EGS planning, production, maintenance of inbred lines and Quality Assurance/Quality Control (QA/QC)

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CIMMYT

Seed Production of Improved Maize Varieties: Best Practices

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Outline

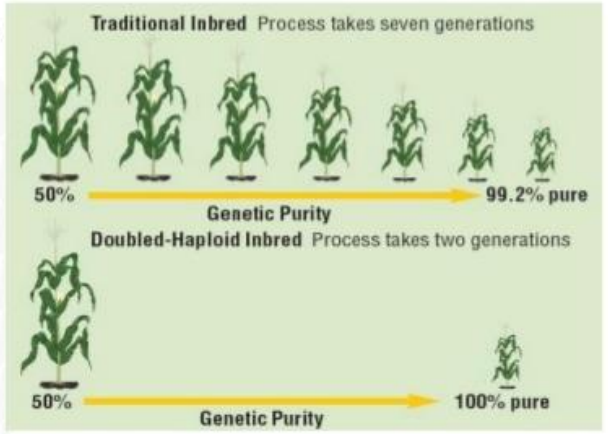
- What are inbred lines?
- Maintenance: Meaning, purpose
- How to maintain inbred lines
- Procedures to maintain breeder's stock and breeder's seed
- Methods to maintain/increase inbred lines by partners
- EGS Planning and production
- Quality Assurance/Quality Control (QA/QC)
  - Conventional method
  - Molecular method

What are inbred lines?

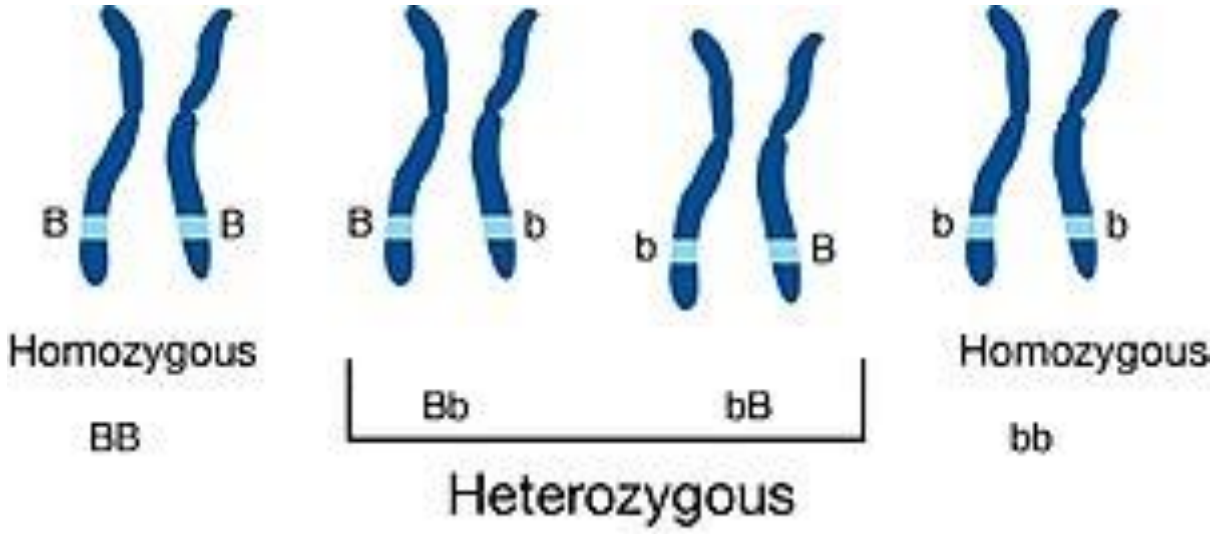
- Inbred lines are **homozygous** genotypes produced **by repeated selfing with selection over several generations** or using **doubled haploid** (DH) technique.

Conventional vs DH Inbred Line Development

- Produced by repeated generations of selfing
- In each generation, heterozygosity reduces by 50%
- Resulting inbred lines s are highly homozygous but not 100%
- DH technique – a quicker method to obtain 100% pure inbred lines



Generation	S1	S2	S3	S4	S5	S6	S7
Homozygosity (%)	50	75	87.5	93.75	96.875	98.45	99.23
Months	6	12	18	24	30	36	42



Source: wikipedia

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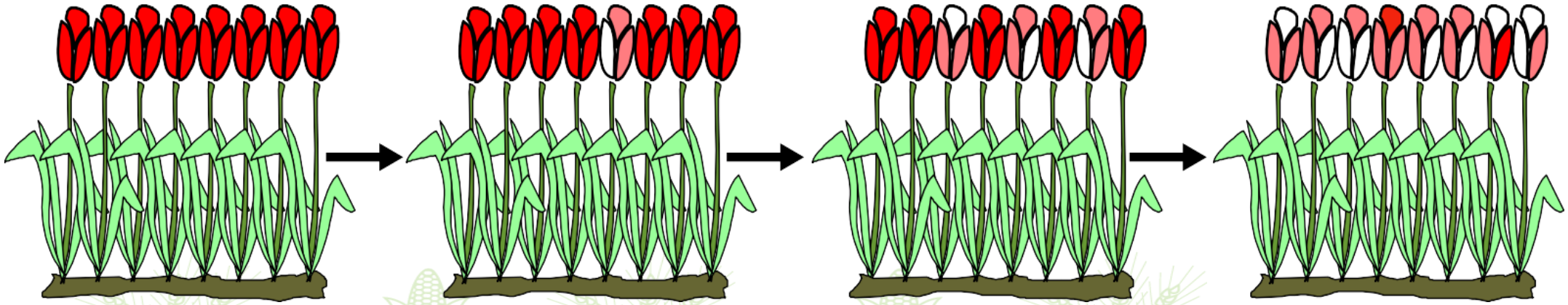
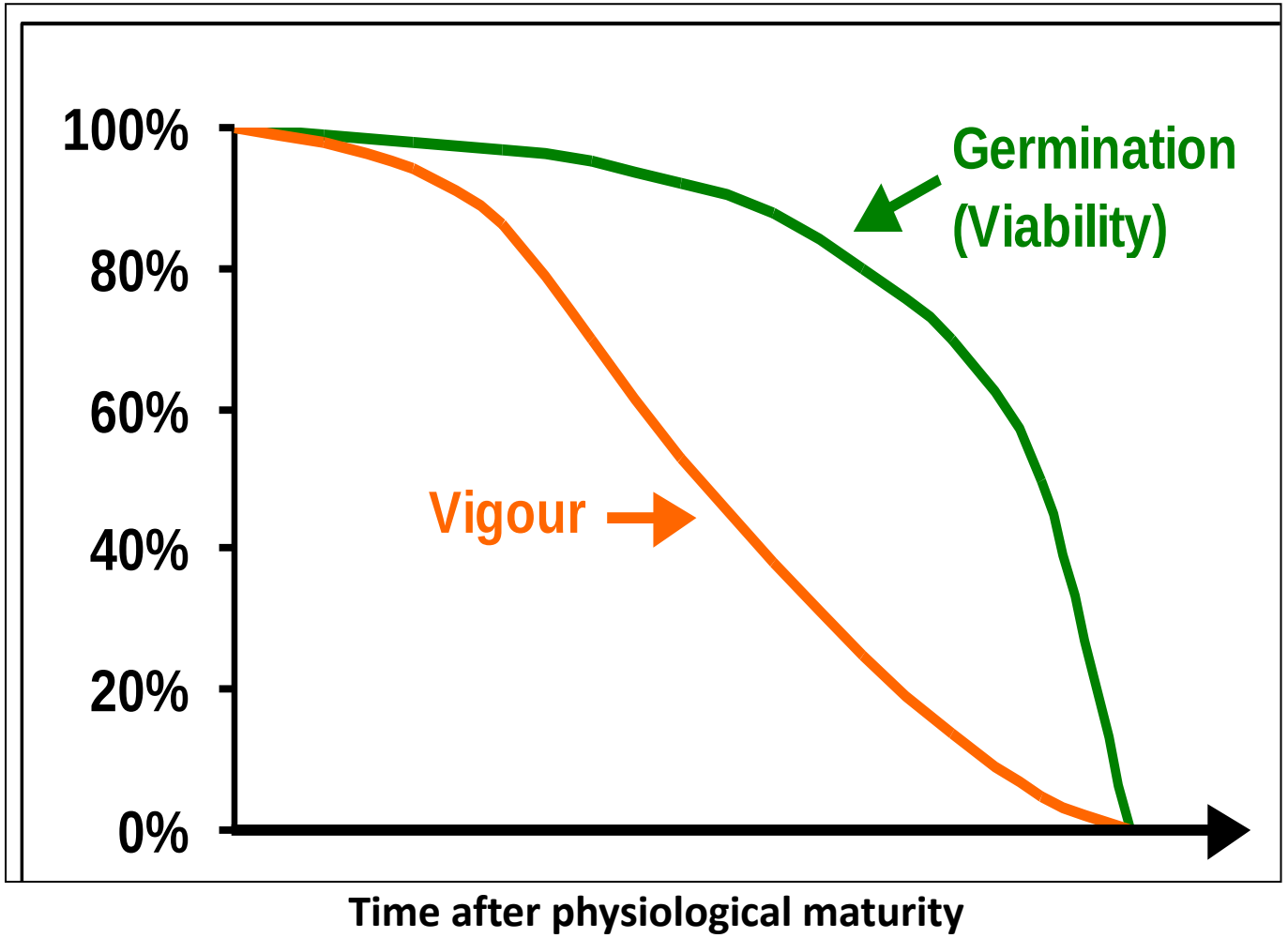
Maintenance: meaning

- **Maintenance is the process of** conserving the genetic composition of a genotype such as inbred lines and OPVs
  - Performance
  - Appearance
  - Genetic integrity of the original lines while limiting rouging expenses.
- **A genotype not maintained well is not stable (genetic deterioration)**
  - The relevant characteristics of a genotype change through the generations.
  - The plant grouping no longer retains the expression of the relevant characteristics of the original variety.



Maintenance: purpose

- To meet the **demand** for the seeds for breeder seed
  - While maintaining, required **quantity** should be increased
- To keep the seeds **viable**
  - The proportion dead seeds increase with length of storage time
- Get **vigor** plants during germination
  - As seed quality deteriorates, the number of abnormal seedlings increases



How to maintain inbred lines

Start with pure parental lines:

when a new hybrid variety is pre-commercial stage, start initiate maintenance from pure seed source.

Breeder's Stock Seed

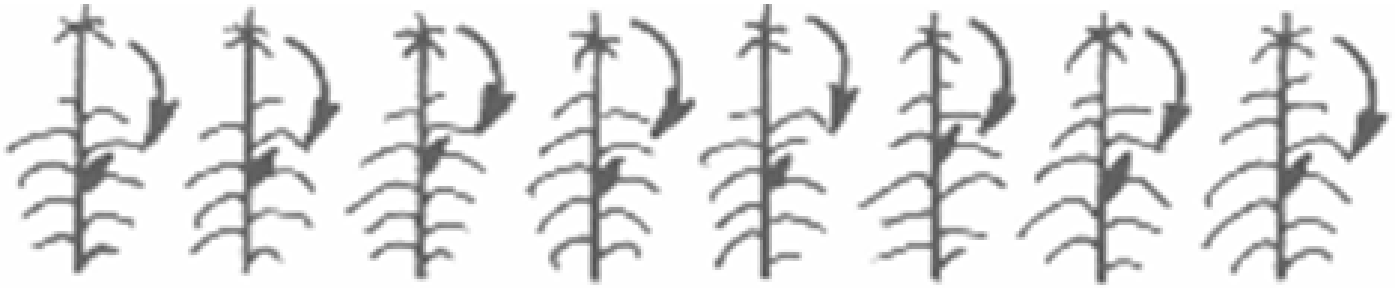
kept in **ETR** that is maintained by the breeder;

Breeder's Seed

increase (by selfing) of the Breeder's Stock seed in ETR and then bulked to produce the Breeder's Seed Generation 1 that can be used to make new increases

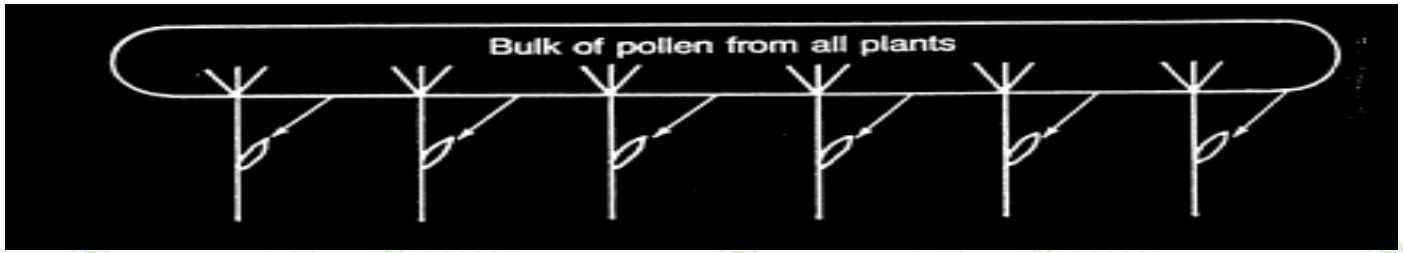
How to maintain inbred lines…

Selfing



Selfing helps in maintaining inbred lines in a pure condition

Sibbing



During sibbing there is probability of mixing off types

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Procedures to Produce Breeder's Stock Seed

- Plant 10 or more ears of F_6/F_7 generation inbred lines/DH lines to rows (ETR)
- Self all plants in a row
- Harvest about 100 ears and keep all ears separately for ETR planting
- **Keep 20 of those ears separately to represent <u>Breeder's Stock</u>**
- Use the other 80 ears to produce the <u>Breeder's Seed Gen 1.</u>

Procedure to produce Breeder's seed (Gen 1)

- **Plant all the 80 ears-to-rows (ETR)**
- **Discard** row with more than **10% contamination**; if less than 10%, do rouging of the "off types"
- **Self** all plants in each rows
- At **harvest**, select and discard any ear showing differences from the inbred line being increased
- **Bulk all the ears to make the <u>Breeder's Seed Generation 1</u>**
- After 3 increases (G1, G2, G3, may be Gen 4) a new source (G1) should be obtained from the breeder

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How to maintain/increase inbred lines from breeder's seed generation 1 by partners

Hand pollination

In order to maintain the genetic purity of the inbred lines at the required level:

- Plant **required rows** of the inbred line using breeder's seed gen 1
- Eliminate **off-type rows (plants)** before flowering
- **Self-pollinate** all plants in selected rows (consistent with the inbred description)
- **Harvest** individually ears from plants with uniform characteristics consistent with the inbred line's description
- **Shell** each ear separately and **bulk** for future use
- Use accurate pedigree records and labels

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Isolation block

- Grow an inbred line in **isolation block** and allow to randomly pollinate
- Ensure proper **isolation distance** from other maize fields
- Eliminate **off-type plants** before flowering
- At harvest **select ears** consistent with the description, and **shell and bulk** for future use
- Use accurate **pedigree records and labels**

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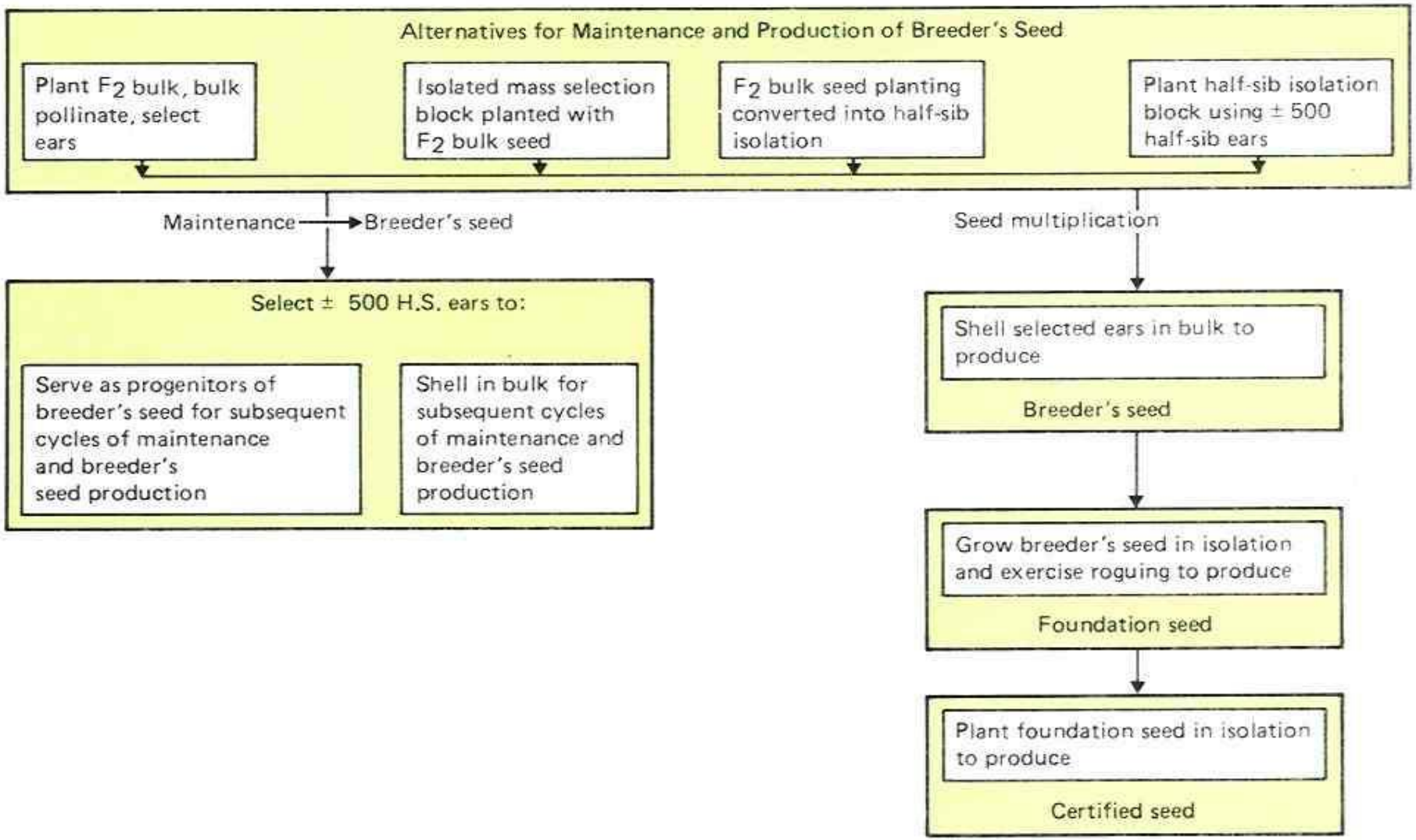
Figure 3. Maintenance and Seed Production of an Open-Pollinated Maize Variety



Seed types

What is seed?

- Entity by which a living thing reproduces itself.
- Seed may be from un- improved or improved plant type/variety.
- Seed can be produced through formal or in-formal system.

Formal Seed Sectors

In Seed Law, seed is produced through known generations/Certification classes, for example in OECD :

- ✓ (1) Breeder,
- ✓(2) Pre-basic,
- ✓ (3) Basic
- ✓ (4) Certified

Prebasic seed also known as Foundation seed

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Standards and requirements for different classes of seed (specific to country seed law, NSA regulation-Tanzania)

Characteristics	(Pb)	Basic seeds	C1
Rotation (min years)	3	2	2
Isolation (min meters)	400	400	300
Off types at & after flowering (max %)	0.1	0.1	0.1
Pollen shading heads in seed parent at flowering (max %)	0.2	0.2	0.5
Pollen shading off type plans in male rows (max %)	Ns	Ns	Ns

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Example (Kenya requirements)

THIRD SCHEDULE (r.9(3))

SEED CLASSES

Code	*Classes*	*Seed Parents*	*Colour of Labels*
Br.	Breeder	Progeny of parental stock	White with a violet strip
Pb	Pre-basic	Progeny of parental stock or breeders seed	White with a violet strip
B.	Basic	Progeny breeders seed or pre-basic seed	White
C.1	Cert. 1st gen.	Progeny of pre-basic seed or basic seed	Blue
C.2	Cert. 2nd gen.	Progeny of basic seed or certified 1st generation seed	Red
Std	Standard Seed	Progeny of certified 2nd generation or certified standard seed or by declaration by the Cabinet Secretary.	Yellow

NOTE:

(i) Potatoes – Stock seed (SS)/minitubers, Pre-basic, basic, C1, C2

(ii) Hybrid Maize – Breeders seed, Pre-basic, basic, C1

(All cases of hybrids).

FOURTH SCHEDULE (r.9(5), 12(4))

FIELD AND LABORATORY STANDARDS

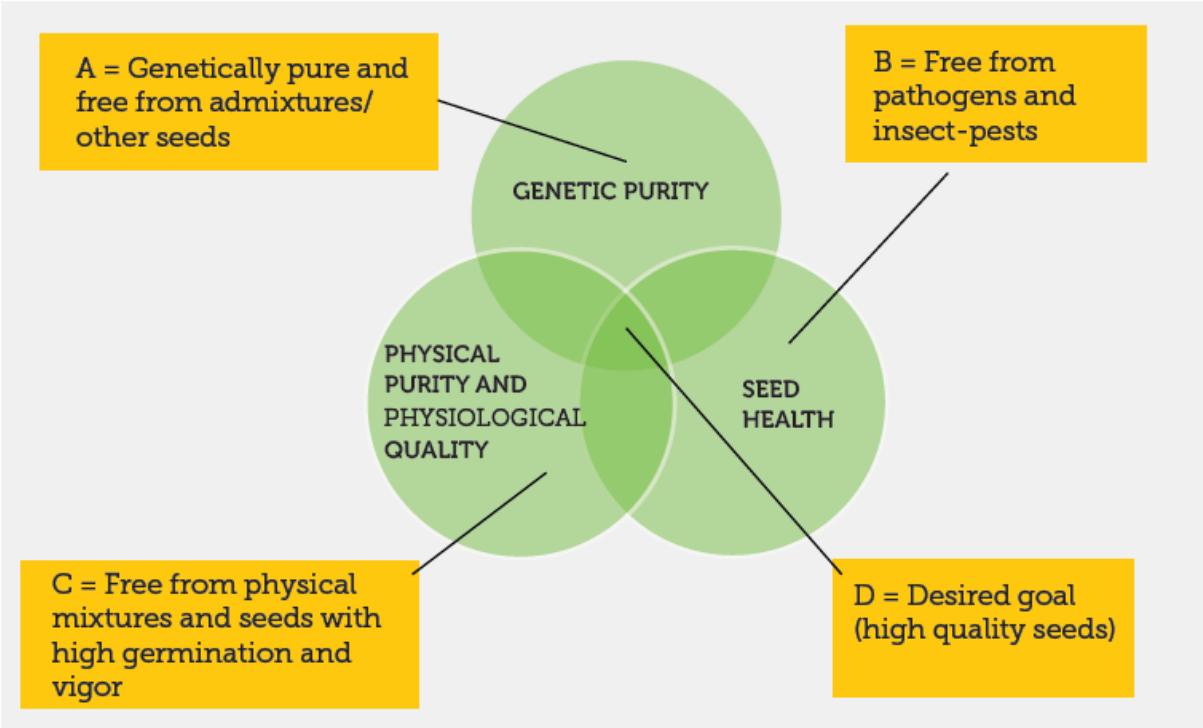
A. FIELD STANDARDS

Species	Isolation, metres (minimum)					Off-types of other cultivars				
	BR	PB	B	C1	C2	BR	PB	B	C1	C2
Cereals						Maximum number per 100 plants (Heads)				
*Maize	400	400	400	200	200	0	0	0	0.5	2
*Sorghum	400	400	400	200	200	0	0	0	1	2
						Maximum number per 100 square metres				
Wheat	10	10	10	4	4	1	1	1	5	6
Barley	10	10	10	4	4	1	1	1	5	6
Triticale	50	50	50	20	20	1	1	1	5	6
Oats	10	10	10	4	4	1	1	1	5	6
Finger Millet	10	10	10	4	4	1	1	1	5	6
*Rice	10	10	10	4	4	1	1	1	5	6
†Hybrid rice	30	30	30	12	12	1^m 1^f	1^m 1^f	1^m 1^f	5^m 5^f	6^m 6^f
Pulses						Maximum number per 100 plants				
Bean	50	50	50	25	25	0	0	0	1	2
*Broad Bean	200	200	200	100	100	0	0	0	1	2

Quality Assurance/Quality Control (QA/QC)

Components of seed quality

- The **success of a maize variety** as well as **economic viability of seed companies** largely depend on the **quality of seed**.
- Seed quality features can be broadly divided into **three components**



Source: Gowda et al., 2017

QA and QC are key in deploying high quality seeds, an important factor for increasing crop yields per unit area.

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Qualtiy Assurance (QA)

- QA is a process focuses on **prevention** of low-quality or genetically impure seed to be mixed with high-quality seed.
- QA starts with **established protocols** on functions, **objectives**, **action plans**, including a set of **quality standards**.
- QA is important for **proactively preventing the mistakes** in the process of creating or maintaining new finished inbred lines, or hybrids.
- For example, in the **process** of seed production,
  - use parental lines from appropriate **source**(s),
  - use the same **protocols** for maintaining these lines,
  - **drying** and **storing** the seed in proper environments,
  - consistent seed **package labeling**, etc.

Quality control (QC)

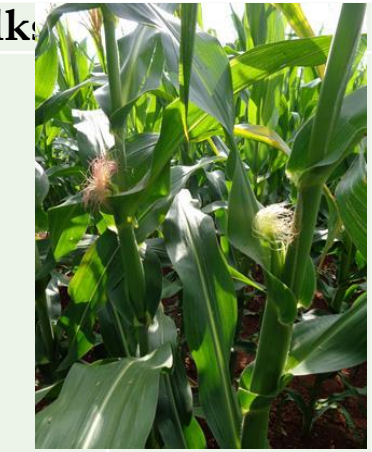
- QC in breeding program is **to identify errors** that may have slipped through Quality Assurance protocols.
- Focuses on the **product**, unlike QA that focuses on the process
- QC involves routinely test breeding materials/seed throughout the process using all available tools
  - Conventional phenotypic and morphological traits
  - Molecular marker techniques

Conventional (morphological) method

- **Conventional method** for assaying the **purity** of seed involving a representative sample of the seed to be marketed.
- Grow out test (**GOT**) serves as a pre-control as well as a 'post-control' test for avoiding genetic contamination.
- Generally, it is a requirement for seed certification of maize hybrids.
- A set of **qualitative and quantitative characteristics** known as "**descriptors**" stringently related to seed quality are used for hybrid or inbred lines identification.

Qualitative and quantitative characteristics – descriptors 1

CML395

Silk


General Information		Ears			Biotic Stress Perfomance	
Adaptation/Agroecological zone	Africa MA/ST				Northern Corn Leaf Blight (1-5)	1.8
Grain colour	white				Grey Leaf spot (1-5)	2
Line Type	convectional				Maize Streak Virus (1-5)	1.8
Agronomic Characteristics					Abiotic Stress Peformance	
Maturity (DAP)	Intermediate				Drought tolerance	.
Grain Yield (t/ha) Opt/Drought	4.27				Low Nitrogen Tolerance	.
Plant height (cm)	184					
Ear Height (cm)	88.7				Key Strengths	
Husk Cover (%)	4.3					
Grain Texture	2					
Anther-Silking Interval (Days)	0.1					
Seed Producibility				**Descriptor for DUS**		
Female Seed Yield	4.3	**Leaf: Anthocyanin colouration of the sheath**	1		Tassel: Anthocyanin colouration of anthers	1
Female Mid-Silk/GDD ^{o}C		Leaf: Width of the blade	10		Tassel: Anthocyanin colouration of glumes(exclude base)	2
Male mid - Anthesis/GDD ^{o}C		Leaf: Leaf surface architecture (leaf just above the upper ear)	.		Tassel: Angle btwn main axis and lateral branches	1
		Stem: Anthocyanin in colouration of brace				

Qualitative and quantitative characteristics – descriptors 2

CML202

Plant

Tassel

Silks

Page 1

General Information	
Adaptation/Agroecological zone	Africa MA/ST
Grain colour	white
Line Type	Convectional
Agronomic Characteristics	
Maturity (DAP)	158 days
Grain Yield (t/ha) Opt/Drought	2.5/
Plant height (cm)	140
Ear Height (cm)	80
Husk Cover (1=good; 9=poor)	1
Grain Texture	Semi Dent
Anther-Silking Interval (Days)	2.5
Seed Producibility	
Female Seed yield (t/ha)	2.5
Female Mid-Silk/GDD °C	883
Male mid - Anthesis/GDD °C	909
Tassel Size/Pollen Shed (Best 1 - 5 poor)	1.3

Ears

Biotic Stress Perfomance	
Northern Corn Leaf Blight (1-5)	3.0
Grey Leaf spot (1-5)	1.9
Maize Streak Virus (1-5)	0.5
CommonRust (1-5)	2.1
Abiotic Stress Peformance	
Drought tolerance	NA
Low Nitrogen Tolerance	NA
Key Strengths	
Good as both male and female parent in seed production	

Descriptor for DUS

Leaf: Anthocyanin colouration of the sheath	very weak	Tassel: Anthocyanin colouration of anthers	weak
Leaf: Width of the blade	9.1 cm, medium	Tassel: Anthocyanin colouration of glumes(exclude base)	weak
Leaf: Leaf surface architecture (leaf just above the upper ear)	slightly recurved	Tassel: Angle btwn main axis and lateral branches	small
Stem: Anthocyanin in colouration of brace roots	absent	Ear: Anthocyanin colouration of the silks	very weak
Stem: Anthocyanin in colouration of internodes	absent	Ear: Colour of the dorsal side of the grain	absent
Stem: Angle between the blade and the stem(leaf above upper ear)	small	Ear: Anthocyanin colouration of the glumes of the cob	absent

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How to record discriptors

Leaf: angle between blade and stem

3. Small

5. Medium

7. Large

SCALE	1	very small	3	small	5	medium
	7	large	9	very large		

Recorded for leaf just above upper ear at flowering

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Leaf: Anthocyanin coloration of sheath (leaf on the ear)

Recorded from flowering to milk stage

Absent or very weak

Weak

Medium

SCALE

1 absent or very weak	3 weak	5 medium
7 strong	9 very strong	

Leaf: Width of blade leaf on the ear

Recorded at milk stage; leaf of upper most ear measured in cm

Narrow *Medium* *Wide*

SCALE

1 very narrow	3 narrow	5 medium
7 wide	9 very wide	

Tassel: angle between main axis and lateral branches

Record at 50% pollen shed

Small

Medium

Large

SCALE

1 very small	3 small	5 medium
7 large	9 very large	

Tassel: Anthocyanin coloration of glumes excluding base

Recorded before pollen shed; middle third of main axis

Absent or very weak

Weak

Medium

SCALE

1 absent or very weak	3 weak	5 medium
7 strong	9 very strong	

Tassel: Anthocyanin coloration of anthers

Record at 50% anthesis; from middle third of main axis on fresh anthers

Absent or very weak

Weak

Medium

SCALE

1 absent or very weak	3 weak	5 medium
7 strong	9 very strong	

Silk: intensity of anthocyanin coloration of silks

Record at 50% silking; from upper most ear

1. Very weak

3. Weak

5. Medium

SCALE

1	absent or very weak	3	weak
5	medium	7	strong
9	very strong		

Stem: Anthocyanin coloration of internodes

Recorded at flowering to milk stage; from middle of the plant

Absent or very weak

Weak

Medium

SCALE

1	absent or very weak	3	weak	5	medium
7	strong	9	very strong		

Stem: anthocyanin coloration of brace roots

Recorded at milk stage

Absent or very weak

Weak

Medium

SCALE

1 absent or very weak	3 weak	5 medium
7 strong	9 very strong	

Ear: shape

Record at hard grain stage; from upper most ear

Conical

Conico-cylindrical

Cylindrical

SCALE 1 conical 2 conico-cylindrical 3 cylindrical

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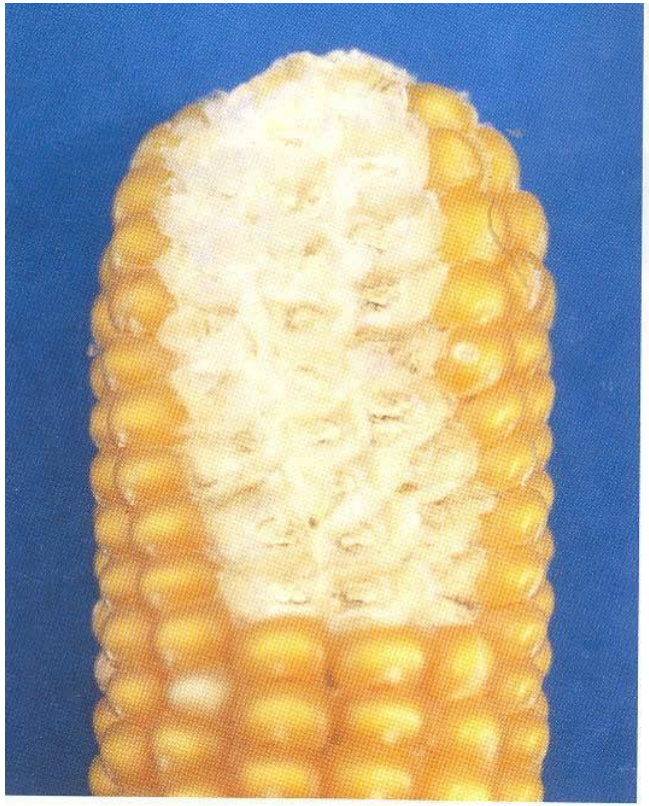
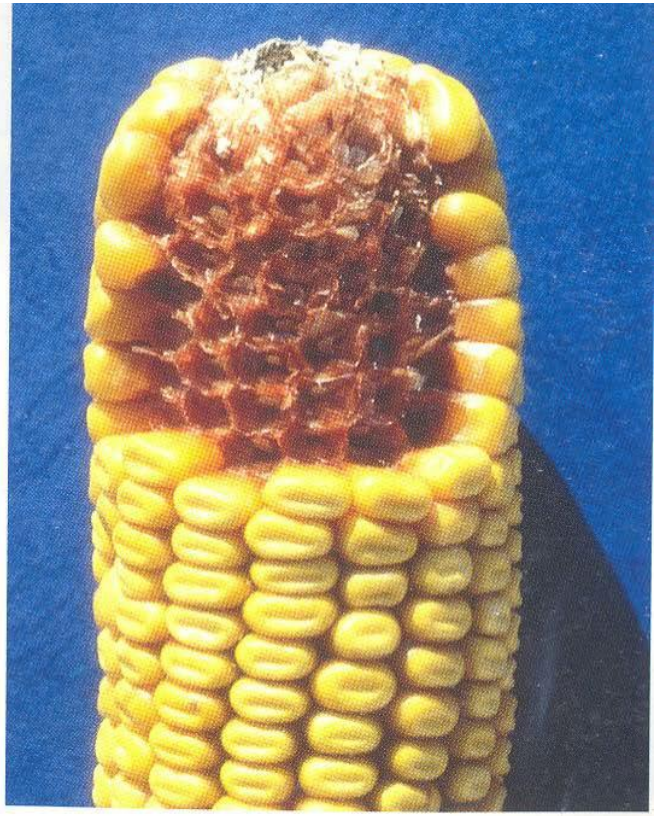
Ear: anthocyanin coloration of glumes of the cob

Record at hard grain stage



Absent



Present

SCALE 1 absent 9 present

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Ear: color of dorsal side of grain

Measure at hard grain stage

Yellow

Yellow Orange

Orange

Red Orange

SCALE

1 white	2 yellowish white	3 yellow
4 Yellow orange	5 orange	6 red orange
7 red	8 dark red	9 blue black

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Sample Result-Descriptor

Characteristics	CML395	Score
First leaf: anthocyanin colouration of sheath	weak to medium	3
First leaf: Shape of the tip of first leaf	round	1
Leaf: undulation of the margin of the blade	Absent	1
Leaf: angle between blade and stem (on leaf just above upper ear)	small	3
Leaf: curvature attitude of blade (on leaf just above upper ear)	slightly recurved	3
Leaf: anthocyanin colouration of sheath (in middle of plant)	absent	1
Leaf: width of blade (leaf of upper ear) (cm)	10 cm	7
Stem: degree of zig-zag	absent/v.slight	1
Stem: anthocyanin colouration of brace roots	weak	3
Stem: anthocyanin colouration of internodes	weak	3
Tassel: time of anthesis (on middle third of main axis, 50 % of plants) (Days at Kiboko)	66	7
Tassel: anthocyanin colouration at base of glume (in middle third of main axis)	Absent	1
Tassel: anthocyanin colouration of glumes excluding base (in middle third of main axis)	very weak to weak	3
Tassel: anthocyanin colouration of anthers (in middle third of main axis on fresh anthers	Absent	1
Tassel: density of spikelets (in middle third of main axis)	medium to dense	5
Tassel: angle between main axis and lateral branches (in lower third of tassel)	very small	1
Tassel: attitude of lateral branches (in lower third of tassel)	straight	1
Ear: anthocyanin colouration of silks	present	2
Ear: intensity of anthocyanin colouration of the silks	weak to medium	5
Ear: length of peduncle (cm)	long(7) 10.8cm	7
Ear: length without husk (cm)	medium(5) 15.0cm	5
Ear: diameter without husk (in middle) (cm)	short(3) 4.5cm	3
Ear: length of husk above tip of the ear	long(7) 6.5cm	7
Ear: shape	conical	3
Ear: number of rows of grains	medium(5) 12-14	5
Ear: type of grain (in middle third of ear)	flint(2)	2
Ear: colour of top of grain	white	1
Ear: colour of dorsal side of grain	white	1
Ear: anthocyanin colouration of glumes of cob	absent	1

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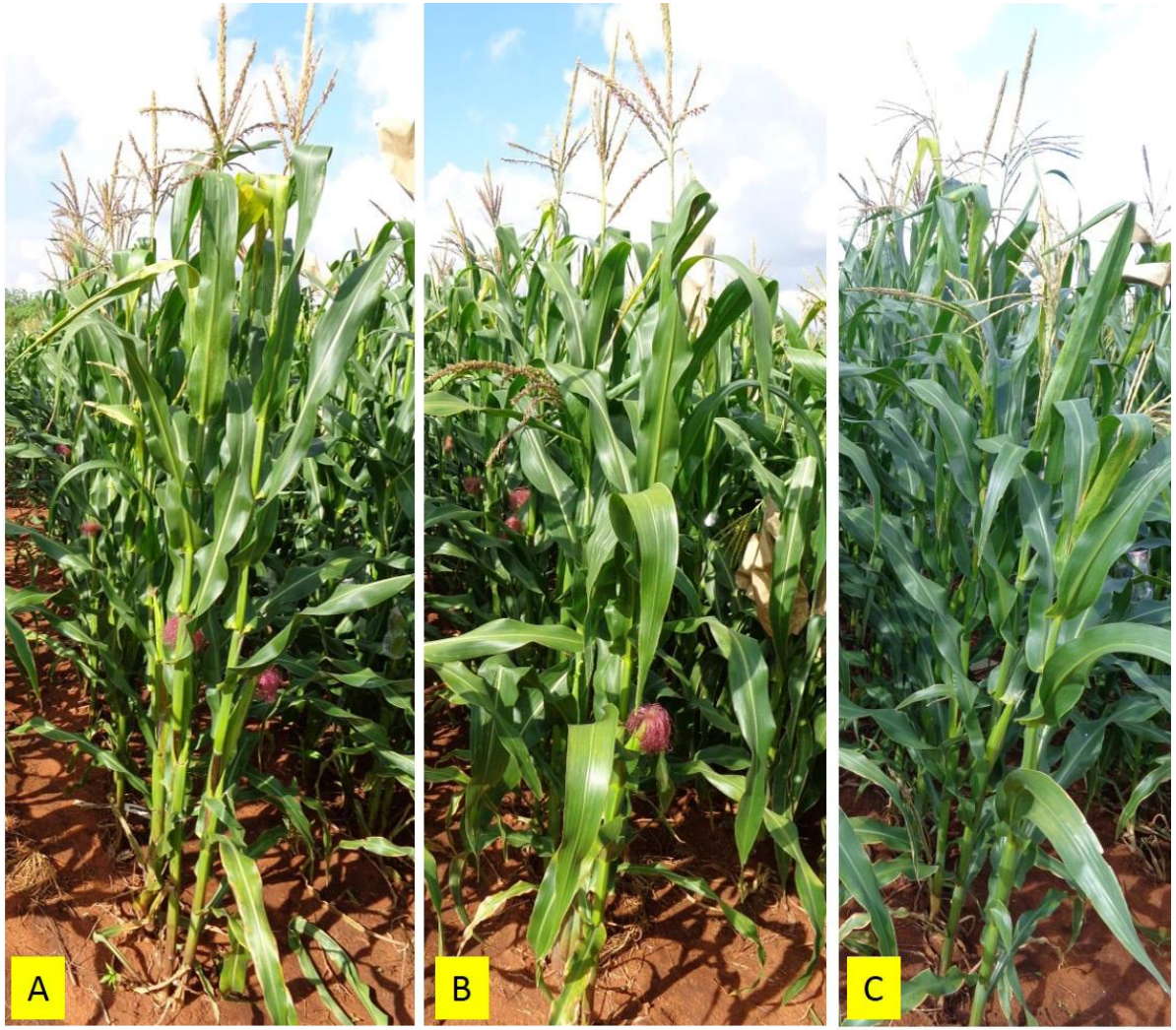
Grow-out test – Conventional method



Morphological comparison, through GOT at Kiboko, Kenya (2016), of a CIMMYT inbred line from three different seed sources (A, B and C). While A (seed from a reference sample) and B were similar in phenotype, C was not true-to-type.

Use of molecular markers

- Different types of molecular markers are used to determine genetic relationships or diversity (variations) of inbred lines and varieties
- **QA & QC issues in seed production**
  - **Parental lines**
    - Genetic purity or homogeneity
    - Genetic identity among different seed sources (growers), batches or origins
  - **Hybrids**
    - Genetic uniformity across grower's vs seed batches/origins
    - Parentage verification (parent-offspring tests)

Genetic purity in parental lines - SNPs

SNP	Parental Line 1			Parental Line 2		
	Source_A	Source_B	Source_C	Source_A	Source_B	Source_C
SNP_01	C:C	C:C	C:C	C:C	C:C	C:C
SNP_02	A:G	A:A	A:A	A:A	A:A	A:A
SNP_03	T:T	T:T	A:T	A:A	A:A	A:T
SNP_04	A:A	A:A	A:A	C:C	C:C	C:C
SNP_05	A:G	G:G	G:G	G:G	A:G	G:G
SNP_06	C:C	C:C	C:C	G:G	G:G	C:G
SNP_07	A:A	A:A	A:T	T:T	A:T	T:T
SNP_08	A:A	A:A	A:A	A:A	A:A	A:A
SNP_09	T:T	T:T	T:T	C:C	C:C	C:T
SNP_10	A:T	A:A	A:A	A:A	A:A	A:A
Purity (%)	70	100	80	100	80	70
Results	**Reject**	**Accept**	**Reject**	**Accept**	**Reject**	**Reject**

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Genetic identity - SNPs

SNP	Original source	Line 1_A	Line 1_B	Original source	Line 2_A	Line 2_B
SNP_11	C:C	C:C	C:C	G:G	G:G	G:G
SNP_12	A:A	A:G	A:A	C:C	C:C	C:C
SNP_13	A:A	T:T	A:A	A:A	A:A	G:G
SNP_14	C:C	C:G	C:C	T:T	T:T	T:T
SNP_15	G:G	A:G	G:G	G:G	G:G	C:C
SNP_16	G:G	C:C	G:G	C:C	C:C	A:G
SNP_17	T:T	A:T	T:T	A:A	A:A	A:A
SNP_18	A:A	A:A	A:A	T:T	T:T	T:T
SNP_19	C:C	C:C	C:C	G:G	G:G	T:T
SNP_20	A:A	A:T	A:A	A:A	A:A	A:A
Identity (%)	100	30	100	100	100	60
Results		Reject	Accept		Accept	Reject

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Conclusion

- The relevant characteristics of a genotype change through the generations.
- **Proper maintenance methods preserves** the genetic composition of a genotype such as inbred lines and OPVs
- QA/QC are crucial to ensure the production of high-quality seed which is key for increased productivity and economic feasibility of seed companies
- QA focuses on prevention while QC **on identifying errors** that may have slipped through QA protocols.
- Morphological and molecular methods can be use for quality control

Thank you for your interest!