IAEA/RCA Coordination meeting to review the progress of field trials within the framework of the project RAS/5/070 " Mutation Breeding and Supportive Techniques for Development of Bioenergy Crops"

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GENETIC IMPROVEMENT OF *Brassica carinata* and SWEET SORGHUM FOR BIOFUEL USING INDUCED MUTATIONS

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Development and evaluation of early maturing mutation in *Brassica carinata* for Bioenergy Crops in India

Work Plan

- **2014-15 : M_1 Generation**

  - **Parent Variety:**

    1. **Pusa Swarnim : 1000 Gy : 2000 Plants**
    2. **Kiran: : 1000Gy : 2000Plants**

- **2015-16: M_2 Generation**

  : Pusa Swarnim : 32567 Plants

  : Kiran : 24892 Plants

  : M_3 generation in off season nursery

- **2016-17 : M_4 generation**

  : Preliminary Yield Trial

2014-15: Parent Variety : Kiran: 1000Gy

Parent Variety : Kiran : Late 180 days

Parent Variety : Pusa Swarnim

Flowering Stage

Pod Filling Stage

2015-16
Segregation for early and late flowering in M_2 Generation

2015-16 off season nursery
M3 generation : True Breeding Early Mutant

Comparison of parent variety and early and dwarf mutation

Parent and early flowering mutants of *B. carinata* in M_4 generation

Maturity : 185 days **125 days**

2016-17
Preliminary Yield trial of early mutation in M_4 Generation

Quantitative traits of parents and mutants

Genotype	Plant height (cm)	Total branches	Total Siliqua	Seeds/Siliqua	Yield/ plant (g)	Oil content
Kiran	267	100-122	907-1076	13	45	37.3
Early mutant 1	133	99-117	923-1107	14	57	38.4
Early Mutant 2	144	103-120	1045-1183	15	52	37.7
Pusa Swarnim	278	98-125	894	14	41	36.5
Early mutant 1	139	95-114	964	15	45	37.2
Early mutant 2	128	99-116	987	16	47	36.9

Future work:

2017-18

- **Multilocation Yield trial of early mutants under marginal Land**
- **Characterisation of early mutants for seed and oil yield under marginal land**

GENETIC IMPROVEMENT OF SWEET SORGHUM FOR BIOFUEL USING INDUCED MUTATIONS

Sorghum improvement:

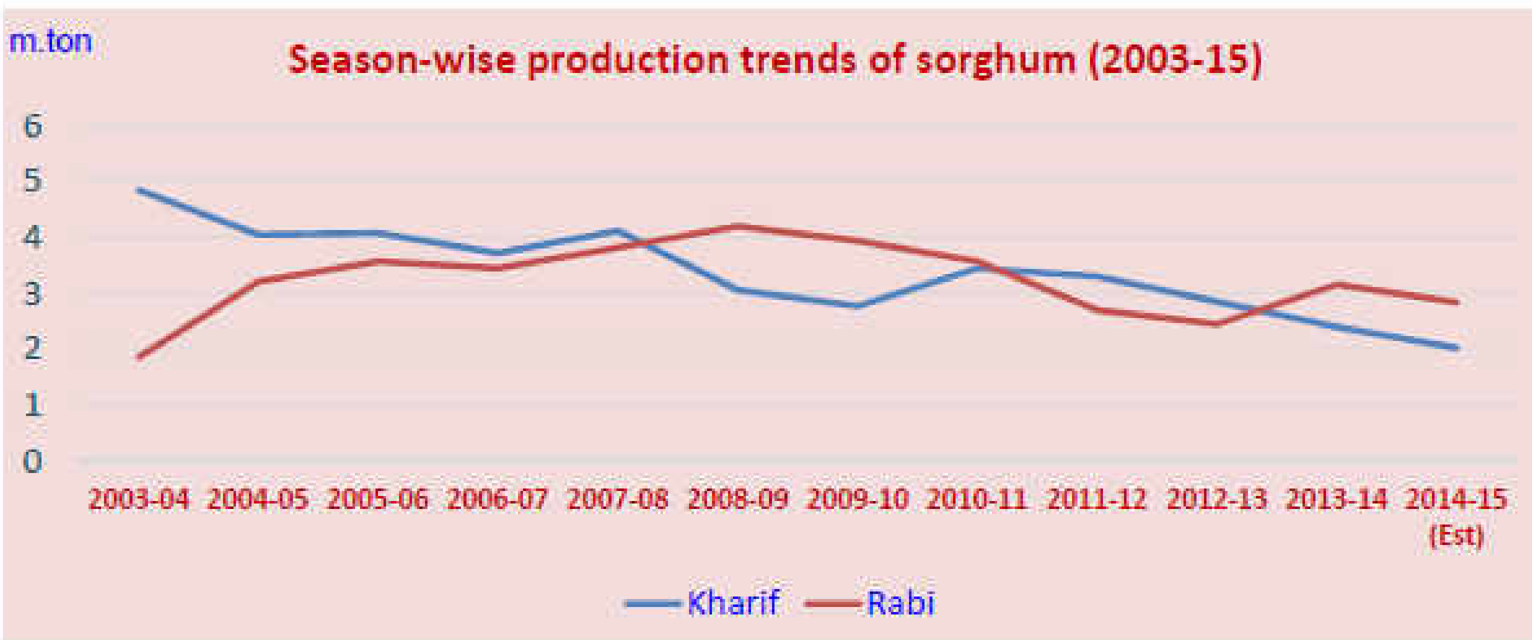
- **Global sorghum area is 44 mha with production of 68.93 mill.tons**
- **Indian Sorghum grown on area 7.38mha with production of 6.48 m. tons contributing 18.21% to the world production.**
- **It grows over wide range of temperatures and elevations and in different soil types**
- **Rainy sorghum : Industrial purpose- feed, fuel**
- **Post-rainy sorghum: used as staple food/ roti quality**
- **Needs less input cost, drought tolerant, short duration and meets food/fodder/ fuel/ nutritional requirements of the society**
- **Low productivity of 949 kg/ha in India with decreasing area over the decade**

  -Crop grown under receding soil moisture situation

  -Various abiotic (terminal drought, temperature) and biotic (shoot fly, charcoal rot)

- **Identify donors with resistance to biotic and abiotic stresses suitable for rain fed situations.**

Trends in Sorghum Area, Production and Yield (Last decade)



Potential of sweet sorghum as biofuel crop:

- Sorghum is an important crop used for food , feed and fuel
- Employs C4 photosynthesis and produces high biomass as lignocellulose, fermentable juice, or fermentable grain
- Cultivated in temperate-tropical climate with short growth period (3-5 months)
- Sorghum yields a better energy output/input ratio compared to other feedstocks
- Contain 73% moisture with 13% non-structural carbohydrates (sucrose, glucose and fructose)+ 9% Cellulosic fiber and 3% lignin
- Compared to sugar cane, the sucrose content in Sw. sorghum is significantly higher compared to glucose and fructose (90, 4 and 6% respectively)

Biofuel production pathways:

- **Starch-to-ethanol:**

Mold affected grain of rainy season crop can be converted to ethanol as that of corn. According to industry estimates, about 15% of the U.S. grain sorghum crop currently goes into ethanol production

- **Sugar-to-ethanol from sweet sorghum:**

Conversion is similar to Sugar cane, which and the best source of ethanol to date and cost effective compared to other pathways.

This process is commercialized and presently two centralized distilleries are in operation in India

- **Cellulosic ethanol:**

No other crop equals sorghum in conversion owing to low lignin sources and production efficiency. This process draws on the versatility and resilience of sorghum to the varied agro- climatic conditions.

Fiber composition of major ethanol feedstock

Feedstock	Fiber(%)	Cellulose(%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Sweet sorghum	13.0	44.6	27.1	20.7	0.4
Sweet sorghum [2]	-	25.0	22.0	4.0	-
Sweet sorghum bagasse[3]	-	41.3	24.6	14.0	3.7
Sorghum straw	-	32.4	27.0	7.0	0.7
Sugar cane	13.5	41.6	25.1	20.3	4.8
Energy cane	26.7	43.3	23.8	21.7	0.8
Corn stover	-	40.0	28.0	21.0	7.0
Wheat straw	-	38.0	32.0	19.0	8.0
Rice straw	-	36.0	28.0	14.0	20.0

All data on dry weight basis
Cellulose, Hemicelluloses and lignin are % of total fiber

Source: Kim and Day, 2011

Sweet sorghum has great potential for bio-ethanol production

Comparison of Sugarcane and sweet sorghum for biofuel production

		Sugarcane		Sweet Sorghum
Sugar quality	↑	Sucrose	↓	Mixed sugars
Establishment cost	↓	Vegetative propagation	↑	Seed propagation
Sugar yield (% fw)	↑	13 – 15%	↓	8 – 13%
Input requirements	↓	Limited by water, nitrogen	↑	50% water, 60% nitrogen
Scale-up time	↓	Vegetative propagation	↑	Seed propagation
Biomass yield/co-generation (tons/ha)	↓	70 – 90 tons/ha	↑	60 – 100 tons/ha*
Marginal Land	↓	Limited yields on marginal land	↑	Yield potential on marginal land
Ratoon/flexibility	↑	12 – 18 months	?	2 – 3 cuts per year is possible
Season extension	↓	12 – 18 months	↑	Rapid growth cycle, 70 – 120 days
Product development	↓	Perennial, 10 – 16 years	↑	Annual, 3 – 5 years

*Note: Single cut. Does not account for ratoon yield.

Sources: ICRISAT, Wu *et al.* 2008 and Ceres internal data and analysis

Potential Bio-fuel traits in Sweet sorghum

S No	Trait	Range
1	Fresh stalk yields	South India : 40-45 t/ha (Monsoon & summer) Indo-Gangetic belt: 50-55 t/ha
2	Juice brix	16-18% (rainy); 10-14% (post-rainy)
3	Reducing sugars (RS)	2-4%
4	Sucrose (%)	8-11%
5	Juice extraction	40-45%;
6	Juice yield/ha	12-14KL
7	Bagasse (residue) Yield	5-7 t /ha (dry wt basis)

RCA-05/70: Mutation breeding for improving biomass yield of sweet sorghum

- Characterization of Sweet Sorghum cultivars and its suitability to bioethanol production (12-21% sugar)
- Improving cane yield, grain and stalk sugar yields by creating genetic variation using Mutation breeding techniques
- Gamma rays is the potential mutagens to bring about heritable changes in the plant genome
- Identifying and screening for mutants with high biomass, adaption to drought conditions (stay green) with good digestibility (low lignin content)
- Testing elite mutants for foliar diseases, adaption to marginal soils and suitability to different cropping systems (intercropping/rice fallows)

Genetic Improvement of sweet Sorghum
Possible ways…

- **Traditional breeding with available germplasm**
- **Hybrids: grain x sweet stalk or forage sorghum**
- **Breeding for high-energy sorghum (grain+sweet)**
- **Identifying mutations for …..**
  - **Pest and disease resistance**
  - **Altered lignin composition (↑ cellulose bioavail.)**
  - **Altered cell-wall composition**
  - **Improved sugar content**
  - **High biomass**

Breeding plan for the development of sweet sorghum lines using mutation techniques

Year 2015-16:

- **Seed irradiation and growing M1 generation:**
  - **Variety: SSV 84 - a sweet sorghum line (12-15% brix)**
  - **Mutagens: Gamma rays (350Gy)**
- **M1 generation was raised with population size of 670 plants**

Year 2016-17:

- **M2 generation consist of 1785 plants**

Traits looking for:

- **Plant height, stalk diameter, number of leaves, earliness, grain yield**

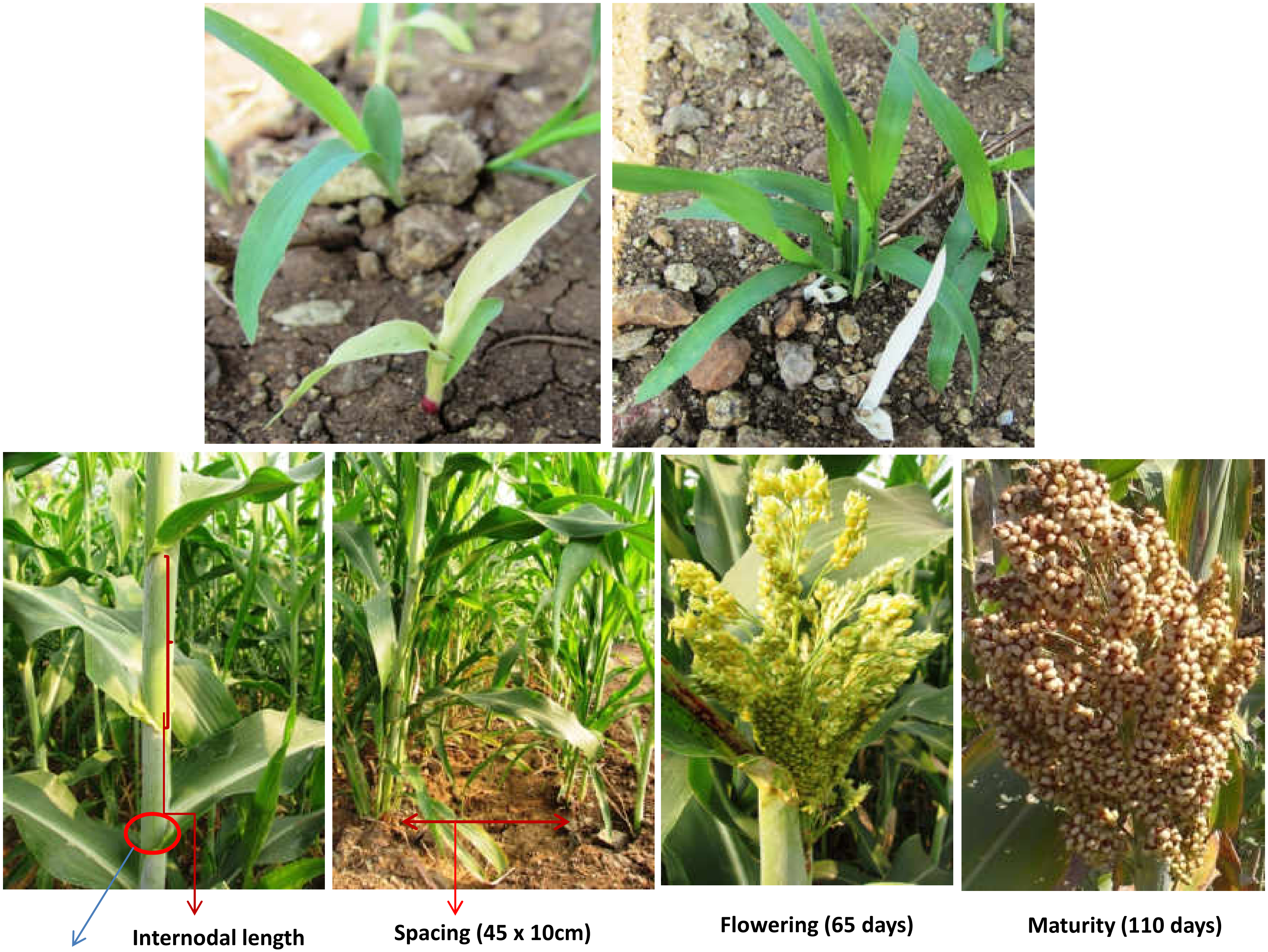
Bioenergy associated traits:

- **biomass, stem juiciness and brix content**
- **Selection were done for these traits & 385 single plant selections were made**

Field view of Sweet sorghum line (SSV84) in M2 generation. Putative mutants showed high biomass with brix value of 18%

Date of sowing: 09/11/16 **Days to maturity: 110**

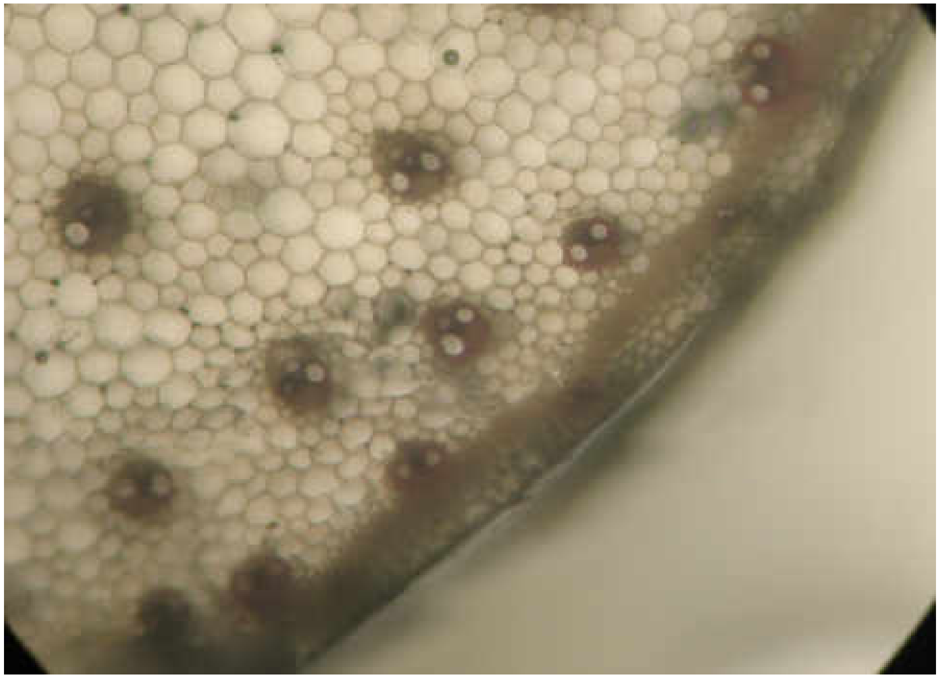
Morphological variants and HY plants of SSV 84 in M2 generation



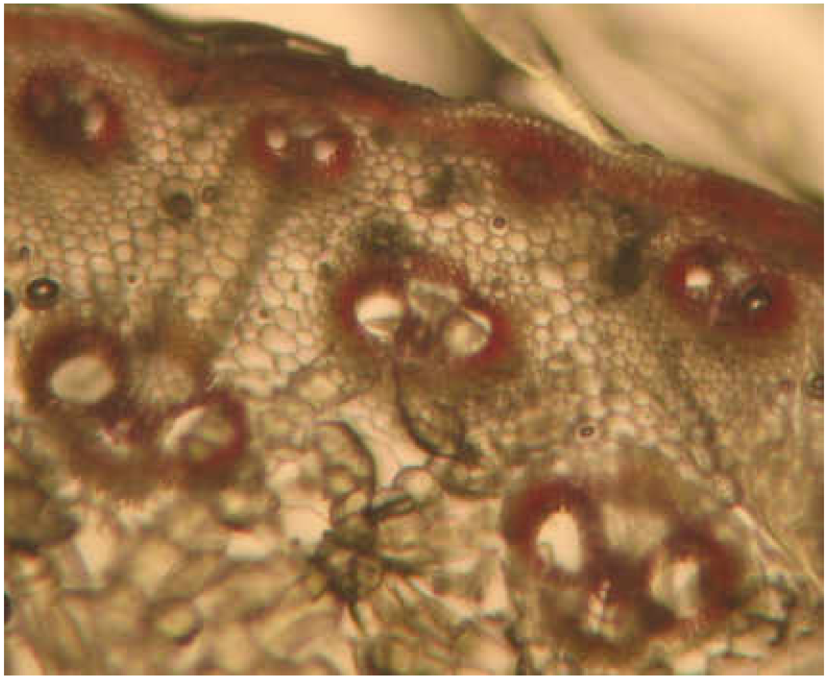
Promising mutants identified in M2 generation

Mutants	Ht	DF	Stem Dia	No. leaves	Int. length	Grain Yld (g)	Brix (%)
SSVM2-1	179	53	1.8	8	18.1	65	15.5
SSVM2-2	166	55	1.8	9	15.5	45	18
SSVM2-3	180	54	1.2	9	14.6	85	16
SSVM2-4	186	55	1.1	8	18.2	68	17.2
SSVM2-5	171	58	1.5	8	17.5	95	14.5
SSVM2-6	180	49	1.5	10	18.4	85	18.2
SSVM2-7	142	55	1.25	8	18.5	45	15.1
SSVM2-8	148	58	1.6	9	21.25	39	14.6
SSVM2-9	164	55	1.4	11	18.1	45	12.5
SSVM2-10	131	59	0.8	8	18.5	85	13.8
SSVM2-11	119	58	1.5	7	17.75	95	12.3
SSVM2-12	148	55	1.2	8	19.5	65	14.2
SSVM2-13	152	54	1.35	9	17.5	23	15.6
SSVM2-14	172	57	1.25	11	17.1	67	18.4
SSVM2-15	160	52	1.75	11	19.5	84	17.5
SSVM2-16	165	55	0.9	10	16.1	51	16.6
SSVM2-17	153	58	1.65	8	15.5	55	14.8
SSVM2-18	164	56	1.35	9	16.75	68	15.5
SSVM2-19	155	59	1.25	7	15.2	95	14.8
SSVM2-20	175	55	1.45	8	16.25	37	17.8

Cross section of Sorghum stem showing reduced lignin



SSV-84 M2 plant with reduced lignin stained with Phloroglucinol



Control (Parent)

Fertile and semi-sterile mutants of SSV-84 in M2 generation

Fertile at milky stage

Fertile at maturity

Semi sterile

High yielding plants of SSV-84 in M2 generation

Future line of work…

- **Identifying elite mutants and testing in yield trials**
- **Confirming their true breeding nature in M3 generation**
- **Biochemical studies of biofuel traits in M3 progeny lines**
- **Identification and selection of elite mutants with high yield and increased brix content and advancing them to replicated yield trials in multi-environmental conditions**

THANK YOU