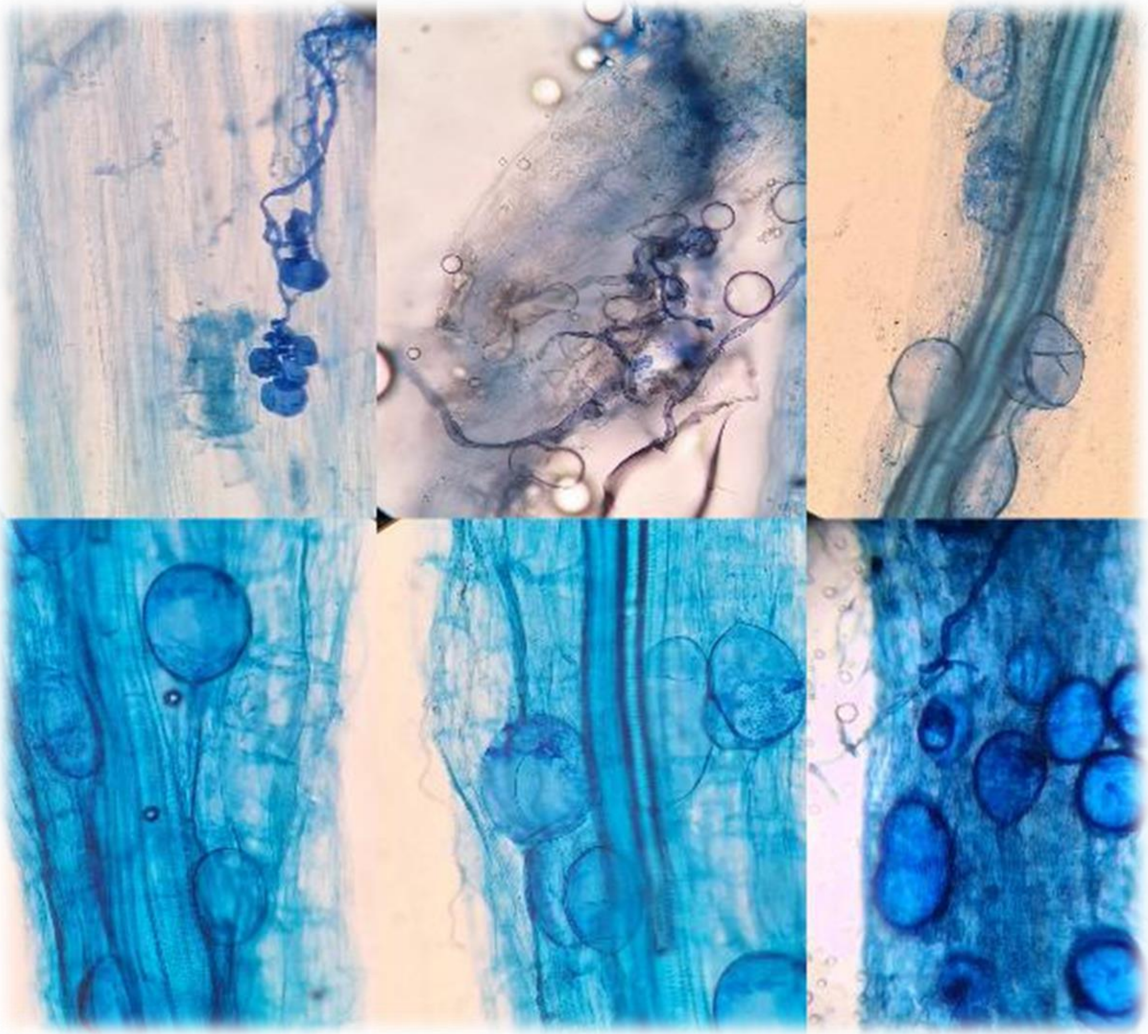
Acidic soil (pH 4.55)

Experimental analyses



Experimental analyses





Results

- **Rhizobia**

↑nodules (25–40%),
↑shoot dry matter (+30%)
↑ root dry matter(20-40%)
↑protein yield (+10%)

- **AMF & co-inoculation**

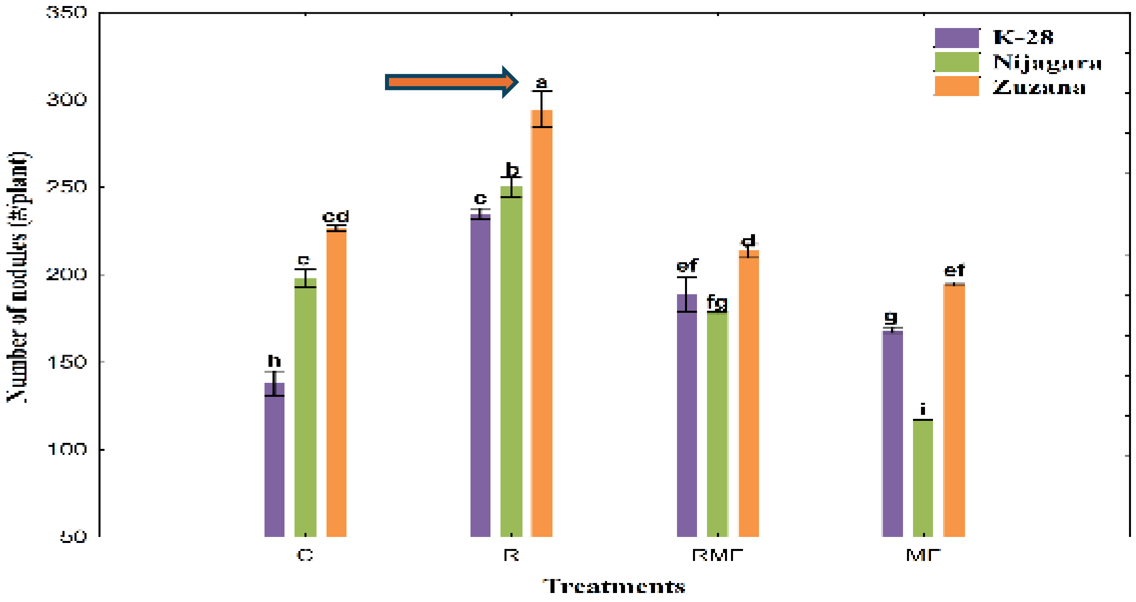
↑mycorrhizal colonization
↑ higher P uptake

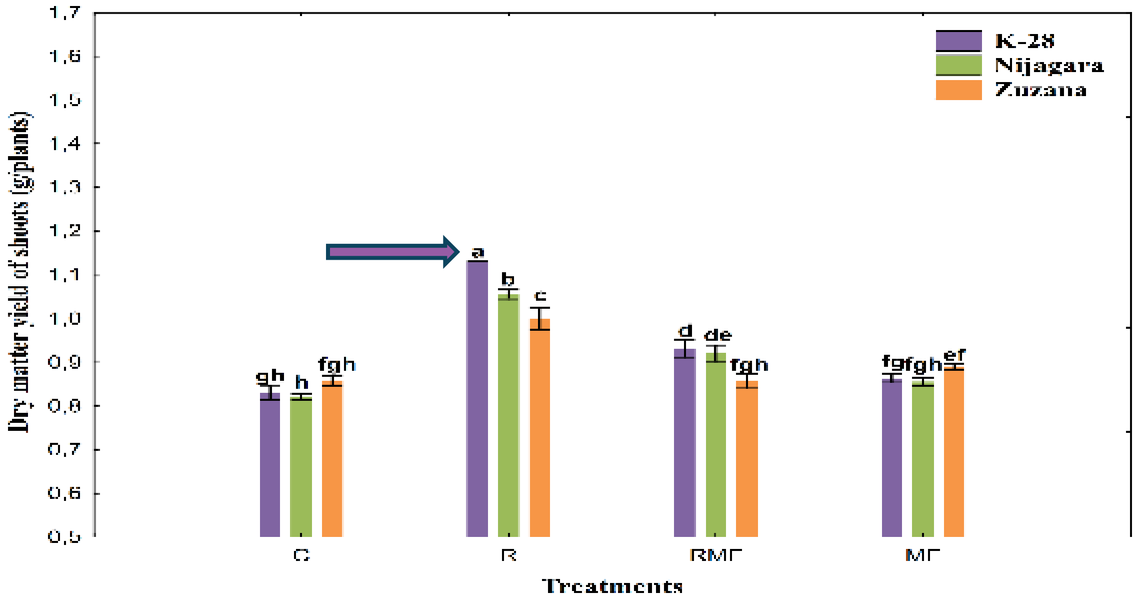
- **All inoculations**

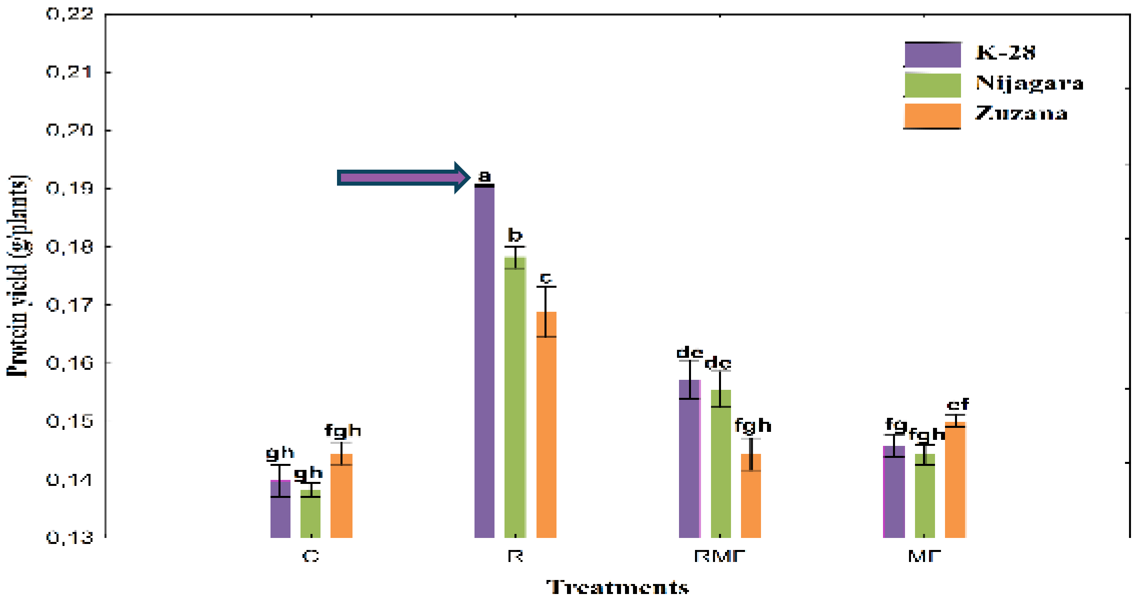
↑shoot dry matter
↑protein yield

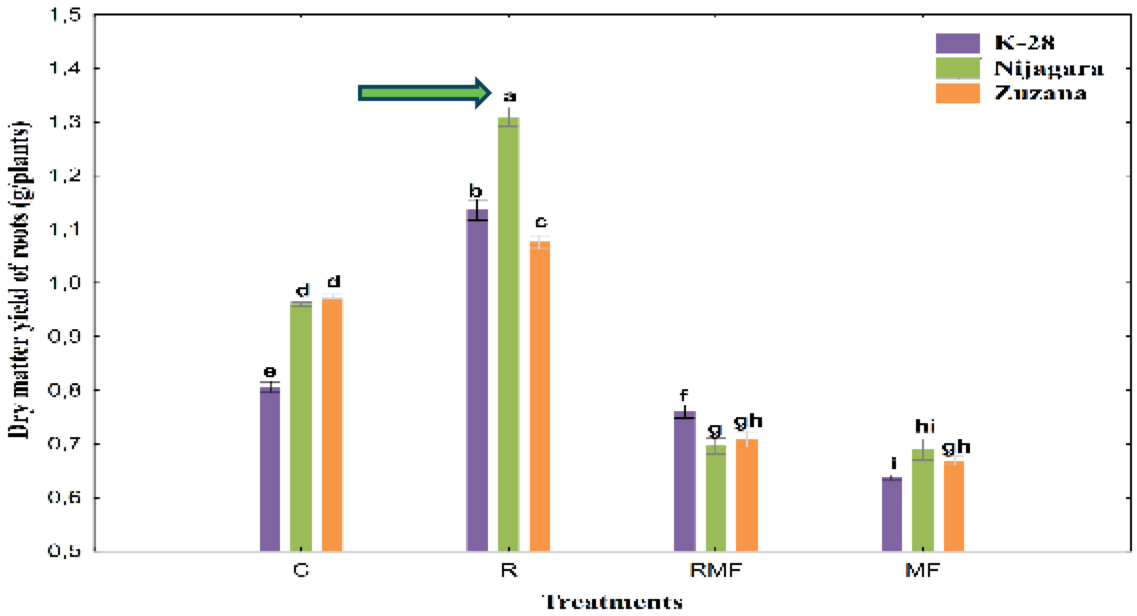
	Number of nodules (#/plants)	Mycorrhizal Colonization (%)	Dry matter of shoots (g/plants)	Dry matter of roots (g/plants)	Protein yield (g/plants)	P content (mg/g DM)
INOCULATION						
C	188±10.2^{b}	45.0±1.67^{b}	0.74±0.01^{d}	0.91±0.02^{b}	0,13±0.005^{d}	3.43±0.15^{c}
R	260±7.73^{a}	41.8±1.42^{b}	1.06±0.02^{a}	1.17±0.03^{a}	0,17±0.011^{a}	4.01±0.17^{b}
RMF	194±5.08^{b}	55.7±2.27^{a}	0.90±0.01^{b}	0.72±0.01^{c}	0,15±0.009^{b}	4.28±0.14^{b}
MF	158 ±8.84^{c}	54.2±1.51 ^{a}	0.87±0.01^{c}	0.67±0.01^{d}	0,14±0.004^{c}	4.82±0.18^{a}
VARIETY						
K-28	182±8.55^{b}	50.0±2.44	0.94±0.03^{a}	0.84±0.04^{c}	0,16±0.021^{a}	4.00±0.20
Nijagara	186±11.1^{b}	47.9±1.52	0.91±0.02^{b}	0.91±0.06^{a}	0,15±0.016^{b}	4.14±0.13
Zuzana	234±9.28^{a}	49.5±2.78	0.90±0.02^{b}	0.86±0.04^{b}	0,15±0.012^{b}	4.24±0.25
ANOVA						
Inoculum	*	*	*	*	*	*
Variety	*	ns	*	*	*	ns
Inoculum x Variety	*	ns	*	*	*	ns

Alfalfa to get back into planetary boundaries


Number of nodules (#/plant)
Treatments
C
R
RMF
MF
K-28
Nijagara
Zuzana
350
300
250
200
150
100
50
h
c
cd
c
b
a
ef
fg
d
g
i
ef


Dry matter yield of shoots (g/plants)
Treatments
C
R
RMF
MF
K-28
Nijagara
Zuzana
gh
h
fgh
a
b
c
d
de
fgh
fgfgh
ef


Protein yield (g/plants)
Treatments
C
R
RMF
MF
K-28
Nijagara
Zuzana
gh
gh
fgh
a
b
c
de
de
fgh
fg
fgh
ef


Dry matter yield of roots (g/plants)
Treatments
C
R
RMF
MF
K-28
Nijagara
Zuzana
e
d
d
b
a
c
f
g
gh
i
hi
gh

Key Findings of the Study

- Rhizobia significantly increased the number of nodules, roots and shoots dry matter yield and protein yield.

- AMF and Co-inoculation played a crucial role in improving mycorrhizal colonization, phosphorus uptake.

- The effectiveness of the inoculants was strain-variety specific, meaning different alfalfa varieties responded differently to the applications.

PCA – General overview

-Control (C)

Are with the lowest values

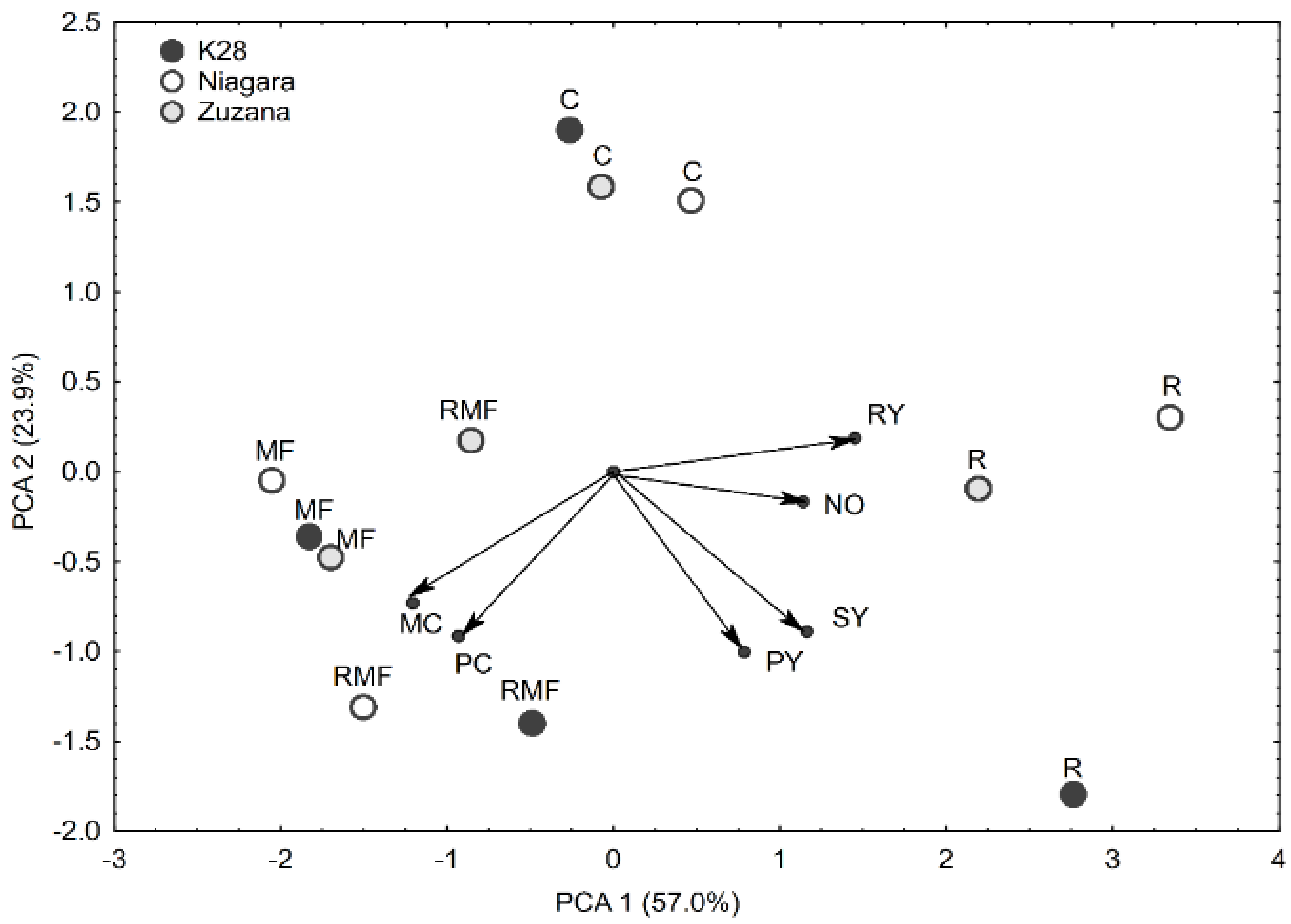
-Rhizobia (R)

Key for Quantity.

-AMF and Co-inoculation (RMF)

Key for Quality.

-Varieties react specifically



Future research should focus

- Field Trials: Conducting studies under agro-ecological conditions to confirm the effectiveness of the inoculation strategy.
- Long-Term Assessment: Evaluating the long-term effects of the inoculation on soil fertility and the sustainability of alfalfa production.
- Breeding/Selection: Selecting more acid-tolerant alfalfa varieties and identifying compatible microbial strains to maximize the benefits of inoculation.

Thank you for your attention

Nedeljko Racić

e-mail: nedeljko.racic@ikbks.com

Institute for Forage Crops, Kruševac

Acknowledgement: Supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia, Project No. 451-03-136/2025-03/200217.