N2Africa rhizobial isolates in Kenya

Progress report

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N2Africa

Putting nitrogen fixation to work for smallholder farmers in Africa

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1 Introduction

This progress report gives details of the research activities related to rhizobial isolates for *Pisum sativum* in Kenya. These activities were undertaken from late 2015 to March 2016. It contains information about:

1. Identification of elite indigenous rhizobia nodulating pea (*Pisum sativum* L.);
2. Preparation and distribution of elite soyabean NAK isolates for regional evaluation;
3. Results from the field trail for evaluation of nine elite pea rhizobial isolates;
4. Quality control of Biofix inoculants samples from the MEA Fertilizer Limited, Nakuru, Kenya.

2 Identification of elite indigenous rhizobia nodulating pea (*Pisum sativum* L.) in vermiculite

Rhizobial isolates were recovered from cultivated legume hosts (Figure 1.1) including *Pisum sativum* (snow pea, garden pea and snap pea), *Vicia faba* (broad bean) and *Phaseolus coccineus* (Runner bean) growing in different agro-ecological zones and from nodules of snow pea inoculated with soils from the same regions namely;

- Agro-Alphine zone of Mt. Kenya (Narumoru);
- High Potential zones surrounding Aberdare Ranges (Kinangop) and Mt. Kenya (Timau) and;
- Semi-arid zones of Laikipia and Naivasha.

Sixty six (66) rhizobial isolates were recovered from nodules of the cultivated legumes and nine (9) from peas inoculated with soils from Central Kenya. They were tested for genetic compatibility and nitrogen fixing ability with snow pea (*Pisum sativum* L.) as the host legume, in greenhouse pots using horticultural grade vermiculite reducing the number of isolates to twenty four (24).

Figure 2.1: Pictures of different legumes from which were collected during the bio-prospecting survey in Laikipia, Naivasha and Narumoru farms.

Sixty six (66) rhizobia were isolated, out of which twenty four (24) rhizobia outperformed the commercial strain USDA 3456 on garden pea and snow pea. They are NAK 388, 389, 391, 392, 393, 395, 396, 390, 394, 397, 409, 417, 434, 439, 440, 448, 450, 451, 452 and 453.

2.1 Performance of indigenous isolates in greenhouse potted soil from Kabete

The effectiveness of the 24 rhizobial isolates were evaluated using two varieties (snow pea var. Oregon Pod II, for export market, and garden pea var. Green Feast, for local consumption) in greenhouse potted soil from Kabete field station. The soil from Kabete was analysed for its nutrient content following Okalebo *et al.* (2002). The isolates were maintained in Yeast Extract Mannitol Agar slants and grown in Yeast Extract Mannitol nutrient broth. The experiment was arranged as a Complete Randomized Design (CRD) replicated four times.

Treatments included the rhizobial isolates, a plus-nitrogen control with no inoculation, a non-inoculated control with no nitrogen and a reference strain (USDA 3456). The pots were sterilized and filled with sterile gravel at the bottom layer and two kilogramme soil. Rhizobia isolates and the reference strain (USDA 3456) were cultured seven days prior to planting to attain approximately 1 x 10^9 cells ml^{-1}. Surface were sown in sterile horticultural grade vermiculite pre-germinated in shallow autoclavable polypropylene tray and then incubated at 28^0C for 2 days until the radicles were 0.5 - 1.0 cm long and ready for planting. At planting, the soil in all pots was fertilized with Sympal, a commercially-available fertilizer blend for legumes (0% N, 23% P, 15% K, 10% Ca, 4% S, 1% Mg and 0.1% Zn) at a rate of 250 kg ha^{-1}. Planting was done by placing three well germinated seeds in each of the well-spaced holes (about 5 cm) in each pot and covering them with the potted soil. Inoculation was done 5 days after planting by dispensing 1 ml of rhizobial broth around the root of each plant in each pot. Emerging seedlings were thinned to two uniform plants per pot after 10 days. Pots were regularly irrigated with tap water.

Destructive sampling was done at 48 days after planting where nodules were recovered from the roots, counted and data on number of nodules per plant recorded. Shoots and nodules were oven dried for 48 hours at 70 ºC and dry weight recorded. The analysis was performed using GENSTAT 14 statistical package (GenStat Release 6.1 Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK). Analysis of variance (ANOVA) was used for evaluation of symbiotic properties of rhizobia. To compare treatment means, Fisher's protected LSD method was used at a significance level of 5%.

2.2 Field evaluation of best performing isolates from the greenhouse potted soil

Nine (9) best performing isolates were selected from the potted soil experiments for further assessment in the field under at the university field station (Table 2.1). The top soils (0–15 cm) from the field site were sampled prior to planting by sampling randomly eight cores per replicate using a 3.5 cm soil auger. They were bulked and sub-sampled. Chemical characterization of soils will be done for nutrient composition as described by Okalebo *et al.* (2002).

Table 2.1: Rhizobia isolates and reference strain to be evaluated with either or both of the pea cultivars.

	Pea cultivar	
	Snow pea	**Garden pea**
Rhizobia isolates	NAK 434	NAK 390
	NAK 417	NAK 450
	NAK 390	NAK 451
	NAK 451	NAK 394
		NAK 439
Standard reference strain	USDA 2447	USDA 2447

The experiment was arranged as a Complete Randomized Design (CRD) with four replicates for each pea cultivar (snow pea var. Oregon Pod II and garden pea var. Green Feast). Each plot (3 m by 3 m) consisted of five rows spaced 0.6 m apart. Plots were 0.5 m apart and the distance between the varieties was 1 m. Inoculation treatments included indigenous isolates, two non-inoculated controls with and without N and a standard reference strain (USDA 2447).

All plots received a basal dressing of DAP (18.46.0) at a rate of 130 kg ha^{-1} to provide a starter N at a rate of 20 kg N ha^{-1} and P at a rate of 60 kg P ha^{-1}. The + N treatment receive DAP at the same rate (130 kg ha^{-1}) and an additional 130 kg ha^{-1} urea, split in two doses (pre-planting and pre-flowering) to provide N at the rate of 80 kg ha^{-1} and P at 60 kg P ha^{-1} rate. These were applied in the furrow and incorporated to a depth of 3 cm. The seeds were inoculated with liquid-based inoculant using a solution of gum arabic (40%, w/v), as a sticker, and planted immediately at spacing of 10 cm between plants.

The first sampling will be done at 40 days from planting at flowering stage when nodules will be recovered as well as biomass and at crop maturity, for biomass and grain yields. counted and number of nodules per plant recorded. Shoots and grains will be oven dried for 48 hours at 70 ºC and dry weights recorded. The analysis will be performed using GENSTAT 14 statistical package (GenStat Release 6.1). Analysis of variance (ANOVA) will be used for evaluation of symbiotic properties of rhizobia and treatment means comparison will be determined using the Fisher's protected LSD method at a significance level of 5%.

2.3 Results: performance of selected isolates in greenhouse potted Kabete soil

The chemical characteristics of soil from Kabete used in the greenhouse are indicated in Table 2.2. The soil was strongly acidic with low P.

Table 2.2: Chemical characteristics of the greenhouse potted soil collected at Kabete.

	pH	C	N	P
Chemical characteristics soil collected at Kabete	H2O	----------%----------		(ppm)
	4.76	2.49	0.32	10.75

Inoculation of pea cultivars with different rhizobia isolates did not significantly differ in terms of nodulation. Inoculation with the reference strain USDA 3456 did not nodulate the pea cultivars (Table 2.3). There were no interactions observed between the rhizobia isolates and the pea cultivars in relation to nodulation as indicated in Table 2.3. The non-inoculated control had no nodule, indicating absence compatible native rhizobia for pea.

Table 2.3: Nodule number for pea cultivars inoculated with rhizobia isolates grown in potted soil from Kabete.

Treatment	Snow pea	Garden pea
NAK 395	20	0
NAK 397	19	22
NAK 393	15	14
NAK 392	14	0
NAK 394	12	9
NAK 409	10	0
PROF	4	0
NAK 390	3	14
MINUS N	0	0
NAK 391	0	1
NAK 434	0	0
PLUS N	0	0
NAK 396	0	7
USDA 3456	0	0
Lsd	21	24
p-value	0.258	0.546
p-value for rhizobial inoculation		0.186
p-value for variety (rhizobial inoculation)		0.325
p-value for rhizobial inoculation × variety		0.755

Rhizobia inoculations were significantly different (P=0.014) in relation to plant biomass (Table 2.4). A significantly difference (P<.001) between the two varieties was observed with snow pea fixing more nitrogen than garden pea. Biomass yield also showed a significant interaction (P=0.002) between the rhizobia treatments and the pea cultivars, suggesting a host-strain specificity (Table 2.4).

For example, NAK 434 and 394 gave a higher plant biomass with garden pea as compared to snow pea and NAK 393 and NAK 392 gave a higher plant biomass with snow pea as compared to garden pea (Table 2.4). NAK 397 performed equally well with both pea cultivars in relation to plant biomass. The reference strain, USDA 3456, performed poorly with both pea cultivars in relation to both nodule number and biomass yield, which raises concern of the inoculants being distributed to farmers. As a result, this strain has been replaced by MIRCEN. The N-fertilized treatment gave the highest plant biomass yield, indicating that none of the strains met the N required by the pea crop.

Table 2.4: Plant biomass for pea cultivars grown in potted Kabete soil under greenhouse conditions.

Treatment	Plant biomass for snow pea	Plant biomass for garden pea
	Shoot dry weight/plant (g)	
PLUS N	2.97 a	2.70 a
NAK 393	2.52 ab	0.76 cde
NAK 397	2.49 ab	1.33 bc
NAK 392	2.28 abc	0.59 cde
NAK 396	2.28 abc	0.95 bcde
NAK 395	2.16 abc	0.94 cde
NAK 390	1.48 bcd	0.65 cde
USDA 3456	1.44 bcd	0.98 bcd
NAK 394	1.13 cd	1.28 bc
NAK 391	1.08 cd	1.02 bcd
NAK 434	0.75 d	1.71 b
NAK 409	0.52 d	0.48 de
PROF	0.45 d	0.70 cde
MINUS N	0.27 d	0.21 e
Lsd	1.26	0.77
p-value	0.002	<0.001
p-value for rhizobial inoculation		0.014
p-value for variety (rhizobial inoculation)		<.001
p-value for rhizobial inoculation × variety		0.002

2.4 Results: performance of selected isolates in greenhouse potted Njambi-ini soil

Nodulation of both pea cultivars was significantly influenced (P=0.004) by the different rhizobia inoculations. NAK 451, 448, 450, 434 and 390 gave the highest nodule number, respectively, while NAK 391, 440, 396 and USDA 2447 (replacement) gave the least, respectively. Rhizobia inoculation did not yield any varietal difference and no significant interactions between the cultivars and the rhizobia isolates in relation to nodulation as indicated in Table 2.5. The N-fertilized treatment gave high nodule count.

Table 2.5: Nodule number for pea cultivars inoculated with rhizobia isolates grown in potted soil from Njambi-ini.

Treatment	Nodule number for garden pea	Nodule number for snow pea
Plus N	30	10 bcde
Minus N	3	5 bcde
USDA 2447	22	1 de
NAK 390	20	37 ab
NAK 391	0	0 e
NAK 392	24	20 bcde
NAK 394	31	8 bcde
NAK 395	27	18 bcde
NAK 396	17	6 bcde
NAK 417	13	35 ab
NAK 434	23	34 abc
NAK 439	52	1 de
NAK 440	0	3 cde
NAK 448	37	17 bcde
NAK 450	33	31 abcd
NAK 451	41	58 a
LSD	30	32
p-value	0.078	0.04
p-value of N-source		0.002
p-value of variety		0.615
p-value of N-source × variety		0.074
p-value for rhizobial inoculation		0.004

Rhizobia inoculation did not significantly differ in relation to biomass yield with both pea cultivars and there were no significant interactions between the rhizobia inoculation and the pea varieties as shown in Table 2.6. However, garden pea showed a significant difference between the rhizobia isolates, indicating their varying symbiotic capability (Table 2.6). NAK 439 and 450 gave a higher plant biomass yield compared to N-fertilized treatment with garden pea and snow pea, respectively, indicating their superior performance in fixing nitrogen. The biomass yield between the pea cultivars was significantly different ($P<0.001$), with plant biomass varying from 6.55 g plant^{-1} to 5.34 g plant^{-1} for snow pea and garden pea, respectively.

Table 2.6: Plant biomass for pea varieties grown under uncontrolled conditions in potted soil collected from Njambi-ini.

Treatment	Plant biomass for garden pea	Plant biomass for snow pea
Plus N	5.009 abc	7.56
Minus N	4.897 cd	5.93
USDA 2447	6.467 ab	5.89
NAK 390	5.463 bcd	7.09
NAK 391	5.624 bcd	6.26
NAK 392	4.969 cd	6.45
NAK 394	5.119 cd	5.34
NAK 395	4.597 d	6.06
NAK 396	4.559 d	6.69
NAK 417	5.303 bcd	6.73
NAK 434	5.543 bcd	6.86
NAK 439	6.879 a	6.82
NAK 440	5.399 bcd	5.87
NAK 448	4.88 cd	6.51
NAK 450	5 cd	7.62
NAK 451	5.154 cd	7.11
LSD	1.208	1.81
p-value	0.03	0.45
p-value for rhizobial inoculation		0.227
p-value for variety (rhizobial inoculation)		<.001
p-value for rhizobial inoculation × variety		0.199

3 Preparation and distribution of elite soyabean rhizobial isolates for regional evaluation (using MEA networks)

The distribution of the superior Kenyan rhizobia isolates, including NAK 84, 89 and 128 forming effective symbiosis with soyabean (Waswa *et al.,* 2014) for further comparison under different agro-ecological zones, is still continuing. Sterilized filter mud based inoculants of the soyabean isolates have been distributed to several countries including Uganda, Rwanda, Tanzania, Zambia and Mozambique. The distribution is done by different institutions in collaboration with MEA fertilizers Limited. Results from these trials will be presented in the next report.

4 Results from the field trail for evaluation of nine (9) elite pea rhizobial isolates

Results from the field trail for evaluation of nine (9) elite pea rhizobial isolates will appear.

5 Quality control of Biofix (commercial rhizobial inoculants) from MEA Fertilizer Limited, Kenya

MIRCEN laboratory at the University of Nairobi has continued with Quality Control assessment of the commercial inoculants produced at the MEA fertilizer Limited, Kenya. Batches produced for use during the Long Rains 2016 (March-August) were sampled on April 21th, 2016. The results are presented in Table 4.6. Two samples were randomly picked from each batch to determine the number of viable rhizobia and contaminants through the drop-plate method described by Miles and Misra (1938). The batches met the minimum standards for viable rhizobia of 1×10^9. However, the inoculants consistently recorded high populations of contaminants, though they were not beyond the threshold of 10^7 in reference to the Colony Forming Units (CFUs) (Table 5.1).

Table 5.1: Quality control results for inoculant batches produced for use during the Long Rains 2016 (March-August).

Crop	Batch number	CFU	Contamination CFU
Pea 50g	20011602P	9.00×10^9	5.50×10^7
Pea 50g	20011602P	6.15×10^9	3.00×10^7
Lucerne 50g	04021602L	4.85×10^9	2.85×10^7
Lucerne 50g	04021602L	7.35×10^9	8.50×10^6
Desmodium 50g	01021602D	6.35×10^9	4.15×10^7
Desmodium 50g	01021602D	6.15×10^9	3.35×10^7
Bean 50g	05031602B	3.35×10^9	4.50×10^7
Bean 50g	05031602B	4.35×10^9	2.50×10^7
French bean	21011602B	7.15×10^9	3.00×10^7
French bean	21011602B	6.50×10^9	3.50×10^7
Green gram 50g	15011602G	7.50×10^9	4.50×10^7
Green gram 50g	15011602G	7.50×10^9	1.65×10^7
Soyabean 50g	05031602S	6.85×10^9	1.65×10^7
Soyabean 50g	05031602S	6.50×10^9	2.00×10^7
Bean 150g	1301102B	3.50×10^9	3.15×10^7
Bean 150g	13011602B	5.15×10^9	1.00×10^7
Bean 20g	04031602B	6.35×10^9	1.00×10^7
Bean 20g	04031602B	5.35×10^9	2.15×10^7
Pea 20g	20011602P	3.65×10^9	2.65×10^7
Pea 20g	20011602P	3.50×10^9	5.50×10^7
Soyabean 150g	18031602S	3.50×10^9	4.15×10^7
Soyabean 150g	18031602S	6.35×10^9	2.85×10^7
Bean 10g	1201102B	6.00×10^9	4.35×10^7
Bean 10g	1101102B	3.15×10^9	4.00×10^7
Green gram 50g	05011602G	8.00×10^9	NIL
Green gram 50g	05011602G	6.50×10^9	NIL

The level of contaminants continues to be a major concern on the quality of MEA BIOFIX fertilizer. MIRCEN has held meetings with laboratory staff, but no improvement has been achieved. However, there is hope once the new factory starts operating. They have also been asked to involve KEMRI or KEPHIS human pathological tests and we are waiting for MEA's response.

6 References

Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (2002). *Laboratory methods for soil and plant analysis. A working manual. 2nd ed. Tropical soil fertility and Biology program*, Nairobi Kenya. TSBF-CIAT and SACRED Africa, Nairobi Kenya, pp. 128.

List of project reports

1. N2Africa Steering Committee Terms of Reference
2. Policy on advanced training grants
3. Rhizobia Strain Isolation and Characterisation Protocol
4. Detailed country-by-country access plan for P and other agro-minerals
5. Workshop Report: Training of Master Trainers on Legume and Inoculant Technologies (Kisumu Hotel, Kisumu, Kenya-24-28 May 2010)
6. Plans for interaction with the Tropical Legumes II project (TLII) and for seed increase on a country-by-country basis
7. Implementation Plan for collaboration between N2Africa and the Soil Health and Market Access Programs of the Alliance for a Green Revolution in Africa (AGRA) plan
8. General approaches and country specific dissemination plans
9. Selected soyabeans, common beans, cowpeas and groundnuts varieties with proven high BNF potential and sufficient seed availability in target impact zones of N2Africa Project
10. Project launch and workshop report
11. Advancing technical skills in rhizobiology: training report
12. Characterisation of the impact zones and mandate areas in the N2Africa project
13. Production and use of rhizobial inoculants in Africa
18. Adaptive research in N2Africa impact zones: Principles, guidelines and implemented research campaigns
19. Quality assurance (QA) protocols based on African capacities and international existing standards developed
20. Collection and maintenance of elite rhizobial strains
21. MSc and PhD status report
22. Production of seed for local distribution by farming communities engaged in the project
23. A report documenting the involvement of women in at least 50% of all farmer-related activities
24. Participatory development of indicators for monitoring and evaluating progress with project activities and their impact
25. Suitable multi-purpose forage and tree legumes for intensive smallholder meat and dairy industries in East and Central Africa N2Africa mandate areas
26. A revised manual for rhizobium methods and standard protocols available on the project website
27. Update on Inoculant production by cooperating laboratories
28. Legume Seed Acquired for Dissemination in the Project Impact Zones
29. Advanced technical skills in rhizobiology: East and Central African, West African and South African Hub
30. Memoranda of Understanding are formalized with key partners along the legume value chains in the impact zones
31. Existing rhizobiology laboratories upgraded
32. N2Africa Baseline report
33. N2Africa Annual country reports 2011
34. Facilitating large-scale dissemination of Biological Nitrogen Fixation

35. Dissemination tools produced
36. Linking legume farmers to markets
37. The role of AGRA and other partners in the project defined and co-funding/financing options for scale-up of inoculum (banks, AGRA, industry) identified
38. Progress Towards Achieving the Vision of Success of N2Africa
39. Quantifying the impact of the N2Africa project on Biological Nitrogen Fixation
40. Training agro-dealers in accessing, managing and distributing information on inoculant use
41. Opportunities for N2Africa in Ethiopia
42. N2Africa Project Progress Report Month 30
43. Review & Planning meeting Zimbabwe
44. Howard G. Buffett Foundation – N2Africa June 2012 Interim Report
45. Number of Extension Events Organized per Season per Country
46. N2Africa narrative reports Month 30
47. Background information on agronomy, farming systems and ongoing projects on grain legumes in Uganda
48. Opportunities for N2Africa in Tanzania
49. Background information on agronomy, farming systems and ongoing projects on grain legumes in Ethiopia
50. Special Events on the Role of Legumes in Household Nutrition and Value-Added Processing
51. Value chain analyses of grain legumes in N2Africa: Kenya, Rwanda, eastern DRC, Ghana, Nigeria, Mozambique, Malawi and Zimbabwe
52. Background information on agronomy, farming systems and ongoing projects on grain legumes in Tanzania
53. Nutritional benefits of legume consumption at household level in rural sub-Saharan Africa: Literature study
54. N2Africa Project Progress Report Month 42
55. Market Analysis of Inoculant Production and Use
56. Identified soyabean, common bean, cowpea and groundnut varieties with high Biological Nitrogen Fixation potential identified in N2Africa impact zones
57. A N2Africa universal logo representing inoculant quality assurance
58. M&E Workstream report
59. Improving legume inoculants and developing strategic alliances for their advancement
60. Rhizobium collection, testing and the identification of candidate elite strains
61. Evaluation of the progress made towards achieving the Vision of Success in N2Africa
62. Policy recommendation related to inoculant regulation and cross border trade
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64. Linking communities to legume processing initiatives
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68. Review of conditioning factors and constraints to legume adoption and their management in Phase II of N2Africa
69. Report on the milestones in the Supplementary N2Africa grant
70. N2Africa Phase II Launch in Tanzania
71. N2Africa Phase II 6 months report
72. Involvement of women in at least 50% of all farmer related activities
73. N2Africa Final Report of the First Phase: 2009-2013
74. Managing factors that affect the adoption of grain legumes in Uganda in the N2Africa project
75. Managing factors that affect the adoption of grain legumes in Ethiopia in the N2Africa project
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