



RAS5070: Developing Bio-energy Crops to Optimize Marginal Land Productivity through Mutation Breeding and Related Techniques (RCA)

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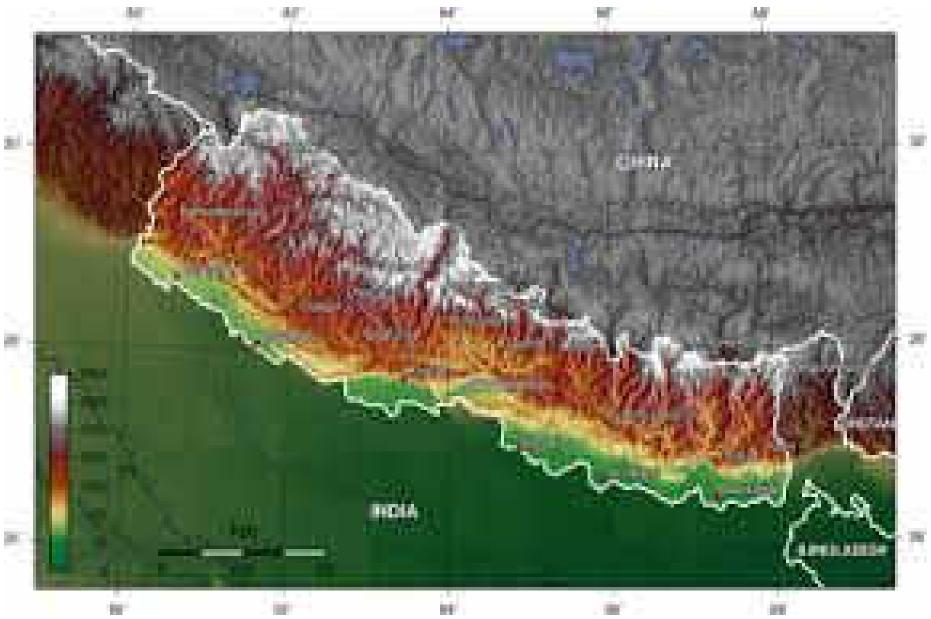
IAEA/RCA Coordination Meeting to Discuss the progress of the field trials

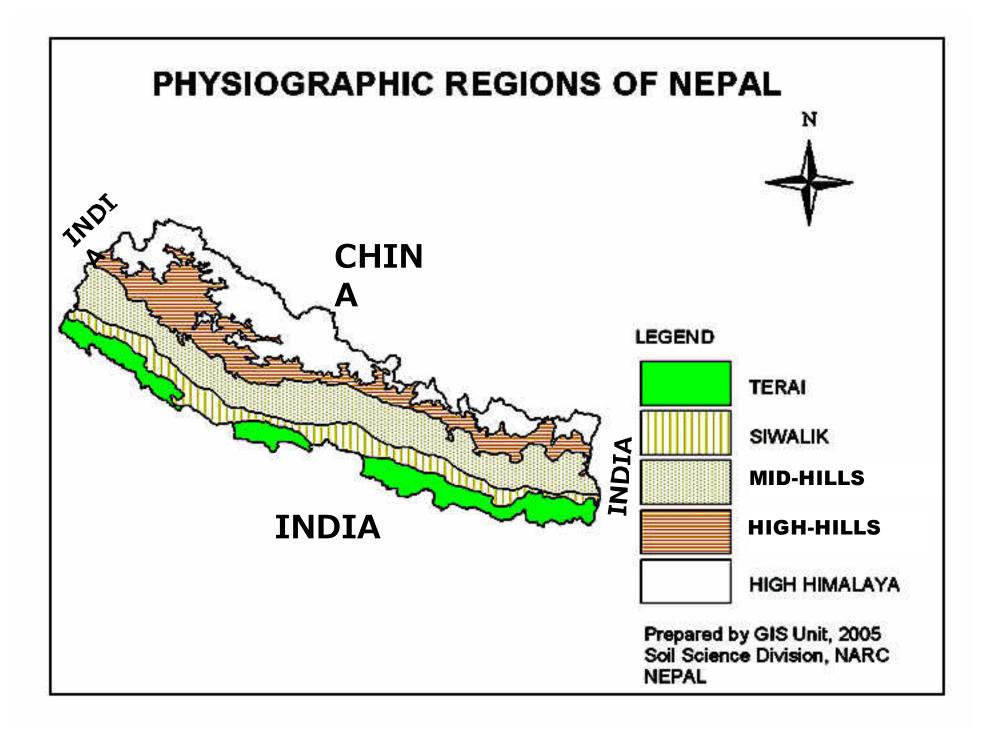
Bindeshwar Prasad Sah Director and NPC-RAS/5/070

National Agricultural Research Institute/ NARC Khumaltar, Lalitpur, Nepal

Nepal is a landlocked country: 3 sides India and 1 side China.

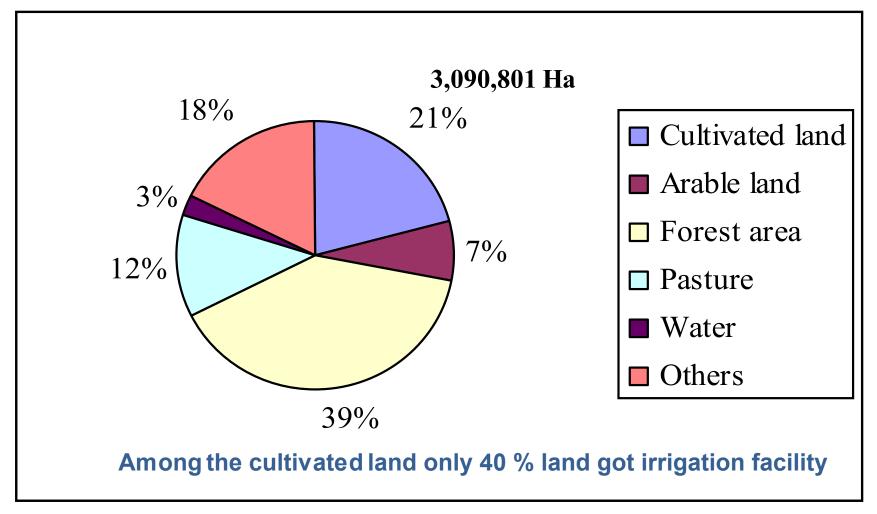




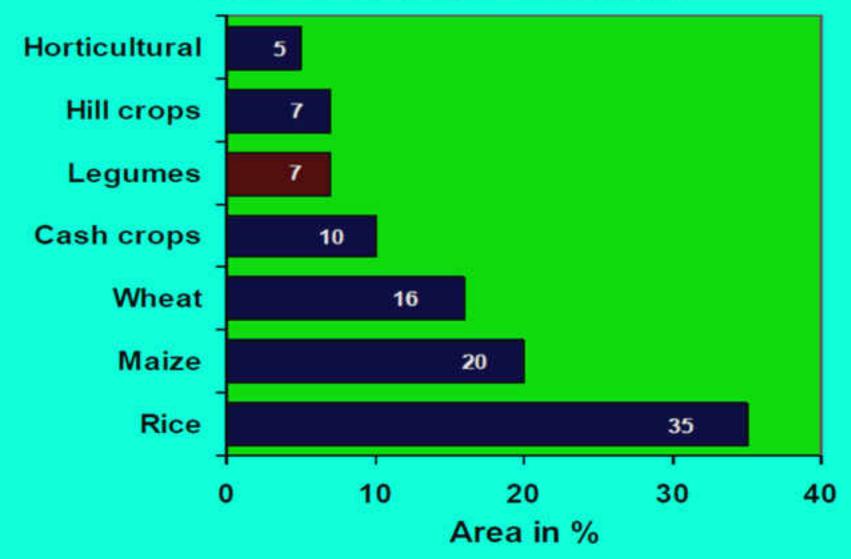


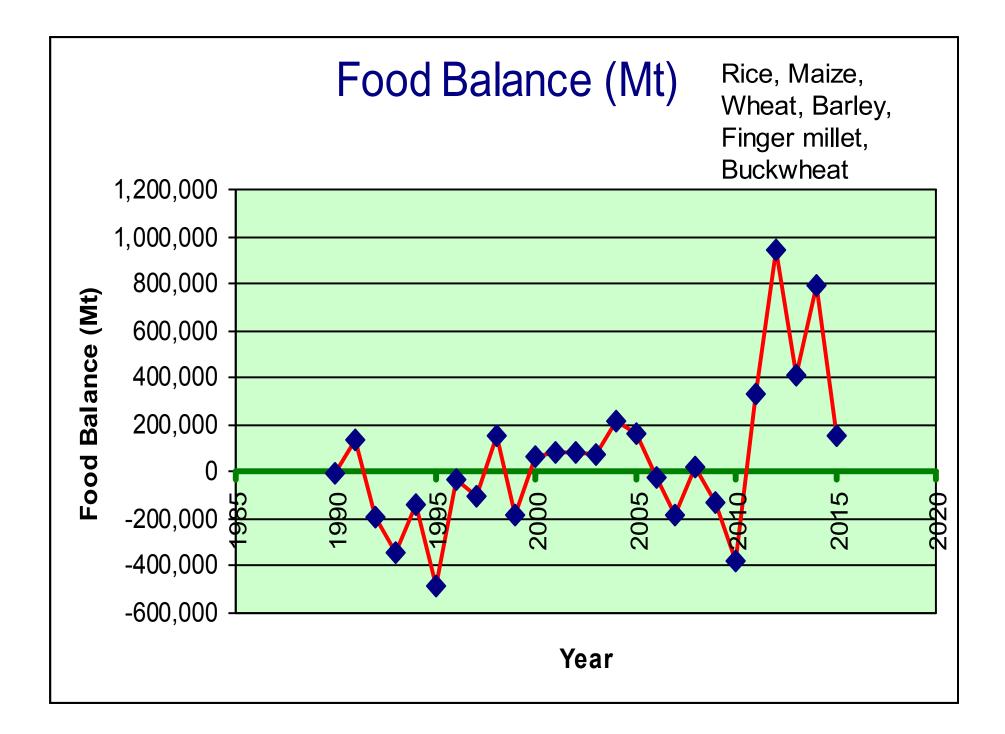
Facts of Nepal

- Total population: 27,539,000
- Population engaged on agriculture : from 93 to 65.6%
- Land coverage: 14,718,100 Hectare

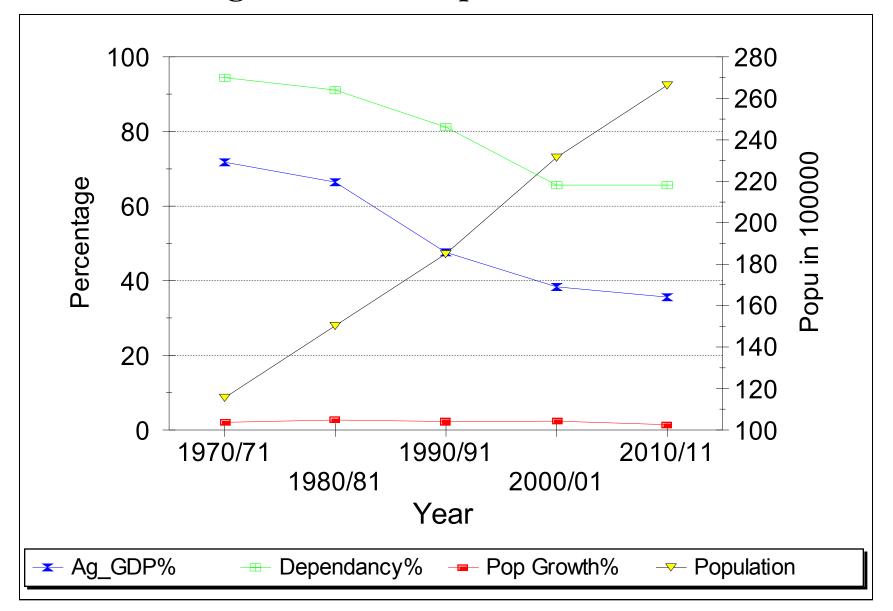


Crop Coverage in Nepal



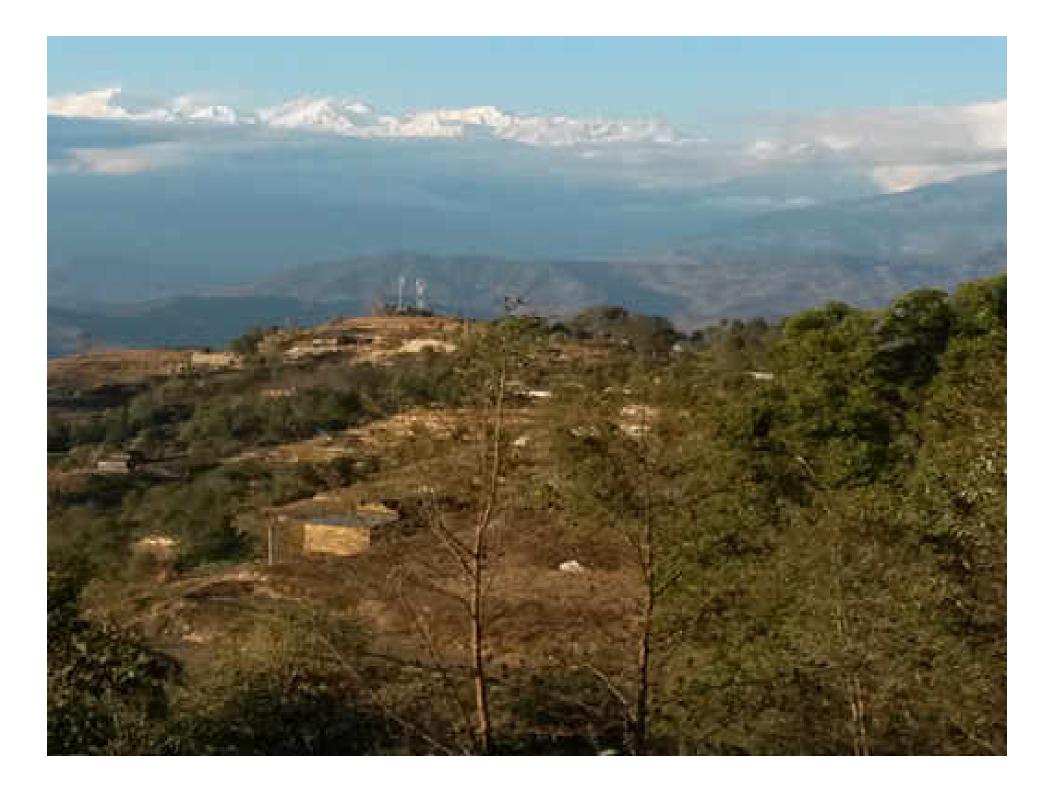


Status of Population Growth, Dependency on Agriculture and share of Ag in GDP in Nepal



Status of Import and Export of Agricultural Commodities

Commodity	Export (Million NRs)	Import (Million NRs)	Export/ Import ratio		
Live animals & their products	22.76	1684.13	1:9		
Plant products	9353.46	17379.40	1:2		
Animal & Plant based fats & oil	314.34	12203.46	1:38		
Processed Plant products	3837.23	13163.42	1: 3.4		
Total	13717.80	44430.17	1: 3.2		









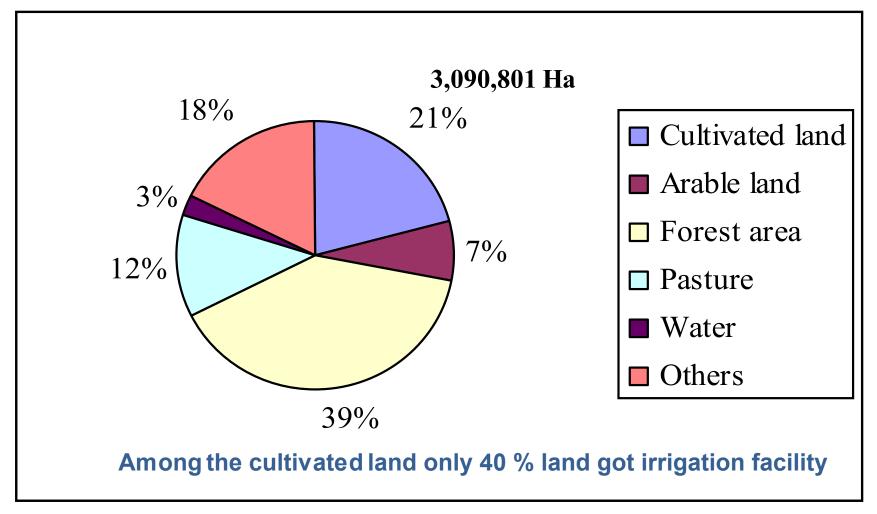


Rice based Major Cropping Pattern:

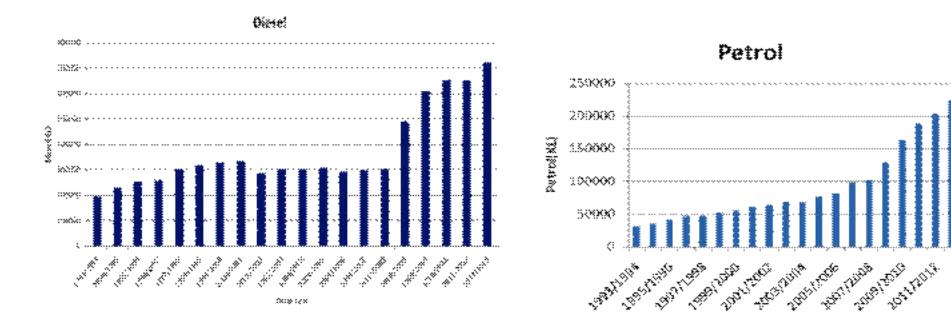
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rice												
Wheat												
Fallow												
Rice												
Lentil												
Fallow												
Rice												
Oilseed												
Fallow												
Sugarcane												
Rice												
Maize												
Fallow												

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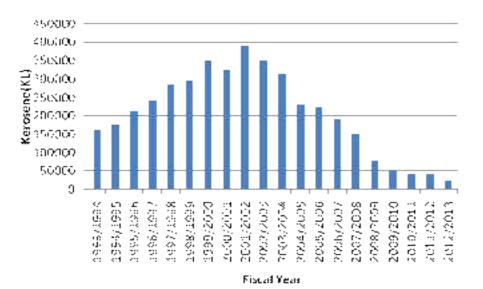


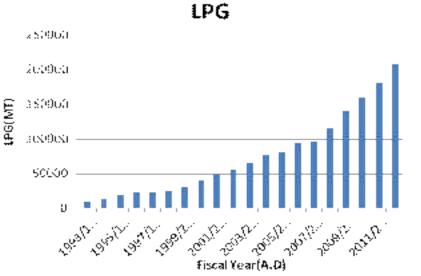
Nepal depends on imported fuel from India and sells petrol, diesel and kerosene at highly subsidized rates, at huge financial cost to the government's Nepal Oil Corporation (NOC).



Fiscal Year(A.D)













Police injured by public during security process for stopping protesting and firing of belongings of Nepal Oil Corporation due to rise in price of fuels











Load-shedding

Load shedding per day:

- 6-8 hours (wet season) i.e. 2190-2920 hours/ 92-122 days per year (wet season)
- 12-16 hours (dry season) i.e. 4380- 5840 hours/ 183-243 days per year (dry season)

Garbage can be converted to bio-fuel



> So, there is a urgent need to solve this fuel crisis

Main Sources of Alternative Energy in Nepal

- 1. Biogas
- 2. Micro-Hydro Power
- 3. Biomass Energy
- 4. Solar Energy
- 5. Wind Energy

Alternative fuel for Vehicle engines

- 1. Compressed natural gas (CNG)
- 2. Liquefied petroleum gas (LPG)
- 3. Alcohol fuels such as methanol (methyl alcohol) and denatured ethanol (ethyl alcohol)
- 4. Bio-diesel
- 5. Electricity (stored in batteries)
- 6. Hydrogen (fuel-cell)
- 7. Solar

Status of Sweet potato in Terai









> Bio-fuel will be one of the most prominent solution.

- In Nepal, for bio-fuel production, Jatropha seeds have been tried, however not been commercialized.
- Other possible commodities are algae, rape seed, sugarcane, sweet potato etc.

Objectives

- To find and improve suitable bio-fuel crop
- To improve soil moisture and fertility for growing bio-fuel crops
- To reduce GHGs
- To preserve ecosystems benefits
- To reduce import of fuels and save national foreign exchange
- To improve national economy

Available resources

• Sugarcane:

- Biotechnology Division
- National Sugarcane Research Program
- > NARC setups

• Sweet potato:

- Biotechnology Division
- National Potato Research Program
- NARC setups

Future plan

Sugarcane:

- Evaluation and selection of mutants having capacity to efficiently utilize soil moisture and fertility for producing higher yield
- Evaluation for higher sugar content and ethanol output

Sweet potato:

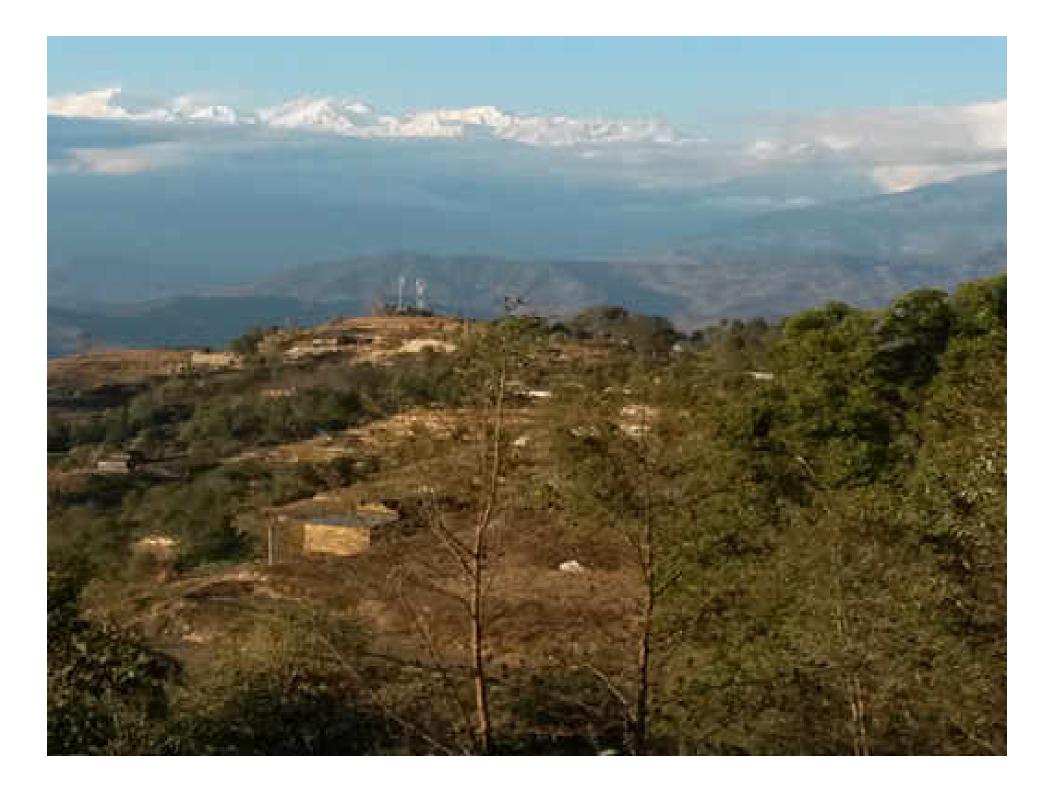
- Collection of available germplasm
- Survey on distribution and cultivation
- Mutagenesis of desirable cultivar

Both:

• Strengthening scientific capacity

Request for assistance

- Facilitate in mutagenesis
- Facilitate for germplasm and scientific exchange
- Support in enhancing scientific capacity



River basin and Mid-hill area













Mid-hills





Multi-purpose crops: food + feed + fuel

Country is facing energy crises

- has great plant diversity that is potential for developing energy source
- plants producing oil and some plants that producing carbohydrate that can be converted to bio-ethanol
- huge amount of unproductive land: called as "marginal land" that cannot be used to grow food crops
- improved agricultural technologies like mutation breeding and nutrient and water management practices can be applied

- productivity of those marginal lands might be improved, especially by growing bio-energy crops
- Bio-energy crops, which will be developed for the marginal areas, therefore have the potential to improve the environment, increase rural incomes and offer a more robust crop
- Through developing potential bio-energy crops with application of nuclear techniques (mutation breeding; N-15 or C-13 isotopic techniques):
 - ✓ expected that crops' productivity will increase and, in turn, it will help to:
 - increase land productivity,
 - protect environment,
 - enhance ecosystem balance, and
 - improve farmers welfare in the region

Objective

 Develop bio-fuel crop for fuel production in marginal and fallow land

Activity

- Collect bio-fuel local commodities and irradiate seeds and plant parts
- Select genotypes with high fuel content
- Motivate for utilization of marginal land for bio-fuel production

Meetings and Outcomes:

The workshop was held from 23 to 27 March 2015 in Vienna, Austria and 16 countries participated.

Outcomes were:

- Fine-tune of Regional project work plan with specific time frames and host countries identified
- National work plans developed and national work team identified
- National priorities on mutation breeding and soil and water management identified
- Current country status on mutation breeding of bio-energy crops presented
- Crops and target traits identified to breed for marginal lands
- IAEA's resources to provide technical support recognized

Outcomes of the meeting during March 2016: Crop: SUGARCANE

Members: Bangladesh/Nepal/Philippines/Thailand

- 1. Background of marginal land problem in the region including soil, water and nutrient stats, current agricultural practices to restore marginal land
- 2. Field studies to address improving land nutrient and water management in marginal: objectives and outputs
- 3. Experimental design (treatments as well as number of replications), type of crops (*i.e.* Sugarcane, Cassava, sweet potato, Kepu, Candle nut, Kenaf, rapeseed, sorghum, *etc.*)

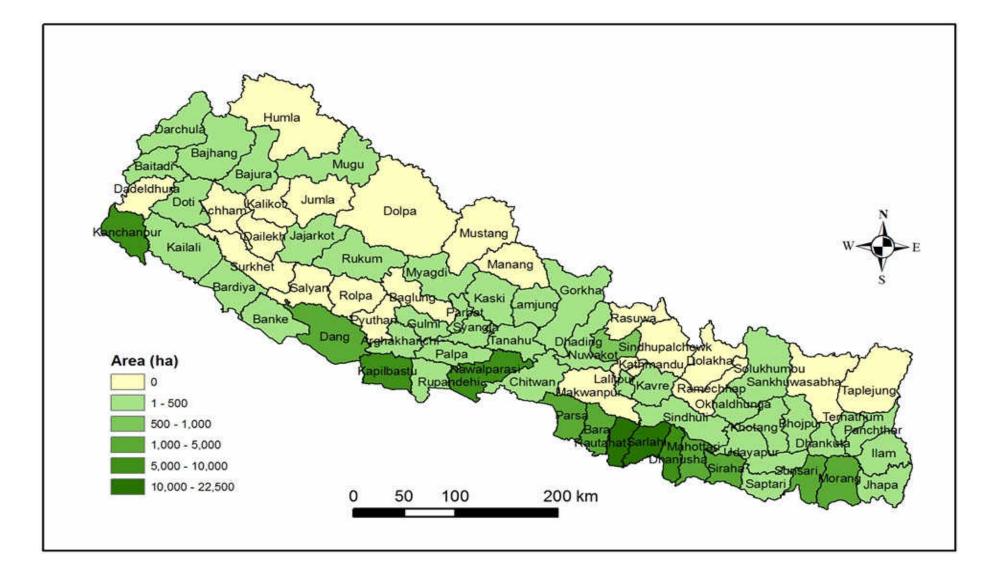
4. Soil, plant and water parameters to be collected and analyzed during the course of the study

5. Duration of the field trials along with starting date

6. Use of Nuclear Techniques such as ¹⁵N to assess nutrient use efficiency, ¹³C to asses soil quality, moisture neutron probe to monitor soil water and ²H and ¹⁸O to quantify evapo-transpiration if any?

7. Team (Soil plus plant breeding) need to be formed

Distribution of sugarcane crop in Nepal



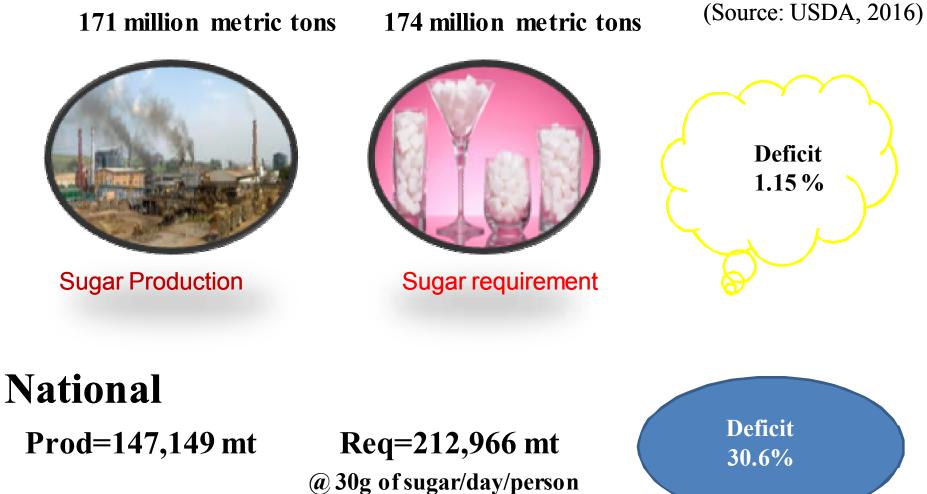
Commercial Sugar Production in Nepal

Details of Sugar Factory, their Crushing Capacity and Sugar Production in Nepal during 2015/16

SN	Name of sugar factory	Address	Cane Crushing Capacity (tons/day)	Total Cane Crushed (tons)	Sugar Production (tons)	Sugar Recovery %
1	Eastern Sugar Mills Ltd.	Amahibelha, Sunsari	2,500	134,150	12,341.9	9.20
2	Everest Sugar and chemicals	Ramnagar, Mahottari	5,000	346,282	33,589.4	9.70
3	Indu Shankar Chini Udhyog Ltd.	Hariwan, Sarlahi	5,000	312,080.5	30,620.9	9.81
4	Annapurna Sugar Mill	Dhankauta, Sarlahi	2,500	114,950	10,690.4	9.30
5	Shri Ram Sugar Mill	Garuda, Rautahat	2,500	89,809.1	8,181.7	9.11
6	Reliance Sugar Industry	Shripur, Bara	2,500	181,000	17,611.2	9.73
7	Mohini Sugar Industry	Nawalparasi	50	-	-	-
8	Indira Sugar and Agro Industry	Nawalparasi	500	36,800	3,128.1	8.50
9	Bagmati Chini Mill Ltd.	Kuriya, Nawalparasi	1,200	66,389.8	6,194.2	9.33
10	Lumbini Chini Udhyog Pvt. Ltd.	Sunwal, Nawaparasi	1,000	34,800	2,784.0	8.00
11	Mahalaxmi Sugar Mill Ltd.	Jawvari, Kapilvastu	2,000	71,700	6,811.7	9.50
12	Mahakali Sugar Mills	Kanchanpur	500	98,300	9,142.0	9.30
13	Bhageswori Chini Udhyog	Kalika, Kanchanpur	1,000	65,800	6,053.7	9.20
14	Himalayan Sugar and Chemicals	Panwari, Siraha	2,500	16,600	-	-
	Total			1,568,661.4	147,149.2	
	Average					9.22

Sugar Status

Global

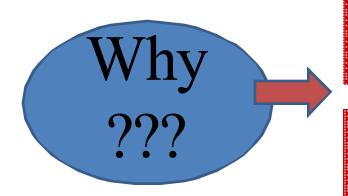


(Source: MoAD, 2015, Indushankar sugar mill, 2015)

- To fulfill the domestic requirements and export promotion of sugars, we must attain at least 70-80 mt/ha national productivity with >11% sugar recovery
- Improved sugarcane varieties and Improved production technologies play an important role in boosting cane productivity.

Major Constraints in sugarcane varieties development in Nepal

• Since 2004 none of the varieties have been released in Nepal



Sugarcane seed fuzz could not be produced

No Congenial environment for natural breeding





 Landraces of sugarcane found in Nepal are not suitable for commercial cultivation

 The landraces are low in sugar content and low productive.

 No any valid external sources of sugarcane germplasm

 Lack of Bilateral and multilateral agreements for germplasm exchange with other countries

Major Constraints in sugarcane production

≻No seed set

➢ Biotic stresses

- Red Rot
- Smut
- Wilt
- Pyrilla
- Root and Shoot Borers
- Whitefly

➢Abiotic Stresses

- Drought
- Flooding/ Waterlogging
- **Nutrient disorder** (deficiency and toxicity)



Red Rot



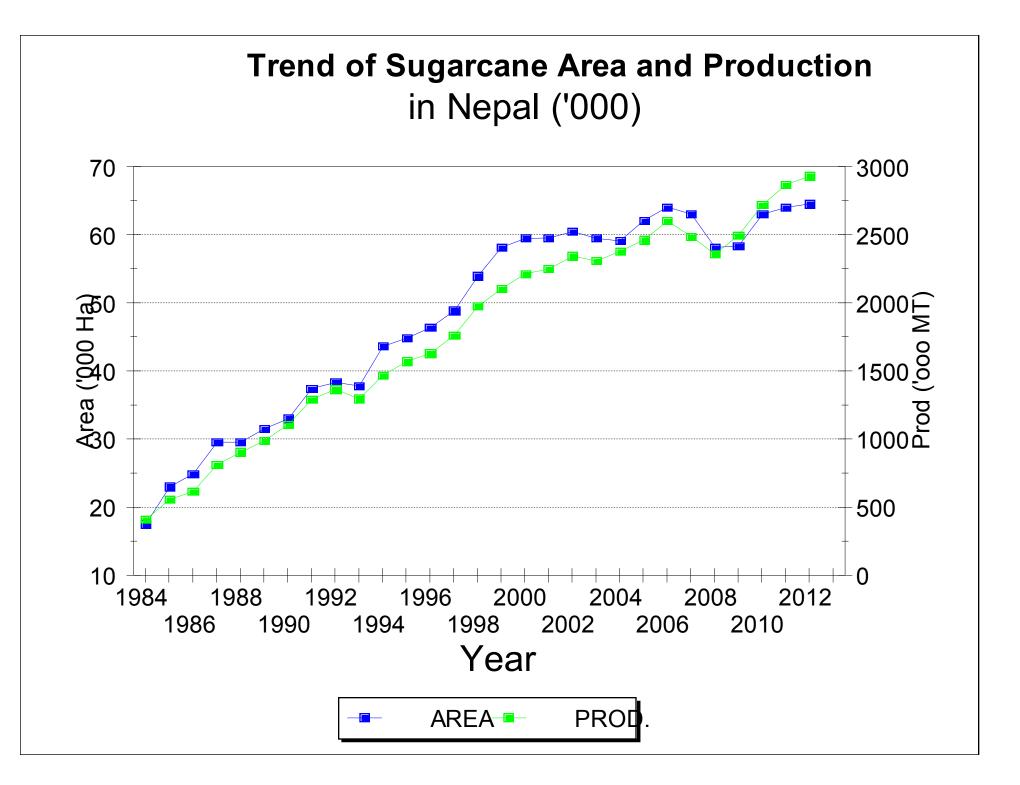
Drought Stress

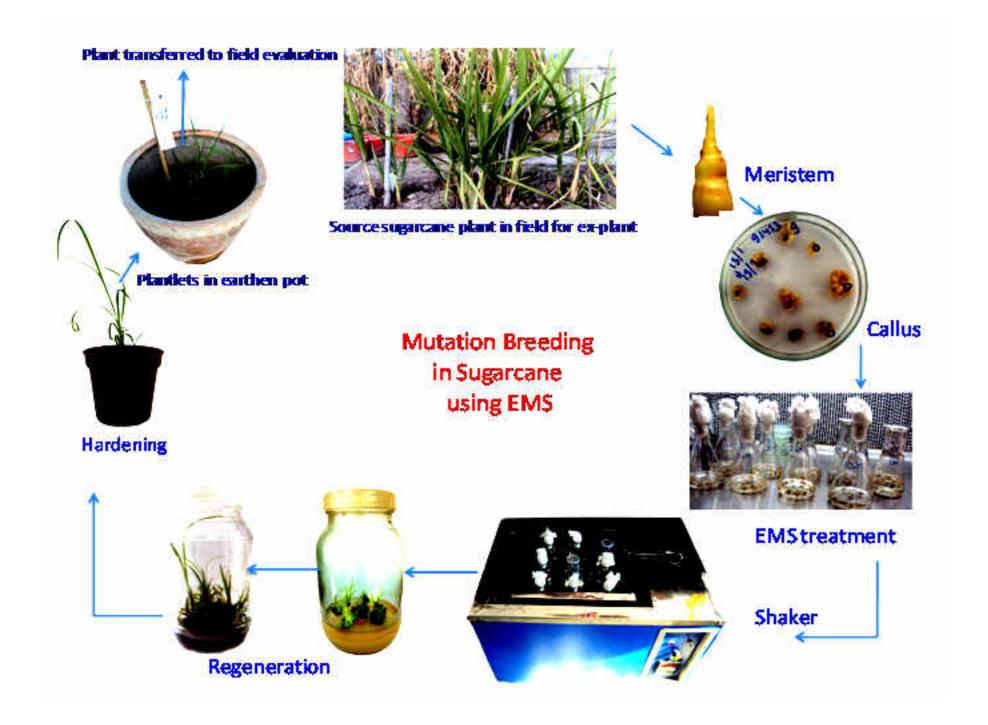


Smut



Flooding





Field Design

Observation nursery

- Plot size: two rows & two meter length with 90cm spacing
- Seed setts: three budded 12 setts per meter length
- Setts treatment: dipping 15 minutes in fungicides carbenzim@1 gm/litre
- Fertilizer recommended: 150:60:40 kg N:P:K/ha
- Other intercultural operations were done as per crop requirement
- Mutant Sugarcane Genotypes: UP 9742, CoP 97182, CoSe 98255 and CoSe 92423

37 Mutant lines grown in the field of SRP, Jeetpur, Bara (Photo at their vegetative stage): variations are clearly visible



Sugarcane cont....

- As a first lot, a total of 36 putative mutant lines of sugarcane, derived from CoSe 92423, had been planted in NSRP, Jitpur for field evaluation.
- Further, five elite sugarcane germplasms, viz. BO-139, BO-141, CO-0233, COJ-85 and COLK-94184 were collected from NSRP, Jeetpur for invitro mutagenesis.

Initial Evaluation Experiments:

- Design: RCBD
- Replication: three
- Plot size: 90 cm spaced 4 row with 4 m length
- Fertilizer: 150:60:40 kg/ha NPK
- Seed setts: three budded 12 setts per meter length
- Setts treatment: dipping 15 minutes in fungicides carbenzim@1gm/litre
- Other intercultural operation was done as per crop requirement

Genotypes	Yield (mt/ha)	Millable canes/ha	Stalk length (m)	Cane diameter (cm)	Single cane weight (kg)
BO 139	116.48	121750	2.61	2.01	0.84
CoSe 1434	79.25	106250	2.60	2.99	0.86
CoSe 98231	87.20	105000	2.31	2.19	0.96
Co 0232	75.58	122500	2.24	2.06	0.76
Co 97016	57.67	76875	2.52	2.13	0.94
BO 147	58.75	70625	2.51	2.15	1.07
CoS 88230	57.42	82500	2.34	2.17	0.93
CoS 8432	46.11	70625	1.59	2.21	0.71
BO 146	109.05	101250	2.71	2.11	0.99
Co 07250	88.92	95000	2.28	2.08	0.86
CoSe 98259	78.02	95625	2.26	2.30	0.95
CoLk 94184	106.26	126250	2.48	1.93	0.84
CoJ 64	52.16	62500	2.23	2.01	0.80
Co 0233	82.58	100000	2.48	2.06	1.02
BO 141	80.56	99375	2.60	1.91	0.84
CoSe 03234	77.56	100625	2.44	2.06	0.94
Co 98014	18.50	33125	2.12	2.21	0.95
UP 0098	67.32	78125	2.56	3.26	0.94
BO 110	83.09	126250	2.63	1.82	0.77
Co 0239	112.91	131875	1.78	2.30	0.83
CoS 96268	82.70	87500	2.47	2.20	0.98
BO 120	63.77	125625	2.35	2.00	0.76
CoJ 88	83.13	91250	2.81	2.11	1.06
UP 0097	64.10	111875	2.16	2.01	0.73
CoSe 96275	82.84	96875	2.42	1.81	0.75
BO 150	88.13	133750	2.64	2.08	1.04
CoP 97182 BD 2	36.16	88750	2.58	2.08	0.92
UP 9742 BD 10	88.49	123125	2.56	1.83	0.89
CV%	10.05	12.80	3.53	5.74	11.39
	17 /0**	20552**	∩ 1 <i>1</i> **	O 10**	0 17**

Table1: Cane yield and yield attributing characters of various genotypes in Initial Evaluation Trial (IET) during 2072/73

- Among them, two BD lines UP 9742 BD 10 from UP 9742 genotype and CoP 97182 BD 2 from CoP 97182 genotype were selected for Initial Evaluation trial based on
 - ✓ cane yield,
 - ✓ tiller numbers,
 - ✓ stalk thickness
 - ✓ stalk length.

- Comparatively, only UP 9742 BD 10 lines performed well and selected for further advanced varietal selection trial from Initial Evaluation Trial.
- But, CoP 97182 BD 2 line was rejected for further field experiment due to low yield, low sugar recovery, thin cane diameter and susceptible to red rot disease.

Evaluation of Selected mutant genotypes under different stress conditions

UP 9742 BD 10

- Evaluation in waterlogged conditions with check
- Evaluation in Drought condition with check



Photo taken at RARS,Parwanipur

Photo taken at NSRP, Jitpur



Photo taken at farmer's field in Rautahat District

- Likewise, sixty four mutagenic lines of sugarcane genotype CoSe 98255 was provided for field evaluation in NSRP in 2015 and planted in field for further evaluation.
- From this observation field trial, only 15 lines were selected from primary evaluation for further Initial Evaluation Experiments. The selected lines were

CoSe 98255 BD 2, CoSe 98255 BD 3. CoSe 98255 BD 6. CoSe 98255 BD 15. CoSe 98255 BD 24. CoSe 98255 BD 28 CoSe 98255 BD 29. CoSe 98255 BD 35. CoSe 98255 BD 38. CoSe 98255 BD 39, CoSe 98255 BD 49. CoSe 98255 BD 52, CoSe 98255 BD 55. CoSe 98255 BD 57. CoSe 98255 BD 58.



Delivering mutant lines to Sugarcane Research Program:



 Among those, genotypes found superior than others in initial evaluation field trials are:

	Mutant line	Yield (mt/ha)
1	CoSe 98255 BD15	101.11
2	CoSe 98255 BD 52	92.00
3	CoSe 98255 BD 38	89.58

* But more aerial roots emerged from stalk nodes



 Similarly, new 56 mutagenic lines of sugarcane genotype CoSe 92423 were generated and delivered to NSRP in 2016. These lines were planted for primary observation evaluation for further selection and experiment.

- The protocol of chemical *in-vitro* mutagenesis in sugarcane has been standardized.
- Till date, 174 putative mutants of sugarcane were induced and delivered to NSRP, Jitpur.
- Identification of the desirable mutation among these lines in sugarcane need to be regenerated more number of mutants.

Current Research Activities

- Varietal improvement research going on in SRP, Jitpur:
 - Collection of Local and Exotic germplasm
 - Disease and Pest screening
 - Selection and characterization (maturity group, different domain)
 - Stability analysis under different environmental condition (GxE interaction study)



Current Research Activities

> Varietal improvement research related with mutation:

Evaluation of mutant putative genotypes





Crop: Sweet potato

 It grows on well-drained soils with good aeration and is well adapted to marginal soils with low nutrient content. In heavy soils, root formation is reduced.

Sweet potato

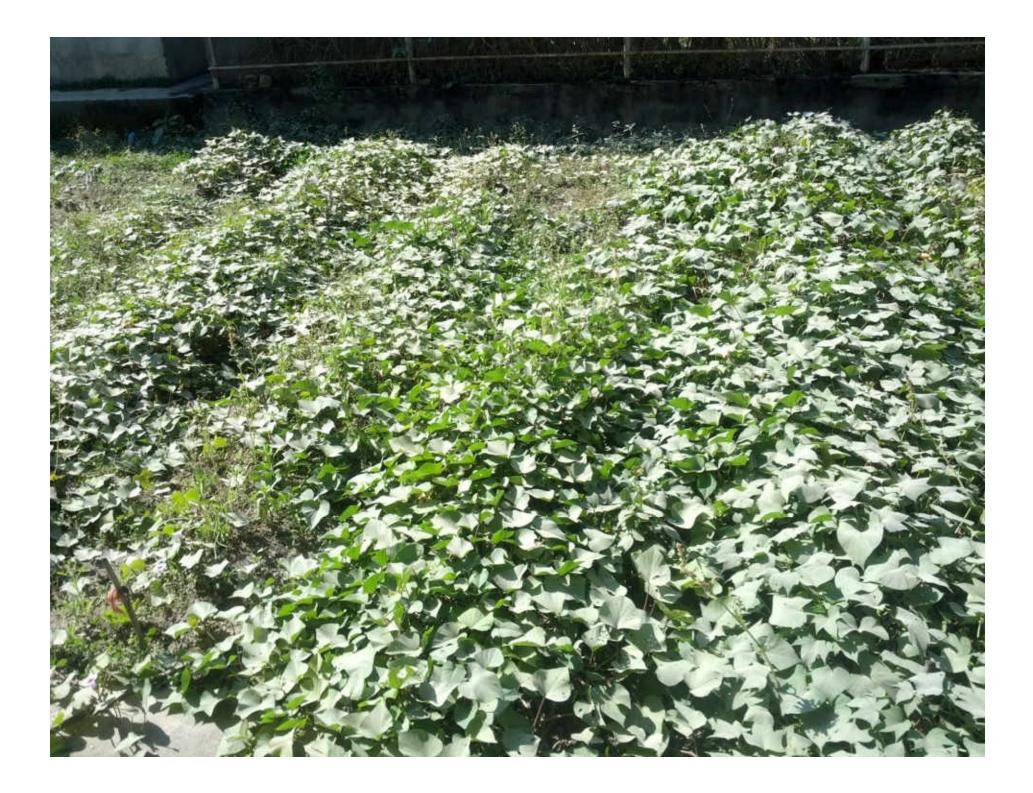
- Sweet potato seedlings are raised in nursery beds and transplanted in marginal lands in hilly area during June-July which is harvested in Oct-Nov., while
- Cuttings are transplanted in Terai areas
- Only grown in river basins soils covered by flood soil, sands, dry areas where no other commodities can be grown
- No any kind of efforts packages of practices are made during the season (irrigation, nutrition, weeding etc.) – only harvesting
- Not even considered as a food security crop

Crop: Sweet potato

- 2016: Collection of germplasm: 47
- 2016: Multiplication
- 2017: Evaluation of germplasm in the field
- 2018: Irradiation of superior and desirable germplasm
- 2018: Descending followed by selection
- 2019: Multiplication of superior mutant
- 2020: Recommendation

Sweet potato in observation nursery showed distinct variation





How to irradiate?

Other candidates:

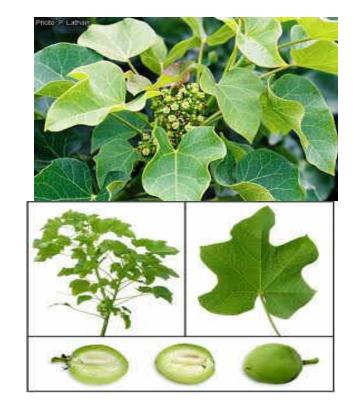
- Castor (*Ricinus communis*) is commonly called as 'adir' in Nepal.
- Castor beans subjected for the extraction of oilcontained 48% of yield.
- The refined oil contained 0.8% free fatty acid (FFA) and 76.258mg KOH/g saponification value which showed that oil was very suitable for bio-diesel production.





Jatropha curcas L.







Summary:

- Only Two meetings (Vienna and Malaysia) were attended
- Only one personnel is being trained

Suggestion:

- Fallow land should be utilized for bio-fuel crop commodities
- NPC should be timely informed for the arrangement of any training
- Team members need to be trained urgently for conducting activities



Sugarcane Researches in Nepal



Coordination meeting to review the **Progress of the Field Trials:**

Soil and Water Use Efficiency Part



Soil Scientist

National Sugarcane Research Program, Jitpur, Bara Nepal Agricultural Research Council

Email: ishankshrestha@gmail.com

Import and consumption of Fertilizer in Nepal In 2014/15 (in Mt)

Fertilizers	Import	Consumption
Nitrogen	83200	71314
Phosphorus	27600	31320
Potassium	4500	2807
Total	115300	105441
urea	140000	128384
DAP	60000	68088
Potash	7500	4678

Fertilizer users

Cereal Crops: >50-70% Vegetable Crops: 30-40% Industrial Crops: < 8% other minor crops: <2%

Current Research Activities

- > Agronomical research:
 - Weed management
 - Integrated Nutrient management
 - Response on spacing on different varieties
 - Ratoonability on different shaving dates
 - Intercropping in sugarcane
 - Plant protection research









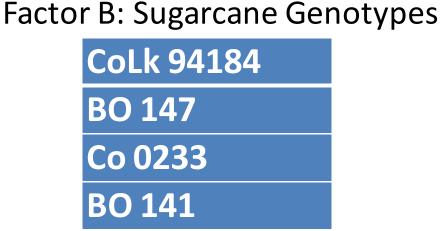


Research in nutrient managements

Activity 1. Identify the nitrogen requirements for various sugarcane genotypes during 2014/15

Factor A: Nitrogen

- 1. 75% RDN*
- 2.100% RDN
- 3. 125% RDN
- 4. 150% RDN



* RDN = Recommended Dose of fertilizers
=150:60:40 kg/ha NPK) for plants
=200:60:40 kg/ha NPK for ratoon crops

Table 1: Yield attributes and cane yield of promising sugarcane genotypes plant cane under different nitrogen levels at NSRP in 2014/15.

Treatments	No of Millable canes /ha	Stalk length (m)	Single cane weight (kg)	Cane yield (mt/ha)
Level of Nitrogen (kg	N/ha)			
75% RDN (112.5)	98207.92 c	2.54	0.92	71.74 c
100% RDN (150)	102156.19 bc	2.49	0.97	82.09 b
125% RDN (187.5)	107602.96 b	2.57	1.00	88.48 a
150% RDN (225)	115548.43 a	2.56	0.98	90.57 a
LSD	6758.93**	NS	NS	5.12**
Sugarcane				
genotypes				
CoLk94184	124831.06a	2.38 b	0.79 b	75.91 b
BO 147	68643.88 c	2.56 a	1.03 a	69.79 c
Co 0233	112583.31 b	2.56 a	1.06 a	93.18 a
BO 141	117457.24 ab	2.65 a	0.98 a	94.01 a
LSD	8933.78**	0.101**	0.177**	3.94**
CV	8.2	6.5	15.1	5.9
Grand mean	105878.9	2.54	0.97	83.22

Table 2: Yield attributes and cane yield of promising sugarcane genotypes ratoon crop under different nitrogen levels at NSRP in 2016

Treatments	No of Millable canes /ha	Stalk length (m)	Single cane weight (kg)	Cane yield (mt/ha)	
Level of Nitrogen (kg N/ha)					
75% RDN (150)	83110b	1.87 b	0.65 b	59.78b	
100% RDN (200)	86640b	1.93 ab	0.76 a	65.87ab	
125% RDN (250)	89050b	1.97 a	0.74 a	69.57a	
150% RDN (300)	100700a	2.0 a	0.76 a	72.37 a	
LSD	9950.94 *	0.08 *	0.047 **	8.22*	
Sugarcane genotypes					
CoLk 94184	107400a	1.91	0.60 b	64.16 b	
BO 147	60480 c	1.95	0.81 a	45.81 c	
Co 0233	96220 ab	1.96	0.77 a	81.72 a	
BO 141	95390 b	1.95	0.74 a	75.89 a	
LSD	11219.9**	NS	0.104 **	8.81**	
CV	12.95	4.1	6.4	12.3	
Grand mean	89879.61	1.94	0.73	66.89	

Conclusion

- Genotypes Co 0233 and BO141
- Higher than recommended dose (150 kg N/ha for plant and 200 kg N/ha for ratoon crops)

Integrated nutrient management:

Activity 3: Integration of chemical fertilizers with Press mud in Sugarcane

- Design: RCBD
- Plot size: 90 cm spaced 5 row with 5 m length
- Fertilizer: 150:60:40 kg/ha NPK
- Seed setts: three budded 12 setts per meter length
- Setts treatment: dipping 15 minutes in fungicides carbenzim@1gm/litre
- Other intercultural operation was done as per crop requirement
- Genotypes: BO 141

Treatments	Combinations
T ₁	120kg N/ha ($\frac{1}{2}$ at basal and $\frac{1}{2}$ in two split doses at 60 and 90 DAP)
T ₂	150kg N/ha ($_2^1$ at basal and $_2^1$ in two split doses at 60 and 90 DAP)
T ₃	200kgN/ha($\frac{1}{2}$ at basal and $\frac{1}{2}$ in two split doses at 60 and 90 DAP)
T ₄	120kg N supplied by Press-mud
T ₅	150kg N supplied by Press-mud
T ₆	200kg N supplied by Press-mud
T ₇	120kg N/ha (50% supplied by urea and 50% by Press-mud)
T ₈	150kg N/ha (50% supplied by urea and 50% by Press-mud)
T ₉	200kg N/ha (50% supplied by urea and 50% by Press-mud)
T ₁₀	0kg N/ha

Treatments	Cane Yield(mt /ha)	No. of millable canes/h	Stalk length(m)	Stalk diame ter	Single cane weight
	,,	a	,	(cm)	(kg)
120kg N/ha ($\frac{1}{2}$ at basal and $\frac{1}{2}$ in two split doses at 60 and 90 DAP)	65.04	79667	1.84	1.77	0.95
150kg N/ha ($\frac{1}{2}$ at basal and $\frac{1}{2}$ in two split doses at 60 and 90 DAP)	59.17	87000	1.79	1.90	0.90
200kgN/ha $(\frac{1}{2}$ at basal and $\frac{1}{2}$ in two split doses at 60 and 90 DAP)	61.25	89000	1.77	1.95	0.99
120kg N supplied by Press-mud	55.76	84667	1.72	1.84	1.00
150kg N supplied by Press-mud	63.77	87000	1.59	1.85	0.95
200kg N supplied by Press-mud	63.42	86000	1.68	1.91	0.91
120kg N/ha (50% supplied by urea and 50% by Press- mud)	72.96	85000	1.85	1.99	1.033
150kg N/ha (50% supplied by urea and 50% by Press- mud)	60.32	84333	1.67	1.72	1.030
200kg N/ha (50% supplied by urea and 50% by Press- mud)	60.00	83000	1.67	1.75	0.96
0kg N/ha	44.25	65667	1.33	1.45	0.75
GM	60.596	83133	1.695	1.81	0.946
CV%	4.98	8.26	9.61	3.2	12.39
FSD(0.05)	* *	* *	**	**	*

Conclusion

 120 kg N/ha supplied in combination with 50% (i.e. 60 kg N/ha) urea and 50% (i.e. 60 kg N/ha) from Press mud

Future Plan

- Nutrient trial will be conducted on sugarcane selected mutant lines along with checks in marginal land from next season starting Sept-Oct 2017
- If N-15 urea is available before planting season, It will be included in the experiment.
- Training on Isotopic techniques is required

Thank you