Research Application Summary

Diversity and symbiotic effectiveness of acid tolerant groundnut rhizobia in Western Kenya soils

Onyango, D.A.[1], Were, B.A.[1], Otinga, A.N.[2], Ogega, J.K.[2] & Okalebo, J.R.[2]
[1]Department of Biological Sciences, Chepkoilel University College, P. O. Box 1125-30100, Eldoret, Kenya
[2]Department of Soil Science, Chepkoilel University College, P. O. Box 1125-30100, Eldoret, Kenya
Corresponding author: akinyidorothy@gmail.com

Abstract

This study was carried out to find suitable strains of rhizobia for use in ground nut production under acid-aluminium soils. Thirty-six rhizobia isolates tolerant to high soil aluminium were identified.. The isolates were gram negative rod cells that reduced nitrate to nitrite, urea to ammonia and utilized citrate. Three isolates A6w, W1w and V2w were selected as the most promising for efficient Biological Nitrogen Fixation (BNF) in groundnut. Upon testing them for symbiotic effectiveness on a groundnut monocrop, V2w and A6w had the best performance compared to Biofix, a commercial strain. These two have been converted to a bio-fertilizer form that is under trial by farmers.

Key words: Acid-aluminium tolerant, BNF, groundnut, rhizobia

Résumé

Cette étude a été menée pour trouver des souches appropriées de rhizobiums pour une utilisation dans la production des arachides dans les sols acide- aluminium. Trente-six isolats des rhizobiums tolérants à la grande teneur en aluminium dans le sol ont été identifiés. Les isolats étaient des cellules en bâtonnets Gram-négatives qui ont réduit les nitrates en nitrites, l'urée en ammoniac et ont utilisé le citrate. Trois isolats A6W, W1w et V2W ont été choisis comme les plus prometteurs pour la fixation de l'azote biologique efficace (BNF) dans les arachides. En les testant pour l'efficacité symbiotique sur une monoculture des arachides, V2W et A6W ont eu la meilleure performance par rapport à Biofix, une souche commerciale. Ces deux ont été convertis en une forme de bio-engrais qui est à l'essai par les agriculteurs.

Mots clés: Tolérant à l'acide-aluminium, BNF, arachide, rhizobiums

Background

Crop production in most smallholder farms in Kenya is characterized by continuous cultivation coupled with low input use, resulting in reduced soil fertility and productivity (Wahome,

2007). Inorganic nitrogen fertilization alleviates N deficiency, but is costly and unaffordable to most resource-poor farmers. An alternative is to use rhizobia, known to improve soil N and foster sustainable agriculture through BNF in legumes (Smartt, 1994). The contribution of BNF to increased grain legume productivity relies on successful root colonization by efficient strains of rhizobia. These can be native to the soil or introduced as commercial inoculants.

Literature Summary

Legumes like groundnut (*Arachis hypogea* L.) can meet all their nutrient N needs from BNF in nodules (Graham and Vance, 2000). However, effective nodulation could fail due to the inability of some rhizobia to persist under edaphic stresses such as acidity and related nutrient deficiencies and metal toxicities (Bayoumi *et al*., 1995; Zahran, 1999). Acid-tolerant rhizobia have been identified (Graham *et al*., 1982), which could perform better under acidic soil conditions (Graham *et al*., 1994). Therefore, identification of effective acid tolerant groundnut rhizobia could benefit groundnut production in East African acid soils.

Study Description

Isolation and characterization of nodule rhizobia from groundnut collected at different localities in western Kenya was done in the laboratory. The symbiotic effectiveness of selected isolates was tested on diverse greenhouse grown groundnut cultivars. The effect of rhizobial inoculation and N on biomass cover and grain yield was determined on a groundnut monocrop at two field sites in Western Kenya. The experiment comprised treatments with or without inoculants of promising rhizobial isolates as well as three lime treatments (calcitic lime, dolomitic lime and no lime). Identity of recovered strains was determined from intrinsic antibiotic resistance patterns after nodule harvest. Competitiveness of isolates will be assessed using 16S rRNA gene diversity. The nitrogen fixed will be quantified using the natural ^{15}N abundance method.

Research Application

This project generated knowledge on the diversity of groundnut nodulating rhizobia from parts of western Kenya. Two superior isolates of indigenous groundnut rhizobia that are tolerant to acidity and aluminium toxicity were identified. The study has demonstrated the potential of the rhizobia inoculantsV2w and A6w to improve groundnut yield in acid soils of western Kenya. These have been converted to a bio-fertilizer form that is under trial by farmers.

Table 1. Effect of high Al on growth of 36 rhizobial isolates grown at pH 4.5 for 4 days in a basal solution containing galactose and arabinose.

Isolate	Aluminium concentration			
	0 µM	130 µM	150 µM	200 µM
A2	12 hrs	60 hrs	84 hrs	-
A3	12 hrs	60 hrs	72 hrs	-
A5	24 hrs	72 hrs	84 hrs	-
A6	24 hrs	48 hrs	48 hrs	60 hrs
A7	48 hrs	84 hrs	-	-
b1	36 hrs	72 hrs	84 hrs	-
B3	12 hrs	72 hrs	84 hrs	-
B4	72 hrs	84 hrs	-	-
B6	60 hrs	84 hrs	-	-
Biofix	12 hrs	60 hrs	60 hrs	72 hrs
C6	72 hrs	84 hrs	-	-
D1	12 hrs	84 hrs	84 hrs	-
D4	12 hrs	72 hrs	84 hrs	-
E1	12 hrs	84 hrs	84 hrs	-
F1	12 hrs	84 hrs	-	-
F4	12 hrs	72 hrs	84 hrs	-
F5	36 hrs	60 hrs	72 hrs	-
F8	12 hrs	60 hrs	84 hrs	-
G1	12 hrs	72 hrs	84 hrs	-
g1	36 hrs	72 hrs	84 hrs	-
G7	12 hrs	84 hrs	-	-
h1	12 hrs	84 hrs	-	-
L5	60 hrs	84 hrs	-	-
M2	12 hrs	84 hrs	84 hrs	-
M3	36 hrs	84 hrs	-	-
M4	36 hrs	72 hrs	84 hrs	-
N3	60 hrs	84 hrs	-	-
n3	48 hrs	72 hrs	72 hrs	84 hrs
S1	48 hrs	84 hrs	-	-
S5	48 hrs	84 hrs	84 hrs	-
S7	48 hrs	84 hrs	-	-
S8	72 hrs	84 hrs	-	-
T4	60 hrs	84 hrs	-	-
T5	60 hrs	84 hrs	-	-
V2	36 hrs	48 hrs	60 hrs	60 hrs
W1	48 hrs	72 hrs	84 hrs	84 hrs

Note: Hrs represents time taken (hours) by each isolate to form visible turbidity in a liquid medium; - means the isolate did not show visible turbidity at the given Al level after four days of incubation.

Acknowledgement

The authors are grateful to the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for financial support.

References

Bayoumi, H., Biro, B., Balazsy, S. and Kecskes, M. 1995. Effects of some environmental factors on *Rhizobium* and *Bradyrhizobium strains*. *Acta Microbiol Immunol Hung.*42 :61–69.

Graham, P.H., Viteri, S.E., Mackie, F., Vargasa, T. and Palacios, A. 1982. Variation in acid soil tolerance among strains of *Rhizobium phaseoli*. *Field Crops Res.* 5:121–128.

Graham, P. H., Draeger, K., Ferrey, M. L., Conroy, M. J., Hammer, B. E., Martinez, E., Naarons, S. R. and Quinto, C. 1994. Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR1899. *Can J Microbiol.* 40:198–207.

Graham, P.H. and Vance, C.P. 2000. Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Res.* 65:95-106.

Smartt, J. 1994. Grain legumes: Evolution and genetic resources. Cambridge University Press, London, UK. pp. 124-139.

Wahome, P.K. 2007. The importance and performance of food grain legumes among the smallholder farmers in Machakos district. M.Sc.Thesis, University of Nairobi, Kenya.

Zahran, H.H. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol . Mol. Biol. Rev.* 63(4):968-989.