Optimizing Biological Nitrogen Fixation Inexpensively as Part of a Sustainable Agriculture Kit (SAK) Strategy to Assist Subsistence Farmers

GIFS Conference, Saskatoon, Canada June 14-16, 2016

Malinda Thilakarathna[1], Tejendra Chapagain[1], Roshan Pudasain[2], Bhawana Ghimire[2], Ram B. Rana[2] and **Manish N. Raizada[1]**

[1] Department of Plant Agriculture, University of Guelph, Guelph, Canada

[2] LI-BIRD, Pokhara, Nepal

Funded by the Canadian International Food Security Research Fund (CIFSRF)

Global Affairs Canada Affaires mondiales Canada

IDRC | CRDI

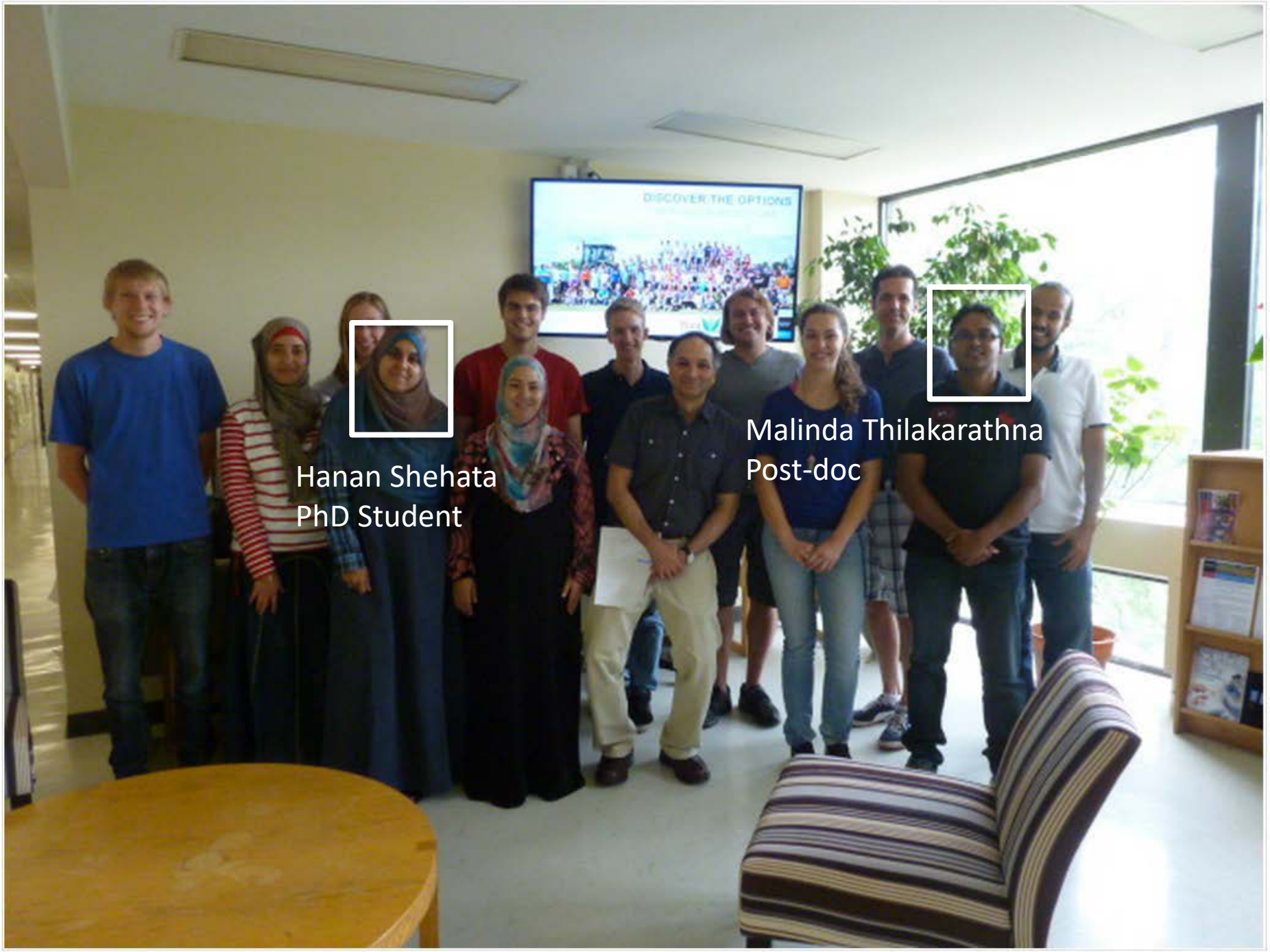
International Development Research Centre

Centre de recherches pour le développement international

Canada

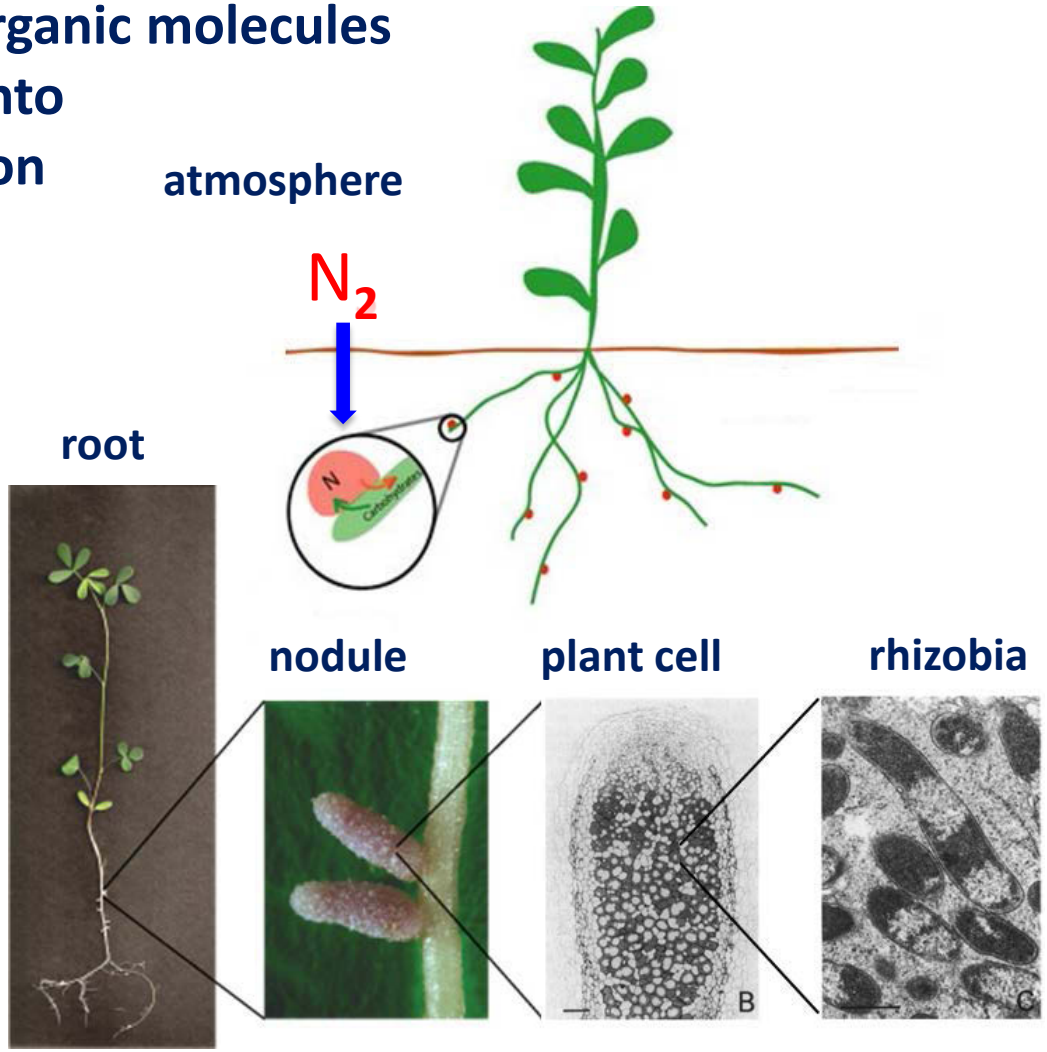
University of Guelph

- 1hr drive from Toronto, Canada
- Canada's oldest and largest agricultural university
- ranked #9 in agricultural research output (globally) and #1 in Canada for inventions per faculty
- ~20,000 students


DISCOVER THE OPTIONS
Hanan Shehata
PhD Student
Malinda Thilakarathna
Post-doc

Globally 75% of malnutrition is in rural areas of which amino acid deficiency is especially problematic. Nitrogen fertilizers are also expensive.

Symbiotic Nitrogen Fixation (SNF) - Rhizobia bacteria inside legume root nodules convert atmospheric N_2 gas into ammonia to <u>build protein</u>, chlorophyll and other organic molecules which can be released into soil during decomposition as <u>organic fertilizer</u>



How can SNF be improved to help farmers, especially smallholder farmers?

Problem 1: Sub-optimal rhizobia in soil
Solution: Coat seeds or spray soil with compatible/improved rhizobia bacteria (technology called "rhizobia inoculant")

Problem 2: Poor crop variety
Solution: Breed/select legumes with improved SNF
(e.g. more active nodules, or resistance to drought stress)

Problem 3: Low micronutrients in soil (or P fertilizer)
Solution: Add fertilizers to the soil (Mo, B)

For all of these, one needs to diagnose the problem and test different possible solutions (e.g. test many rhizobia strains)

Current methods for assessing SNF and their limitations

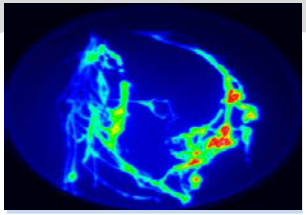
- Dry matter yield method (DM)
- Total N difference method
- Nodule observations
- Acetylene reduction assay (ARA)
- Hydrogen evolution
- Xylem-solute technique
- ^{15}N isotope (%Ndfa)***

Limitations

- Vary in reliability
- Time consuming
- Expensive ($10-20 per sample)
- Can analyze only few samples at a time
- Difficult to examine nodule to nodule variation

We need an efficient, low cost method to measure SNF in developing nations

Outline



1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the *GlnLux* biosensor

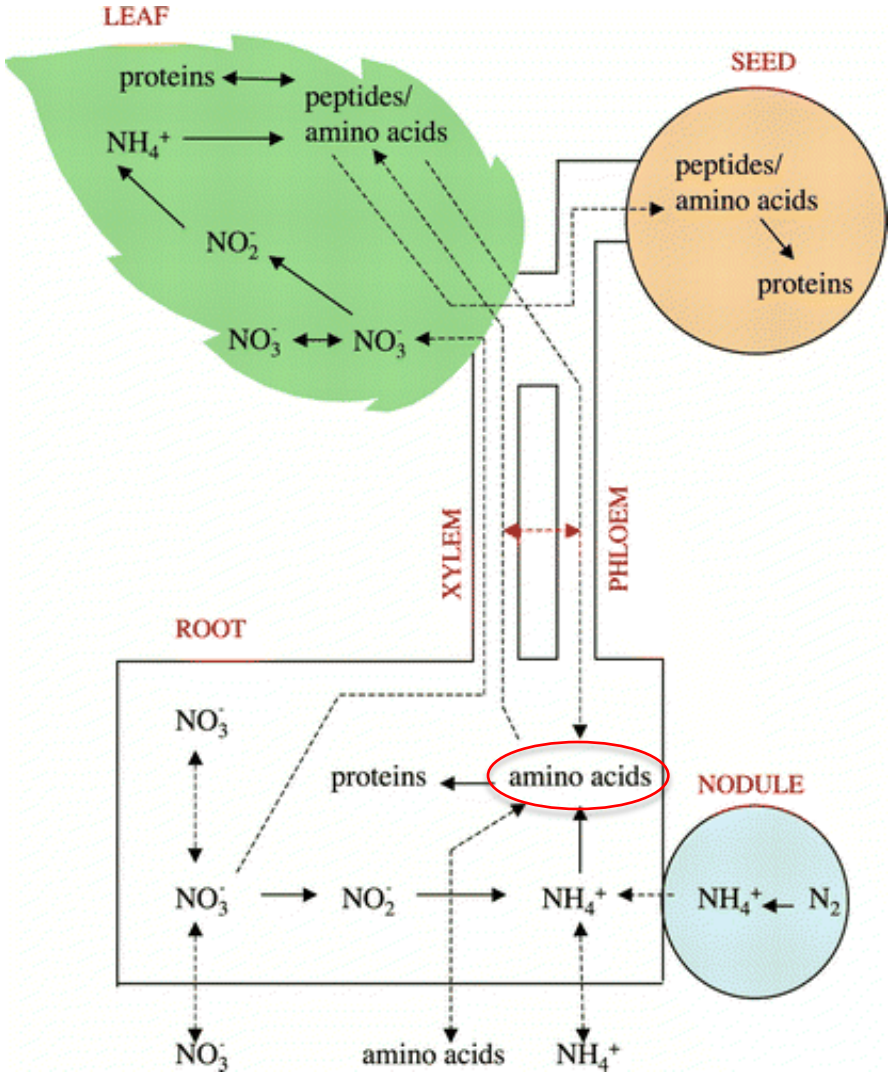
1.2. Detection of SNF in colonies of rhizobia *in vitro*

1.3. Detection of SNF in legumes *in planta*

2. Helping farmers to overcome barriers to maximize legume production

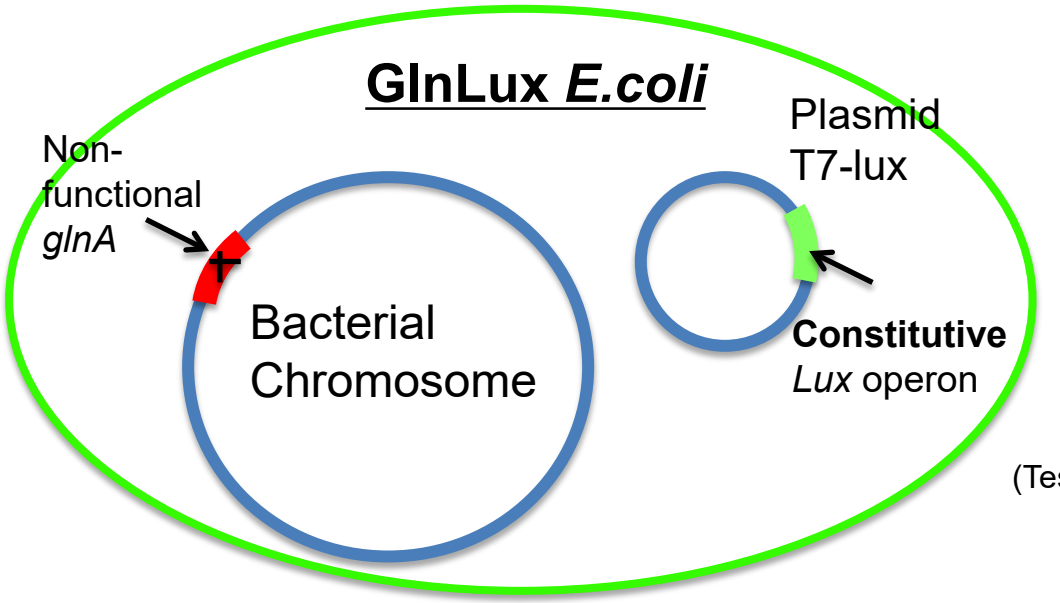
3. The Sustainable Agriculture Kit (SAK) strategy

Legumes – a portion of fixed nitrogen is transferred to leaves as amino acids such as glutamine (Gln)

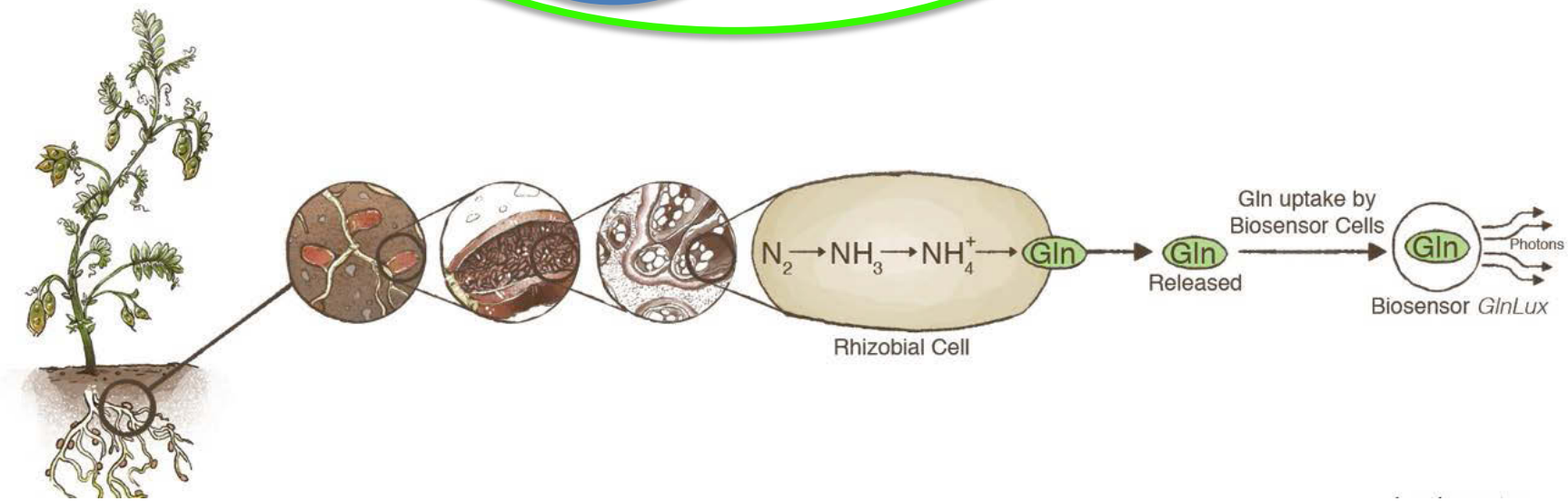


Williams and Miller (2001)

The *GlnLux* biosensor is an *E.coli* auxotroph that detects the amino acid glutamine, grows and releases measurable photons



(Tessaro and Raizada, 2012)



GlnLux Agar Assay for High-Throughput Screening Bacterial Colonies for N-fixation (1-2 h protocol)

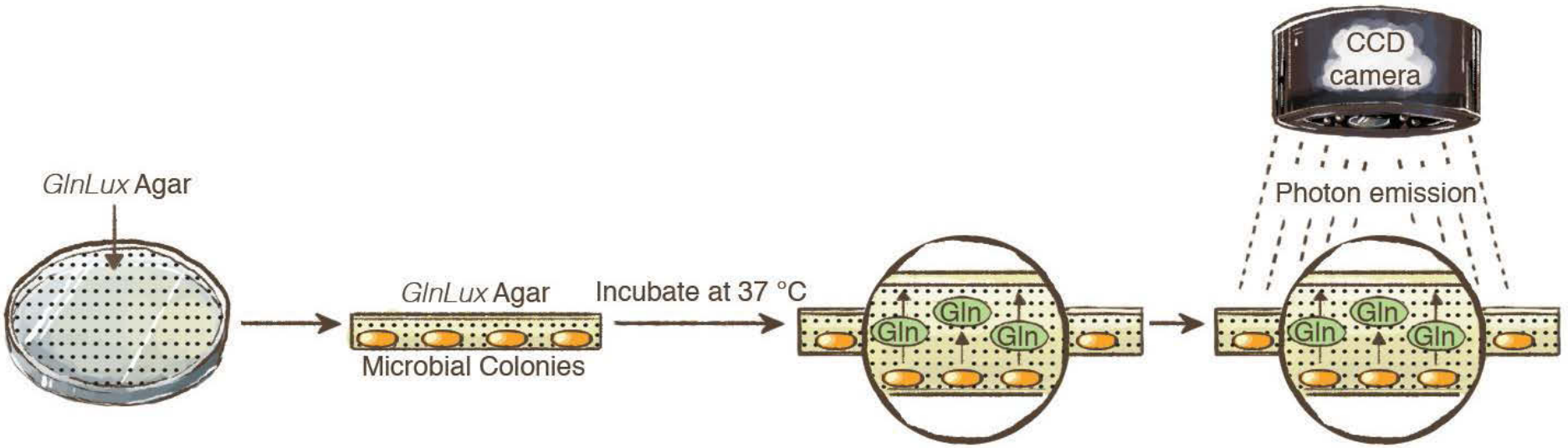
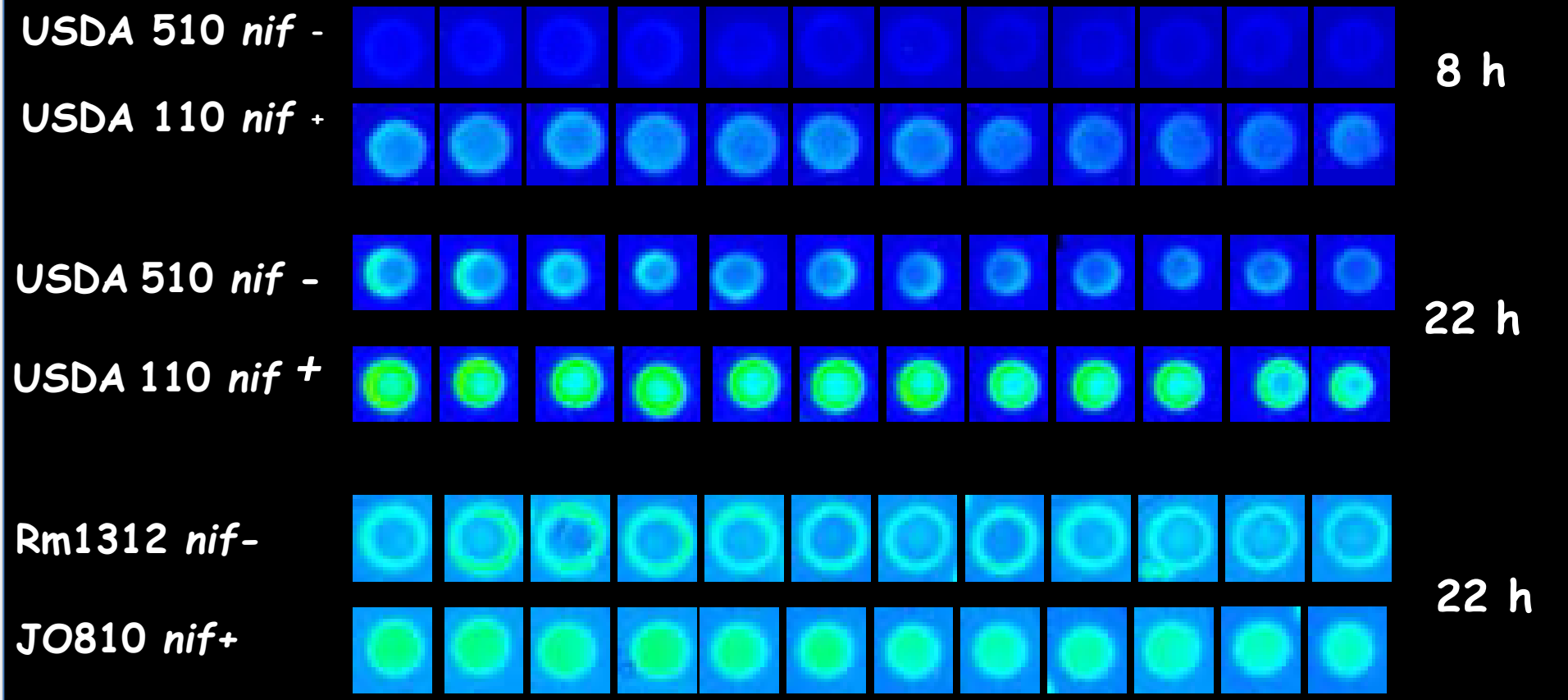


Illustration by Lisa Smith

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation

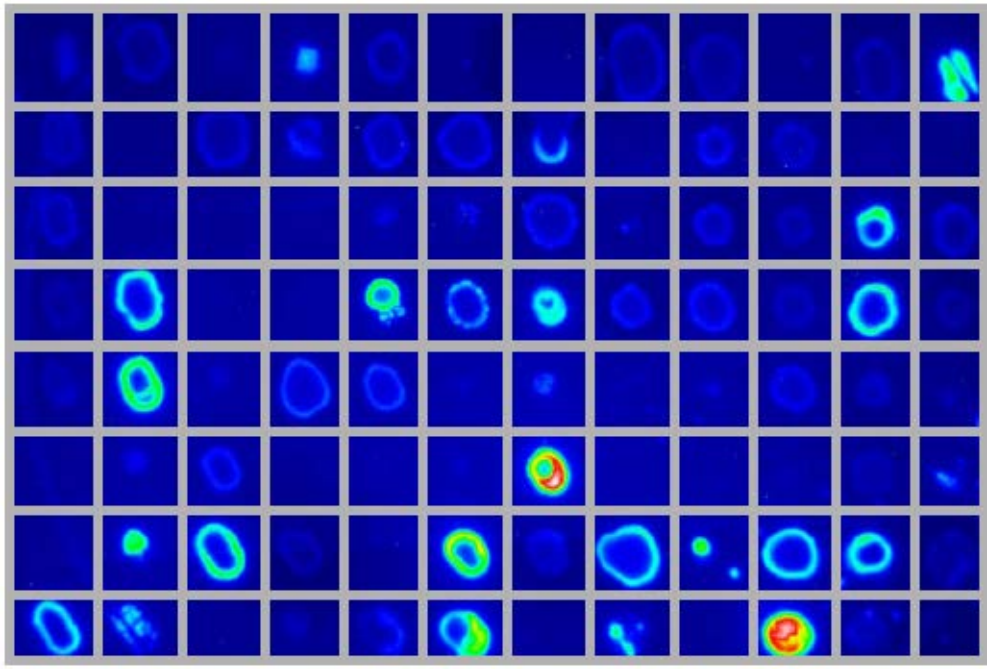
The GlnLux biosensor can detect nitrogen fixation output in single colonies on agar



Colonies of *Bradyrhizobium japonicum* (510,110) and *Sinorhizobium meliloti* (1312, JO810) wild type versus mutant nif strains on GlnLux agar after incubation for 8-22 hrs. Images were taken using CCD camera using a 600 sec exposure.

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation

GlnLux agar technology was used to detect nitrogen fixation in bacterial endophytes isolated from maize seeds



Detection of BNF	Number of endophyte strains
Total GlnLux +	**54** (out of 96)
GlnLux + and ARA or DBH +	53
GlnLux + and ARA or DBH -	1 (possible false positive)
GlnLux - and ARA or DBH +	**5** (possible false negatives)
Total	**59**

ARA
acetylene reduction assay

DBH
Dot blot hybridization with *nif* probe

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation

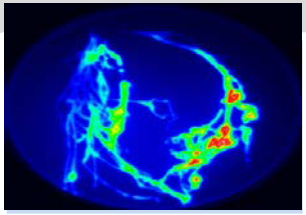
GlnLux assays permit high throughput screening of *in vitro* nitrogen fixation

- Thousands of colonies can be screened in a single day inexpensively and rapidly

- However, there are capital costs

- May enable screening for:
  --new nitrogen fixing bacteria
  --selection of inoculants (e.g. directed evolution) for improved nitrogen fixation under stress conditions or specific niches

Outline



1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the *GlnLux* biosensor

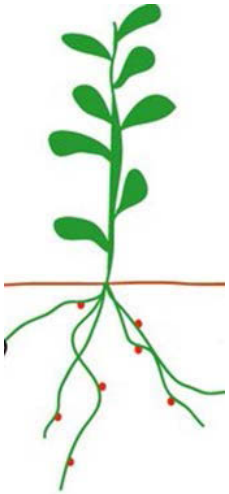
1.2. Detection of SNF in colonies of rhizobia *in vitro*

1.3. Detection of SNF in legumes *in planta*

2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy

Current problem is detecting extent of low activity nodules within a root system (e.g. due to stress): *GlnLux* Agar Assay for Detecting SNF Output at Nodule Scale Resolution (1-2 h protocol) for any Legume Species/Variety-Rhizobia Combination



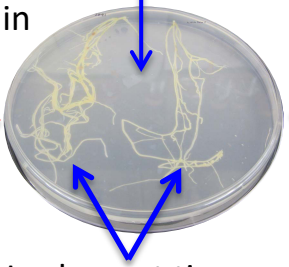
Grow plants

without N

or only starter N

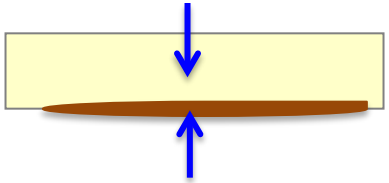
Freeze roots in -80 °C

GlnLux Agar



Leaky root tissue

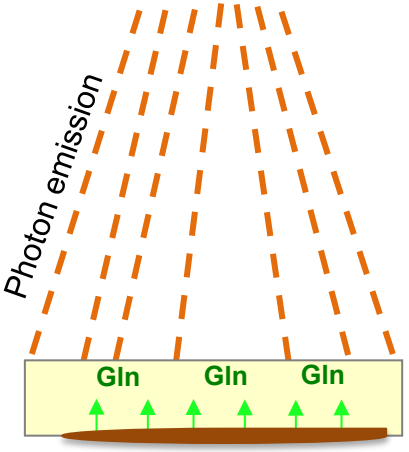
GlnLux-agar

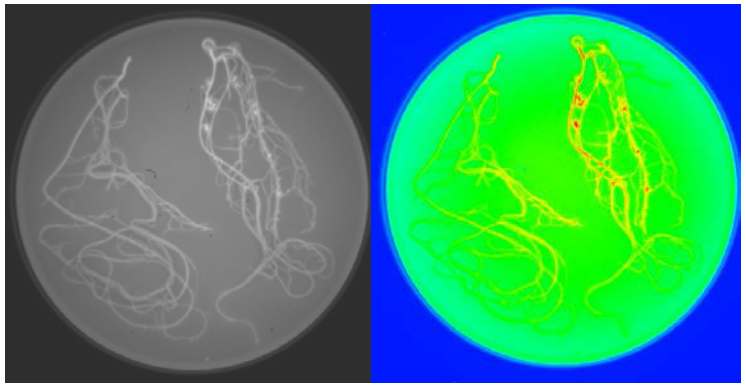


Leaky root tissue

Incubate At 37 °C

Photon emission

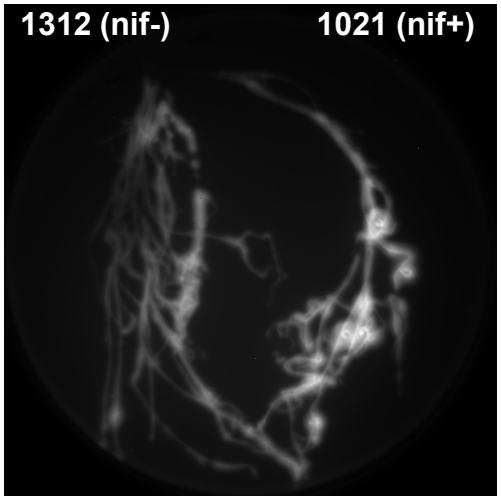


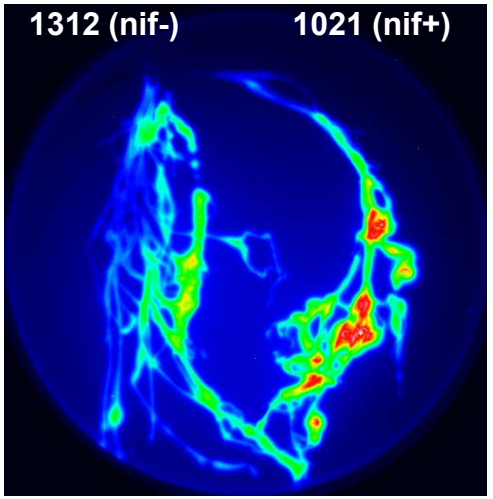


Methodology: Primarily indoors without added N (except starter N)

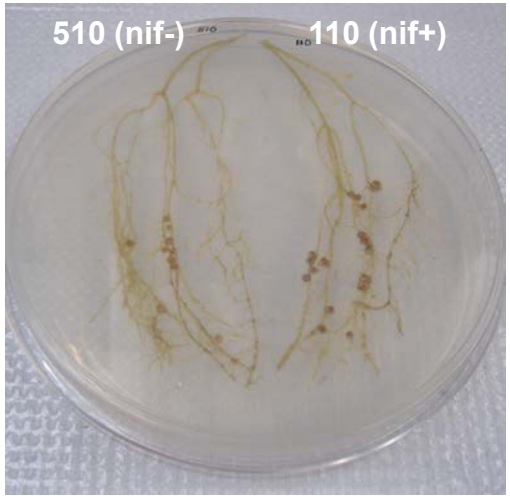
Identification of Active Sites of SNF using Split Root Systems (wild-type vs *nif* mutant rhizobia)

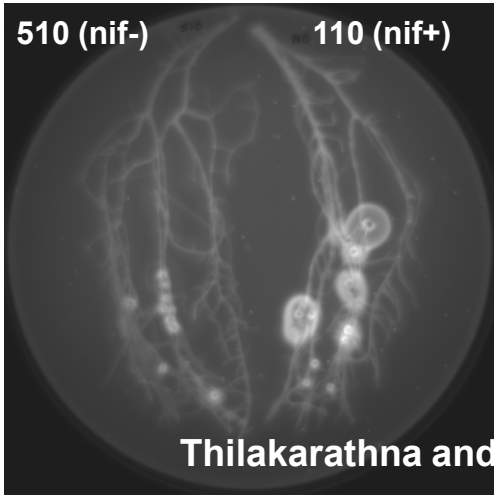
Green peas (*Pisum sativum* L.)

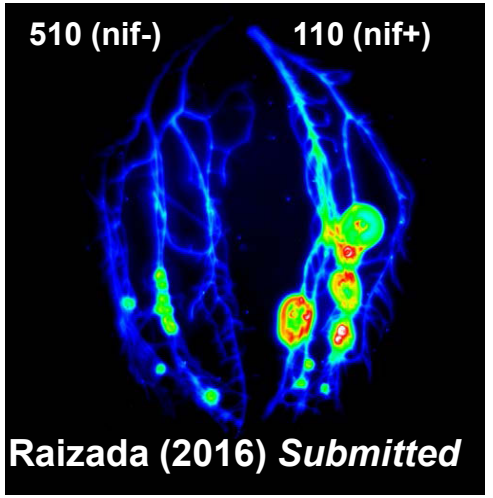




Soybean (*Glycine max* (L.) Merr.)



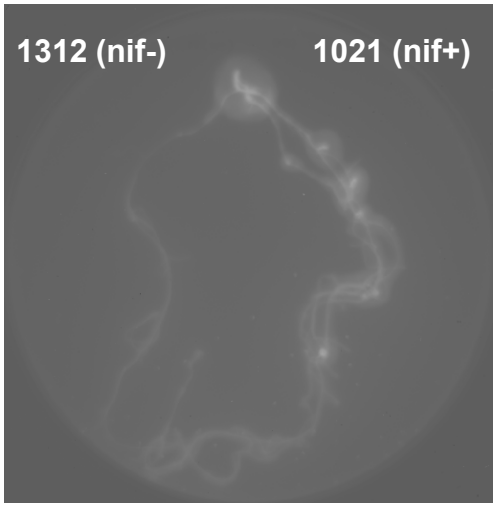


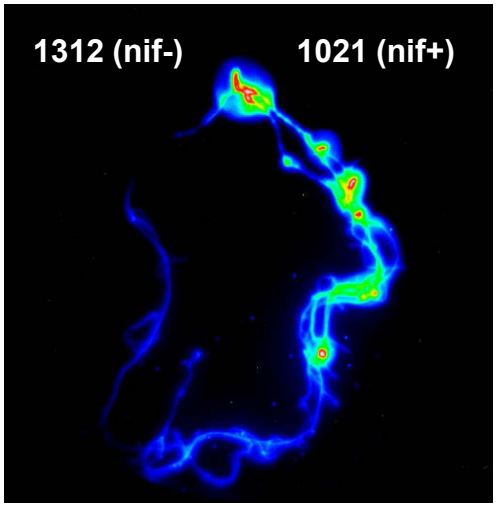


Thilakarathna and Raizada (2016) *Submitted*

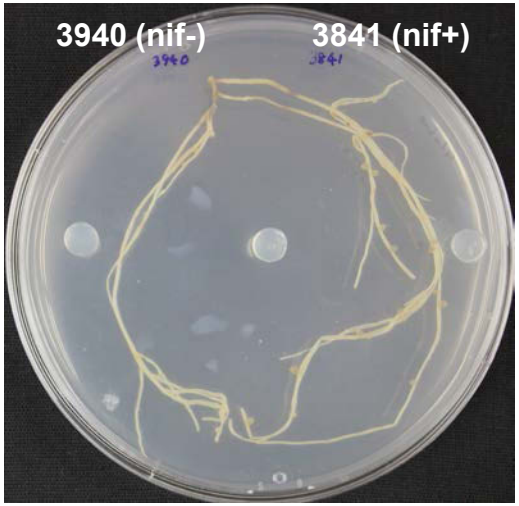
Identification of Active Sites of SNF using Split Root Systems (wild-type vs *nif* mutant rhizobia)

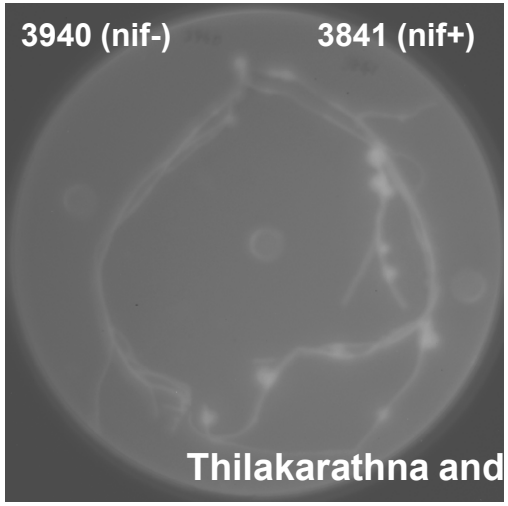
Alfalfa (*Medicago sativa* L.)

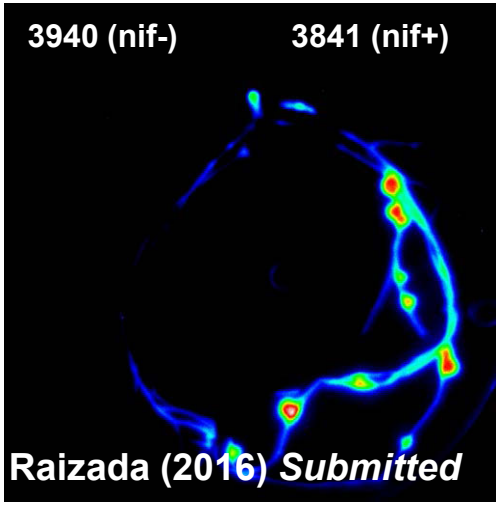




Lentil (*Lens culinaris* Medik.)







Thilakarathna and Raizada (2016) *Submitted*

Most applicable to scientists working with smallholder farmers:

GlnLux 96-well Liquid Assay for *in planta* nitrogen fixation:

Uses a single leaf punch

(3 h protocol, $1 USD per sample)

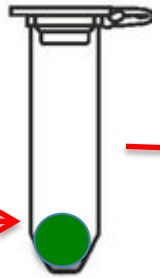
Punch a leaf disk

Grow plants

without N

or only starter N

Freeze in liquid nitrogen



Grind with sand + add protease inhibitor

Centrifugation

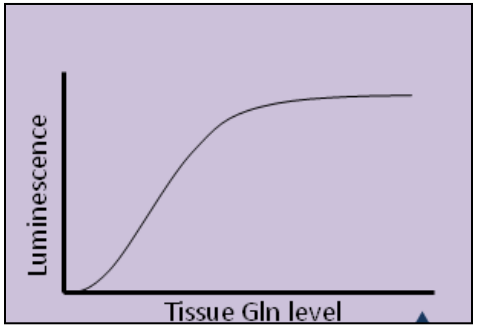
Collect supernatant (dilute 1/100)

M9 minimal medium + plant extracts + *GlnLux* bacteria

Incubate at 37 °C

Allow luminescence to accumulate

Measure Lux



Compare tissue Gln level

Effect of different rhizobia strains on SNF of lentil: *GlnLux* leaf punch liquid assay

b

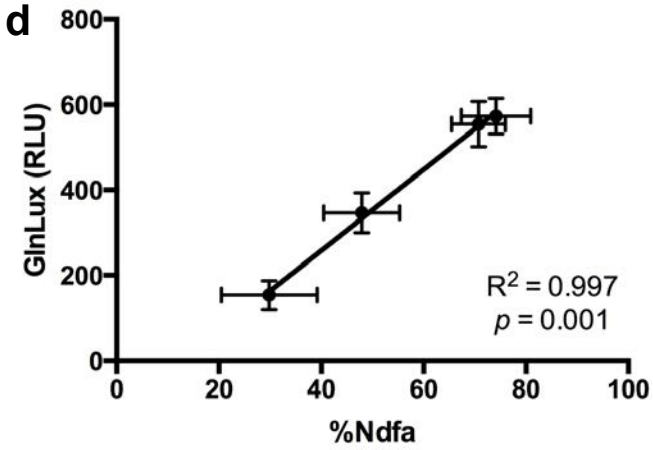
c

Rhizobia strains

Rhizobia strains

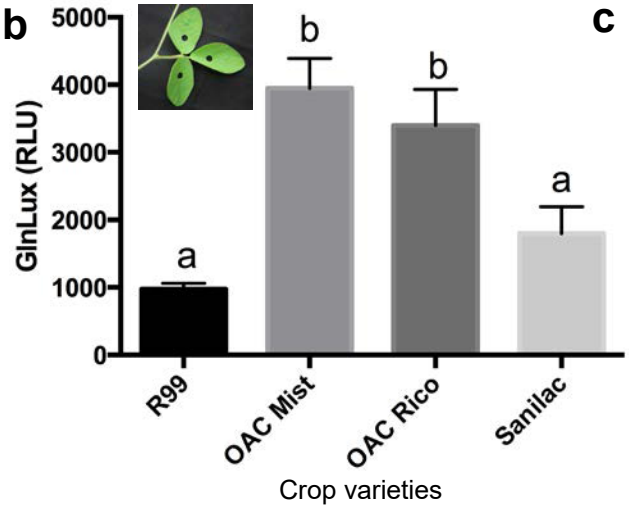
Rhizobia strains

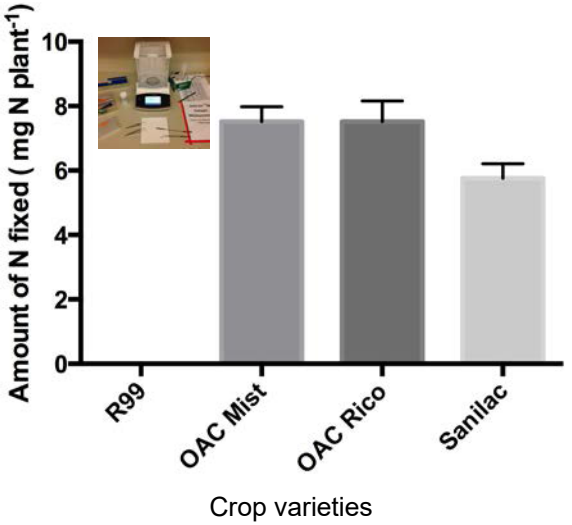
d

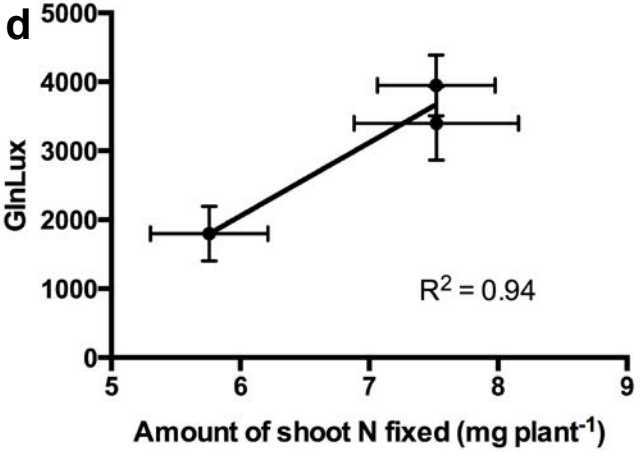


Thilakarathna, Moroz and Raizada (2016) *Submitted*

Effect of different crop varieties on SNF of common bean: *GlnLux* leaf punch liquid assay

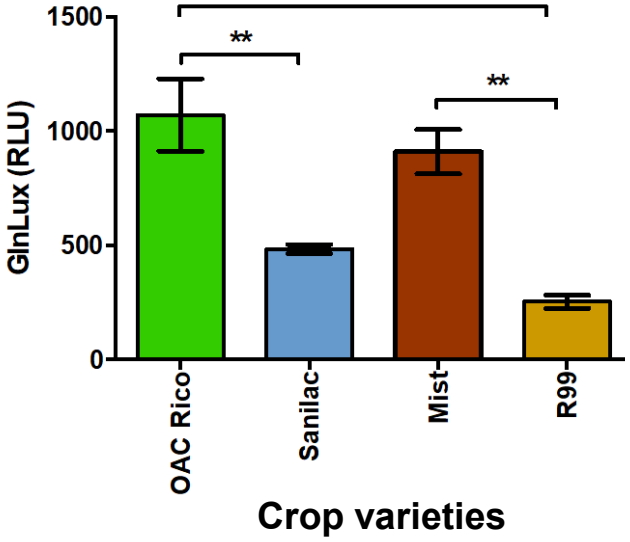


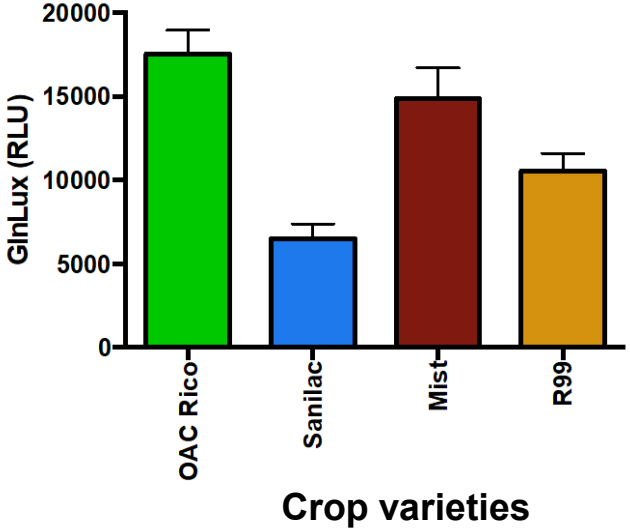


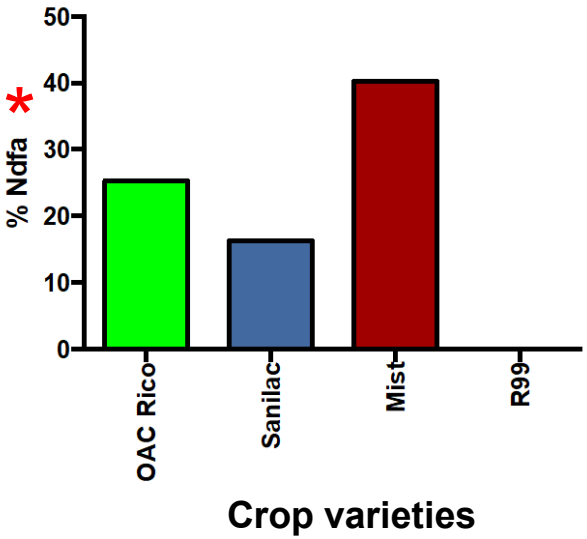


Thilakarathna, Moroz and Raizada (2016) *Submitted*

Relevance of *GlnLux* leaf punch liquid assay from the greenhouse to the field: Common bean







Greenhouse data

Field Data (low N field)

*Mean %Ndfa of bean cultivars under field conditions in 2011 and 2012 (Farid and Navabi 2015)

Conclusions to Part I – GlnLux biosensor

- *GlnLux* biosensor is a **new method to measure SNF output** in non-transgenic ureide- and amide-exporting legumes.
- *Glnlux* agar permits **screening of colonies** of rhizobia for SNF activity, potentially to permit strain improvement.
- *GlnLux* 96-well liquid assay uses **single leaf punches** to measure relative SNF output in plants growing without exogenous N, making it a **rapid, low-cost, high throughput** screening method.
- *GlnLux* agar permits **visualization of active sites** of nitrogen fixation.
- *GlnLux* can be used to **pre-screen** plants inoculated with different **rhizobia candidates** prior to field testing.
- *GlnLux* may be useful for pre-screening of **crop varieties that vary in SNF** prior to field testing.
- *GlnLux* measures Gln only which limits the assay.

Question?

GlnLux may be a good tool for the early stages of legume variety and rhizobia screening, but can be used on smallholder farms to quantitatively diagnose low nitrogen fixation and improved nitrogen fixation?

GlnLux field trials with terrace farmers in Nepal

Trial plots preparation

Soil sampling for nutrient and rhizobia

Bean plots with treatments

Cowpea plot with Canadian rhizobia

Nodulated roots

Rhizobia trial field team

LI-BIRD UNIVERSITY of GUELPH Global Affairs Canada Affaires mondiales Canada IDRC | CRDI International Development Research Centre Centre de recherches pour le développement international Canada CMU CANADIAN MENNONITE UNIVERSITY NARC Nepal Agricultural Research Council anamolBIU

Kaski (Nepal) terrace field trial - Adjusted grain yield (g) demonstrates that rhizobia inoculant response is site-specific and hence an inexpensive diagnostic technology is needed for site-specific recommendations

Common bean

B = boron fertilizer Mo = molybdenum fertilizer

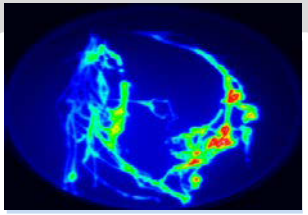
Farmer (anon Number)	T1 Uninoculated	T2 B + Mo micronutrients	T3 Local rhizobia	T4 Canada/US rhizobia	T5 Canada/US rhizobia + B +Mo
22	**194**	292	548	348	559
29	**1076**	447	466	294	518

Cowpea

22	**286**	**259**	**199**	**133**	**170**
39	**96**	87	158	256	273

GlnLux technology has been transferred to Nepal (NGO, LI-BIRD) and we are awaiting final Year 1 *GlnLux* field analysis

Outline



1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the *GlnLux* biosensor

1.2. Detection of SNF in colonies of rhizobia *in vitro*

1.3. Detection of SNF in legumes *in planta*

2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy

Helping farmers to overcome barriers to maximize legume production as part of the **Sustainable Agriculture Kit (SAK) Project on Nepalese terrace farms**

- 1. Funded by a $2.3 million grant from the Canadian International Development Research Centre (IDRC) and Global Affairs Canada (Canadian PI: MN Raizada).
- 2. For more information, to go: www.SAKNepal.org

Global Affairs Canada Affaires mondiales Canada

IDRC | CRDI

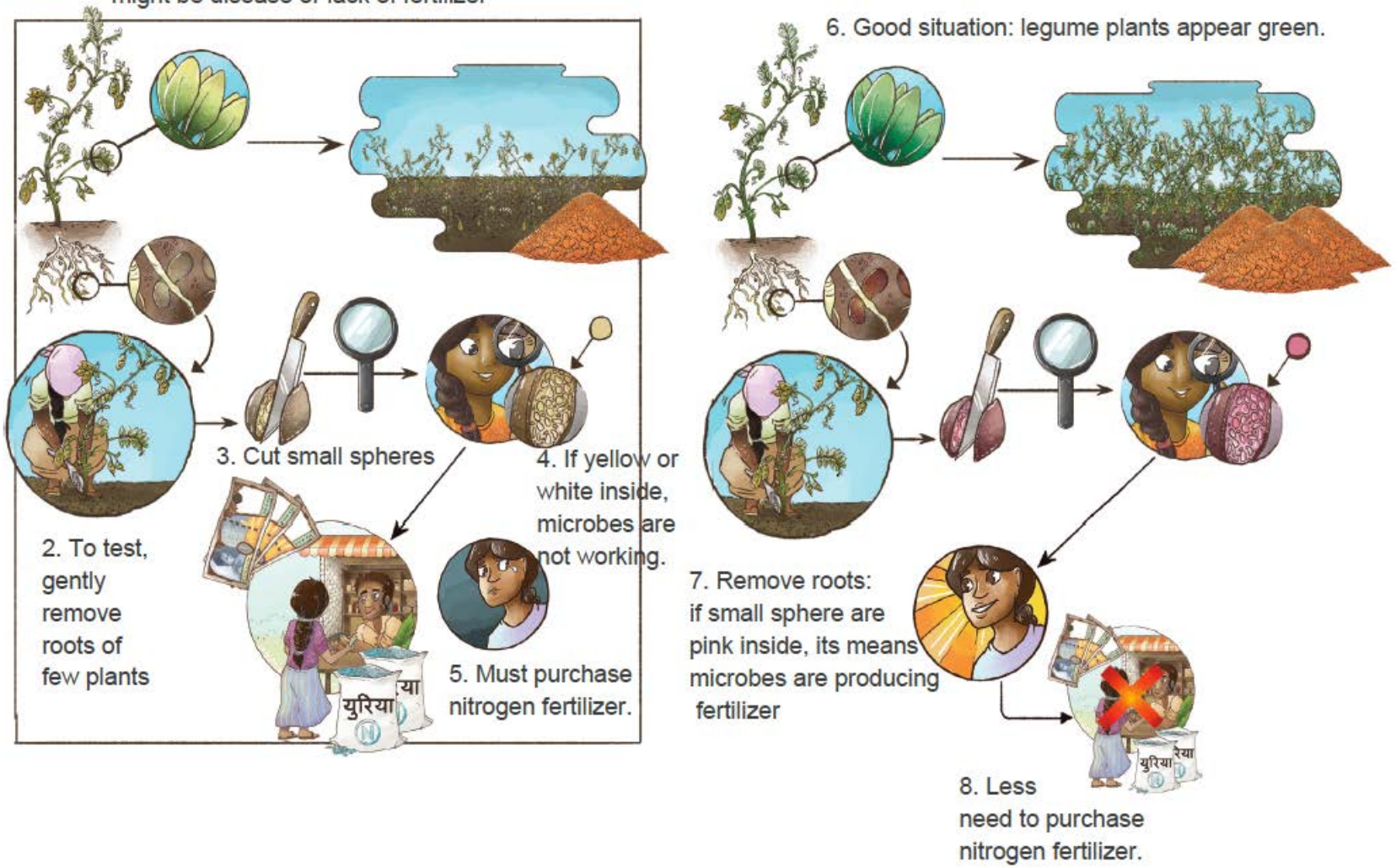
International Development Research Centre
Centre de recherches pour le développement international

Canada

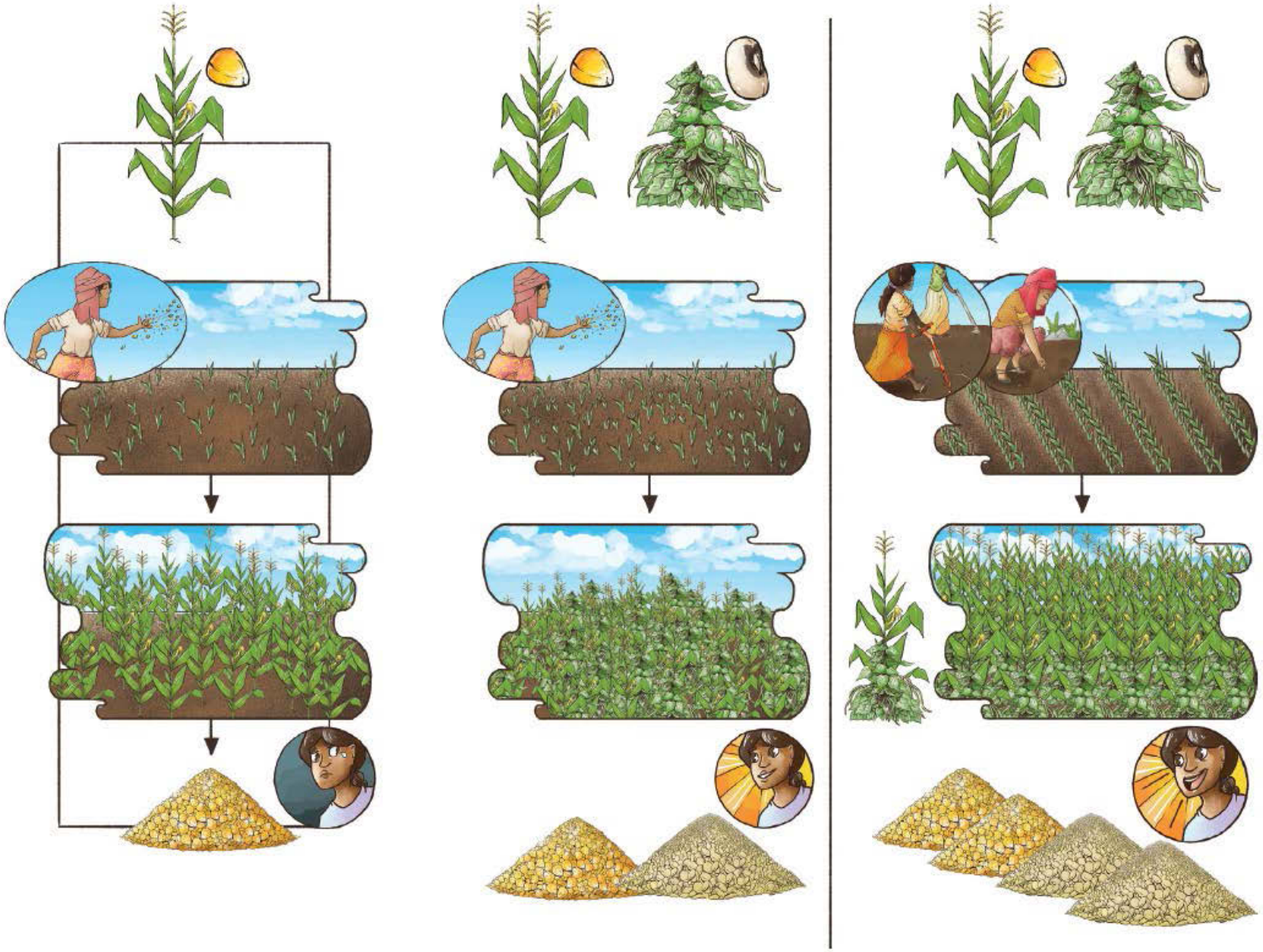
Maximizing the benefits of legumes on smallholder farms

A holistic, farm-systems and human-centered based approach is being undertaken based on farmer-identified opportunities and complaints……

Lesson: If small spheres on legume roots are only yellow inside, they do not contain healthy microbes to make natural nitrogen fertilizer, but a pink colour inside means they are producing fertilizer



Lesson: Sowing maize together with cowpea will yield more profit than maize only.



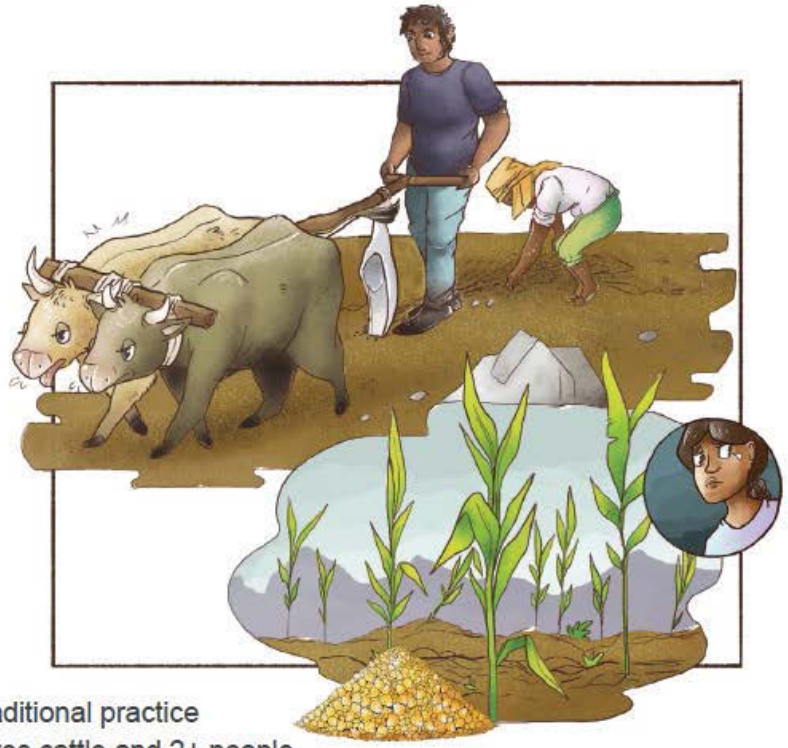
1. Intercropping Trials

A. Seasonal Intercropping Trials (row/mixed intercropping)

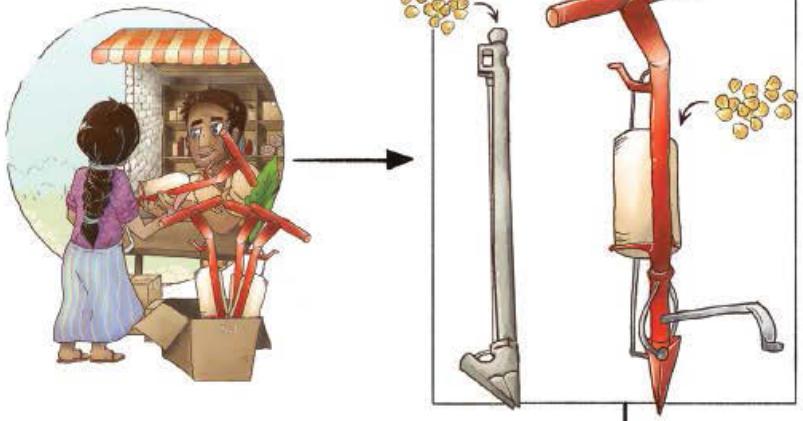
Season 1: Mid-March to Mid-July: Unit plot size: 30 m^2 for both test and non-test plots in split-plots

Combinations		Intercrop (Test) Plot Yield (t/ha)			Non-test Plot Yield (t/ha)	% Increase or Decrease
		Non-legume	Legume	TOTAL	Non-legume	
Maize-makaibodi (Kaski)	Grain	4.10	0.99	5.09	3.40	+ 49.7
	Biomass	11.98	2.00	13.98	9.93	+ 40.7
Maize-makaibodi (Dhading)	Grain	3.97	0.88	4.85	4.06	+ 19.5
	Biomass	5.21	4.93	10.14	5.49	+ 84.8
Maize-suryabodi (Kaski)	Grain	7.96	0.11	8.06	6.48	+24.4
	Biomass	14.21	0.67	14.88	12.44	+ 19.6
Maize-bean (Kaski)	Grain	6.80	0.10	6.90	7.16	- 3.6
	Biomass	12.79	0.89	13.68	12.64	+ 8.2

Lesson: A jab planter reduces people and livestock required to sow seeds

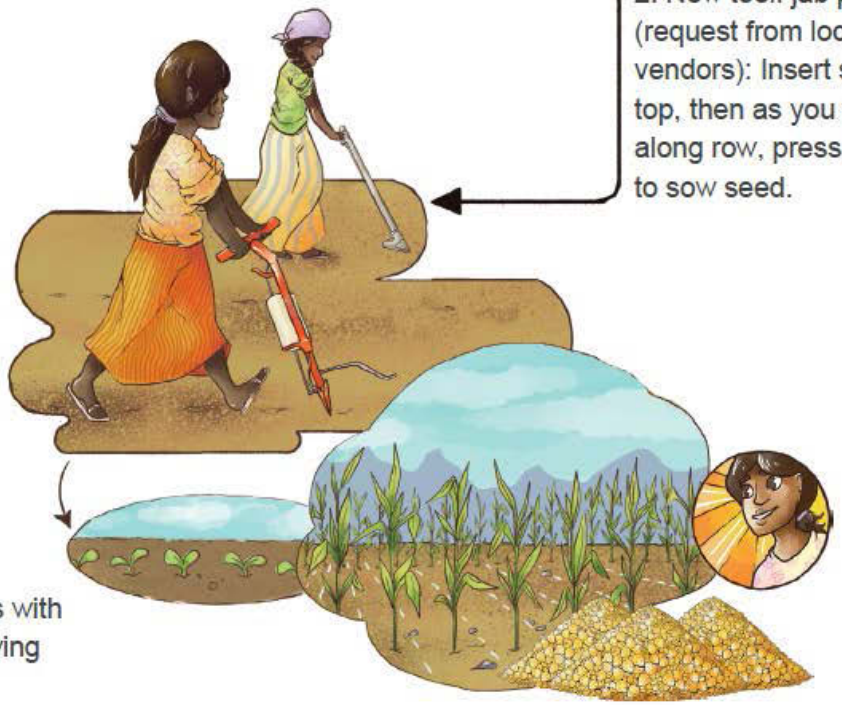


1. Traditional practice requires cattle and 2+ people. Difficult on steep hillside or narrow terrace.



2. New tool: jab planter (request from local vendors): Insert seed at top, then as you walk along row, press down to sow seed.

3. Single person can use

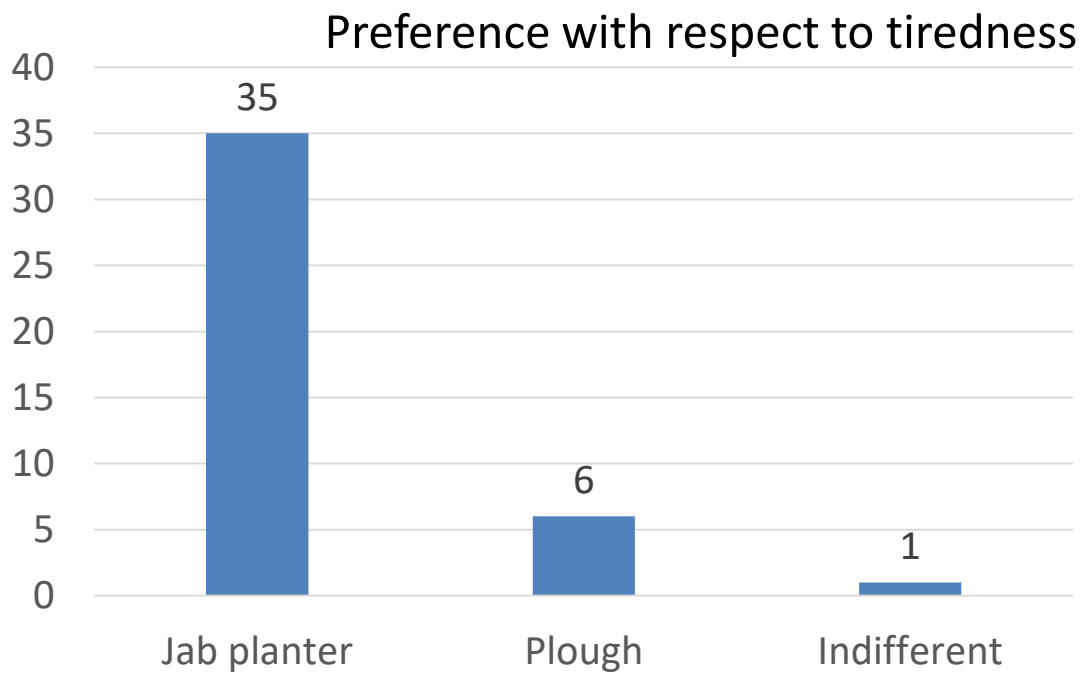


4. Helps with line sowing

Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons

A low cost seed planter (jab planter) to reduce the need for human and livestock labour especially on narrow terraces

A low cost seed planter (jab planter) to reduce the need for human and livestock labour especially on narrow terraces

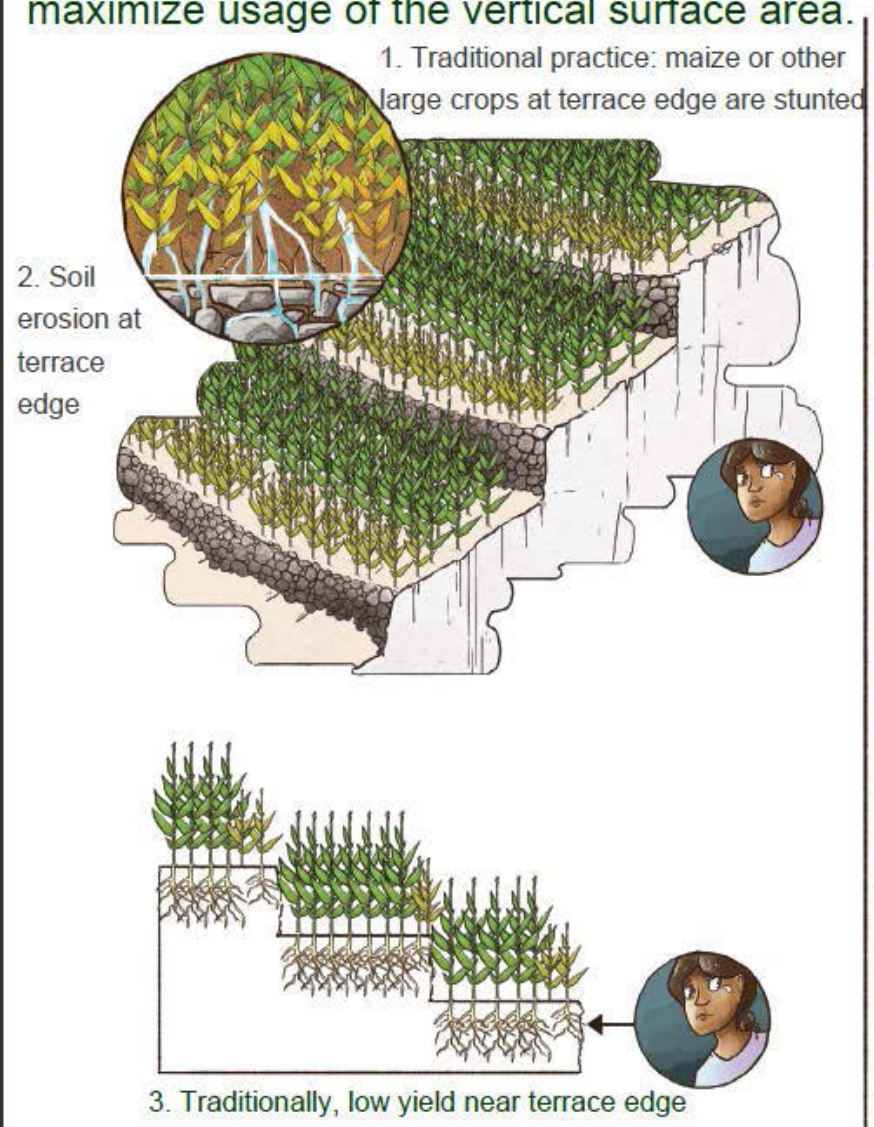


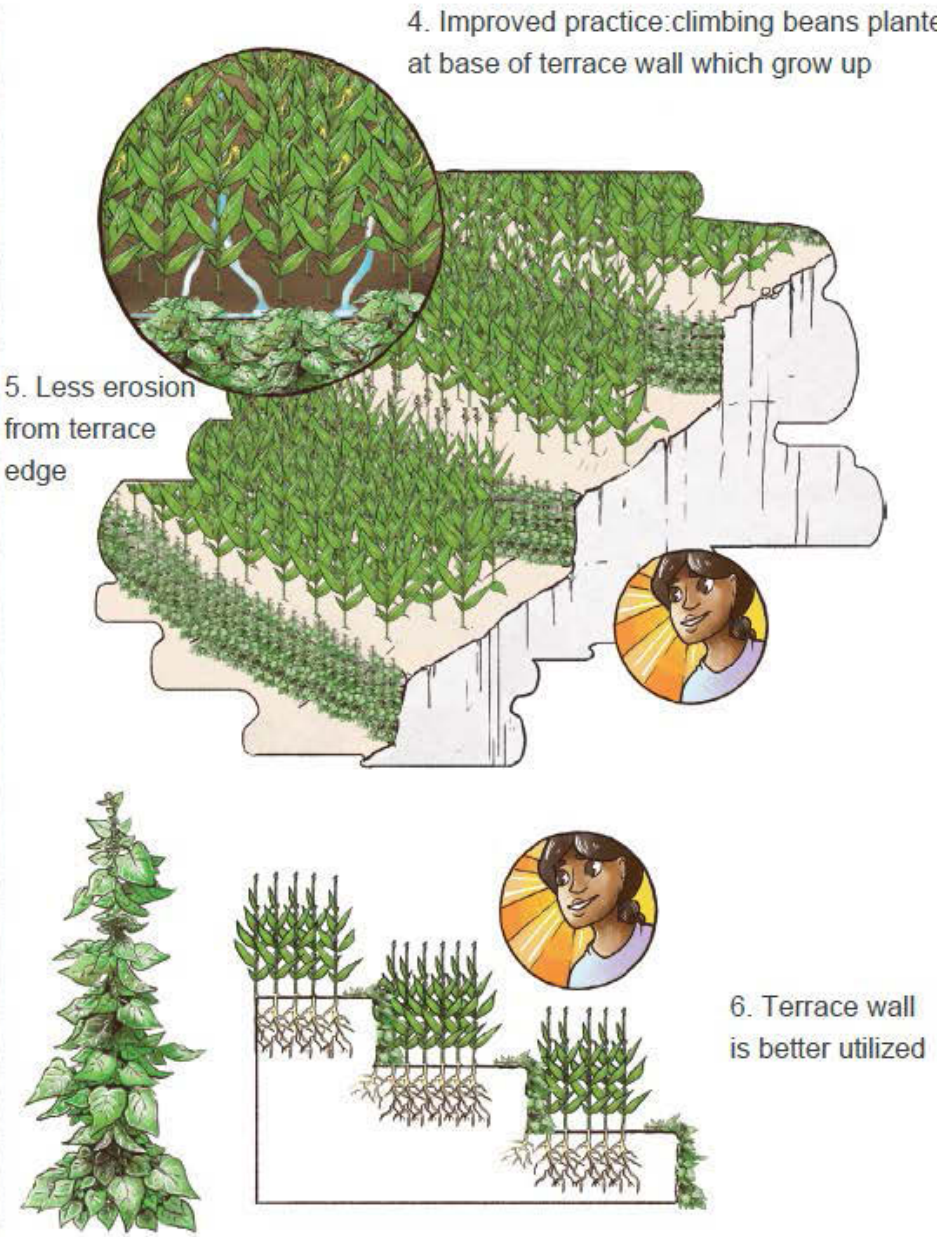
Will you use this in future?	
Yes	39
No	3

Current progress:

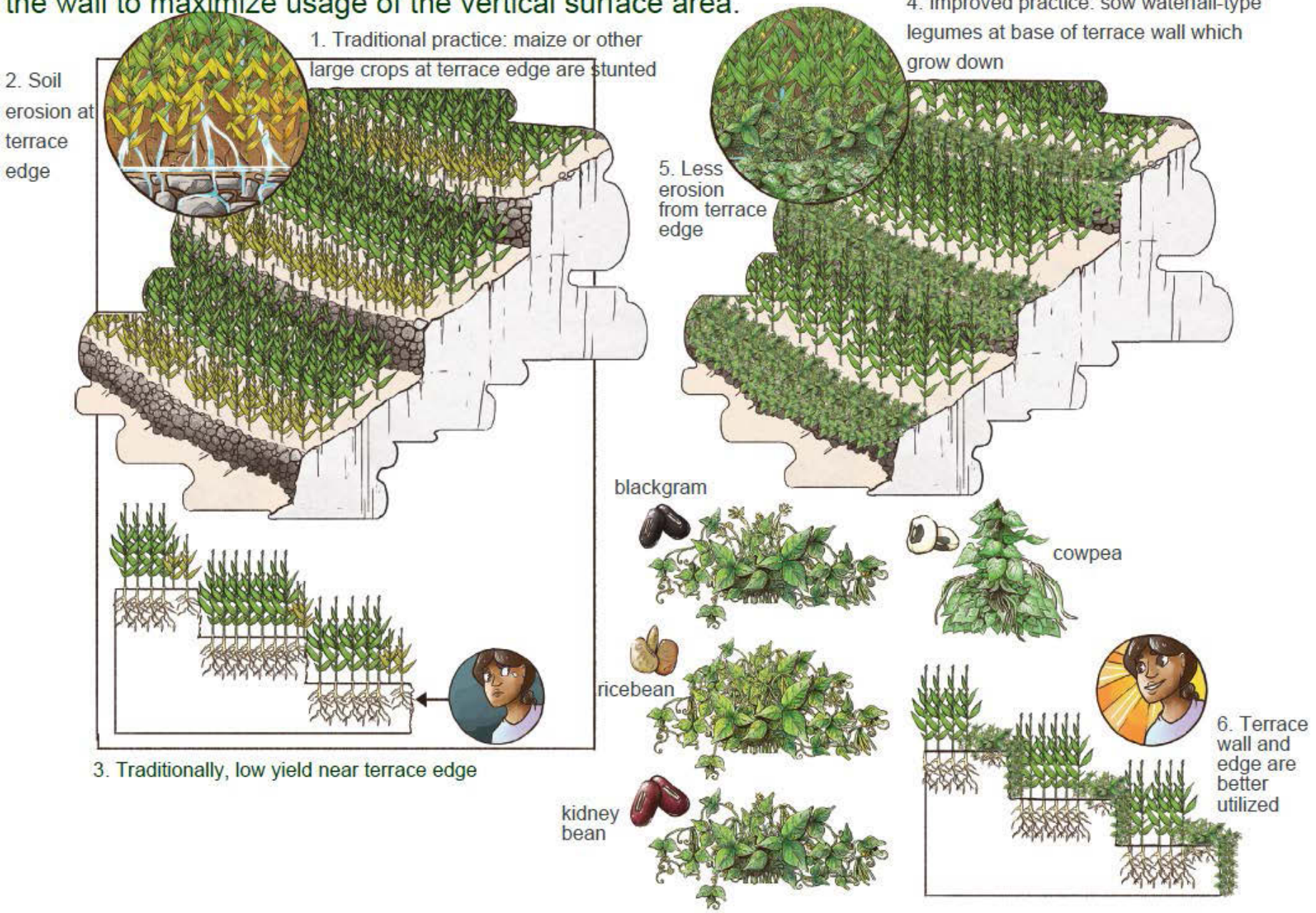
- 100 farmers are currently testing different models

Lesson: Climbing beans can be planted at the base of the terrace wall for growth up the wall to maximize usage of the vertical surface area.





Lesson: Waterfall-type legumes can be planted at the top edge of the terrace wall and grow down the wall to maximize usage of the vertical surface area.



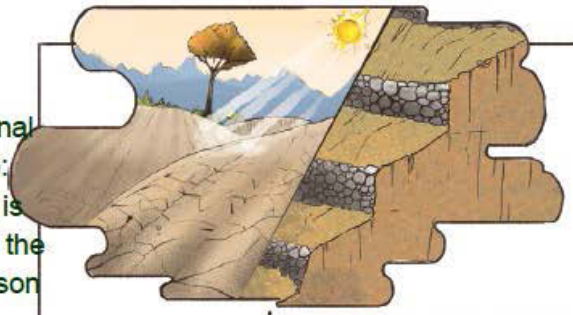
3. Edge Crops: Yield and Income Benefits

Crop	Area (m^2) covered (/plant)	Plant parts	Yield (kg/ha)	Price (Rs/kg)	Gross income (Rs.)	Costs (Rs.)	Income less cost (Rs.)	NET INCOME (CAD)
Rice bean (Kaski)	2.32	Grain	**486**	60	29,160	~10,000	19,160	**$240**
		Biomass	**3367**	Used as fodder for livestock				
Rice bean (Dhading)	1.98	Grain	**374**	60	22,440	~10,000	12,440	**$156**
		Biomass	**535**	Used as fodder for livestock				
Horsegram (Kaski)	0.58	Grain	**315**	90	28,350	~10,000	18,350	**$229**
		Biomass	**2734**	Used as fodder for livestock				
Horsegram (Dhading)	0.48	Grain	**317**	90	28,530	~10,000	18,530	**$232**
		Biomass	**694**	Used as fodder for livestock				
Black gram (Kaski)	0.28	Grain	**248**	90	22,320	~10,000	12,320	**$154**
		Biomass	**584**	Used as fodder for livestock				
Blackgram (Dhading)	0.34	Grain	**256**	90	23,040	~10,000	13,040	**$163**
		Biomass	**636**	Used as fodder for livestock				
Cowpea (Kaski)	1.03	Grain	**288**	60	17,280	~10,000	7,280	**$91**
		Biomass	**2308**	Used as fodder for livestock				

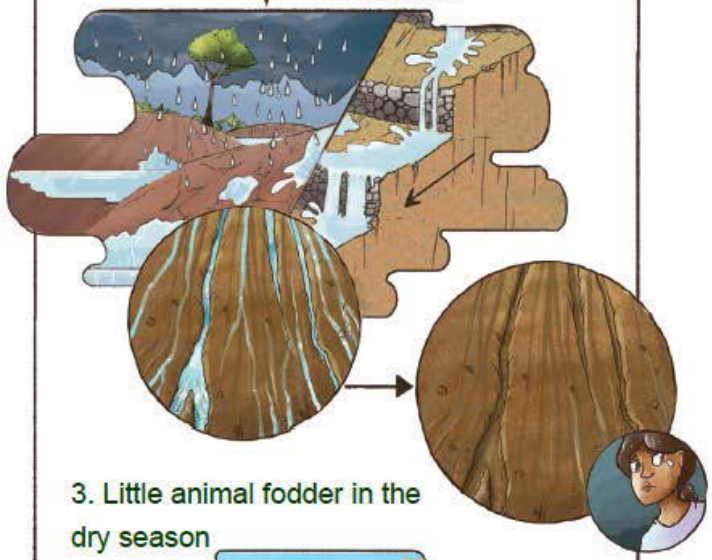


Lesson: Planting vetch in the dry season will reduce soil erosion and provide animal fodder

1. Traditional practice: nothing is sown in the dry season

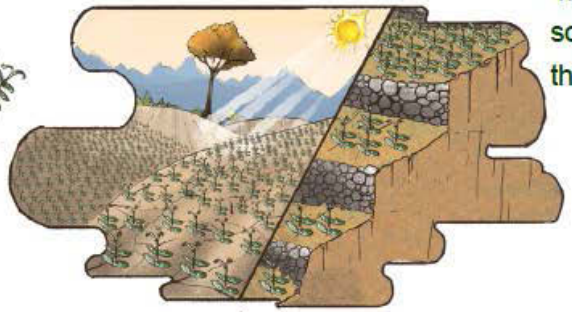


2. Soil erosion when first rains arrive



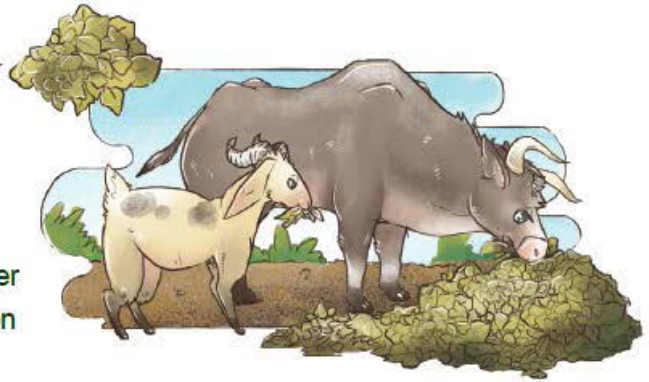
3. Little animal fodder in the dry season

4. Improved practice: sow vetch prior to the beginning of the rainy season



5. Reduced erosion

6. Good animal fodder in dry season



Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons

Lesson: Kneepads can reduce pain at knees and prevent knees from becoming wet or cold such as during weeding

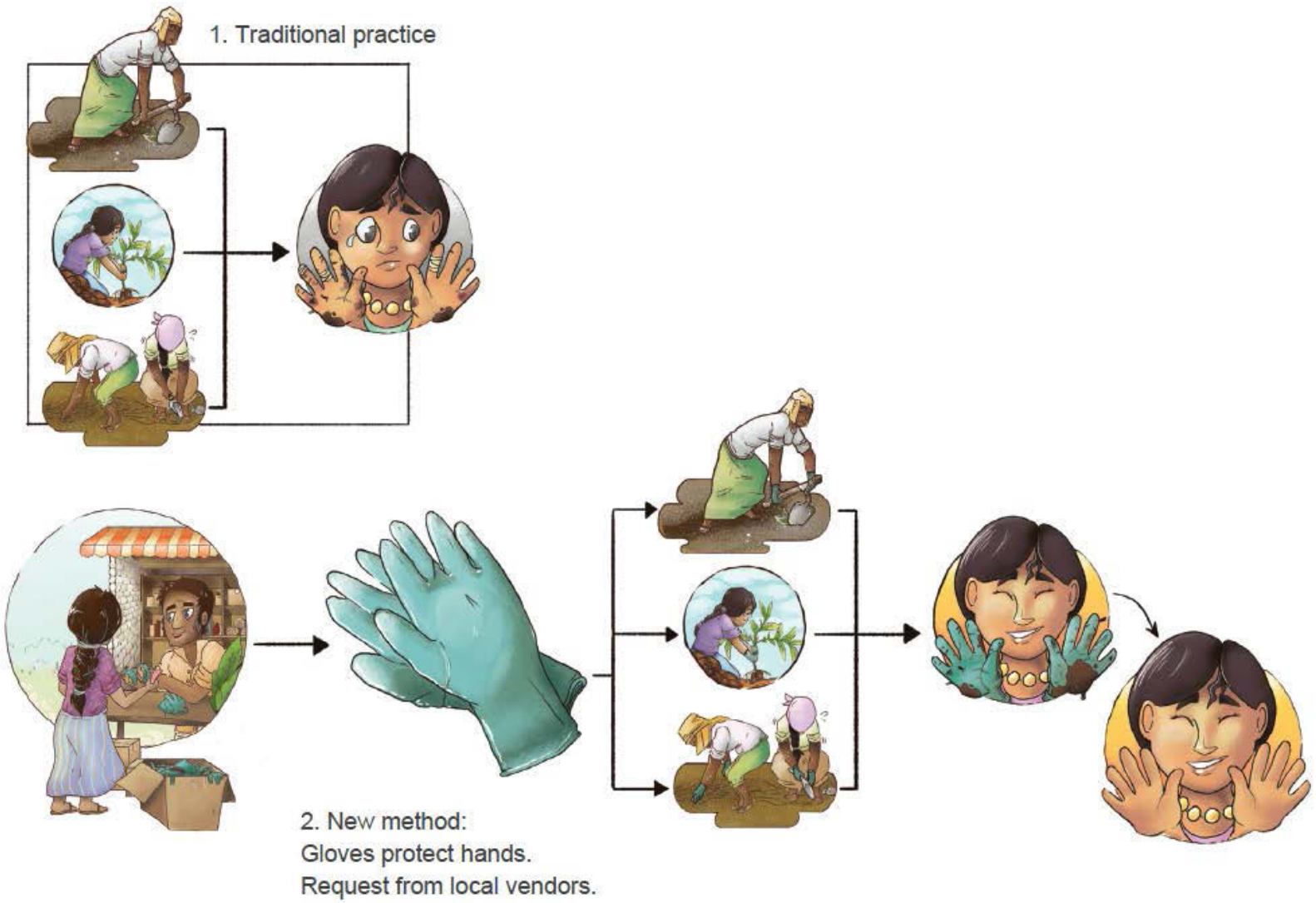
1. Traditional practice causes cold, pain on knees

2. New practice

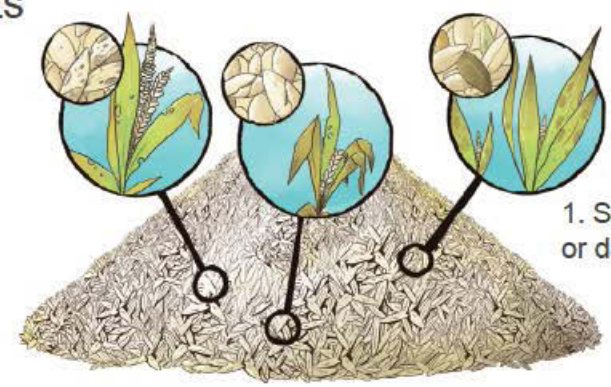
3. Purchase from vendor

Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons

Lesson: Gloves reduce pain and damage to hands.

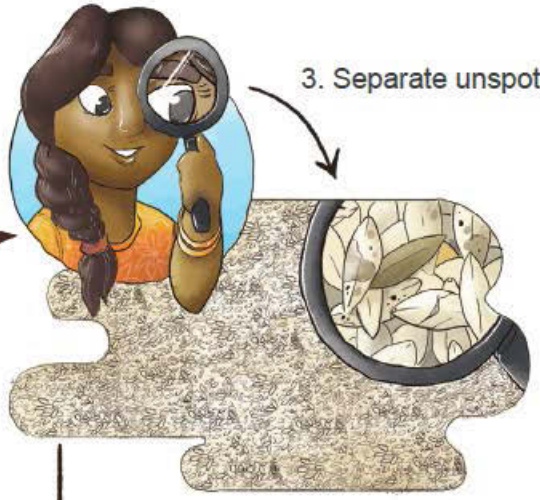


Lesson: Before sowing seeds, use a magnifying glass/sheet to help remove seeds with disease or pests

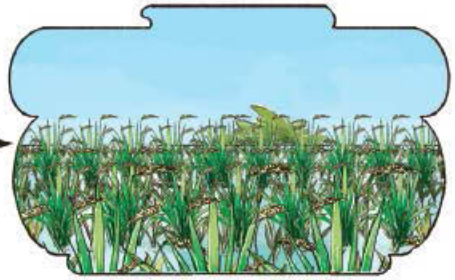


1. Seeds for sowing may have small spots or damage due to insects or mold

2. Purchase magnifying glass/sheet from vendor



3. Separate unspotted, undamaged seed

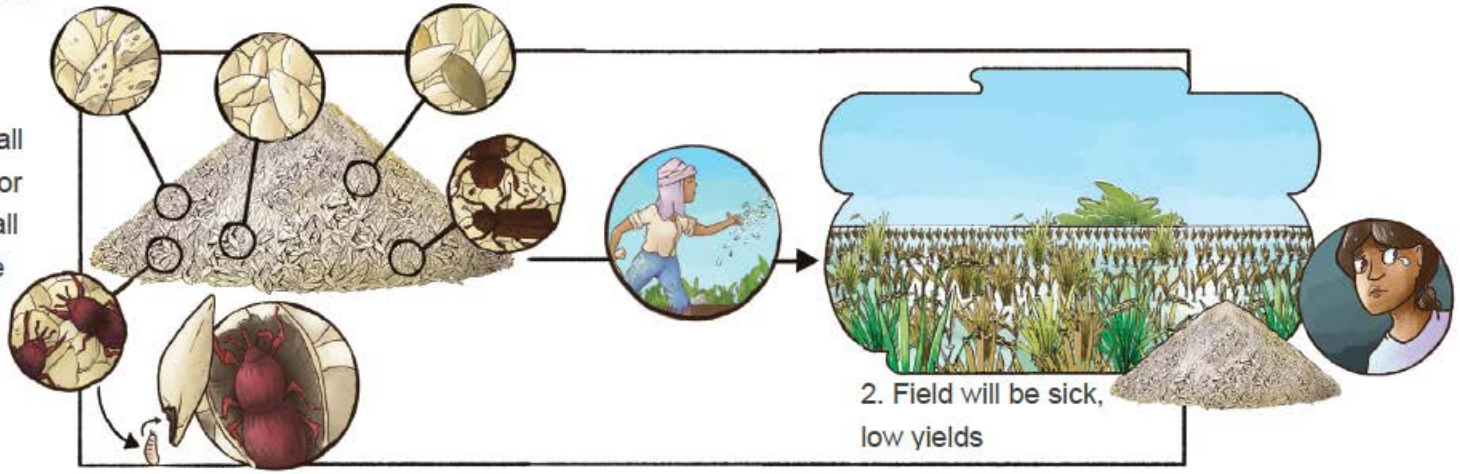


4. Sow healthy seed only

Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons

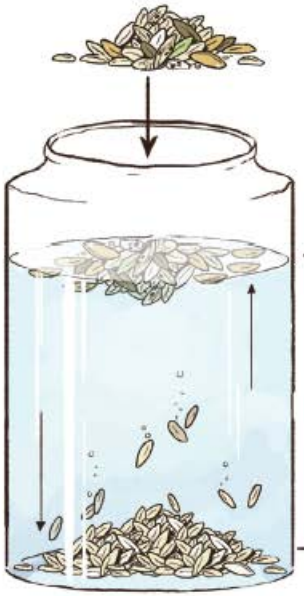
Lesson: Healthy seeds can be easily separated from sick seeds prior to sowing using water floatation

1. Traditional practice: seeds with small disease spots or containing small insects may be missed, and sown in field

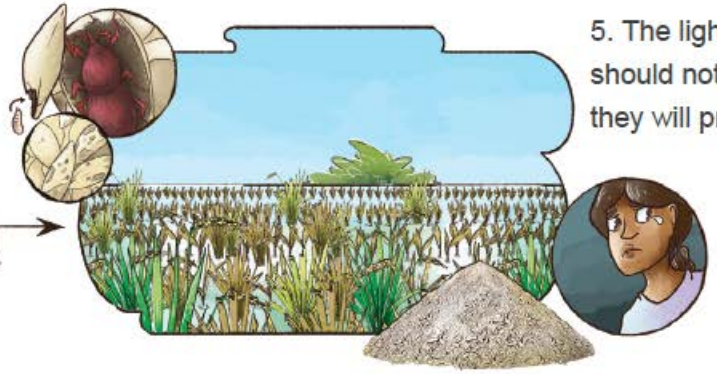


2. Field will be sick, low yields

3. Improved practice: Add seeds to water.



4. Sick seeds are light-weight and will float.



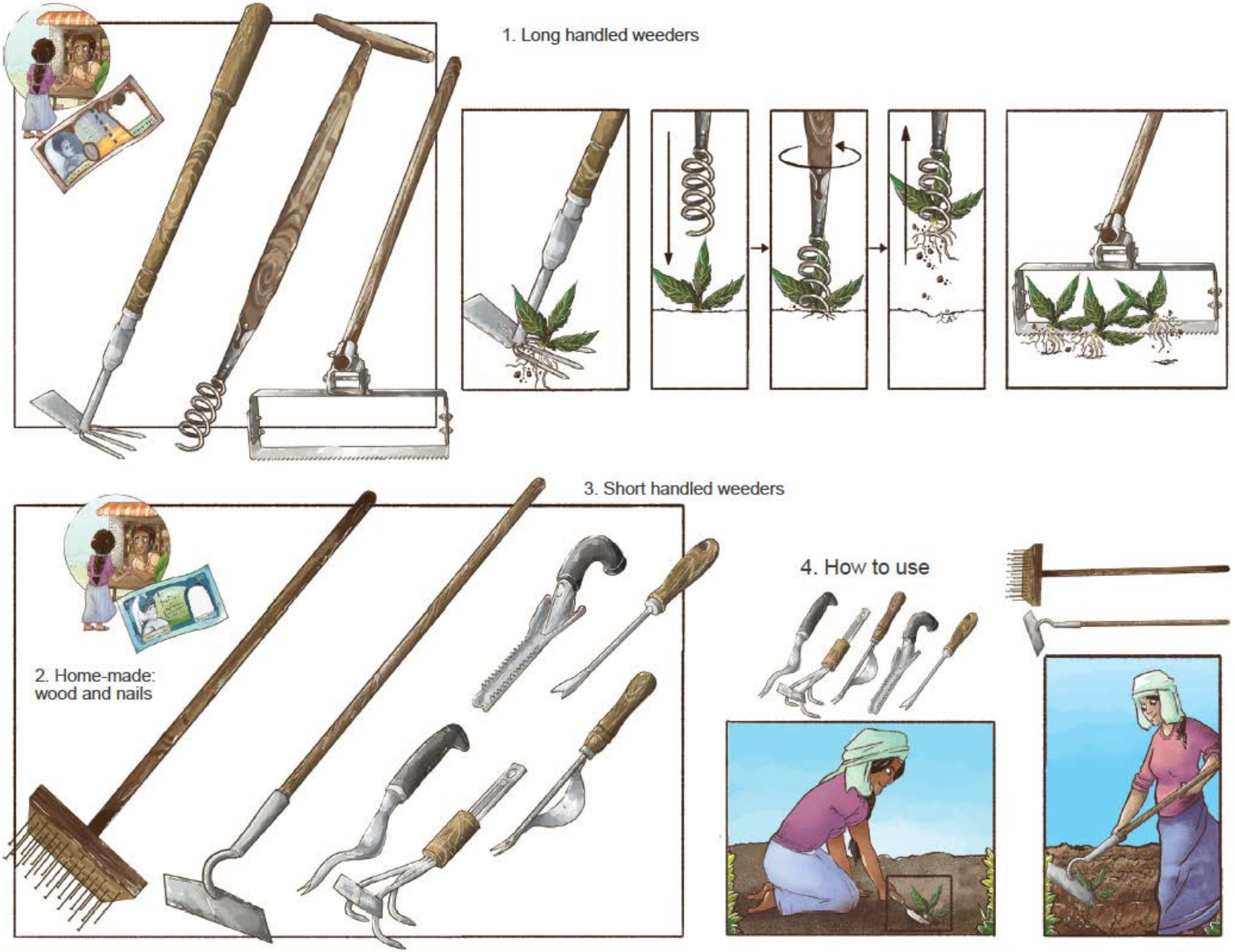
5. The light-weight seeds should not be planted as they will produce a sick field

6. Healthy seeds are heavy and will sink

7. The heavy seeds will produce a healthy field

8. If seed size is large, then salt should be added to jar to better enable seed separation

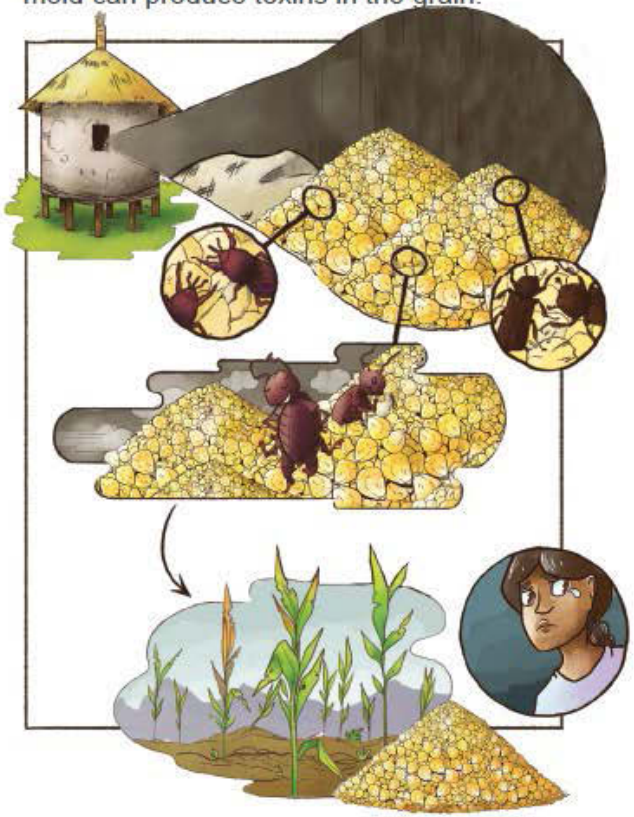
Lesson: New tools to reduce drudgery of hand removal of weeds: Long-handled, medium cost options.



New weeding tool made by local Nepalese blacksmiths, creating local jobs, created using participatory testing with women farmers

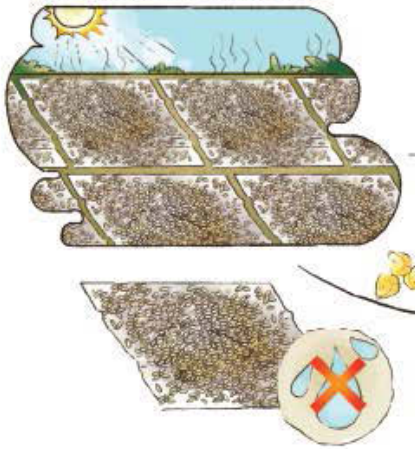
Lesson: Special bags can be used to store grain which reduce oxygen inside bag which prevents insects and fungal molds from surviving, which also reduces toxins.

1. Traditional practice: stored grain is damaged by insects and mold. The mold can produce toxins in the grain.

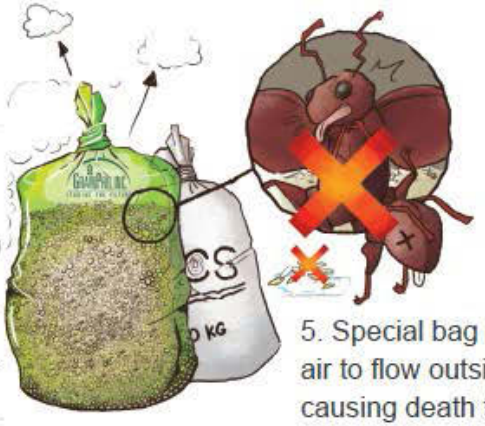


2. New practice

3. Dry grain completely

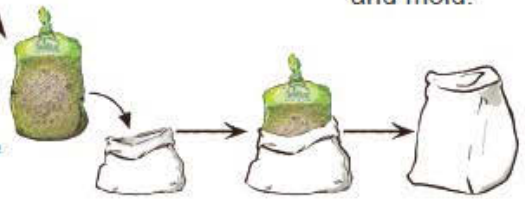


4. Purchase bag from vendor. Put grain in bag, remove air and tie.



5. Special bag causes air to flow outside, causing death to insects and mold.

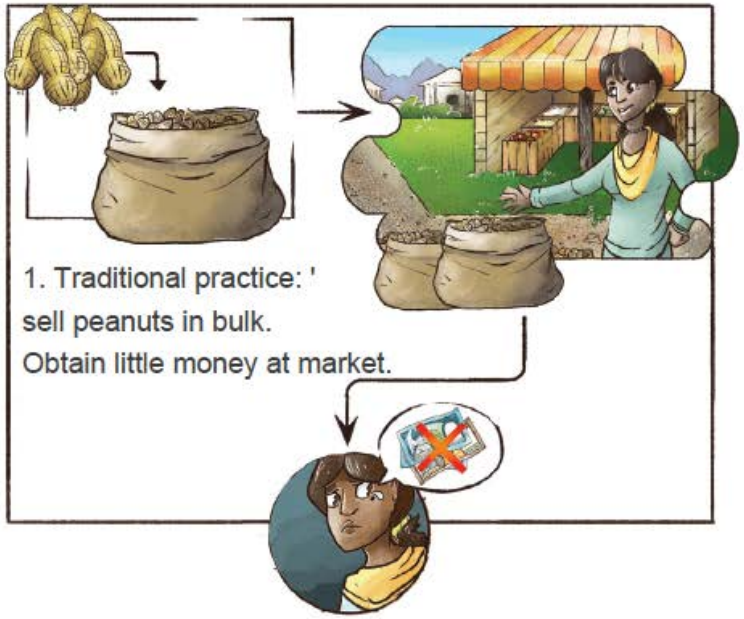
5. Put bag inside a jute bag. Elevate from ground if possible to prevent rodents.



6. High yielder if sown and less toxins in food.

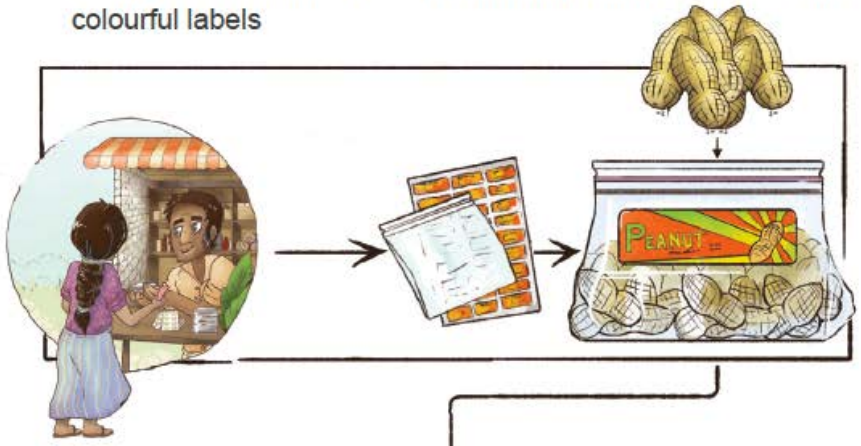
7. Re-use bag many times.

Lesson: Rather than selling harvested products in bulk, it is more profitable to package them beautifully



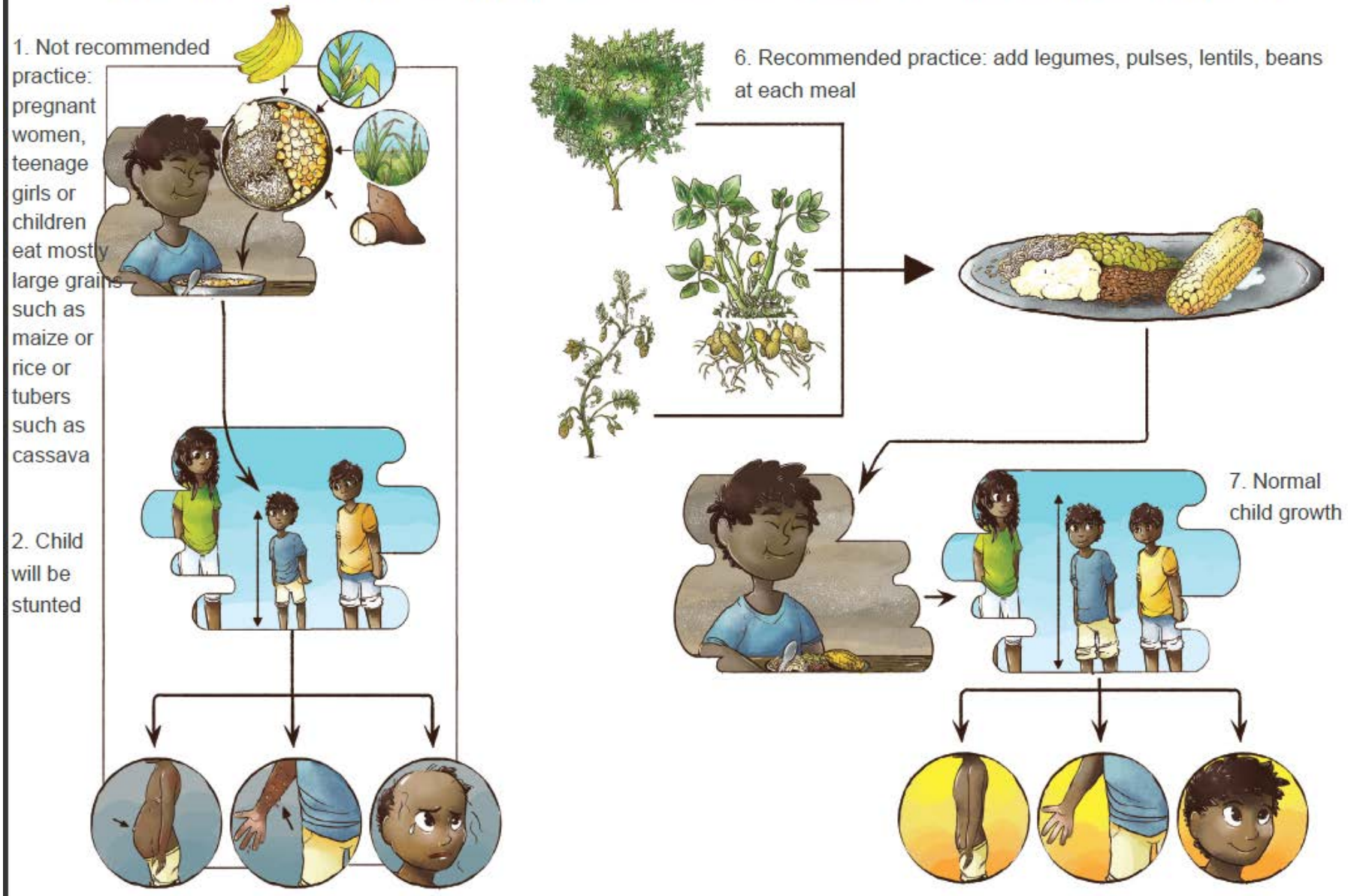
1. Traditional practice: ' sell peanuts in bulk. Obtain little money at market.

2. Improved practice: place peanuts in packages with beautiful, colourful labels

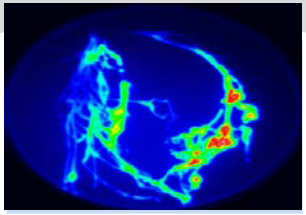


3. Packaged peanuts obtain a higher price at the market

Lesson: People especially pregnant women and children should eat legumes/pulses



Outline



1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the *GlnLux* biosensor

1.2. Detection of SNF in colonies of rhizobia *in vitro*

1.3. Detection of SNF in legumes *in planta*

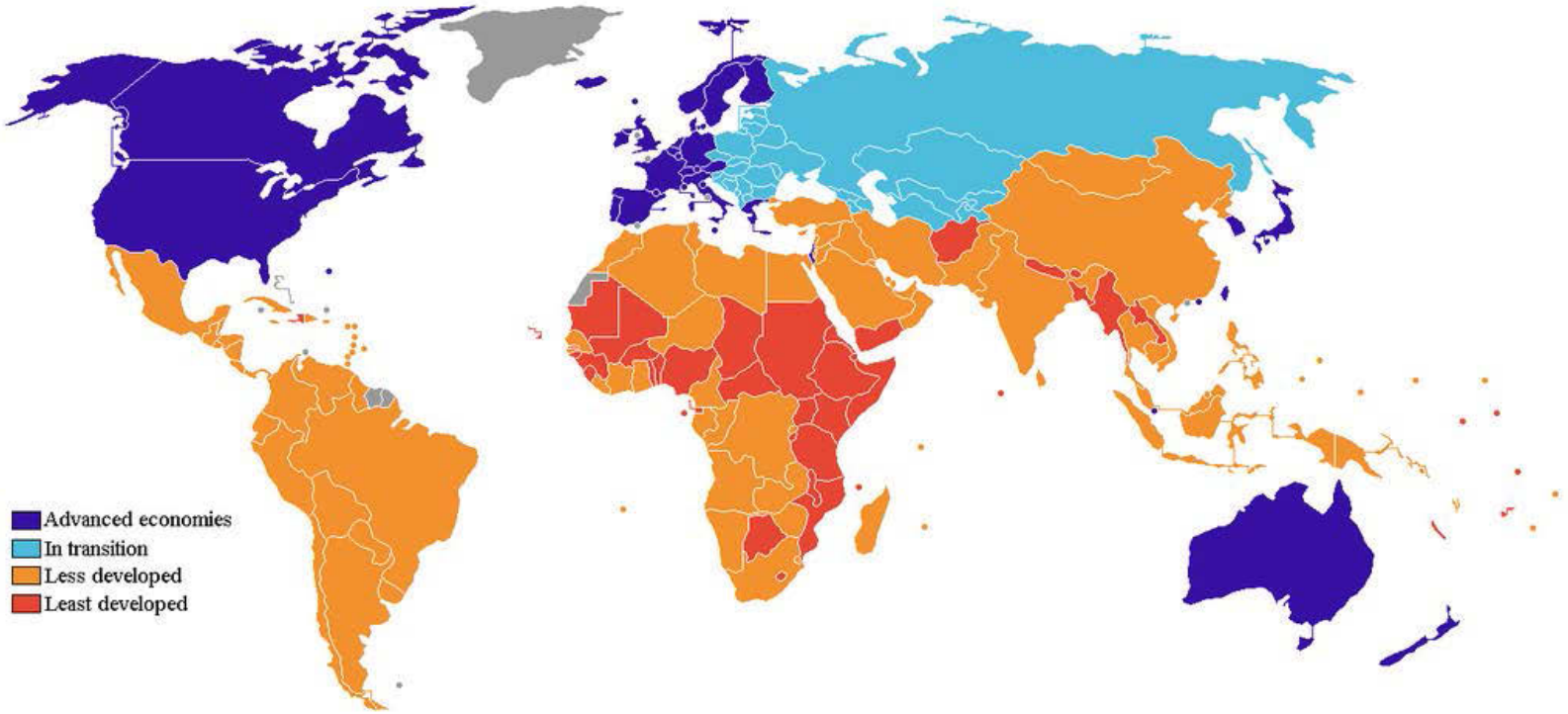
2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy

The Challenge of Scaling Up

Problem: There are ~400 million smallholder farming families (<2Ha) who have little cash income. They live in remote areas, receive little knowledge extension support and have poor access to private sector inputs and markets.

Farmer pilot projects by NGOs and governments are typically not scaled up to impact the ~2 billion people who need them



Challenge of Scaling Up is Enormous in the Remote, Mountainous Regions of Nepal

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Example: Nepal SAK Survey Question: Corn kernels were being removed from cob by labour-intensive methods for women

Variable	Kaski	Dhading
Finger Millet	Stamping with feet:30 Beating with sticks:12	Feet: 10 Beating with sticks:15
Maize	Hand: 29 Cobs placed in sack and beaten with sticks: 29	Hand:15 Cobs placed in sack and beaten with sticks: 8
Cowpea	Beating by sticks: Hand: 21	Beating by sticks:1 Hand: 5
Ricebean	Beating by sticks: Hand:4	Beating by sticks:2
Bean	Beating by sticks: Hand: 13	Beating by sticks:2 Hand: 4
Horsegram	Beating by sticks: 4	Beating by sticks:2

Data collected by LI-BIRD, Nepal (unpublished)

Proposed intervention: simple kernel sheller……

Innovation: A $2 handheld tool to reduce kernels of corn from the cob (corn sheller) reduces female drudgery and prevents kernel breakage

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

THE BEACH
RESTAURANT - BAR
BEACH CLUB

APPETIZERS

Chicken Wings $7.99
Fresh local chicken wings grilled and topped with buffalo, blue cheese or BBQ sauce.

Potato Skins $5.99
Baked potato filled with sour cream and bacon bits.

Onion Rings $4.99
Thick cut onions breaded and served with BBQ or Blue Cheese.

Pepper Poppers $5.99
Made with local peppers, bacon, cream cheese and jalapenos.

Chili Bean Dip $5.99
Special chili sauce served with french fries or nachos.

Grilled Sliced Sausages $7.99
Grilled to temptation served with Mustard, BBQ or Blue Cheese.

Cheese Quesadilla $5.99
Tortilla wrap with Swiss cheese and cheddar melted on the grill.

The Beach Combo $18.99
Tortilla wrap with Swiss cheese and cheddar melted on the grill.

SOUPS & SALADS

Potato Soup $6.99
Freshly baked potatoes sliced & diced with bacon bits and cheddar.

Vegetable Soup $5.99
Fresh vegetables cut and cooked in our flavorful seasoning.

Bean Soup $6.99
Tex Mex style Bean and cheese soup.

Salad Bar $7.99
Price per plate.

MAIN DISHES

Bean & Cheese Burritos $12.99
Our rich and tasty bean and cheese burritos grilled and served with sour cream and vegetables.

Grilled Pork Chops $14.99
Flame grilled with Chili ,BBQ, Jack Daniels sauce.

Fajitas $17.99
Chicken, Beef or Vegetable Fajitas grilled and served with tomatoes, sour cream, grilled onions, lettuce and avocados.

Grilled Chicken Breast $13.99
Fresh chicken grilled to perfection. Add any of these sauces: Jamaican Jerk, Jack Daniels, BBQ or Chili Sauce.

Jack Daniels Ribs ½ $14.99 Full $18.99
Slow cooked to perfection and flamed. The best tasting ribs in town!

Churrasco Select $15.99
Fresh select cut of prime meat grilled to your style.

Fresh Angus Steak $24.99
Grade A. half pound Flat Meat Grilled and served with Chili ,BBQ or Jack Daniels sauce.

Cowboy Steak $25.00
This special plate for only the bravest has 32oz of the best steak.

Sirloin Steak $29.99
The one and only half pound specialty plate, grilled to your taste.

The beach meat lover $29.99
Has a combination of Grilled Chicken, Sausage & half a rack of ribs with Corn on the cob or baked potato.

Served with your choice of fries, baked potato or grilled vegetables.

KIDS MENU

Served with fries or fresh vegetables. Includes juice, milk, or soda.

Chicken Fingers $6.99

Mini Hamburgers $6.99

Mac & Cheese $5.99

Hot Dog Sliced $5.99

Cheese Tortilla $5.99

BURGERS

Bacon Cheese Burger $9.50
Onions, tomatoes & and cheese on a freshly toasted bun.

Doble Swiss Burger $9.50
Ground beef with Swiss cheese and served on fresh rye bread.

Ranch Jalapeno Burger $10.00
Swiss cheese, Jalapenos, Ranch sauce and served on fresh bread.

Onion Burger $10.00
Caramelized onions, Swiss cheese and served on fresh bread.

Veggie Burger $9.50
Vegetarian patty with onions, tomato, and cheese on a fresh bun.

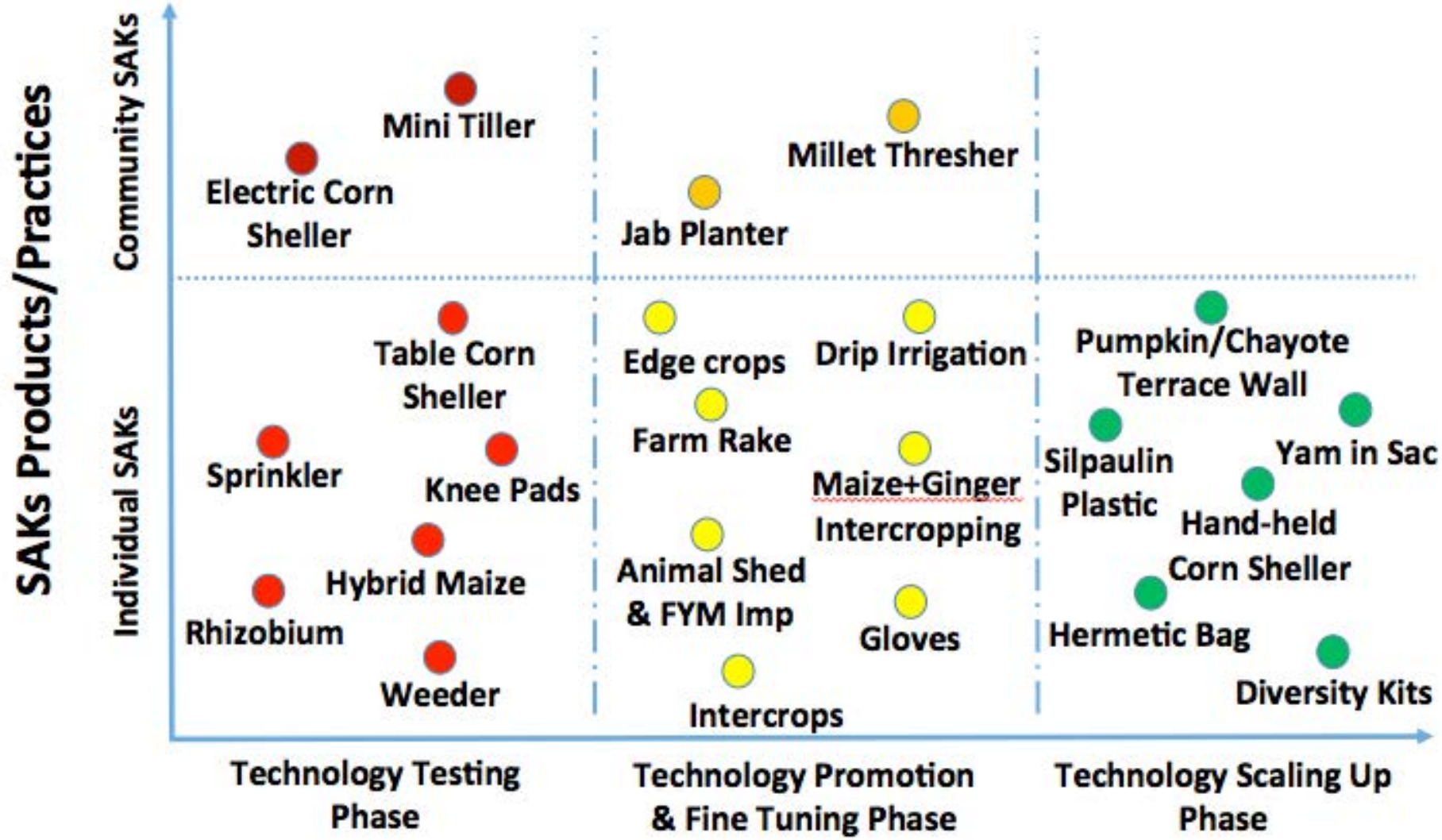
Grilled Chicken Burger $10.99
Grilled chicken with cheese, onions and tomato on fresh a bun.

Salmon Burger $15.99
Salmon fillet with onions, tomato, and cheese on a fresh bun.

The Beach Sandwich $11.99
Imported Churasco cut with swiss cheese, onions and mushrooms.

All our burgers are served with your choice of fries, baked potato, mashed potatoes or grilled vegetables.

Stage of scaling up of the SAK Menu in Nepal (growing)



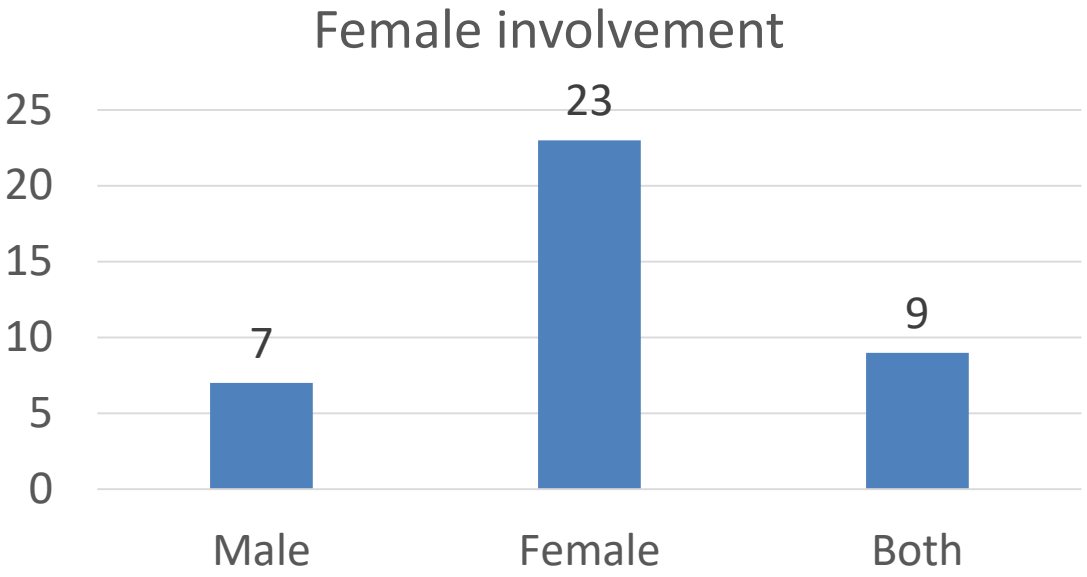
Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Survey of test farmers on the $2 corn sheller



Results:

- One woman can remove up to 80 kg of grain in a single day
- 11,000 corn shellers have already been procured with ~15,000 more planned by 2017

Trials and Demonstrations: An Overview

SN	Trial	Year 1: 2015			Year 2: 2016	
		Total Trials	Test Farmers	% Female	Total Trials	Test Farmers
Practices						
1.	Intercropping trials	12	77	62	6	80
2.	Wall crops	3	68	53	2	40
3.	Edge crops	4	46	67	2	40
4.	Cropping sequence	5	89	90	2	40
5.	Inverse slope	1	7	57	0	0
6.	Rhizobium trials	4	132	43	4	80
7.	Biochar	3	5	80	0	0
8.	Dry season forage	1	-	-	1	-
9.	Seed treatment trials	-	-	-	2	4
SUB-TOTAL		**33**	**424**	**61 (Ave.)**	**19**	**284**
Demonstrations						
10.	FYM Improvement	1	49	51	1	60
11.	Drip irrigation + poly-house	1	41	56	1	18
12.	Hybrid maize seed production	1	5	-	1	10
SUB-TOTAL		**3**	**95**	**53 (Ave.)**	**3**	**88**
Products (Tools and Supplies)						
13.	Tools and equipment	9	478	55	8	530
14.	Composite seeds	1	377	88	1	350
SUB-TOTAL		**10**	**660**	**70 (Ave.)**	**9**	**660**

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Step 4. Use participatory surveys with test farmers to rank best innovations for scaling up

Participatory Champion SAKs Identification Exercise @ Majthana, Nepal

Women Participants: 17

Men Participants: 12

Place Conducted:

Gairi Saura, Kaski

SAKs Interventions:

26 SAKs options

Results of Champion SAKs Identification Exercise By Women Farmers @ Majthana

SAK Products/ Practices	Score	SAK Products/ Practices	Score
$2 Hand Corn Sheller	54	Free - Terrace Wall Crop (Yam, Chayote, Pumpkin, Cowpea)	54
Free - Yam in Sacks	54	Rhizobium Trials	24
$2 Composite Vegetable Kits	54	$200 - Mini Tiller	26
$1 Hermetic Bags	54	$10 - Table Corn Sheller	26
Free - Maize+Ginger+Soybean	54	Animal Shed+FYM Improvement	34
Free - Edge Crops (rice bean, horse gram, blackgram)	54	etc etc (~18 more)	

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Step 4. Use participatory surveys with test farmers to rank best innovations for scaling up

Step 5. Procure and sell individual items from the regional menu to consumer farmers using pre-existing snackfood/cigarette/alcohol dealers into village stalls, using the NGO-spinoff company

Little stalls in the most remote villages around the world sell snacks, cigarettes and alcohol via pre-existing distributors

Nepal, Dhading village stall
(photo: M Raizada)

Current SAKNepal Private Sector Vendors (2016)

District	Peri-urban snackfood dealers	Small machinery dealers	Farmer cooperatives	Agrovet Dealers	Total
Chitwan	2	3	3	3	11
Nawalparasi	3	2	2	3	10
Tanahu	2	3	3	2	10
Dhading	2	2	3	4	11
Gorkha	2	3	3	5	13
Kaski	2	2	4	4	12
Parbat	2	2	3	3	10
Baglung	2	1	3	3	9
Myagdi	2	1	3	2	8
Lamjung	2	2	3	3	10
TOTAL	**21**	**21**	**30**	**32**	**104**

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

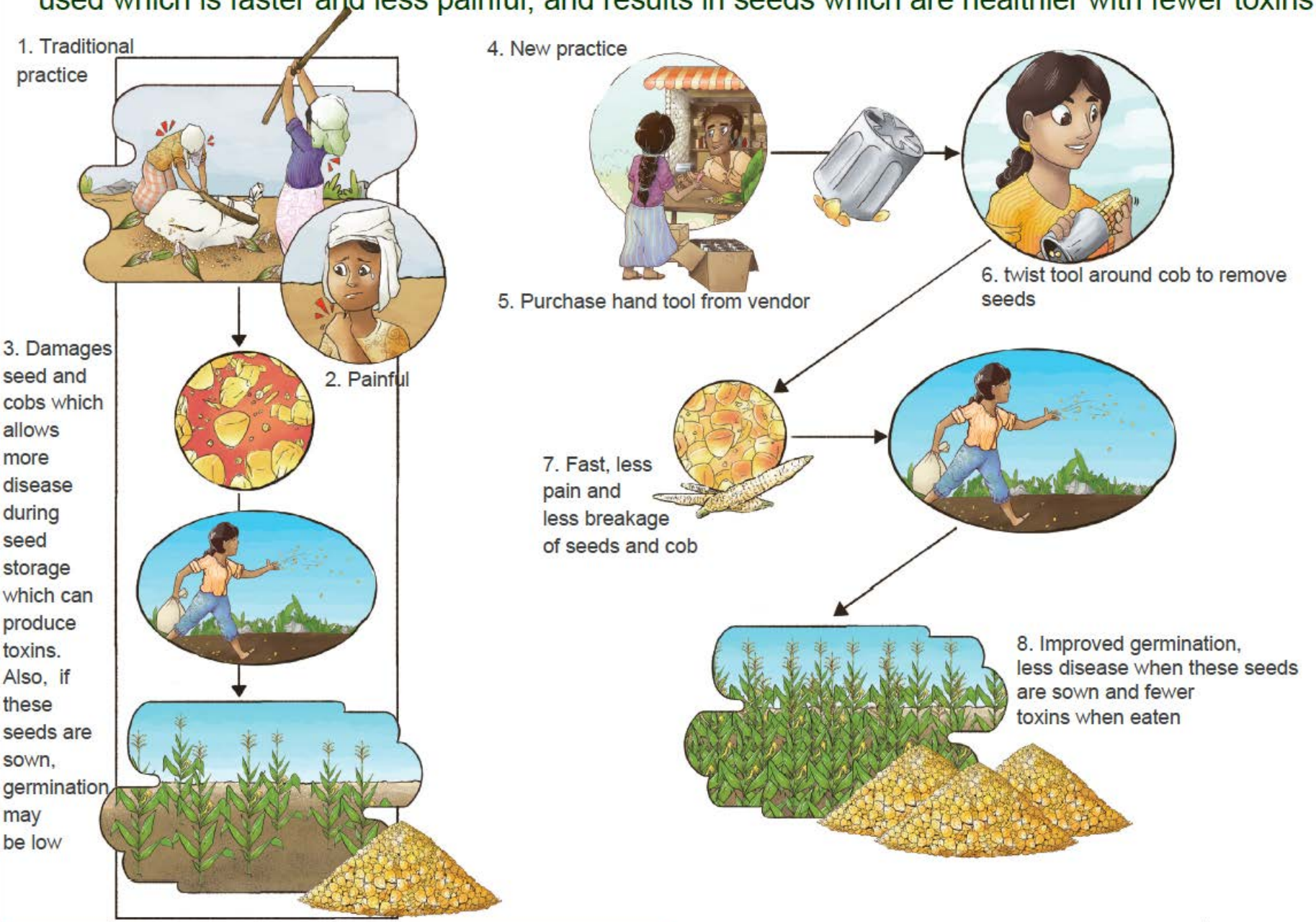
Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Step 4. Use participatory surveys with test farmers to rank best innovations for scaling up

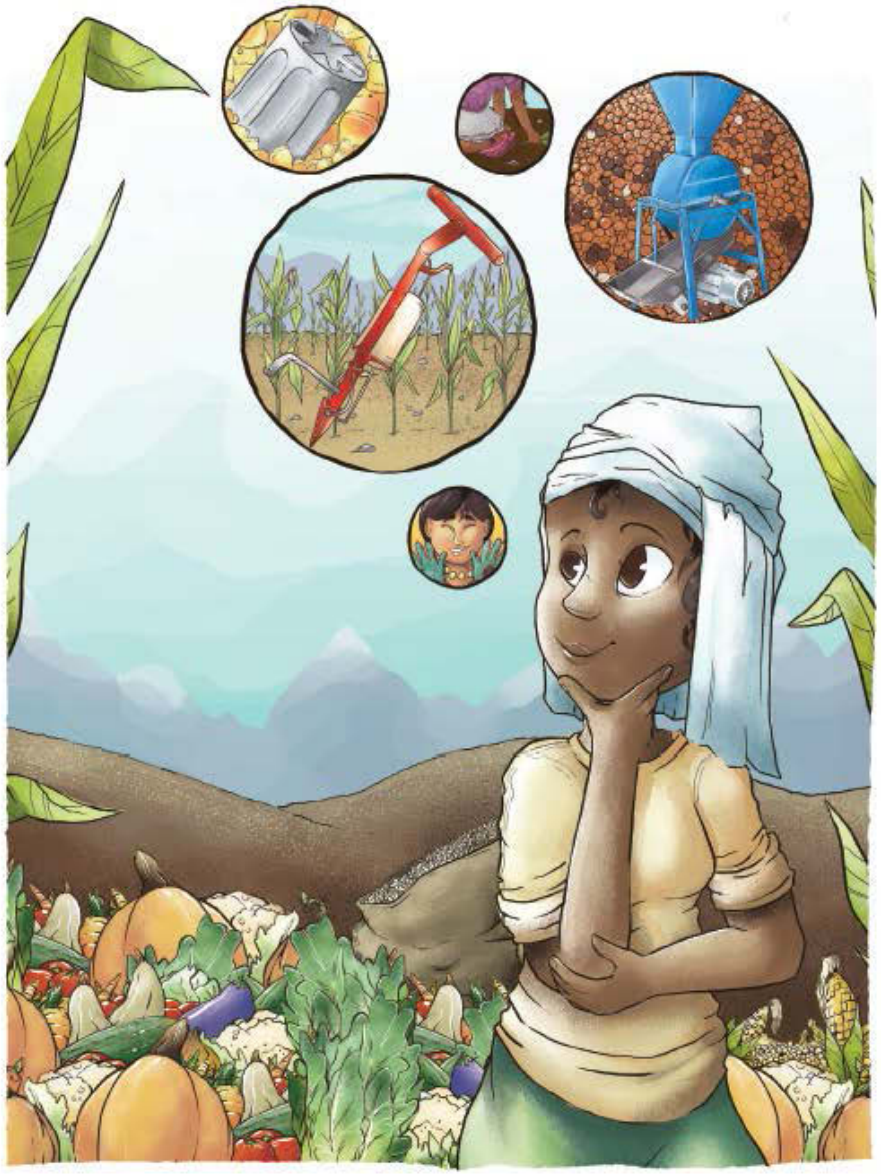
Step 5. Procure and sell individual items from the regional menu to consumer farmers using pre-existing snackfood/cigarette/alcohol dealers into village stalls, using the NGO-spinoff company

Step 6. Accompany each product with instructions in picture format, and a menu of practices in picture booklets, to communicate with illiterate women farmers

Lesson: Instead of removing grains of maize by beating sacks with a stick, a hand tool can be used which is faster and less painful, and results in seeds which are healthier with fewer toxins



Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons



- 190 pages
- 150 lessons
- Individual lessons accompany SAK products
- Smaller customized farmer booklets
- Free, downloadable

A Picture Book of Best Practices for Subsistence Farmers: South Asian version

June 2016

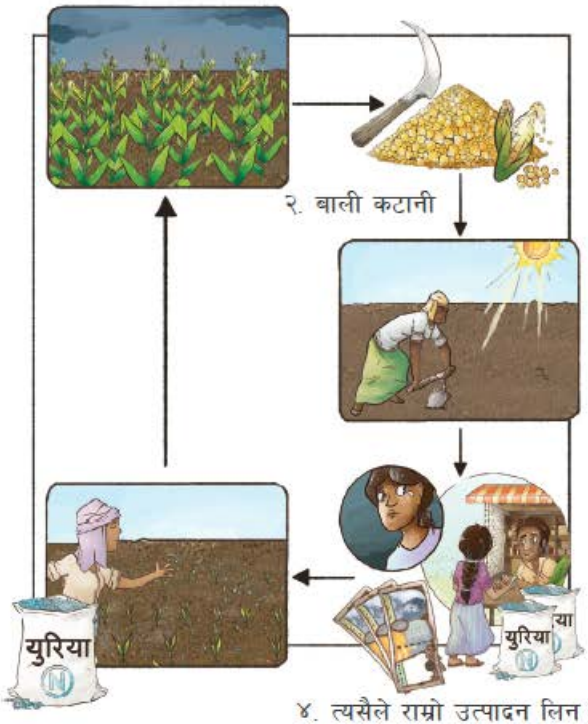
Manish N. Raizada, Ph.D.
University of Guelph
Illustrations by Lisa Smith
University of Guelph

Global Affairs Canada
Affaires mondiales Canada
IDRC International Development Research Centre
CRDI Centre de recherches pour le développement international

This book was made possible through generous funding from the Canadian International Food Security Research Fund (CIFSRF) from the International Development Research Centre (IDRC, Ottawa) and Global Affairs Canada. We thank our collaborators at LI-BIRD and Anamolbiu in Nepal and at the Canadian Mennonite University.

पाठ : कोसे तथा दलहन बालीहरुको जरामा मसिना गाँठाहरु हुन्छन् जसमा भएका उपयोगी जीवाणुहरुले प्राकृतिक नाइट्रोजन मल बनाउँदछ जसले गर्दा कम मात्रामा कृत्रिम मलको खरीद गरे पुग्दछ ।

१. गलत तरिका : सबै मौसममा मकै, गहुँ, धान, कादोको एकल बालीका रुपमा लगाउने र कोसा तथा दलहन बालीहरु नलगाउने



२. बाली कटानी

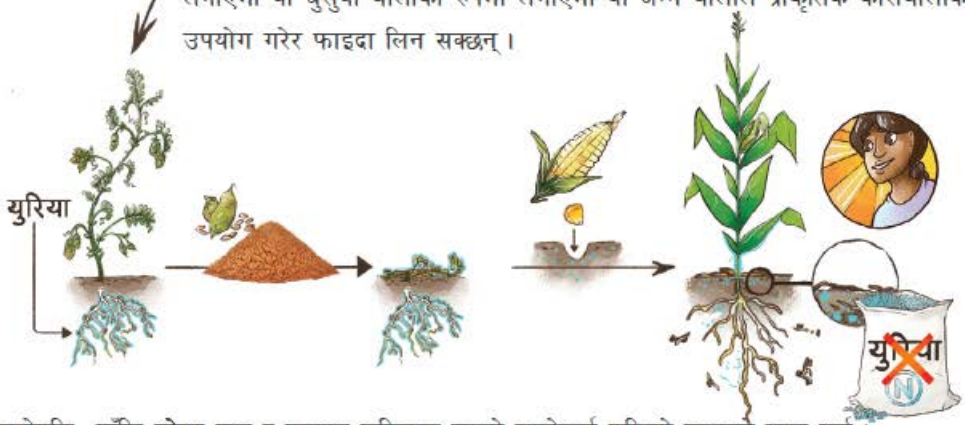
३. फेरी खाद्यन्न बाली लगाउने

४. त्यसैले राम्रो उत्पादन लिन म हगो नाइट्रोजन मल खरीद गर्नु पर्ने हुन्छ ।

५. सुधारिएको तरिका : कोसे वा दलहन बालीहरु घुसुवा बालीको रुपमा लगाउने अथवा अर्को मौसममा लगाउने ।

६. कोसे तथा दलहन बालीहरुको जरामा मसिना गुलाफी रङ्गका गाँठाहरु हुन्छन् जसमा प्र ाकृतिक मल बनाउने आँखाले देख्न नसकिने जीवाणु हुन्छन् । -फोटोमा देखाइएको जस्तै निलो तर यी जिवाणुलाई आँखाले देख्न सकिदैन ।

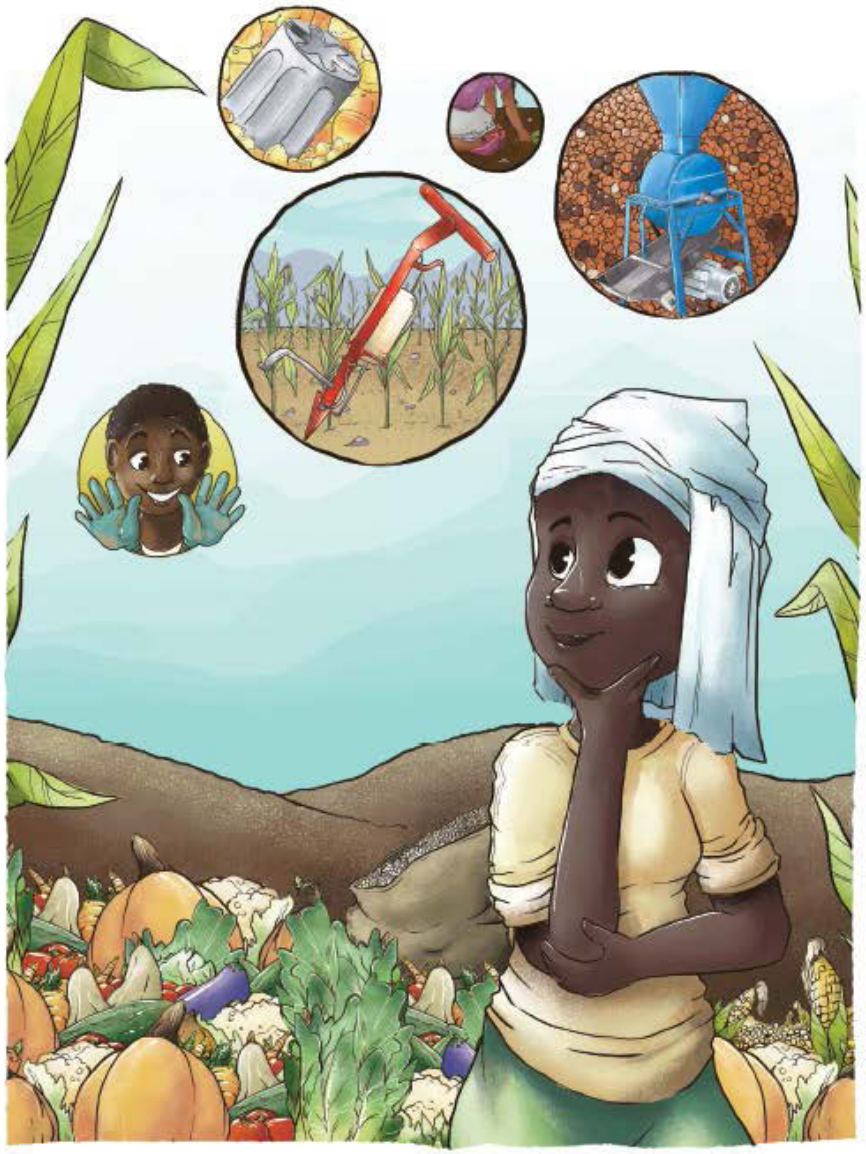
८. अर्को मौसममा कोसे तथा दलहन बाली लगाएको ठाउँमा मकै, धान, गहुँ वा कोदो सोही लगाएमा वा घुसुवा बालीको रुपमा लगाएमा यी अन्न बालीले प्राकृतिक कोसेबालीको मल उपयोग गरेर फाइदा लिन सक्छन् ।



७. कोसेबालीको दाना भित्राइसकेपछि बाँकि रहेका जरा र पातहरु कुहिन्छन् जसले माटोलाई मलिलो बनाउने काम गर्छ ।

९. यसको साथै कम मात्रामा नाइट्रोजन मलको खरीद गरी पैसा जोगाउन सकिन्छ ।

Participatory editing of the SAK Picture Book with 56 female farmers lead by Rachana Devkota in Nepal



- **African version completed**

Other versions in progress

- **East/Southeast Asia**
- **Latin America**
- **North Africa/Middle East**
- **Users can download individual lessons and add own text translations, and create custom booklets**

A Picture Book of Best Practices for Subsistence Farmers: Afro-Caribbean version

June 2016

Manish N. Raizada, Ph.D.
University of Guelph
Illustrations by Lisa Smith
University of Guelph

Global Affairs Canada | Affaires mondiales Canada

IDRC International Development Research Centre | CRDI Centre de recherches pour le développement international

This book was made possible through generous funding from the Canadian International Food Security Research Fund (CIFSRF) from the International Development Research Centre (IDRC, Ottawa) and Global Affairs Canada. We thank our collaborators at LI-BIRD and Anamolbiu in Nepal and at the Canadian Mennonite University.

Sustainable Agriculture Kit (SAK) Methodology

Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Step 4. Use participatory surveys with test farmers to rank best innovations for scaling up

Step 5. Procure and sell individual items from the regional menu to consumer farmers using pre-existing snackfood/cigarette/alcohol dealers into village stalls, using the NGO-spinoff company

Step 6. Accompany each product with instructions in picture format, and a menu of practices in picture booklets, to communicate with illiterate women farmers

Step 7. Use mobile phones to obtain feedback from consumer farmers on efficacy and improvements needed

The cell phone penetration rate in Nepal is 83%

SAKNepal Project Goal: To reach 100,000 people (25,000 households) by early 2018 with private and public sector partners

Global Affairs Canada
Affaires mondiales Canada

IDRC | CRDI
International Development Research Centre
Centre de recherches pour le développement international

Canada

University of Guelph
Changing Lives Improving Life

UNIVERSITY of GUELPH

LI-BIRD

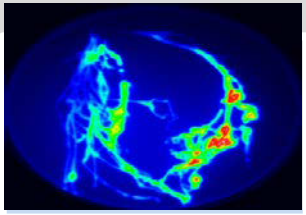
anamolbiu

NARC
Nepal Agricultural Research Council

PLANTPRODUCTS

XiteBio®

Conclusion



1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the *GlnLux* biosensor

1.2. Detection of SNF in colonies of rhizobia *in vitro*

1.3. Detection of SNF in legumes *in planta*

2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy

SAKNepal Acknowledgements

- **Funded by the Canadian International Food Security Research Fund (CIFSRF) (IDRC and Global Affairs Canada**
- Dr. Kevin Tiessen, **IDRC**
- Dr. Tejendra Chapagain, SAKNepal coordinator and agronomist, Univ of Guelph
- Roshan Pusasaini, SAKNepal coordinator, Nepal
- Bhawana Ghimire, LI-BIRD, Nepal and LI-BIRD Staff
- Dr. Ram Rana, LI-BIRD, Nepal
- Lisa Smith, SAK Picture Book graphic designer
- Anamolbiu staff (Nepal)
- Canadian private sector collaborators: PlantProducts (Ontario), XiteBio (Manitoba), Agriculex (Guelph)
- Post doctoral fellow: Dr. Malinda Thilakarathna
- Graduate students: Hanan Shehata, Rachana Devkota, Kamal Khadka, Finlay Small, Eamonn McGuinty, Michael Tessaro
- Gryphon Therault-Loubier, SAKNepal website coordinator
- Undergraduate research assistants: Jaclyn Clark (now MSc), Austin Bruch, Sara Wyngaarden, Caleb Niemeyer, Nick Moroz, Sophia Watts, Myla Manser
- Faculty advisors: Prof. Cate Dewey, Prof. Ralph Martin, Prof. Helen Hambly, Prof. Alastair Summerlee, Prof. Ali Navabi and other graduate committee members
- 400 Guelph undergraduate students: Canadian Youth Agrifood Food Trade Ambassadors (CYAFTA)

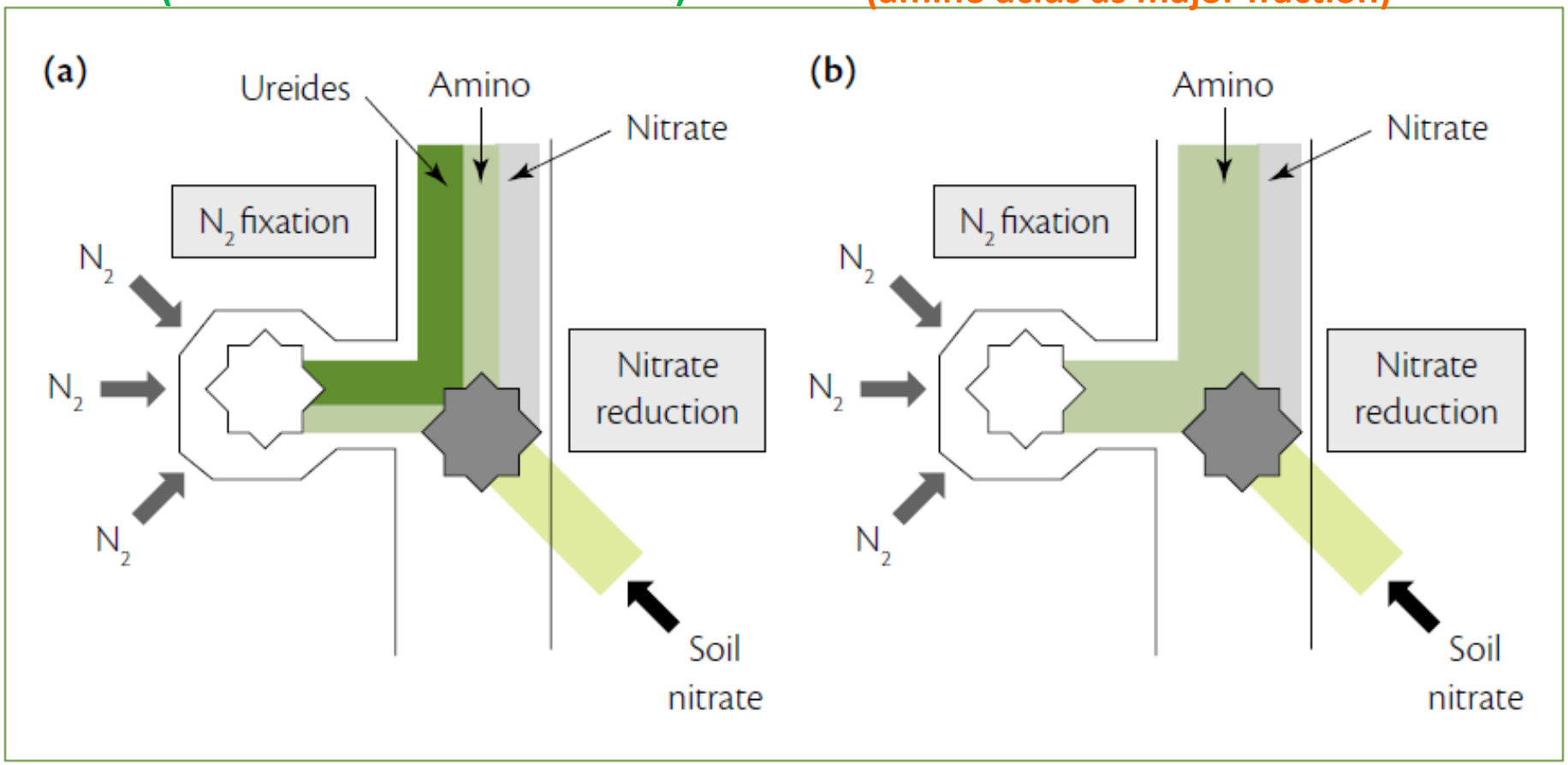
- Prof. Manish N. Raizada: raizada@uoguelph.ca

- Picture books available starting Jul2016: www.SAKBook.org
- Visit SAKNepal website: www.SAKNepal.org
- Sign up for Twitter: @SAK_nepal (>7500 followers)

Extra slides

Ureide exporters
(amino acids as minor fraction)

Amide exporters
(amino acids as major fraction)



* Soybean
* Common bean
* Kidney bean
* Cowpea
* Pigeon pea
* Black gram

* Lentils
* Pea
* Groundnuts
* Chickpea
* Clover
* Alfalfa

GlnLux 96-well Assay to Measure Relative SNF Output from Rhizobia Liquid Cultures (3 h protocol, 10-20 cents per sample)

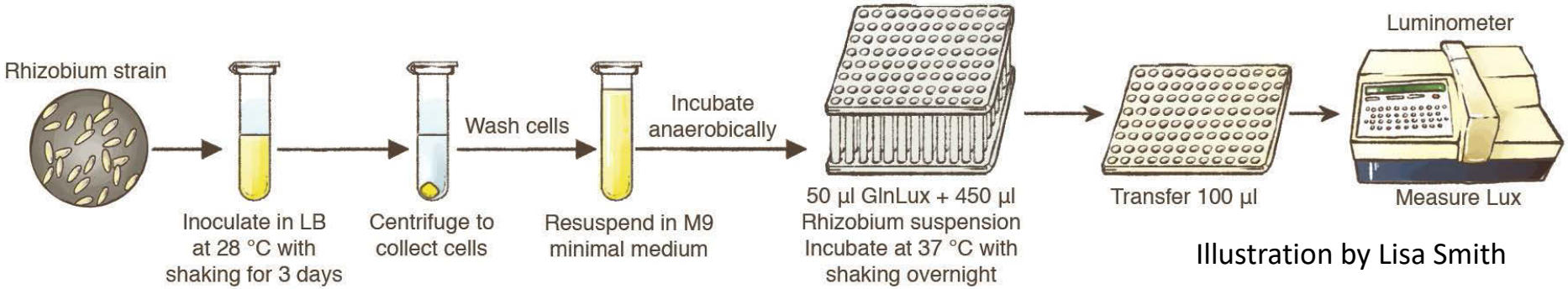
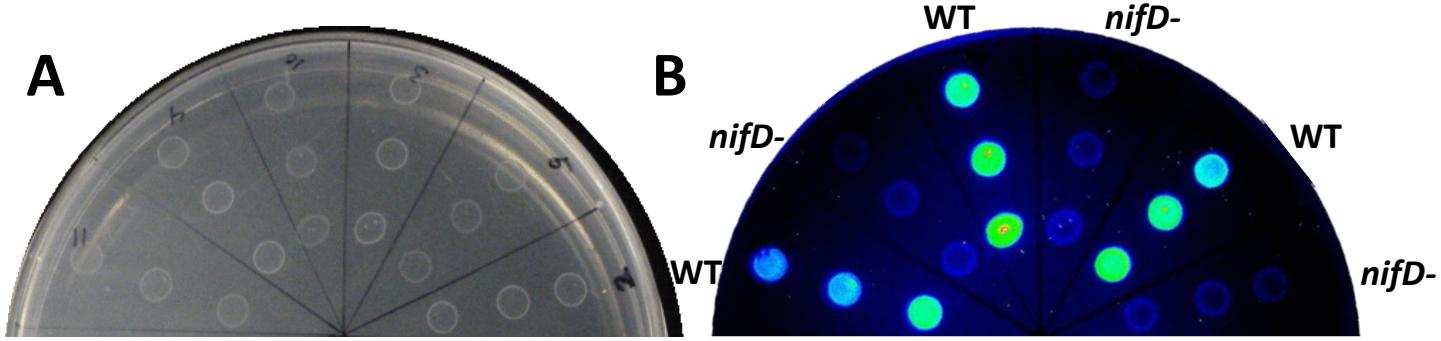


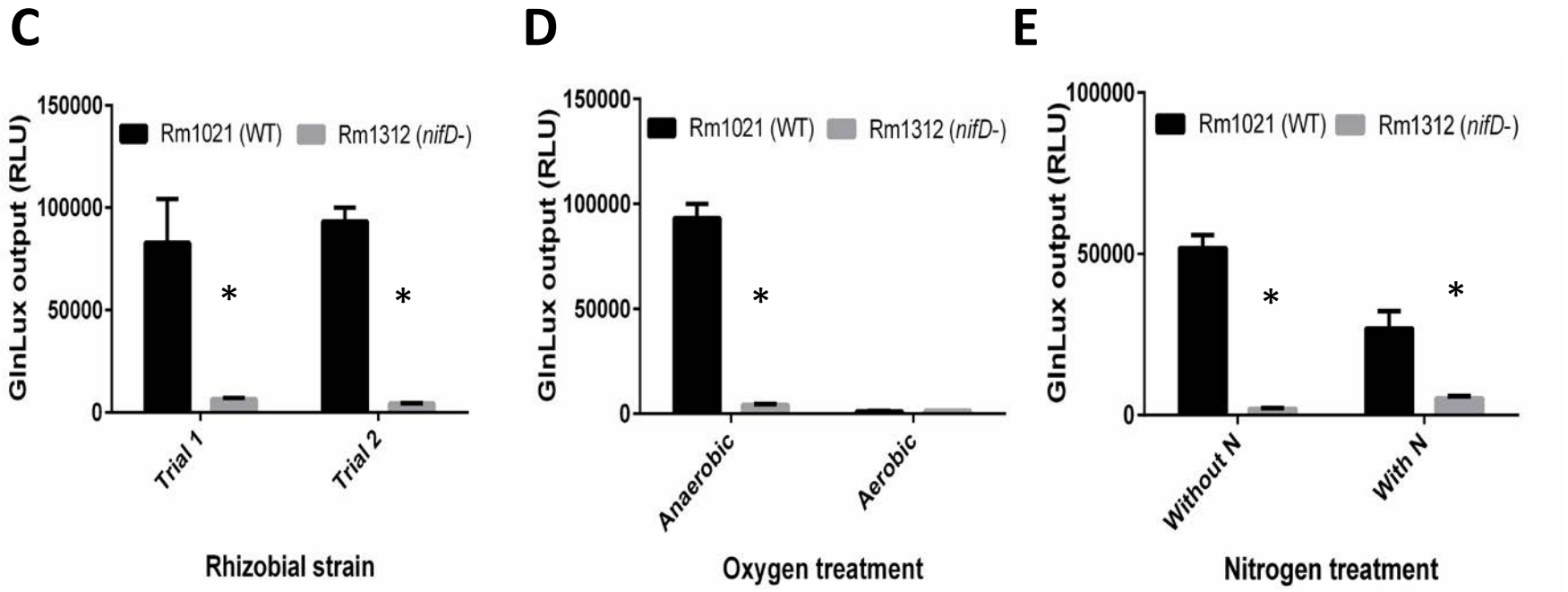
Illustration by Lisa Smith

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation

GlnLux agar can detect N-fixation activity from rhizobia colonies plates on *GlnLux* agar (*Sinorhizobium meliloti)*

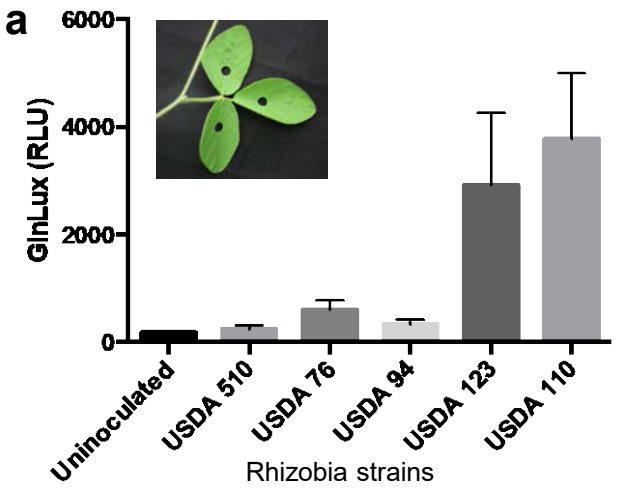


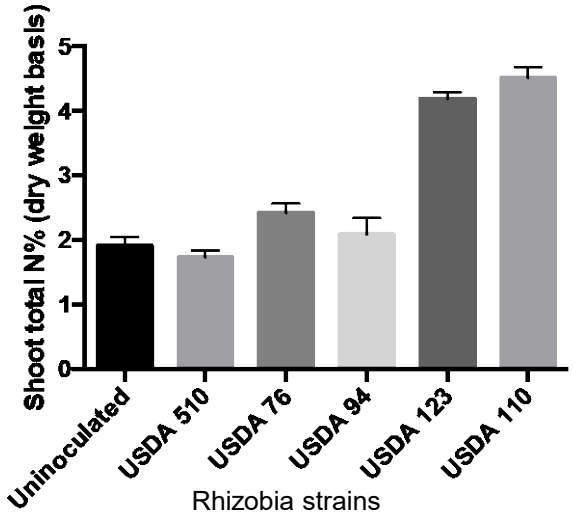
GlnLux cells co-incubated with rhizobia in 96-well liquid culture plates can measure how N fixation responds to the environment

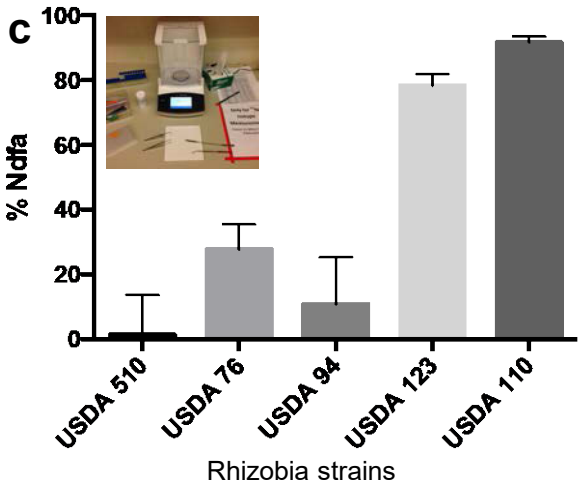


Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation

Effect of different rhizobia strains on SNF of soybean: *GlnLux* leaf punch and agar assays

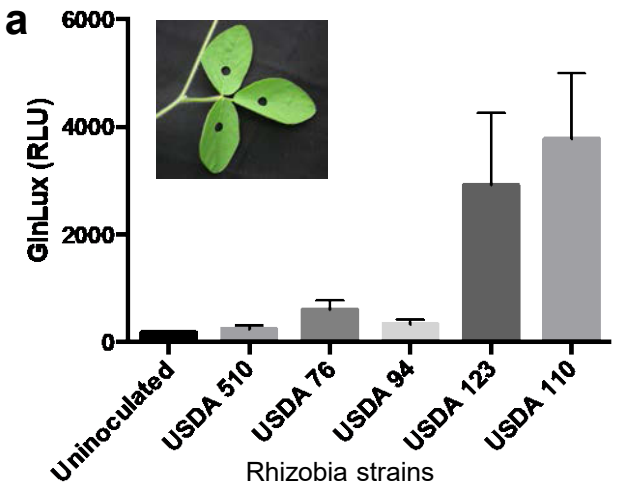


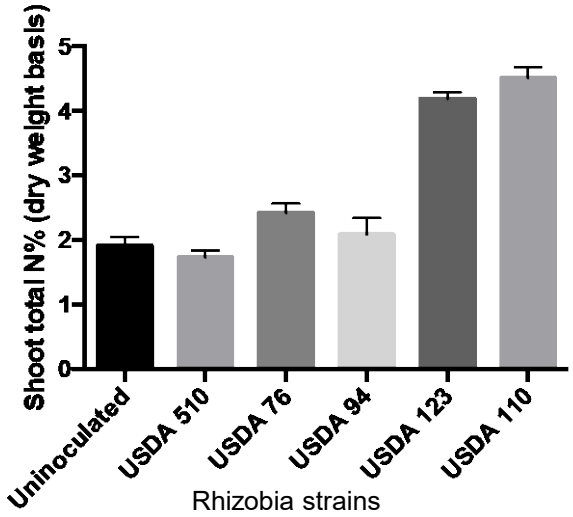


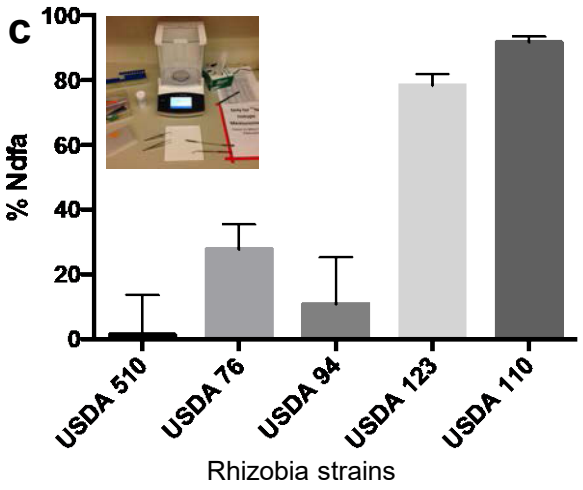


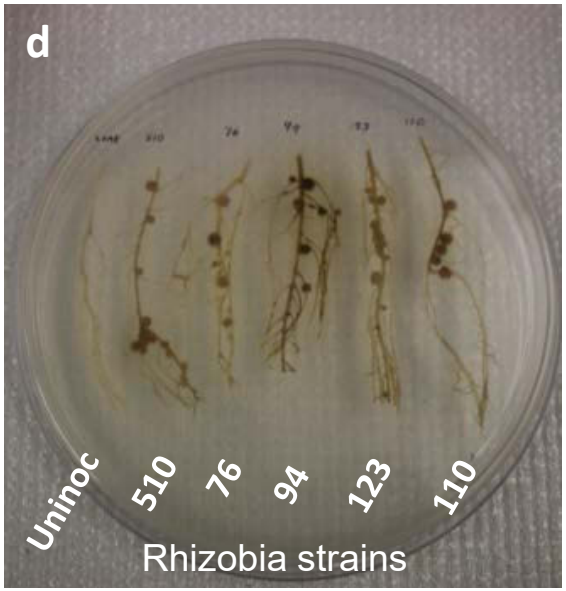
Thilakarathna, Moroz and Raizada (2016) *Submitted*

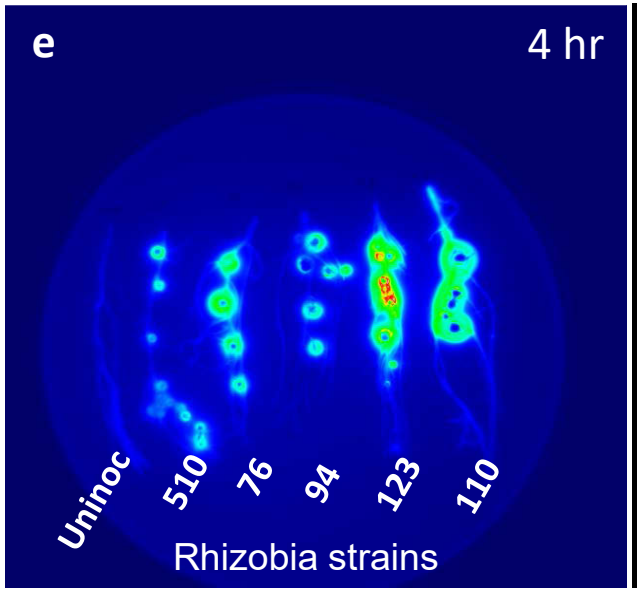
Effect of different rhizobia strains on SNF of soybean: *GlnLux* leaf punch and agar assays

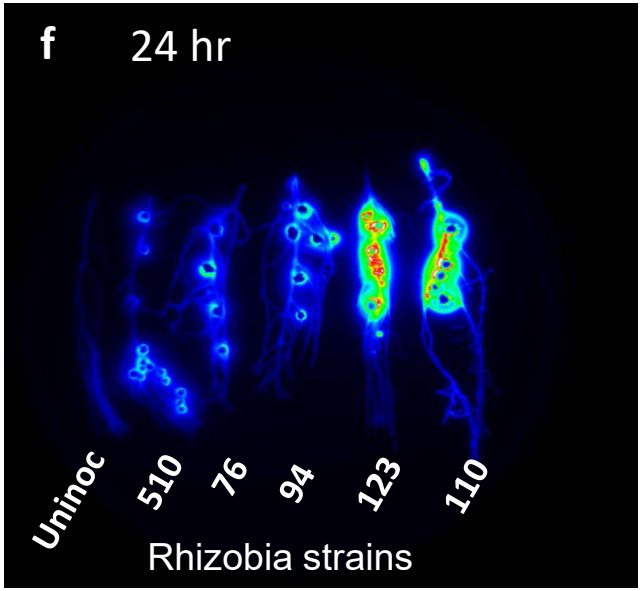












Thilakarathna, Moroz and Raizada (2016) Submitted

Effect of different rhizobia strains on SNF of lentil: *GlnLux* imaging of roots

a 

b 

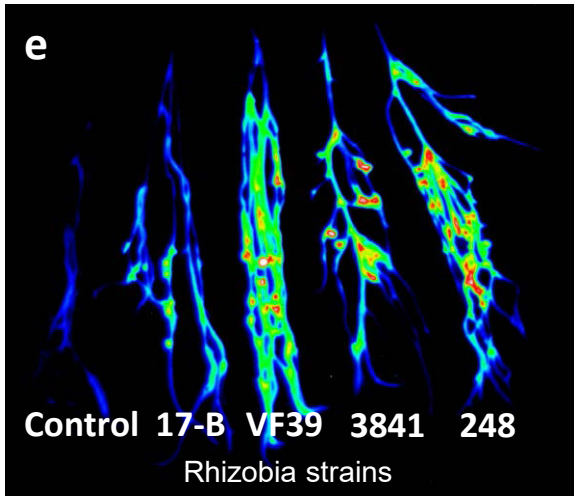
Rhizobia strains

Rhizobia strains

Light image

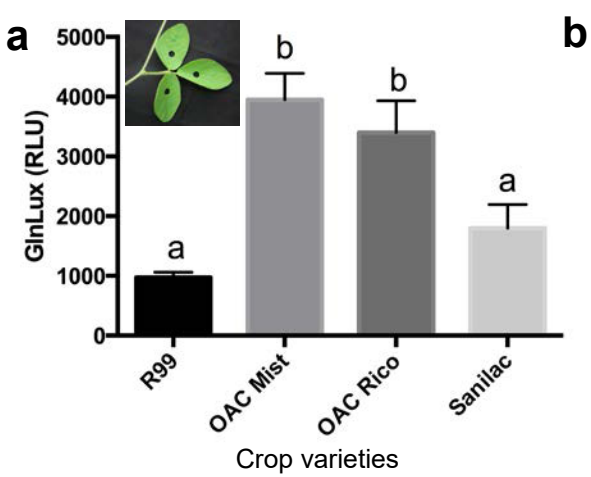
White lux image

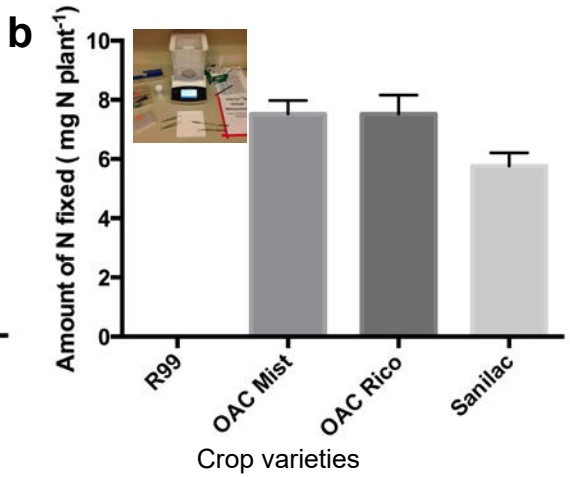
False-colored lux image



Thilakarathna, Moroz and Raizada (2016) *Submitted*

Effect of different crop varieties on SNF of common bean: *GlnLux* imaging of roots





Thilakarathna, Moroz and Raizada (2016) *Submitted*

Light image | **White lux image** | **False-colored lux image**