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Genetic molecular markers and their deployment in public sector plant breeding programs: Challenges and opportunities

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(EiB-CIMMYT, Kenya)

15th September 2021

Challenge of feeding the planet: Malthusianism

An Essay on the Principle of Population

"Population growth will always tend to outrun the food supply and that betterment of humankind is impossible without stern limits on reproduction."

- Famine is inevitable: Population grows geometrically and food production arithmetically.
- Slowing the growth of population is the only possibility to prevent starvation

History (so far) has proven Malthus wrong . . .

Thomas Robert Malthus (1766-1834)

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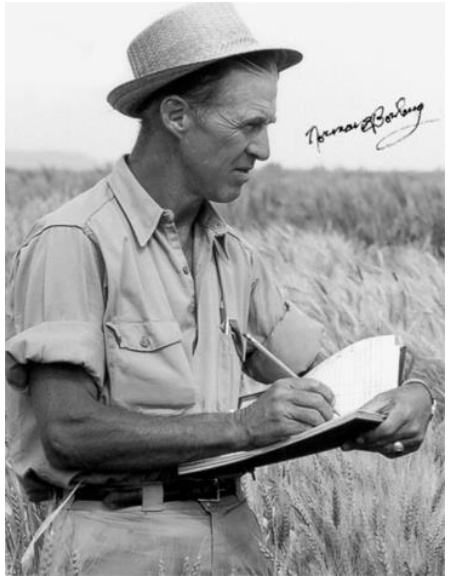
The Green Revolution:

Consultative Group for International Agricultural Research (CGIAR) & National Agricultural Research System (NARS)

- Development and adoption of new improved varieties
- Application of better agricultural techniques
  - Irrigation
  - Mechanization
  - Use of fertilizer
  - Use of pesticides



Dr. Norman Borlaug (1914-2009)

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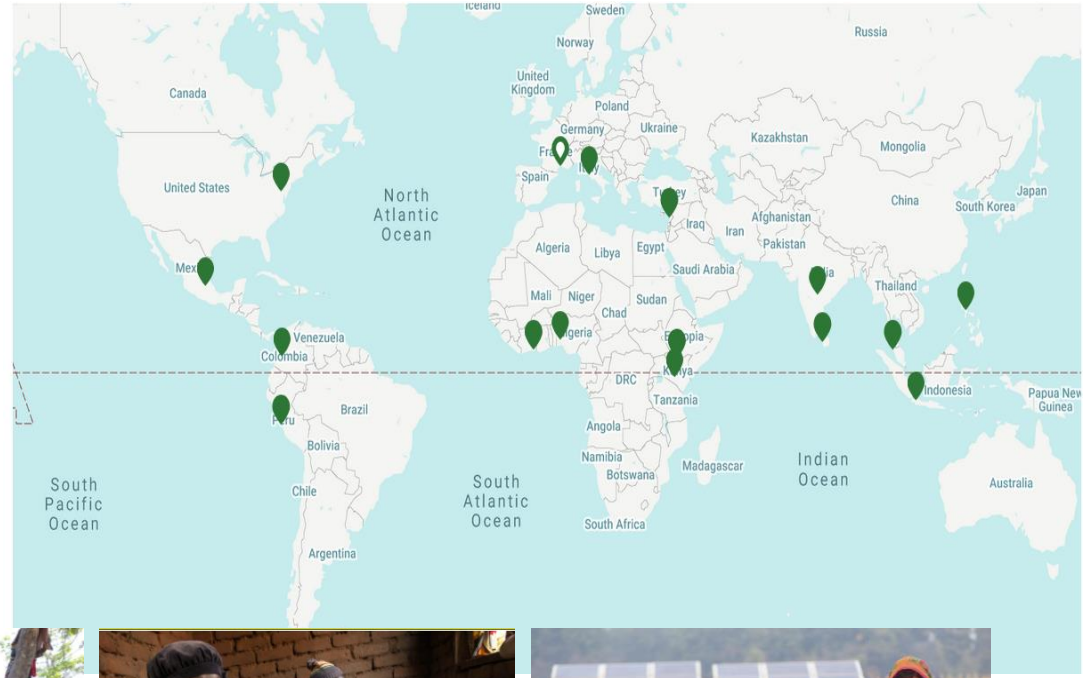
Consultative Group for International Agricultural Research (CGIAR)



https://www.cgiar.org/

15 top-class research centers

Present in 108 countries

> 50 Years experience

> 3,000 partners

> 770,000 germplasm accessions

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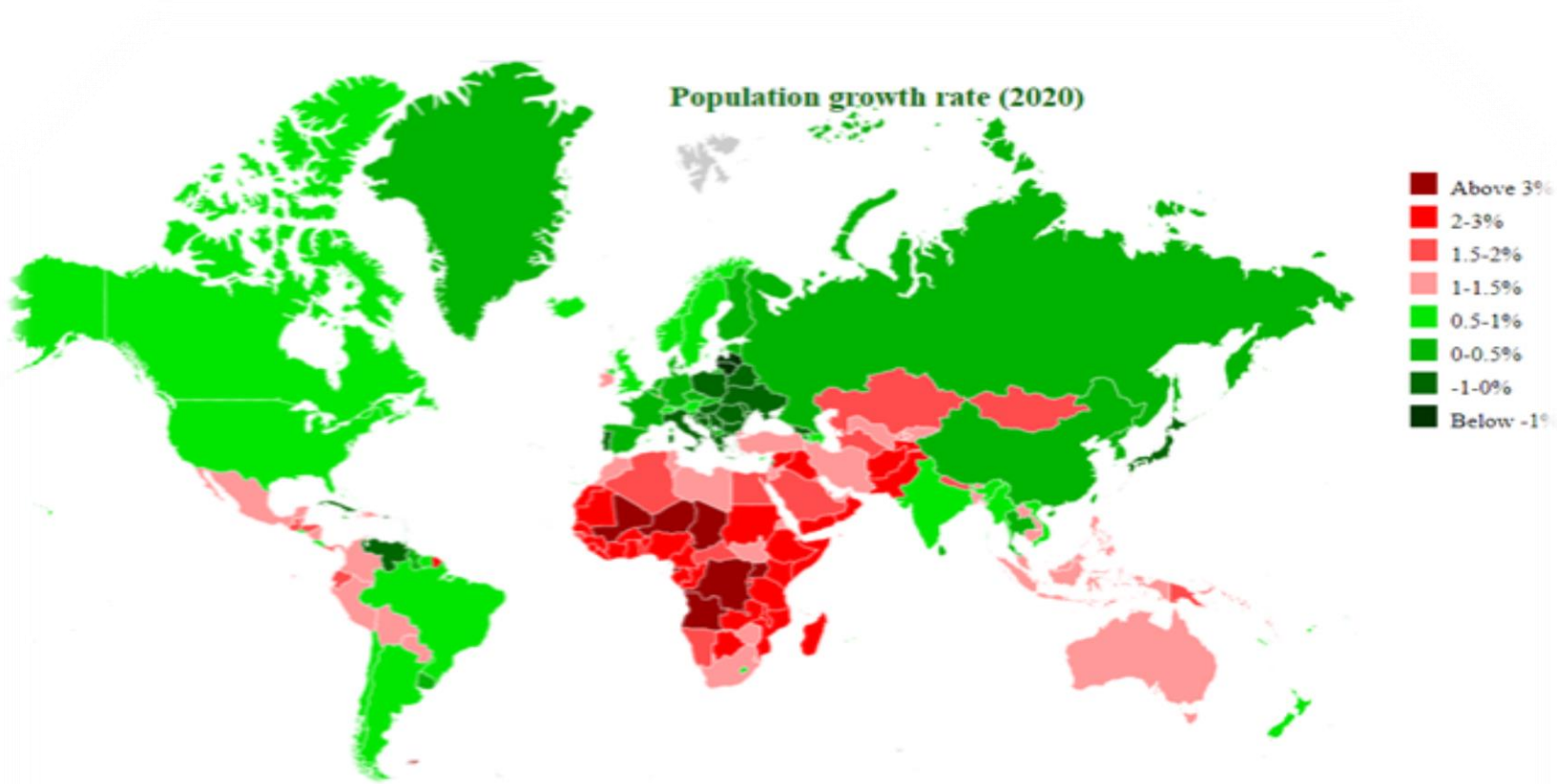
Human population growth: The road ahead for food security



- Asia is the world's largest continent (44.57 m km^2) followed by Africa (30.06 million sq. km)
- Both continents are home of more than two-third world population
- More than 8 out of 10 people in the world will live in Asia or Africa by 2100

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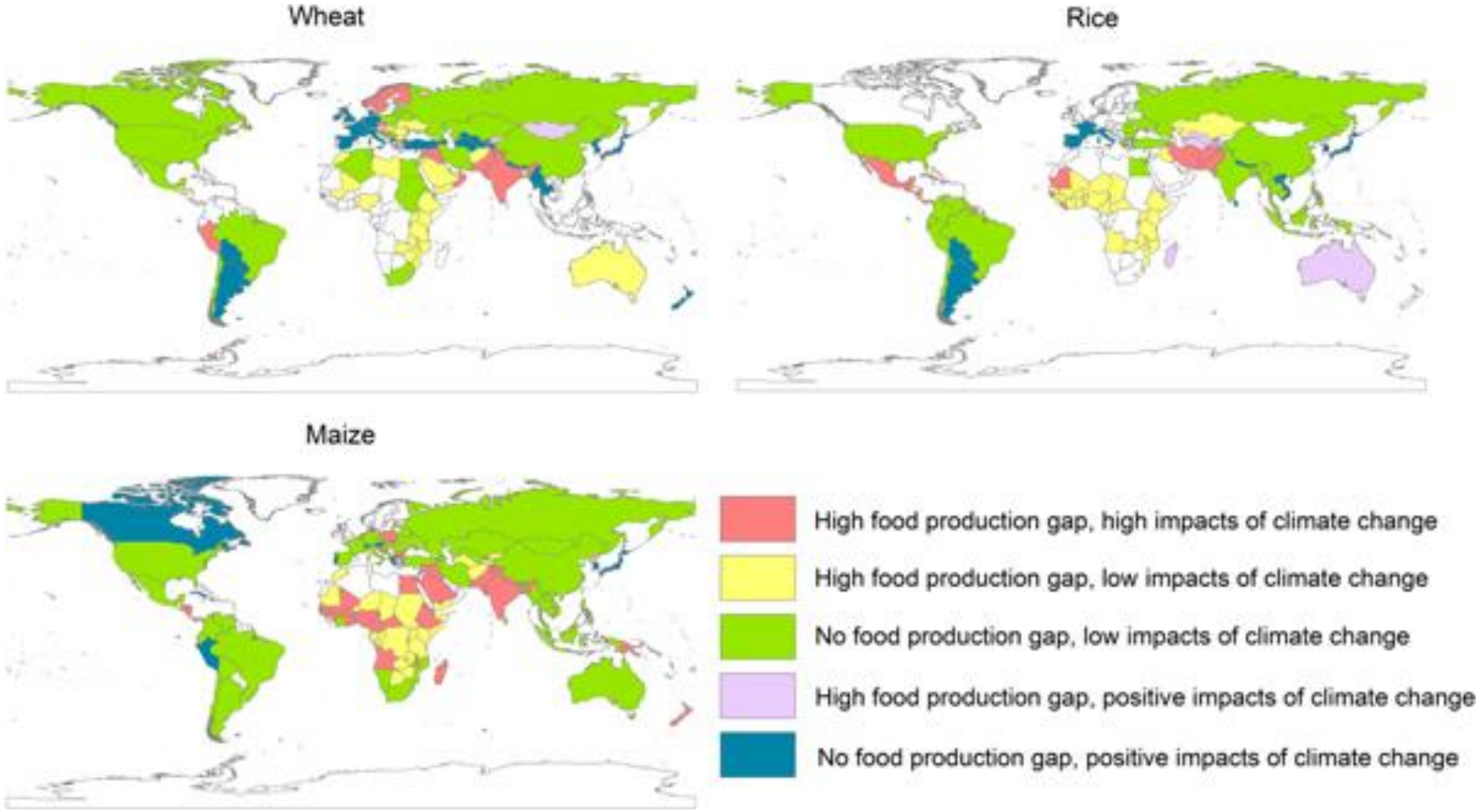
Climate change: Challenge of feeding the planet this century



Source: Aggarwal et al. 2019

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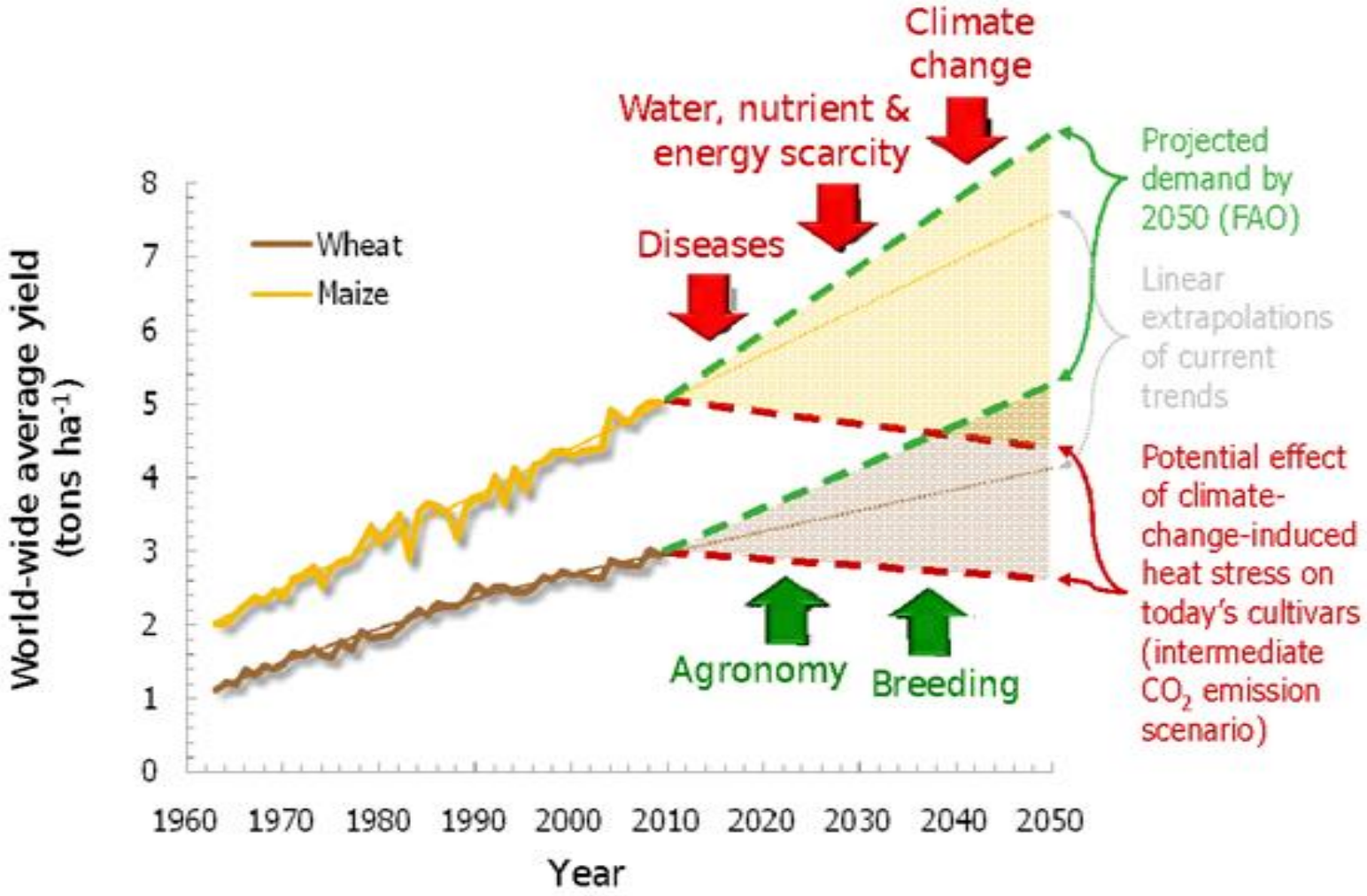
Genetic gain increase model

Annualised production increases must be >1.7% to prevent catastrophic price rises

Genetic gain should be within the context of well-developed **product profiles**, which should drive where gain is needed and where maintenance of essential traits is adequate.



Source: CIMMYT

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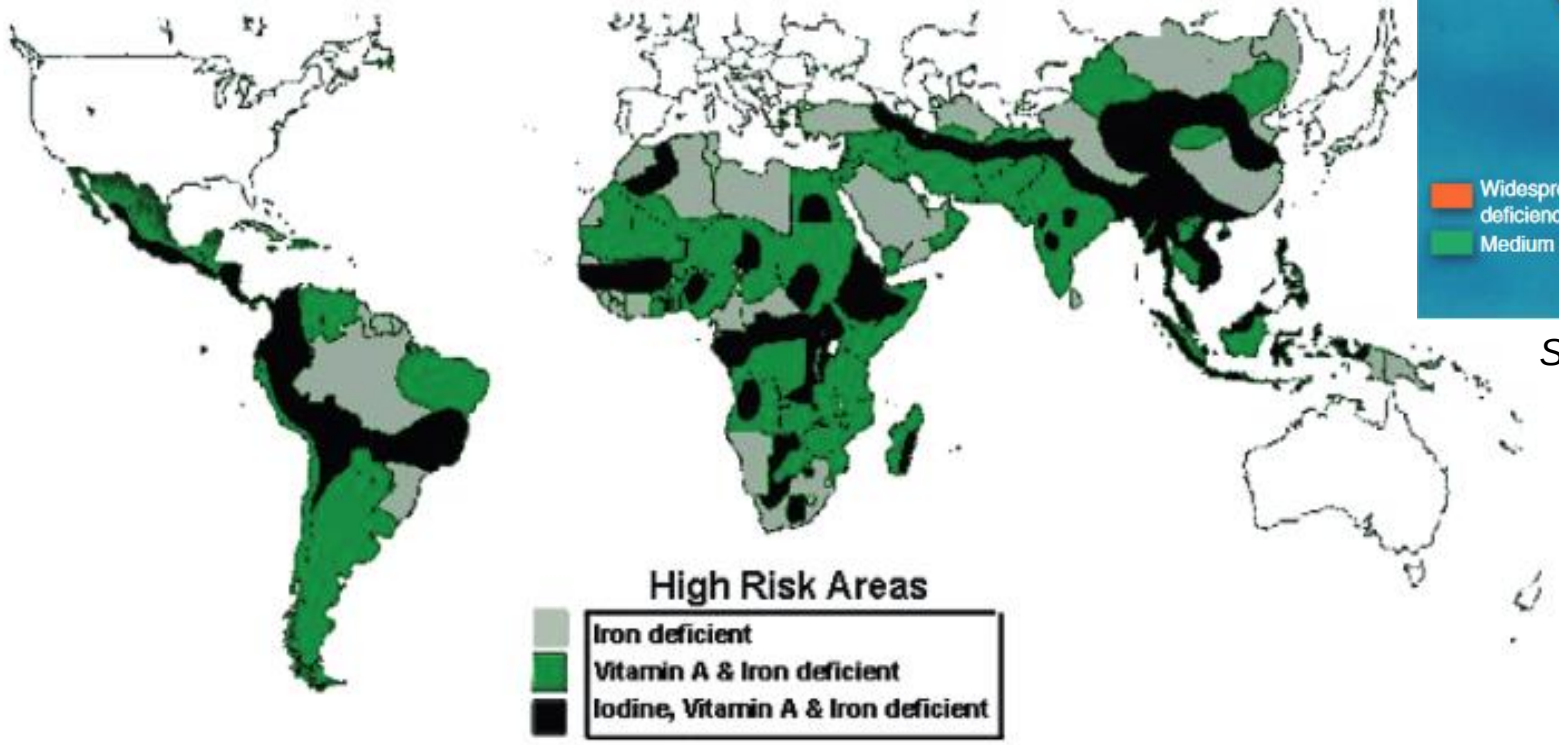
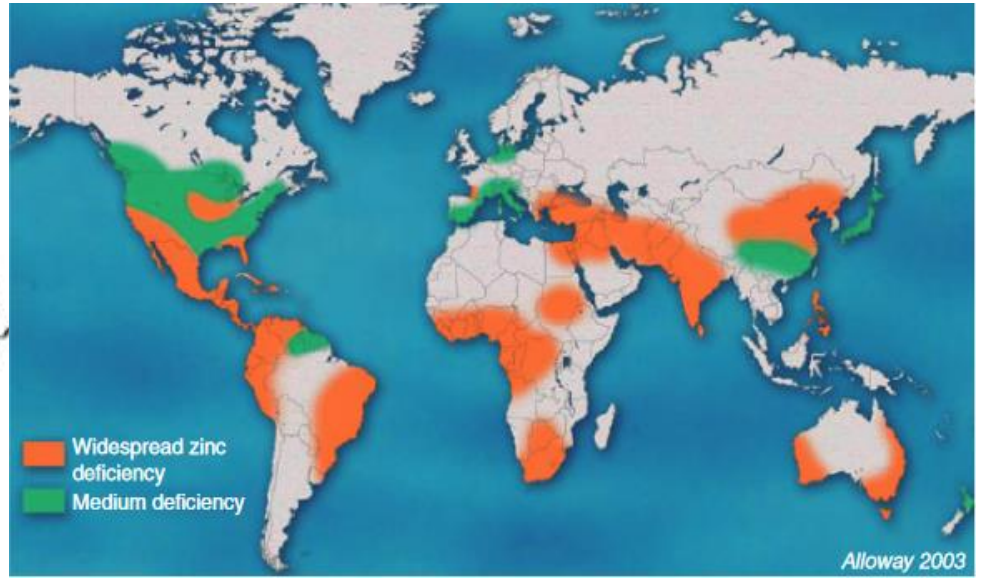
Micronutrient deficiency: *Major Public Health Problem*

Essential micronutrient deficiency:

Vitamin A (VAD), Iron (Fe) and Zinc (Zn)





Source: Alloway 2008

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Selected milestones in molecular markers technology

- 1980's : 1st major effort to produce a human genetic map, mainly used RFLPs (Hybridization based)
- In mid 1980s- Polymerase Chain Reaction (PCR) technique allowed scientists to make millions of copies of a scarce sample of DNA.
- 1990's : Shift to microsatellites –More informative and easier to type
- 2000's : Movement to SNPs – because requirement for very high density of markers
- Late 2000's: Shift to High through put SNP makers
- 2010's: Automation for genotyping

Technology is very rapidly changing

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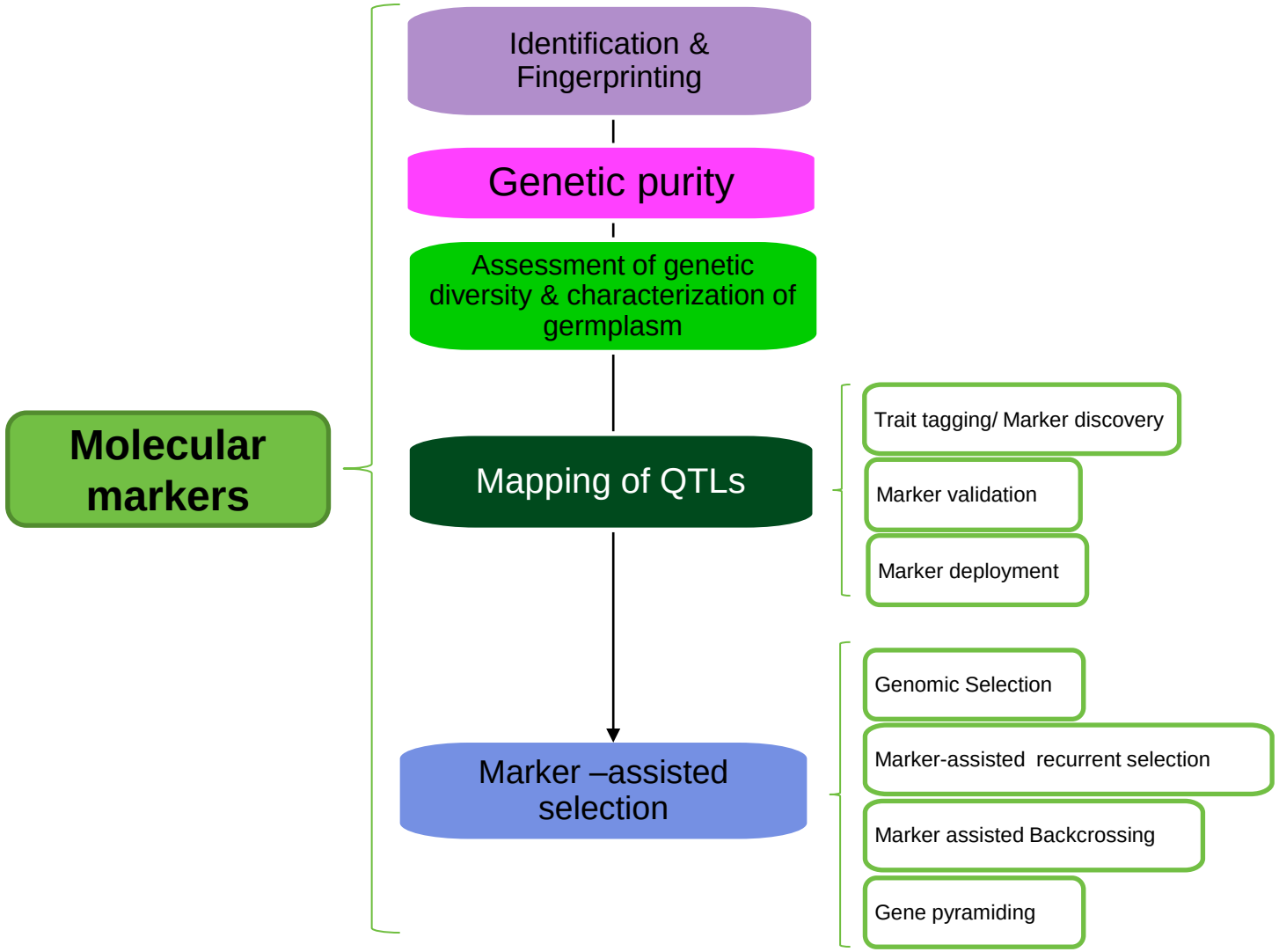
Integration of molecular markers in public plant breeding programs



Garcia-Oliveira (2021)

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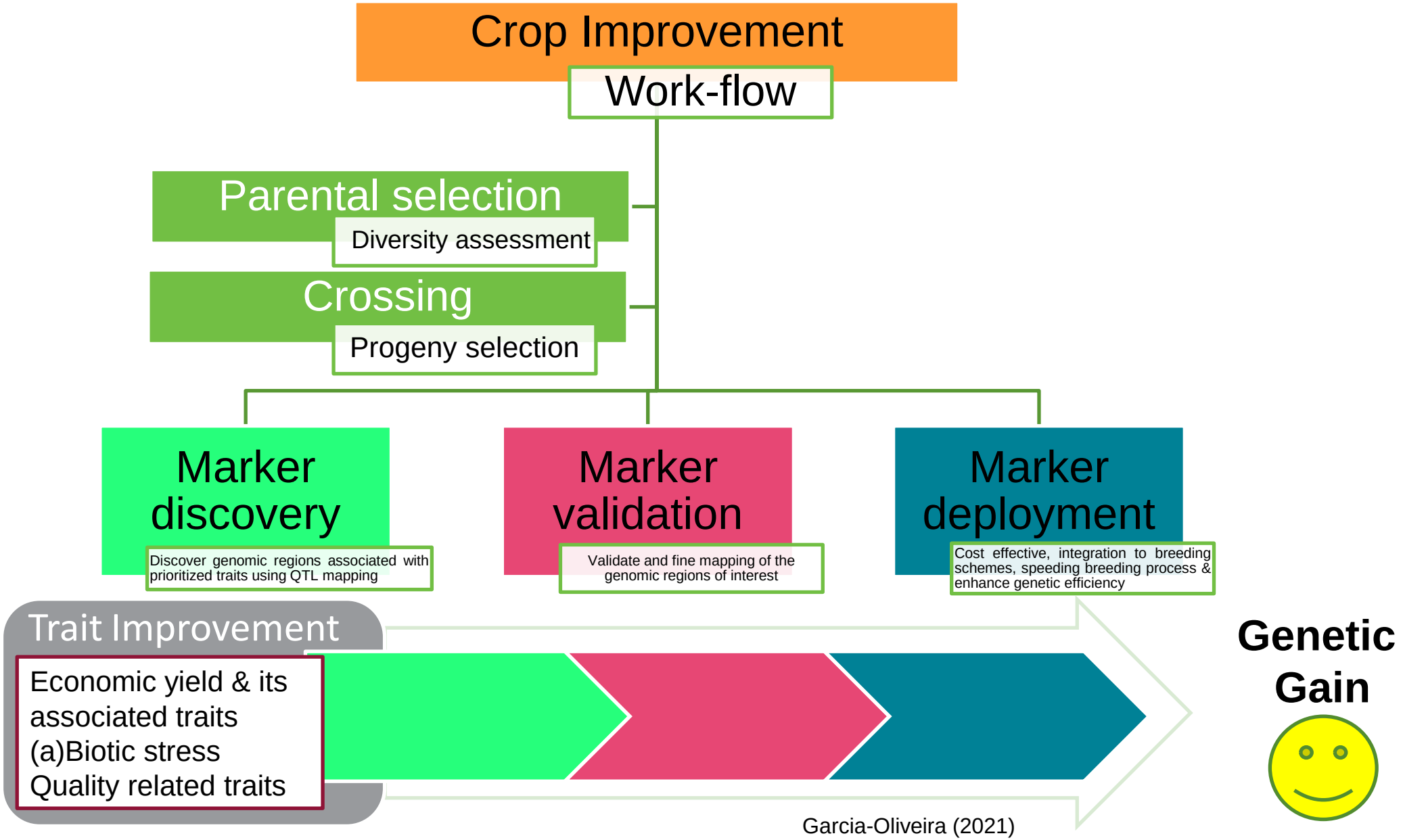
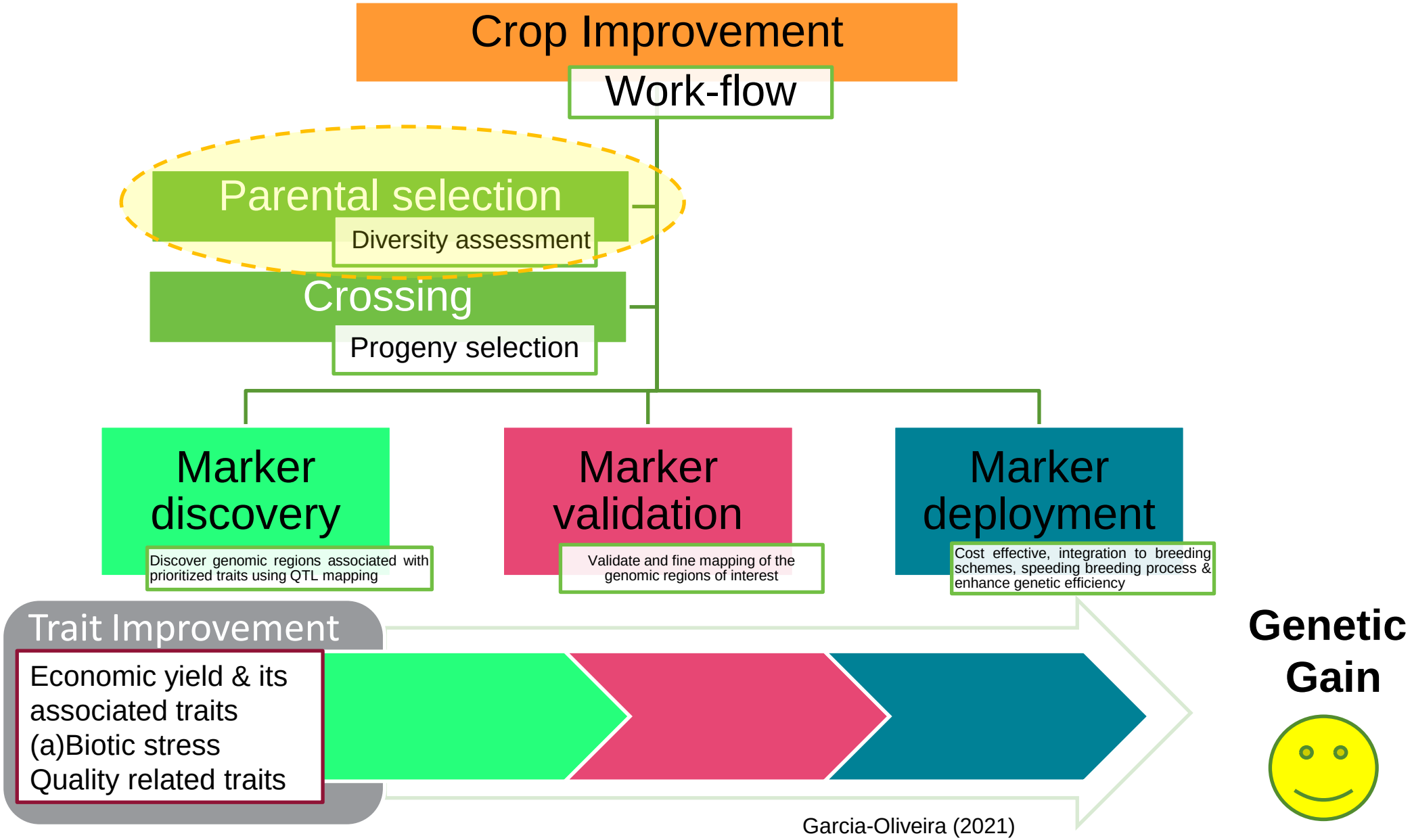
Molecular markers work-flow in crop improvement



Garcia-Oliveira (2021)

Molecular markers work-flow in crop improvement



Garcia-Oliveira (2021)

Case Study-IITA Cassava: Cultivar's identification & molecular characterization

- Cassava (*Manihot esculenta*) is widely grown in Africa, Asia, and Latin America where it is consumed as a food and utilized as an industrial crop primarily for starch.
- Biotic stresses particularly viral diseases [Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD)] and insect pests such as Cassava Green Mite (CGM) inflict a large yield penalty and impact the availability of clean planting material.

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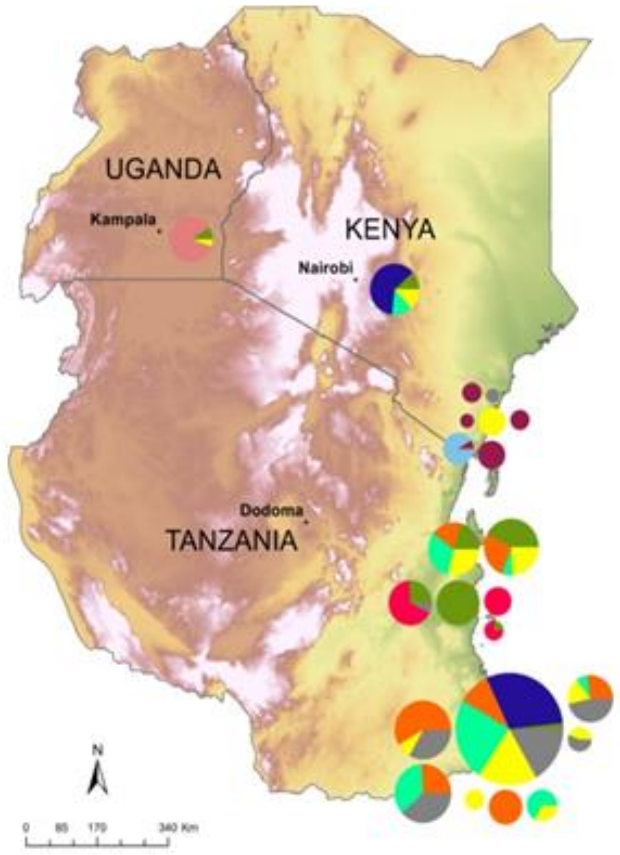
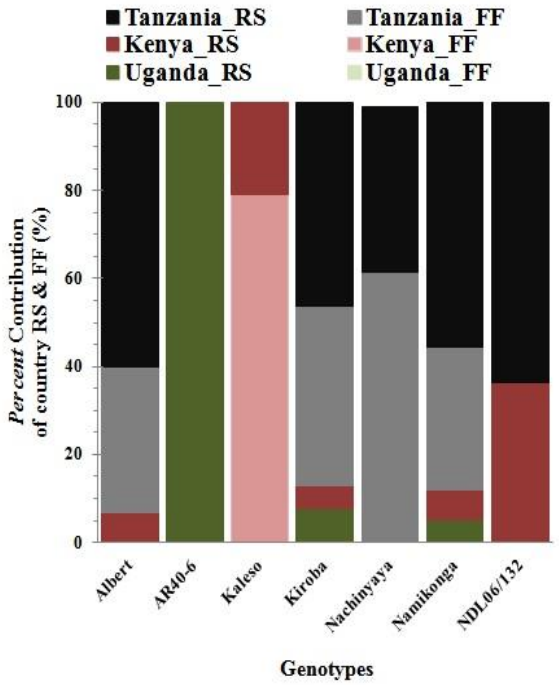
Case Study-IITA Cassava cultivars identification



- A field survey was undertaken with NARS team to identify and characterize prominent cassava cultivars from east African countries namely Kenya, Tanzania and Uganda
- 268 samples of cassava leaves together with stakes were collected from the farmer's field and research stations representing eight, fourteen and one locations in Kenya, Tanzania and Uganda, respectively.



- 15,992 SNP markers with known position on the cassava reference genome (http://www.phytozome.net/cassava) were used for further analysis.

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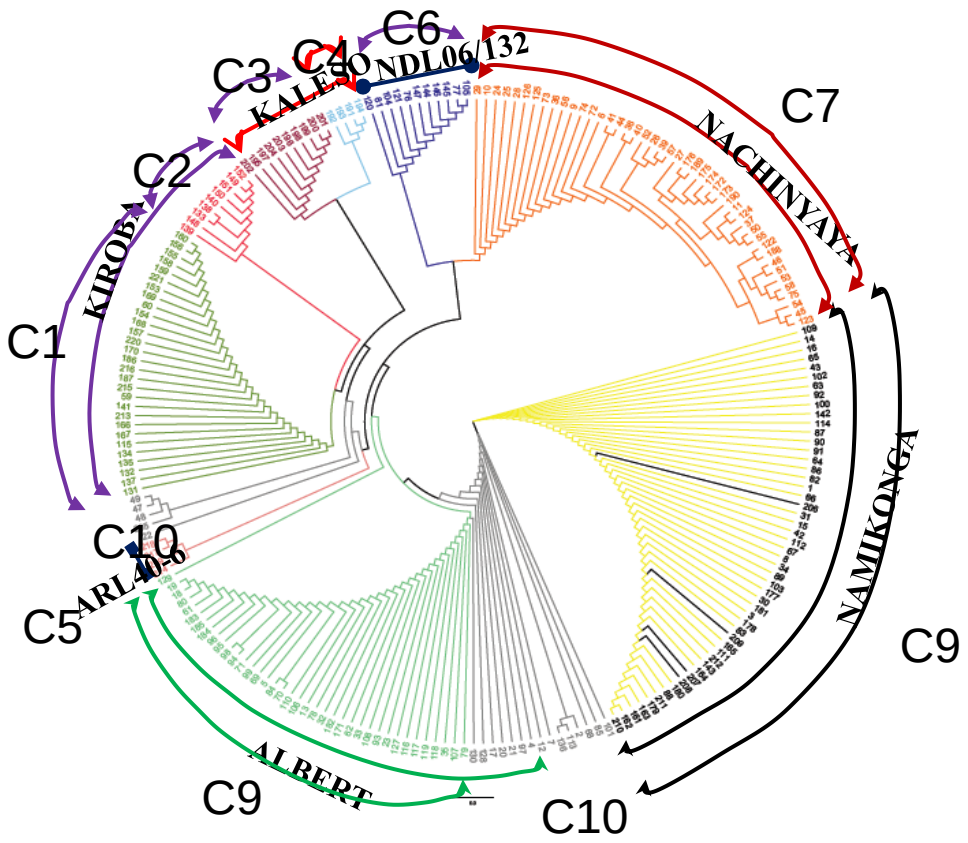
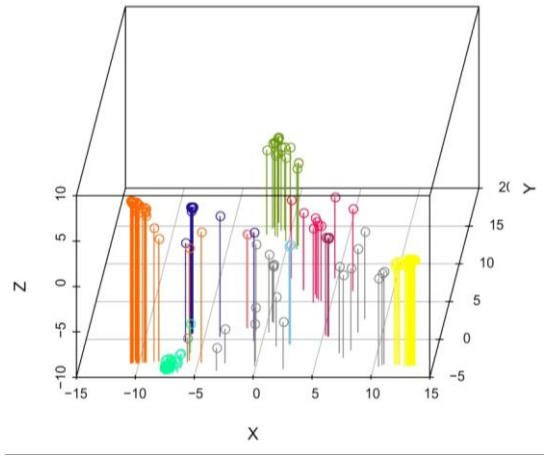
Case Study : Molecular characterization…



- The phylogenetic analysis and Principal coordinate analysis allowed us to group all samples in well distinguished groups (cultivar's).



- Some cultivars described by breeders were misrepresented by farmers or at research stations.
- Finally, our findings suggest that breeders need to be more conscious in future for decision making in cassava genetic improvement.

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Case Study-IITA Maize Improvement Program

Genetic diversity and population structure of early and extra-early maturing maize germplasm adapted to sub-Saharan Africa

- We used 439 early and extra-early maize inbred lines developed from three narrow-based and twenty-seven broad-based populations by the IITA Maize Improvement Program (IITA-MIP).
- These inbreds were genotyped using 9642 DArTseq-based single nucleotide polymorphism (SNP) markers distributed uniformly throughout the maize genome.


Badu-Apraku et al. BMC Plant Biology (2021) 21:96
https://doi.org/10.1186/s12870-021-02829-6

BMC Plant Biology

RESEARCH ARTICLE Open Access

Genetic diversity and population structure of early and extra-early maturing maize germplasm adapted to sub-Saharan Africa

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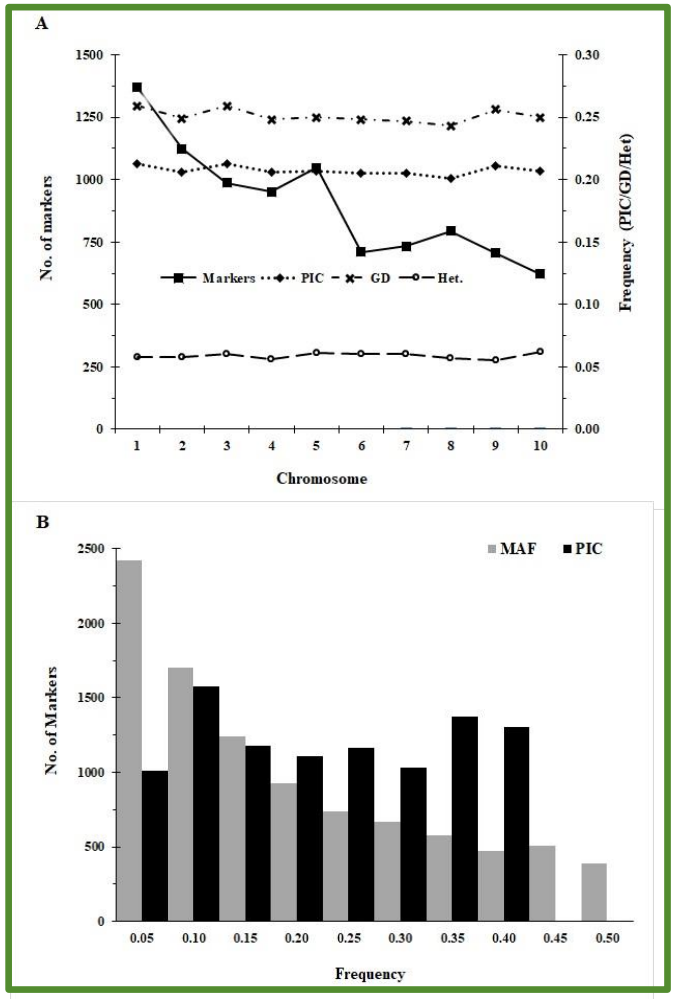
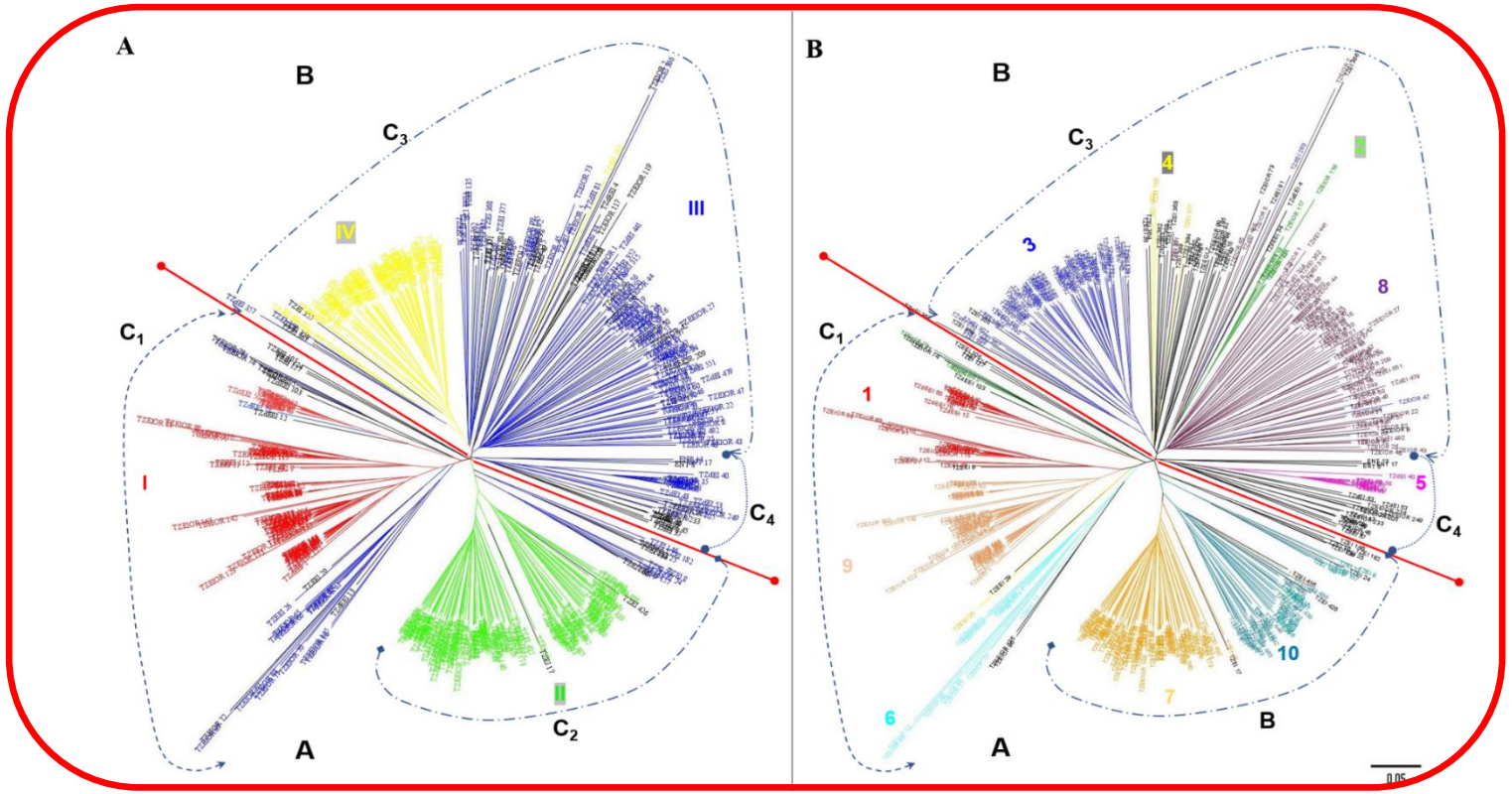
Case Study-IITA MIP: Genetic diversity & structure analysis





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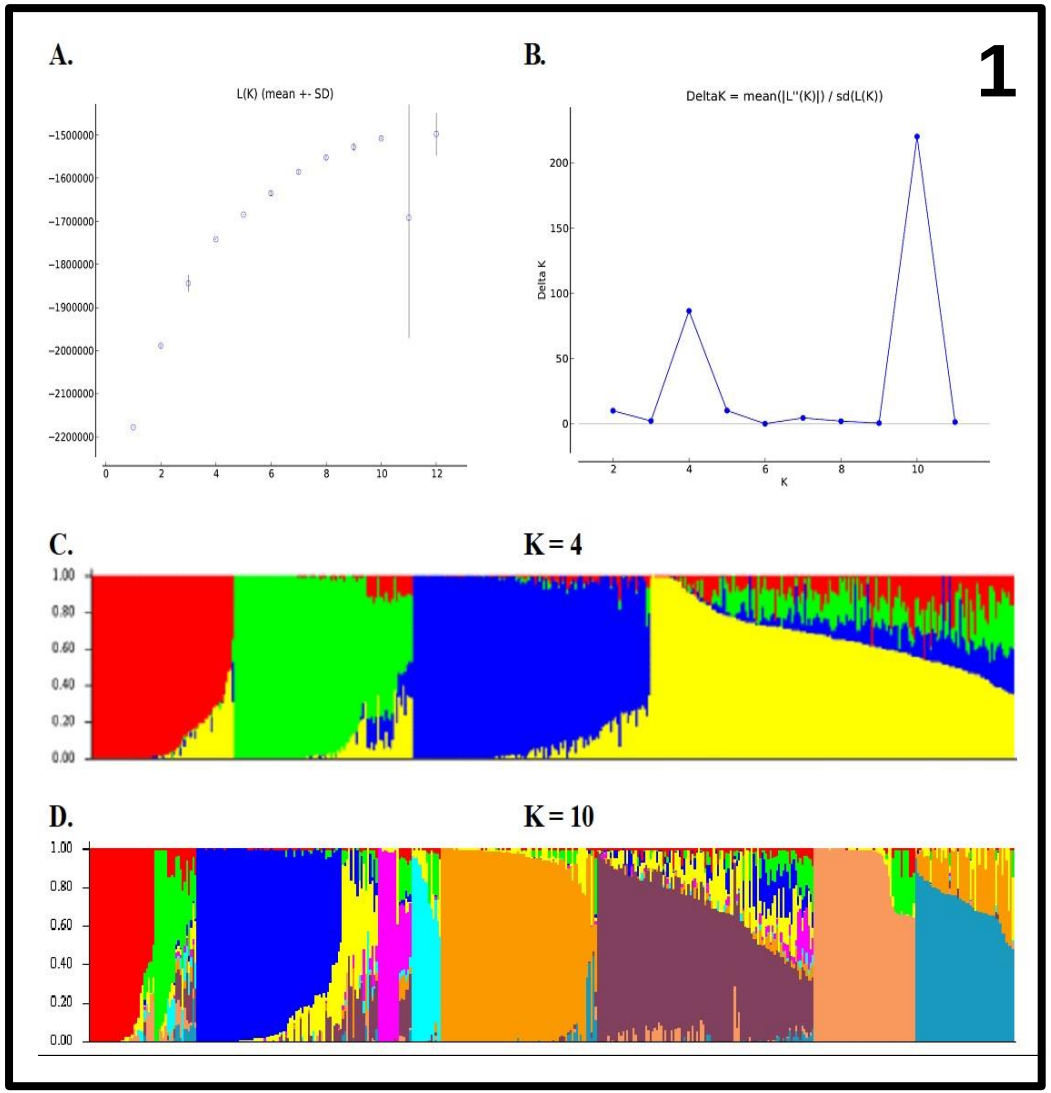
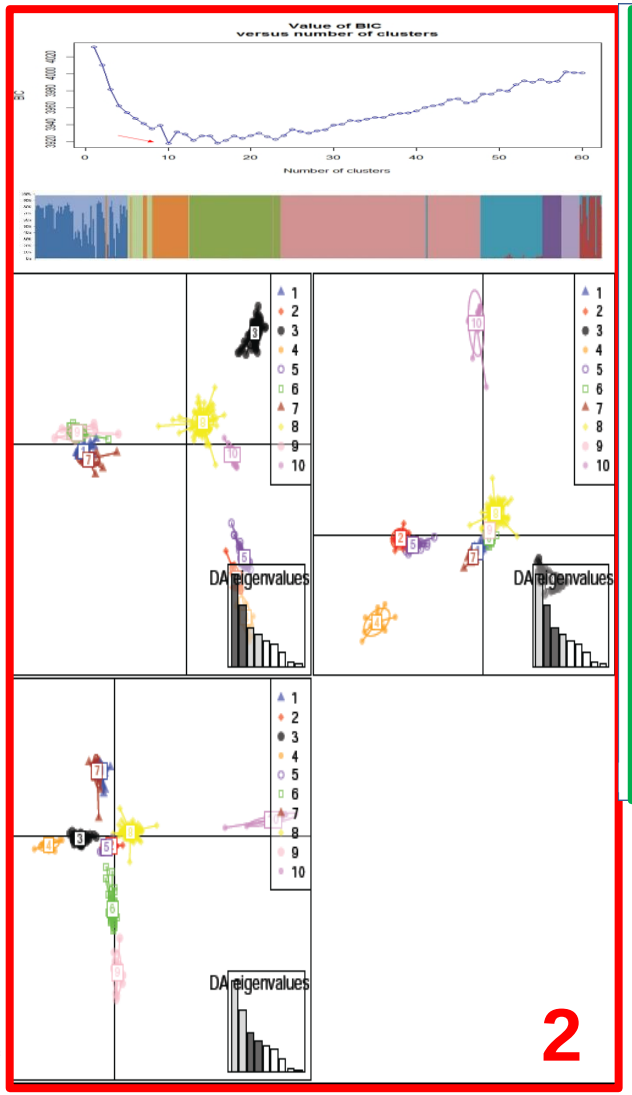
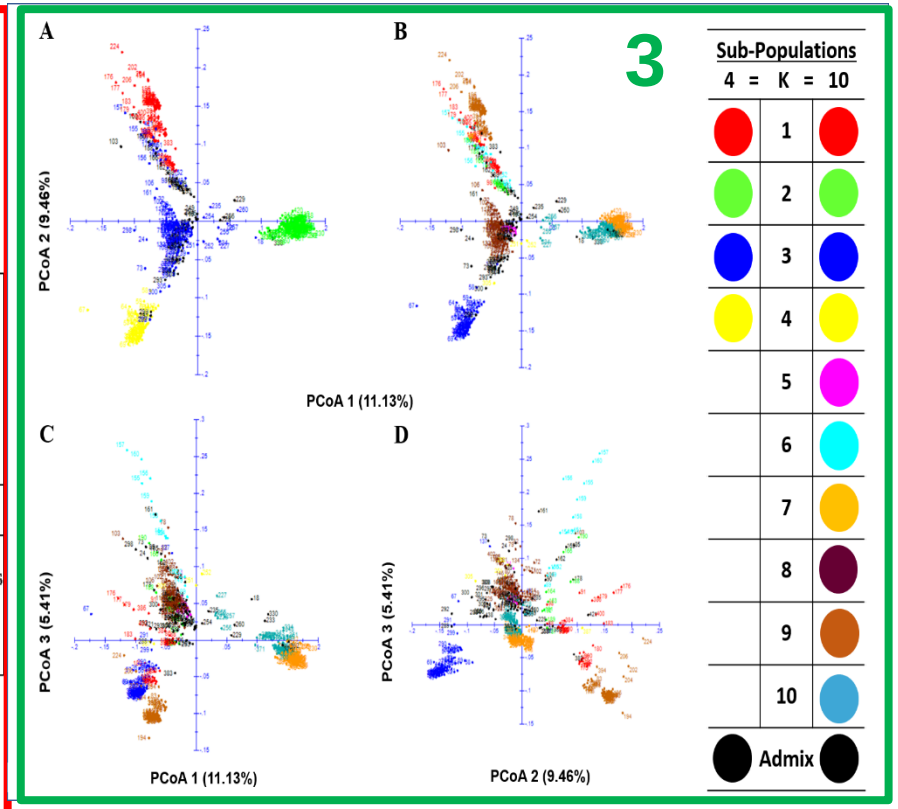
Case Study-IITA MIP: Genetic structure analysis







1 Sub-population by STRUCTURE analysis

2. Discriminant Analysis Principal Components (DAPC)

3. Principal Coordinate Analysis (PCoA)

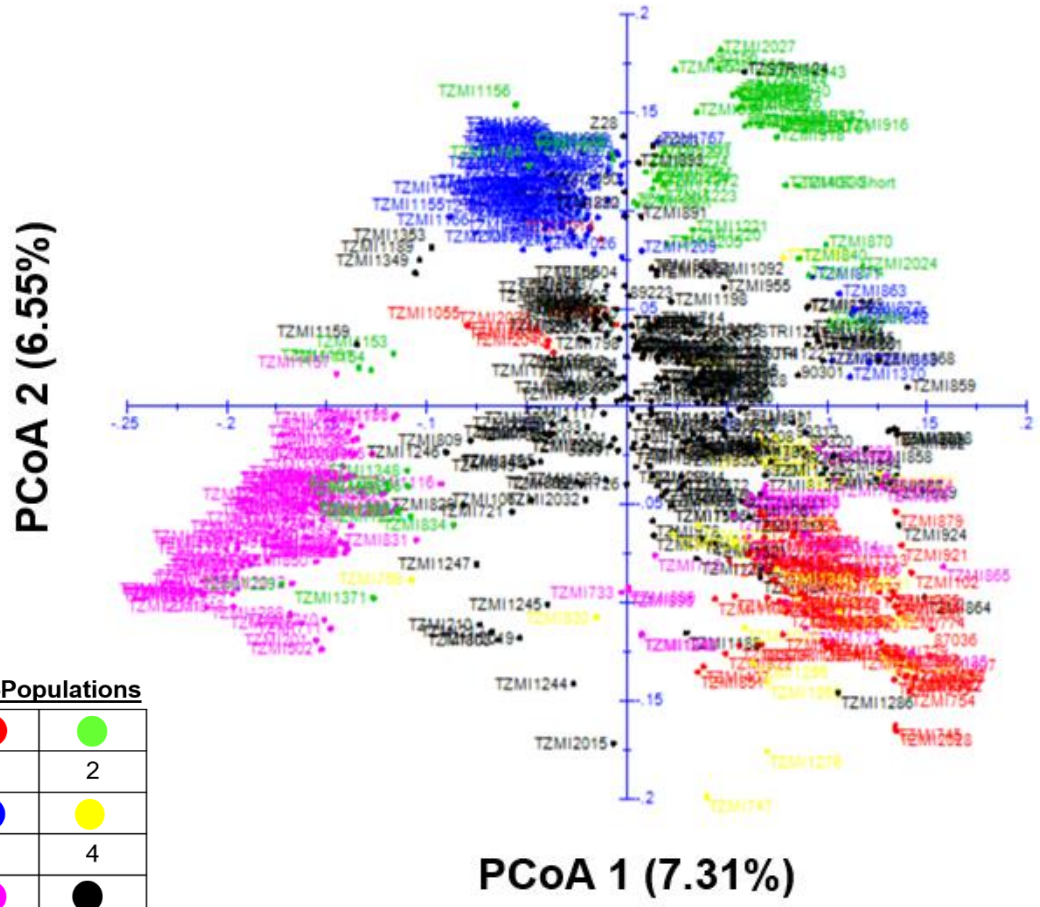
Case Study-IITA MIP: Genetic diversity & structure analysis

Genetic diversity and population structure of intermediate to late maturing inbred lines of IITA maize breeding program using SNP markers

- In this study, 623 intermediate to late maturing maize inbred lines generated from the IITA West Africa maize breeding program were used.
- A total of 9,425 DArT single nucleotide polymorphism (SNP) markers obtained by genotyping by sequencing (GBS) procedure.
- Multiple clustering methods were employed for better understanding the distribution of genetic diversity across the population.

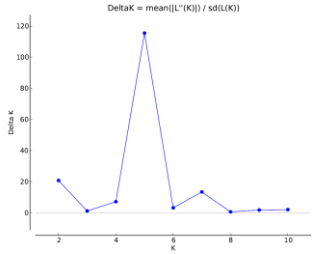
Case Study-IITA MIP: Genetic structure analysis

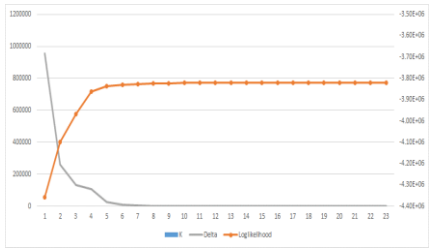
Intermediate to late maturing maize inbred lines

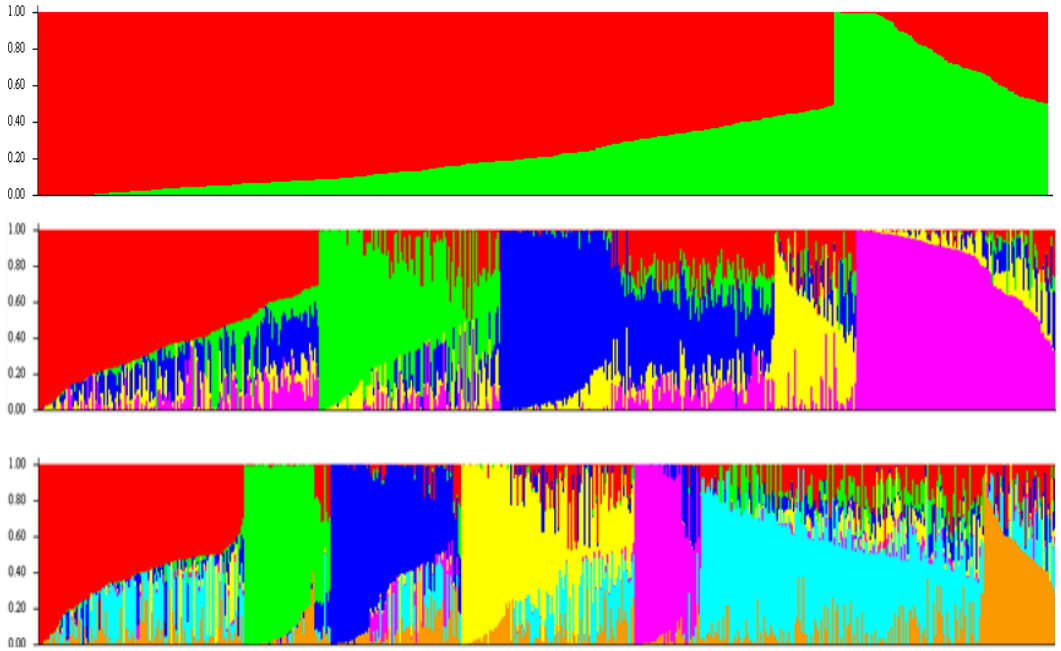


Sub-Populations

1	2
3	4
5	Admix







K=2

K=5

K=7

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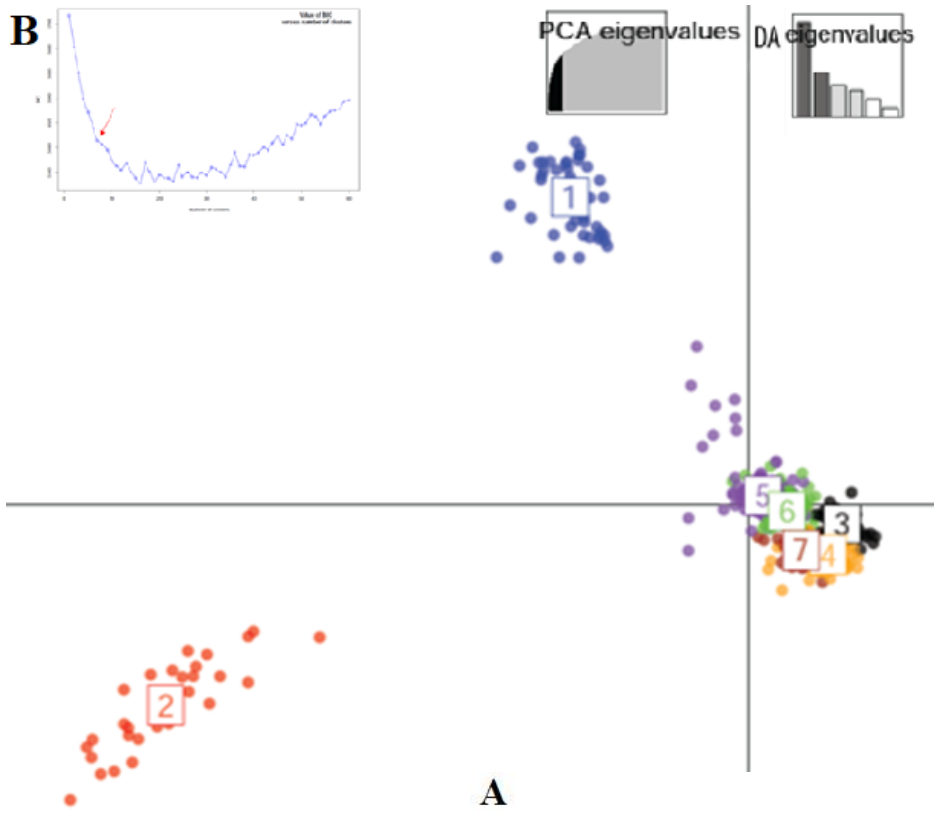
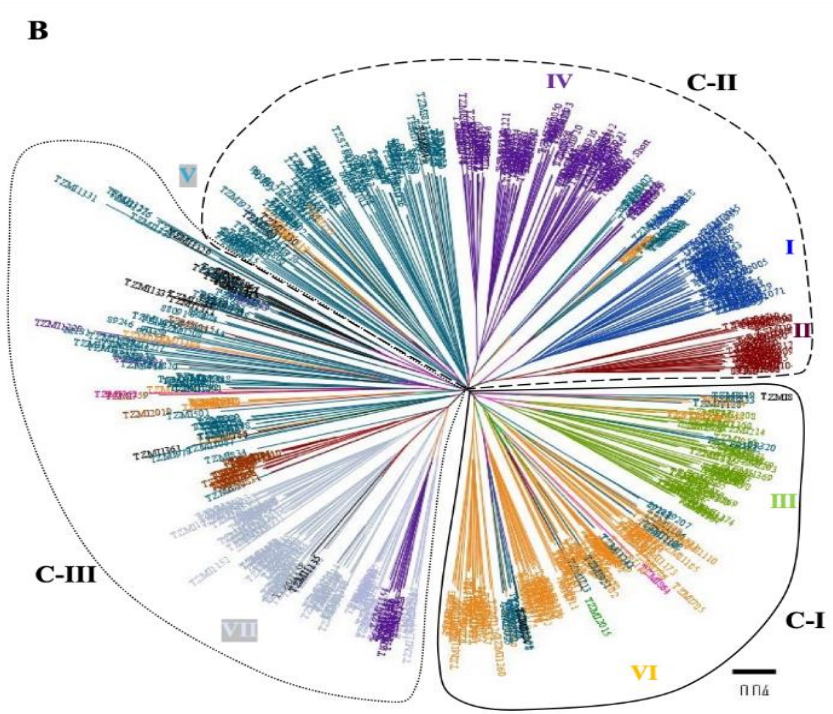
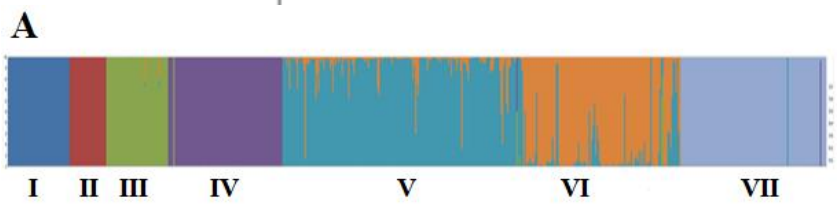
Case Study-IITA MIP: Genetic structure analysis

Intermediate to late maturing maize inbred lines







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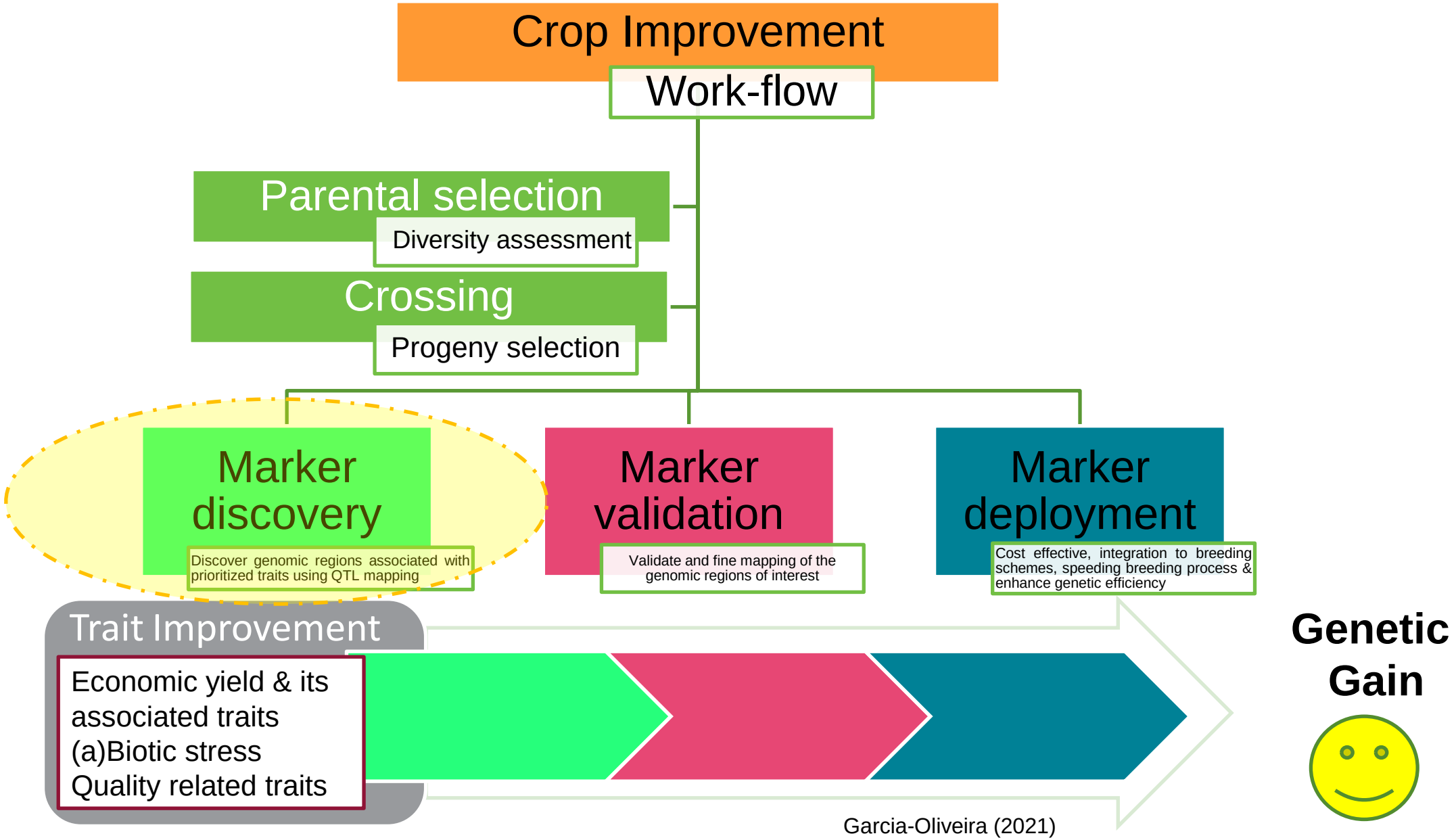
Conclusion:

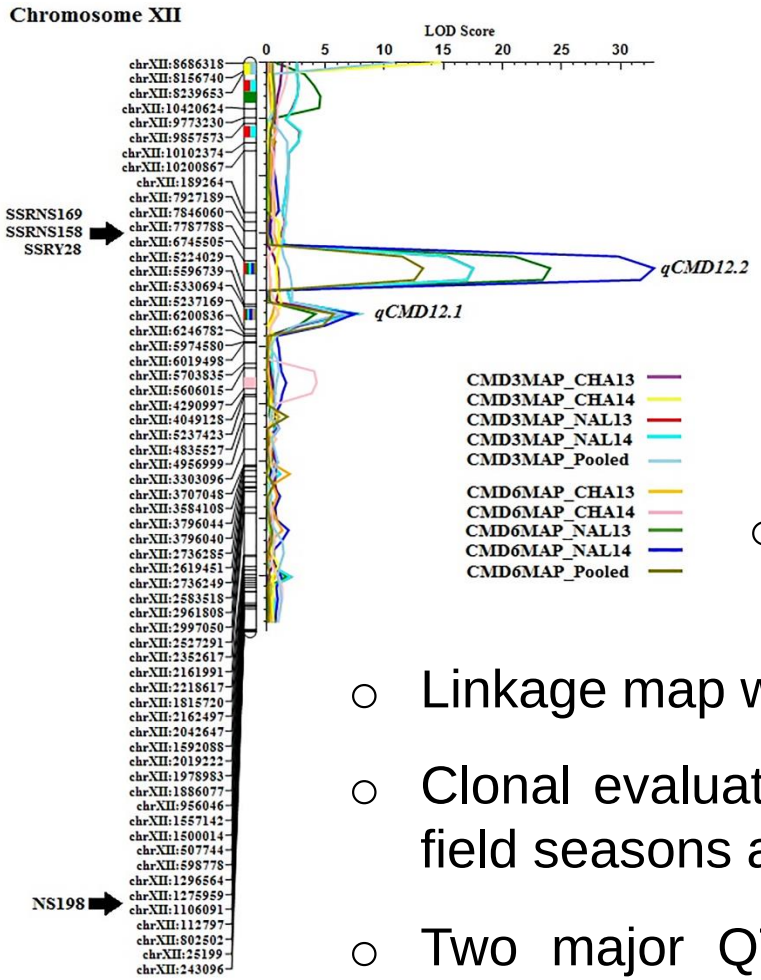
- The sub-populations/groups classification based on different complementary approaches using SNP markers data largely agree with pedigree information.
- Moreover, it also revealed the complexity of genetic pattern in tropical maize which indicates the mixed genetic constitution of the source germplasm.
- Molecular markers together with seed yield seems to be the best strategy to define heterotic patterns for future hybrid breeding practice and management of germplasm.

Molecular markers work-flow in crop improvement

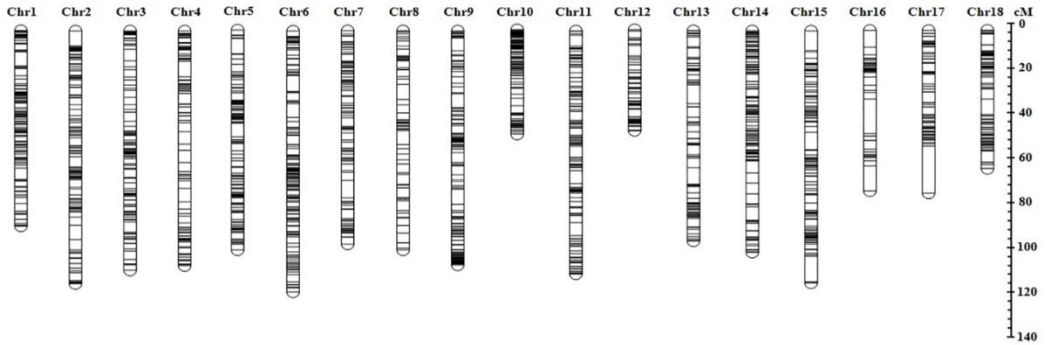


Garcia-Oliveira (2021)

Case Study-IITA Cassava: QTL mapping for cassava mosaic disease (CMD) resistance



Garcia-Oliveira et al. 2020 PlosOne



- A full-sib mapping population was derived from the genotypes AR40-6 and Albert.
- Linkage map was constructed with 2125 SNP markers using GBS approach.
- Clonal evaluation trials were conducted in alpha-lattice design for two consecutive field seasons at Chambezi and Naliendele, Tanzania.
- Two major QTL or multi-allelic variants influencing CMD resistance within the locality of the *CMD2* locus were identified.

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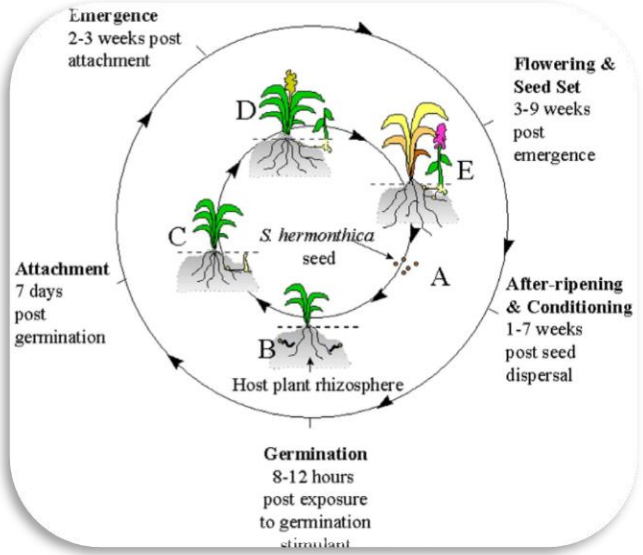
Case Study-IITA MIP: GWAS of *Striga* resistance in early maturing white tropical maize inbred lines

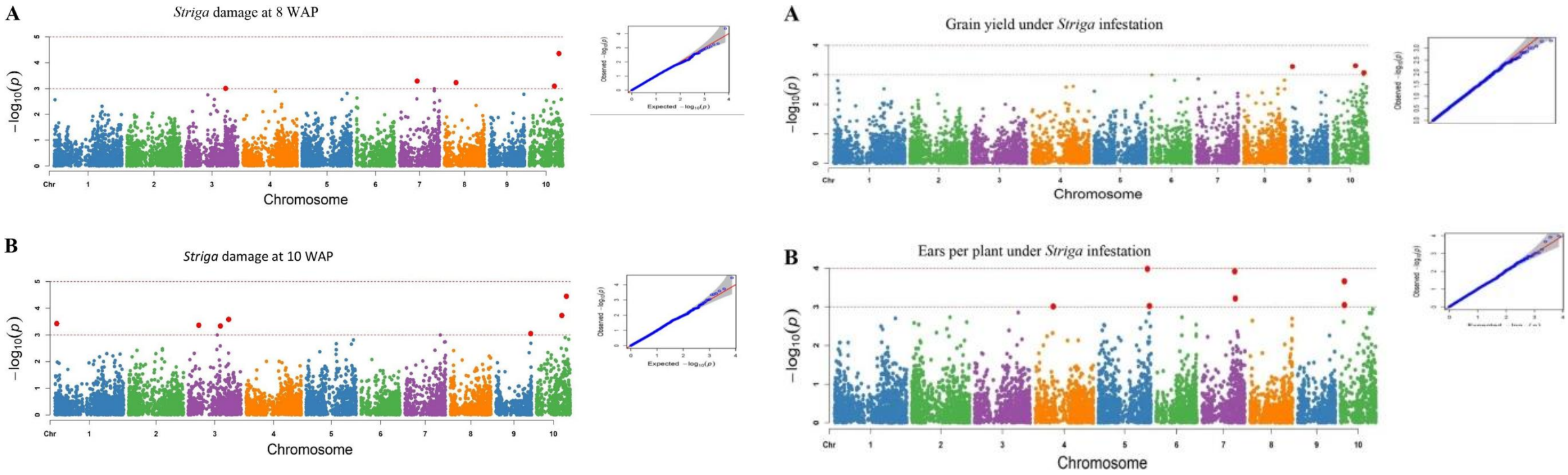
- *Striga hermonthica* (Benth.) parasitism militates against increased maize production and productivity in sub-Saharan Africa (SSA).
- We used a panel of 132 early-maturing maize inbreds were phenotyped for key agronomic traits under *Striga*-infested and *Striga*-free conditions.
- The inbred lines were genotyped using 47,440 DArTseq markers from which 7224 markers were retained for population structure analysis and genome-wide association study (GWAS).

Yacoubou et al. 2020. *Plant Breeding*

Adewale et al. 2021 *BMC Plant Biology*



Case Study-IITA MIP: GWAS of *Striga* resistance ….



Manhattan and Q-Q plots of the SNP-based associations mapping for Striga damage at 8 WAP and 10 WAP (left) and grain yield and ears per plant (right) under artificial Striga infestation

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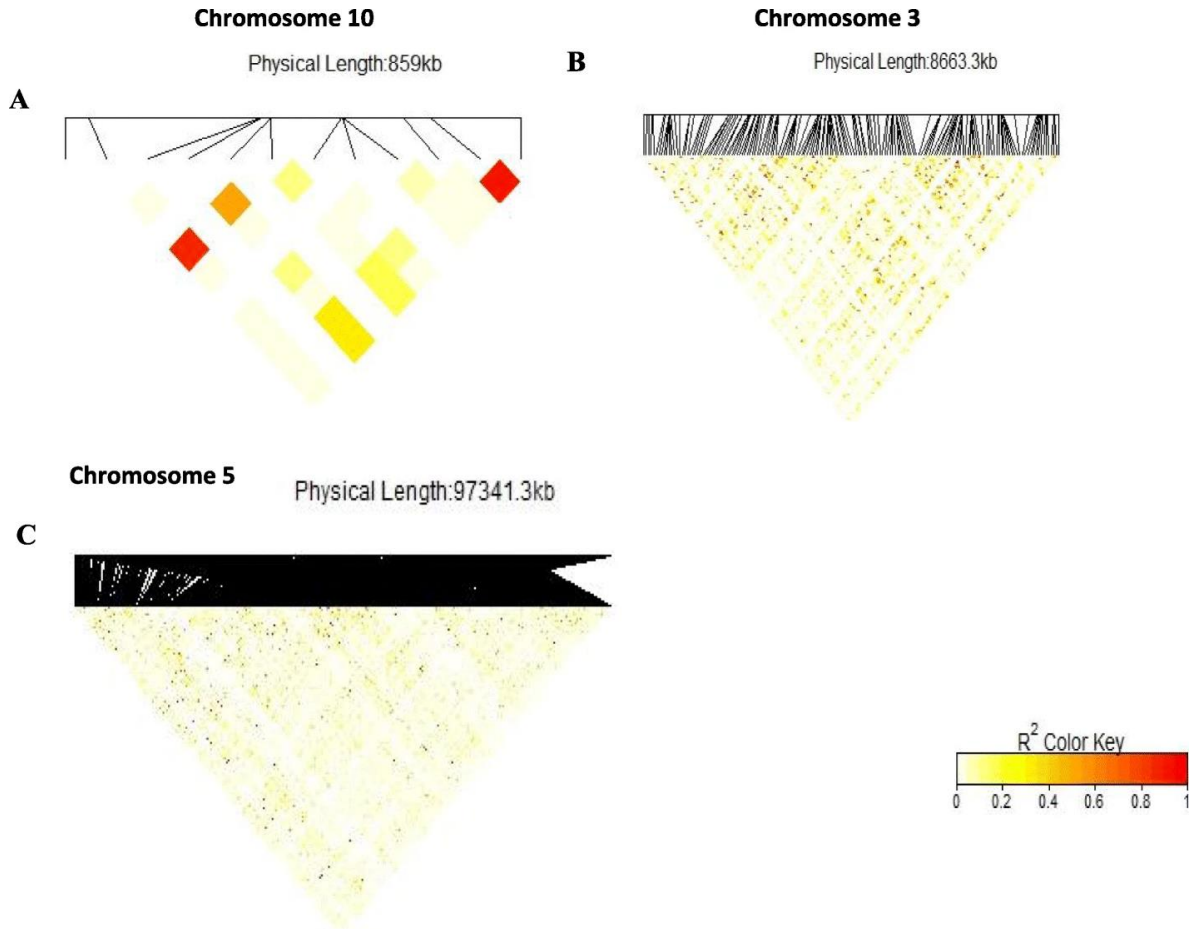
Case Study-IITA MIP: GWAS of *Striga* resistance ….



Local LD block surrounding (**a**) *amt5* gene on chromosome 10. (**b**) ereb13 gene on chromosome 3. (**c**) *lg2* gene on chromosome 3. The square lattice panel represents the extent of LD based on r^2. The R^2 color key indicates the degree of significant association

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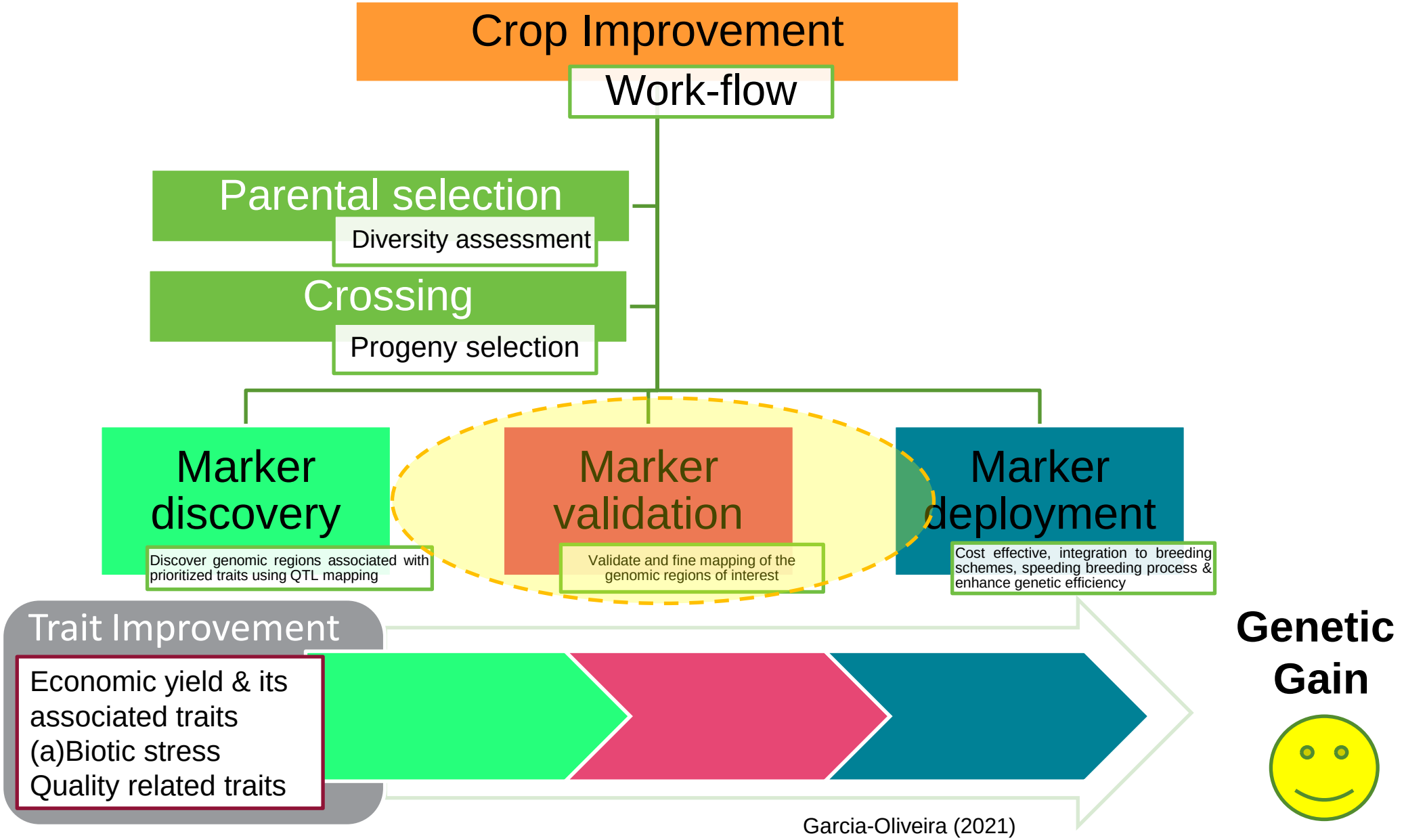
Case Study-IITA MIP: GWAS of *Striga* resistance ….

- Three markers physically located close to the putative genes GRMZM2G164743 (bin 10.05), GRMZM2G060216 (bin 3.06) and GRMZM2G103085 (bin 5.07) were detected.
- These markers were linked to grain yield, *Striga* damage at 8 and 10 WAP and number of ears per plant under *Striga* infestation, explaining 9 to 42% of the phenotypic variance.
- Furthermore, the S9_154,978,426 locus on chromosome 9 was found at 2.61 Mb close to the *ZmCCD1* gene known to be associated with the reduction of strigolactone production in the maize roots.
- Validation of the identified loci is in progress for marker-assisted selection (MAS) to accelerate genetic enhancement of maize for *Striga* resistance in the tropics, particularly in SSA

Molecular markers work-flow in crop improvement



Garcia-Oliveira (2021)

Case Study-IITA MIP: Carotenoid's variability in medium-late maturing maize inbred lines

Traits	Range		Mean (µg/g)	Std Dev
	Minimum	Maximum		
Lutein	0.58	20.87	5.36	4.50
Zeaxanthin	0.36	30.61	11.13	7.84
α-Carotene	0.00	3.28	0.49	0.66
β-Carotene	0.88	21.91	6.75	4.32
β-cryptoxanthin	0.08	10.47	3.72	2.72
Total Carotenoids	7.41	50.11	28.41	8.91
Total PVA Carotenoids	1.34	22.30	8.87	3.90

Note: Total Carotenoids represent sum of the values of Lutein, Zeaxanthin, α-carotene, β-carotene and β-cryptoxanthin whereas Total PVA Carotenoids represent sum of the values of β-carotene plus one-half each of α-carotene and β-cryptoxanthin

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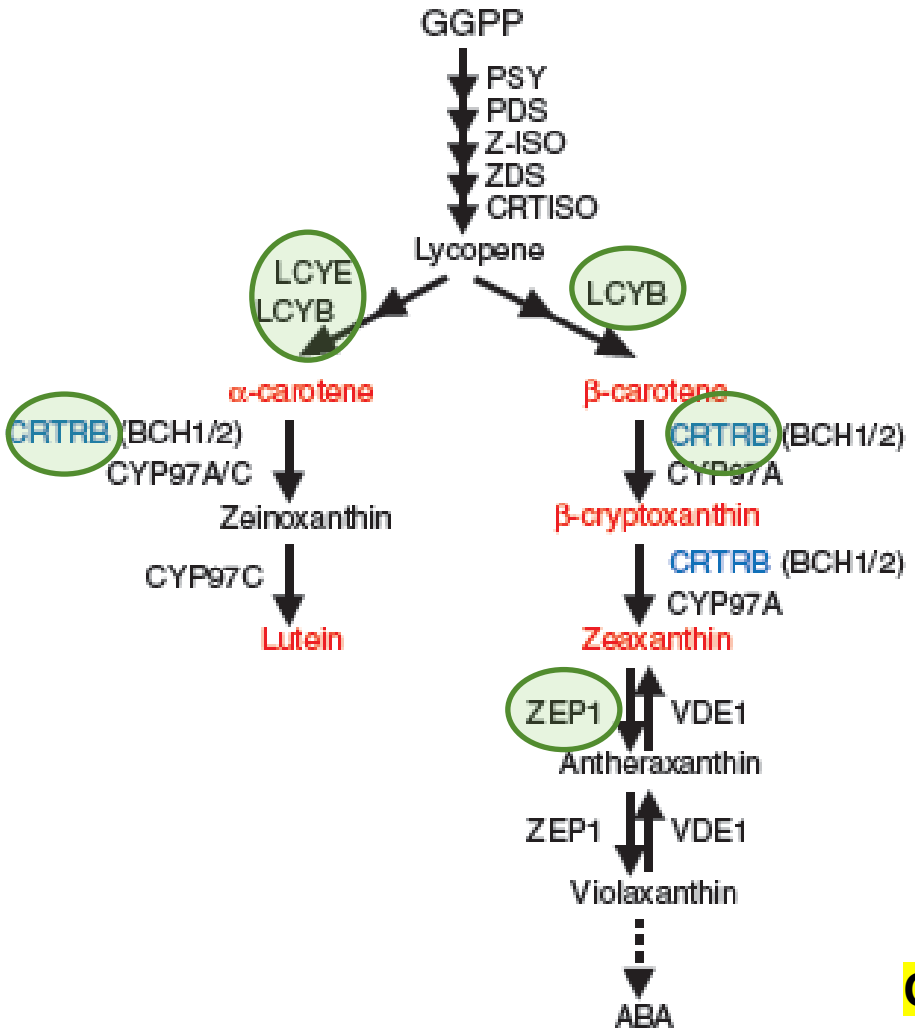
Case Study-IITA MIP: Markers validations for carotenoid's content in tropical maize inbred lines



- The marker, crtRB1-5'TE & crtRB1-3'TE had significant effect on PVA content in maize grain.
- New marker, Zep-SNP(801) showed significant association with α-carotene content.
- Increasing number of favourable alleles exhibited substantial effect on PVA content especially β-carotene.
- High PVA lines were present across the five sub-groups which were grouped by SSR markers

Gebremeskel et al. 2018 Journal of Cereal Science

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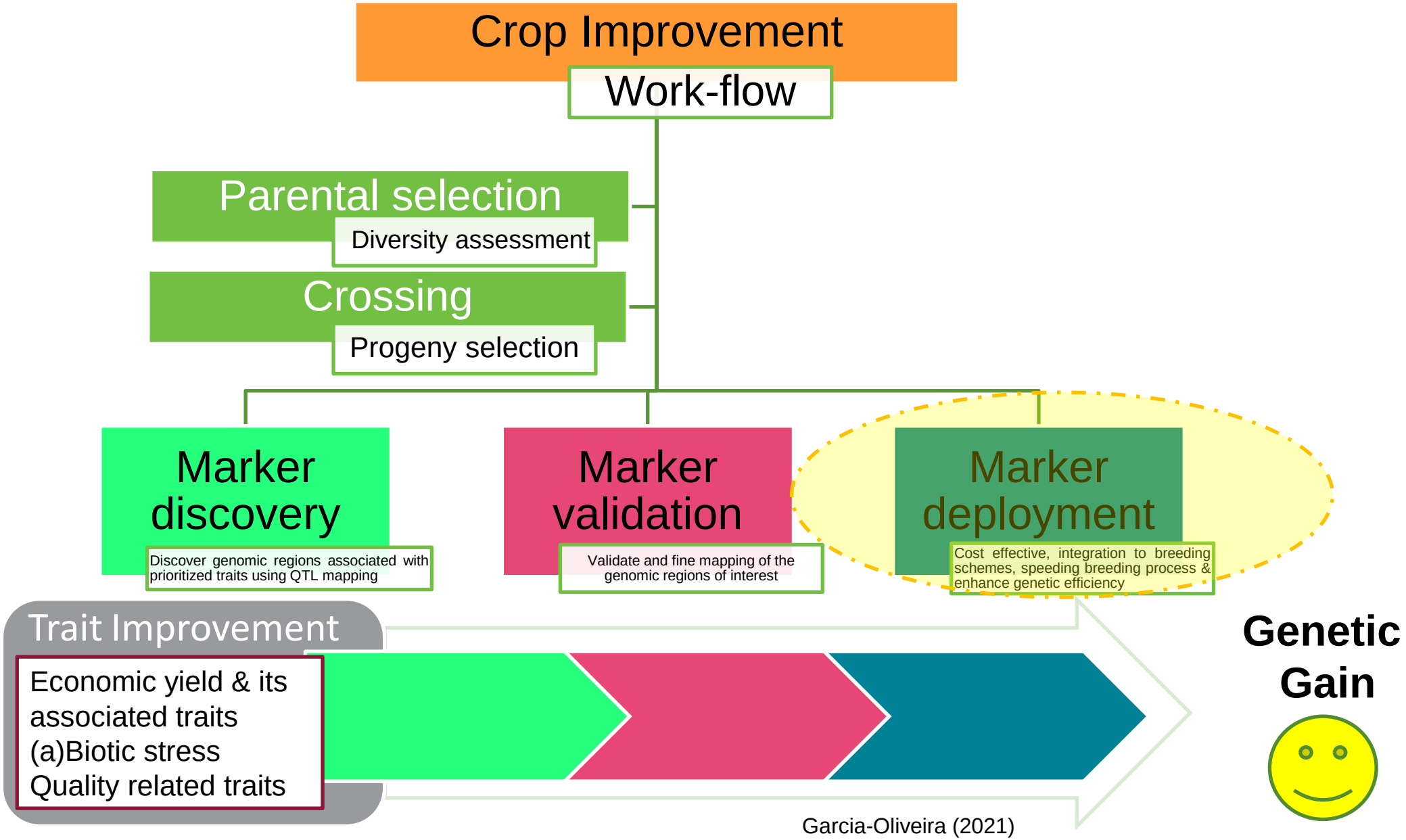
Molecular markers work-flow in crop improvement



Garcia-Oliveira (2021)

Case Study-IITA MIP: Marker-assisted recurrent selection (MARS) for PVA in OPV

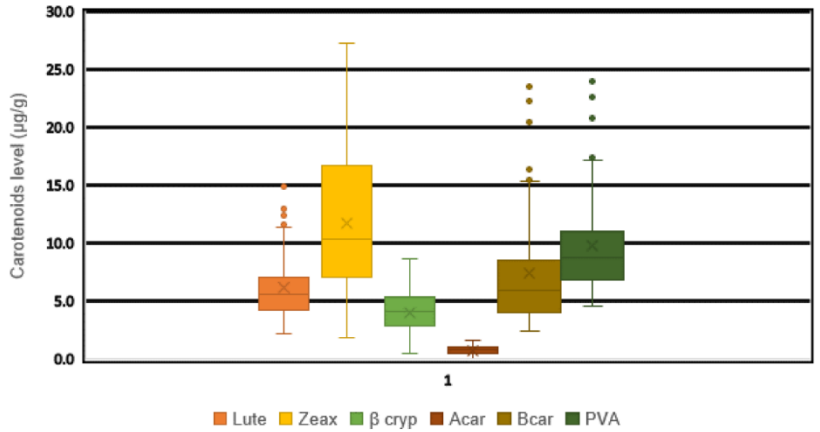


Figure 3. Distribution of the different carotenoids across the S4 inbred lines derived from HGA.

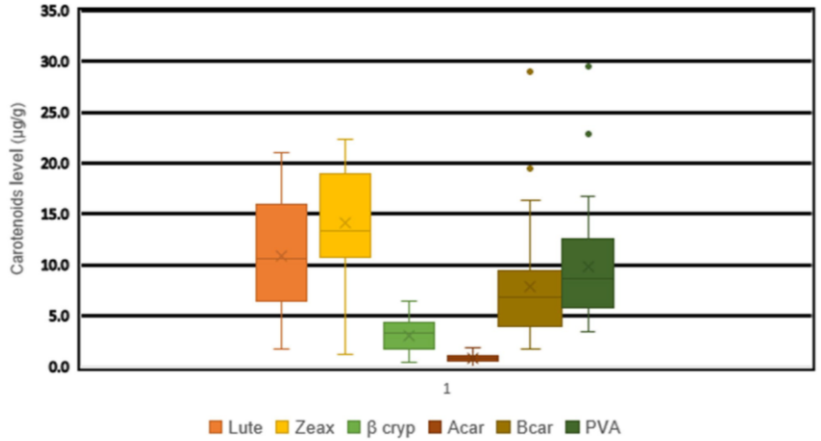


Figure 4. Distribution of the different carotenoids across the S4 inbred lines derived from HGB.

Cycles	# Samples [1]	Missing data (%)[2]	% Polymorphic loci[3]	# Effective alleles[4]	Observed hetrozygosity[5]	Expected hetrozygosity[6]	Fixation Index[7]
HGAC0	60	4.6	93	1.6 ± 0.026	0.33 ± 0.015	0.32 ± 0.014	0.01 ± 0.018
HGAC1	60	3.9	94	1.6 ± 0.027	0.29 ± 0.013	0.33 ± 0.013	0.11 ± 0.019
HGAC2	60	5.5	93	1.5 ± 0.027	0.24 ± 0.013	0.27 ± 0.013	0.13 ± 0.022
Mean	60	4.7	93	1.5 ± 0.016	0.29 ± 0.008	0.31 ± 0.008	0.08 ± 0.012

Cycles	# Samples[1]	Missing data (%)[2]	% Polymorphic loci[3]	# Effective alleles[4]	Observed hetrozygosity[5]	Expected hetrozygosity[6]	Fixation Index[7]
HGBC0	60	3.7	88.6	1.5 ± 0.027	0.25 ± 0.015	0.27 ± 0.014	0.081 ± 0.02
HGBC1	60	6.7	93.4	1.6 ± 0.027	0.3 ± 0.014	0.32 ± 0.013	0.054 ± 0.01
HGBC2	60	5.8	94.6	1.5 ± 0.026	0.26 ± 0.014	0.29 ± 0.013	0.083 ± 0.021
Mean	60	5.4	92.2	1.5 ± 0.016	0.27 ± 0.008	0.29 ± 0.008	0.07 ± 0.011

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Marker based enrichment of provitamin A content in two tropical maize synthetics

Dejene Kebede[2,3], Wende Mengesha[1*], Abebe Menkir[1], Ayodeji Abe[3], Ana Luisa Garcia-Oliveira[4] & Melaku Gedil[1]

CGIAR Excellence in Breeding Platform

IITA scientists develop multiple stress tolerant maize hybrids with high levels of Pro-Vitamin A

Vitamin A deficiency is a major health problem in sub-Saharan Africa (SSA). *Striga hermonthica* (Giant Witchweed) and drought are two major constraints to maize production in the sub-region.

Cobs of some Pro-Vitamin A maize hybrids.

The new PVA hybrids out-yielded the commercial PVA top-cross hybrid check; TZEE-Y Pop STR C5 x TZEEI 58 had a yield of 1205 across stress environments and 2611 kg/ha across non-stress environments. These interesting results

open a great opportunity for breeding and releasing PVA maize hybrids with 50 percent higher levels of PVA than the target of 15 µg/g set by the HarvestPlus Challenge Program. Commercialization of these hybrids should contribute to food security and improved nutrition in West and Central Africa.

The collaborators on this research are Principal Scientist/Maize Breeder and Geneticist, Badu-Apraku; Research Administrative Manager, Abidemi O. Talabi; and Molecular Geneticists including Ana Luísa Garcia-Oliveira and Melaku Gedil.

CGIAR Excellence in Breeding Platform

Why low level of integration of molecular markers in breeding programs (BPs) ?

- **Establishment of genotyping facilities with all breeding programs is costly.**
- **High cost of genotyping due to underutilization of genotyping facilities.**
- Lack of comprehensive understanding of the potential of molecular markers in a breeding programs.
- Issues related qualified human resources availability/cost effective.
- Fragmented information: Unavailability of single platform
- Lack of physical infrastructure in developing countries such as uninterrupted power supply, marker data analysis tools etc.
- Inability to deliver high-quality genotypic data within short period.

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Cost effective genotyping: Shared genotyping services

- Genotyping as an integrative service
  - Genotyping as part of routine breeding operation

- Regional support- One CGIAR
  - Multi-crops support
  - Planning drives costs down where genotyping should be part of routine breeding actions

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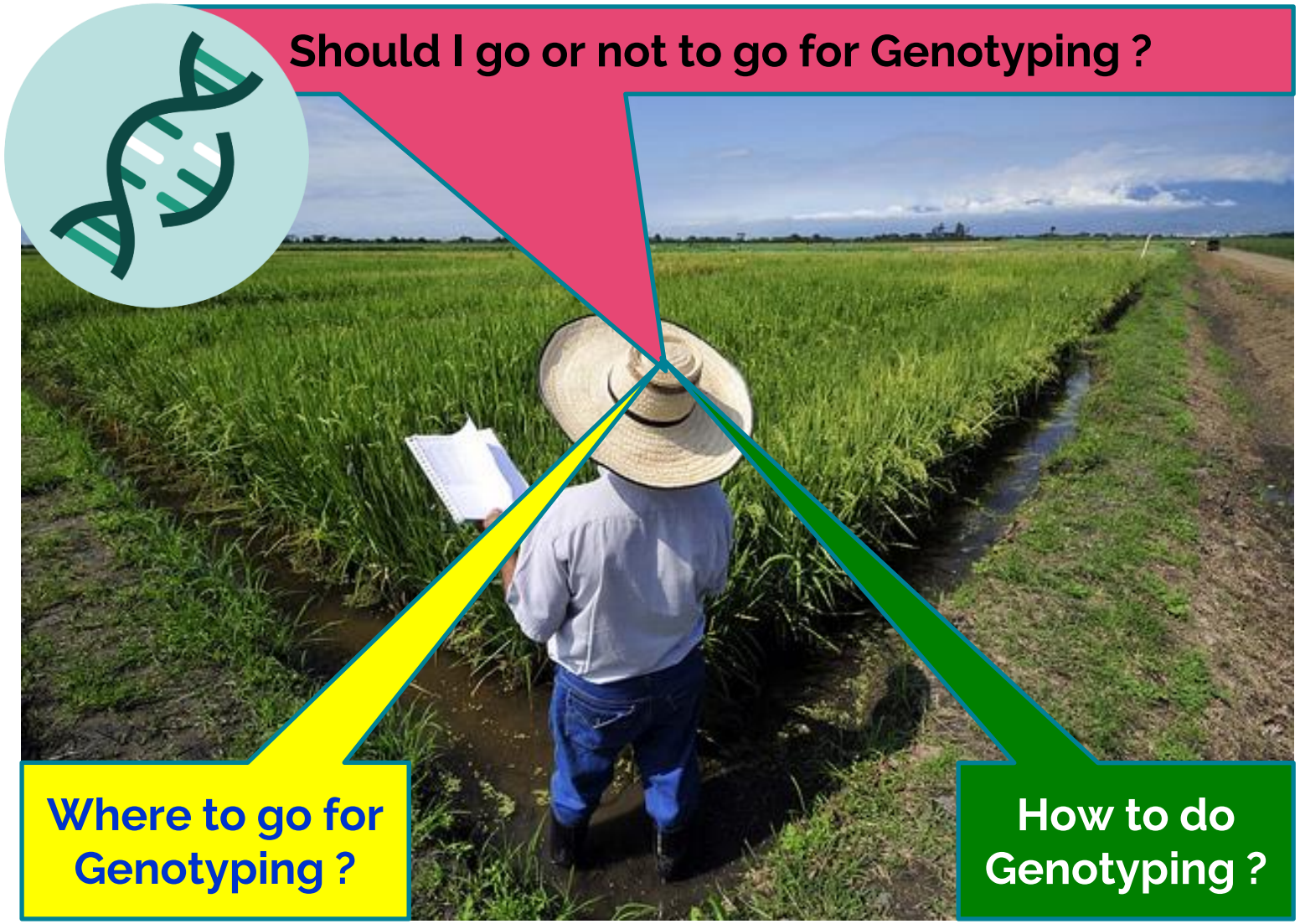
Integration of molecular markers: Contribute for a change in mind set of breeders



CGIAR Excellence in Breeding Platform

Efficient & effective application of genomic technology in public sector breeding programs

Do or not to do: Genotyping ?

Assist breeder with assessment of breeding program, if and where genotyping is appropriate

- ✓ At which breeding stage do you integrate genotyping?
- ✓ What breeding materials/populations do you have?
- ✓ What's your purpose for genotyping?
- ✓ **Are markers available for the trait of interest?**
- ✓ Which genotyping platform is suitable?
- ✓ What sample size do you have?
- ✓ Budget

Where to do: Genotyping ?

Search for lowest cost solutions for broader applications

How to do: Genotyping ?

Mediate genotyping process between source and end service

CGIAR Excellence in Breeding Platform

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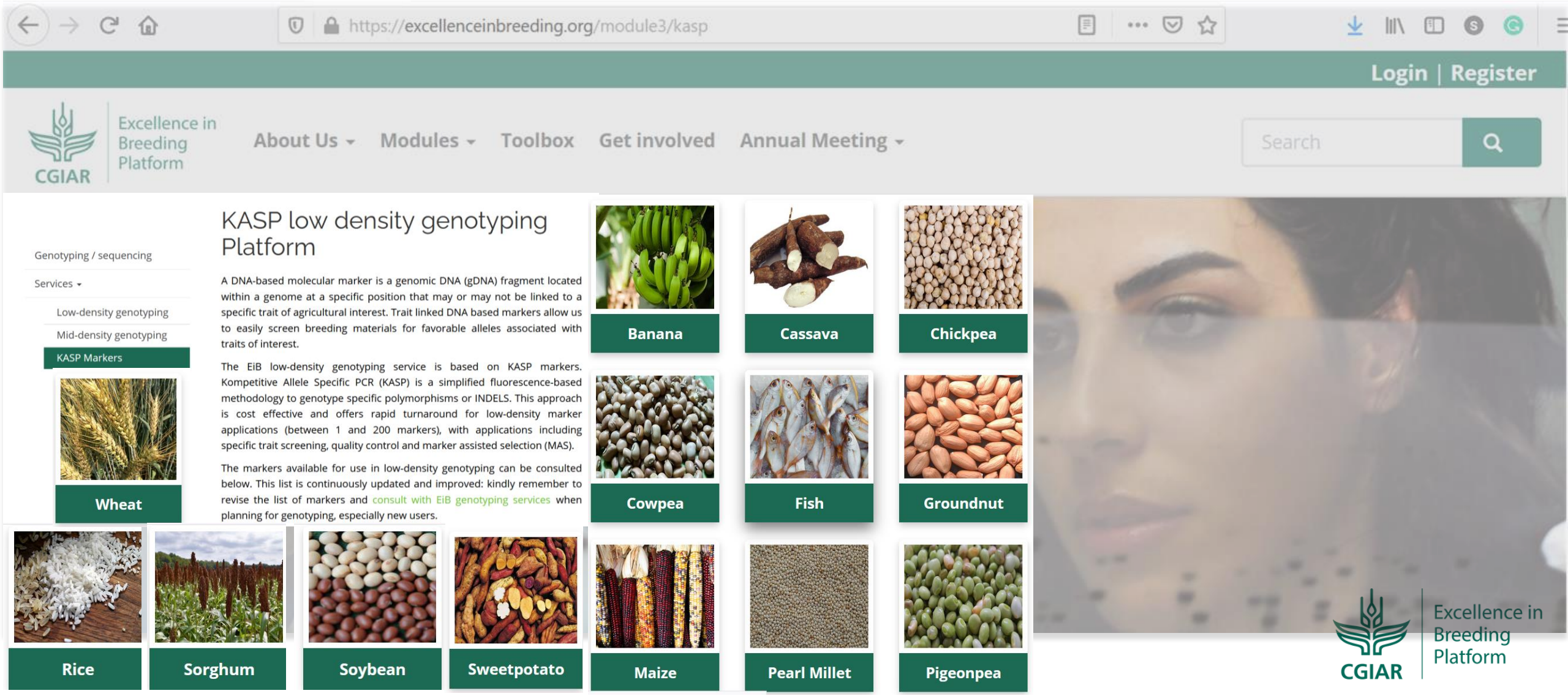
Value for public sector BPs in Developing countries



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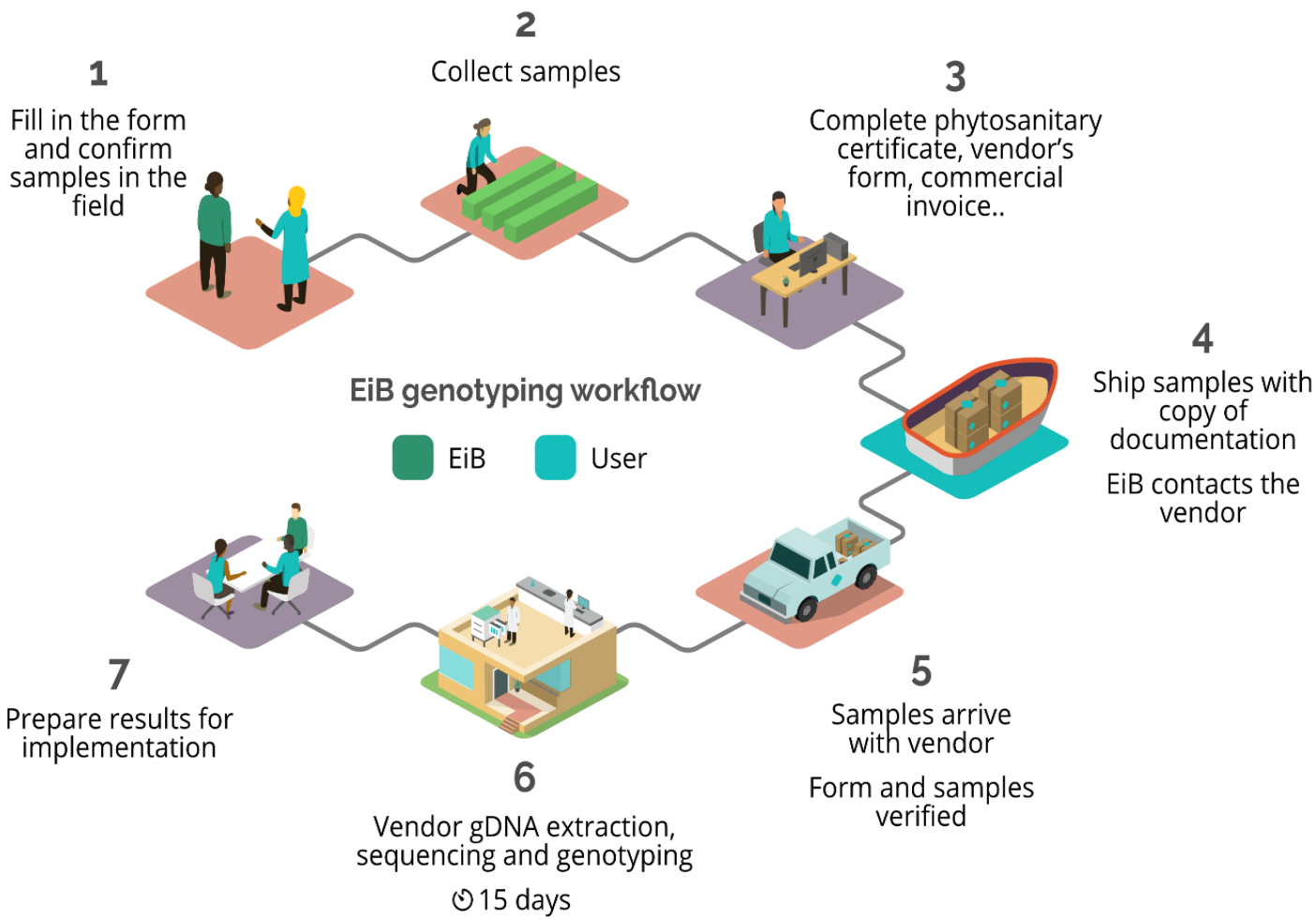
Molecular markers automation:



Source: Ana Luisa Garcia-Oliveira; Sam Storr

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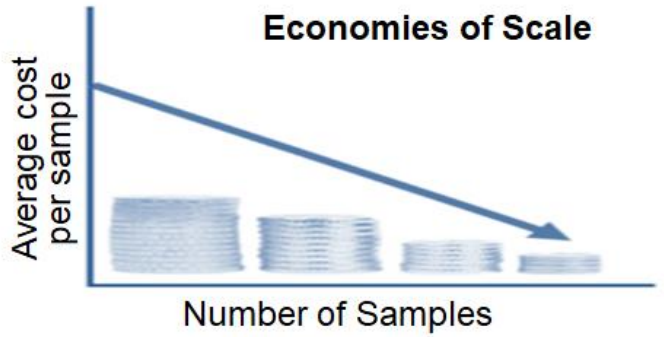
Genotyping & Sequencing – Enabling tools

- Develop capacity for genotype data application among breeding teams.
- Ensure delivery of high-quality genotypic data that can be readily interpreted and utilized.
- Assist members to implement forward marker selection, genomic selection and appropriate quality assurance in breeding programs.
- Identify & implement more efficient genotyping workflow to drive the cost of genotyping down.
- Develop schedule sample flow with genotyping laboratories and optimize the delivery of data back to the breeding programs



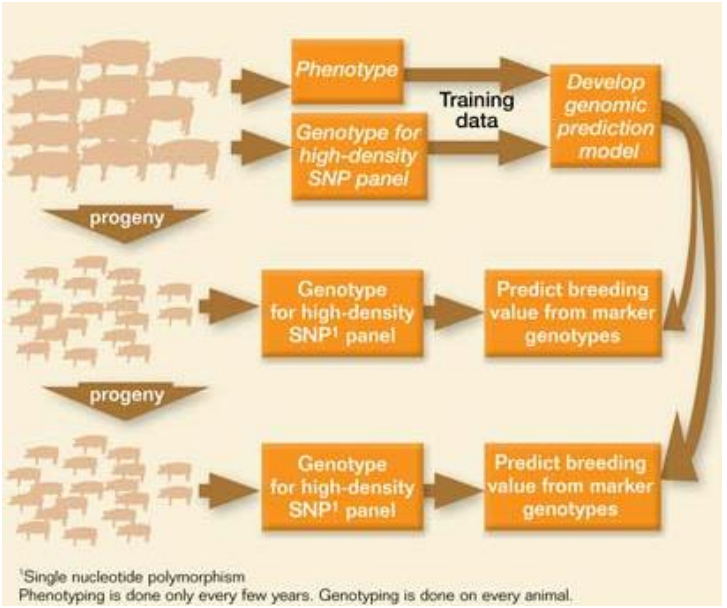
Moving forward

Marker availability

Genomic Selection

Use of mid density marker panels

e.g.



Rothschild et al. (2010)

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Mid-density genotyping:

Mid-density genotyping service

Services

Genotyping / sequencing tools and services

Low-cost access to world class genotyping services for CGIAR and partner breeding centers working on the majority of CGIAR mandate crops

The EiB mid-density genotyping service is a DArTAg genotyping method primarily suited to genomic selection applications, but that can also be used for diversity studies, material fingerprinting or background recovery in marker assisted selection (MAS) to complement low-density genotyping.

The service is targeted at CGIAR and NARS breeding institutions, aggregating demand across institutions to offer genotyping at a cost of US $10-11 per sample, and a turnaround time of 10-15 days.

Mid-density genotyping service

Contact:
CIMMYT-EiB-Genotyping@CGIAR.org

Crop / status	Panel name	Vendor	Marker density	Status
Rice	1K RiCA (v4)	Agriplex/ DArT	1K	Implemented
Cowpea	Cowpea DArTag EiB (1.0)	DArT	2.6K	Implemented
Potato	Potato DArTag EiB (1.0)	DArT	2.1K	Implemented
Wheat	Wheat DArTag EiB 2.4K (1.0)	DArT	2.4K	Implemented
	Wheat DArTag EiB 3.9K (2.0)	DArT	3.9K	Validation
Maize	Maize DArTag EiB (2.0)	DArT	3.5K	Validation
Sorghum	Sorghum DArTag EiB (1.0)	DArT	3.5K	Design
Pigeonpea	Pigeonpea DArTag EiB (1.0)	DArT	2K	Design
Groundnut	Groundnut DArTag EiB (1.0)	DArT	2.5K	Design
Finger Millet	TBA	DArT	2K	Planning
Cassava	TBA	DArT	3-4K	Planning

Refer : https://excellenceinbreeding.org/toolbox/services/wheat-mid-density-genotyping-services

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'The integration of new technologies into public plant breeding programs can make a powerful step change in agricultural productivity when aligned with principles of quantitative and Mendelian genetics."

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For questions & inquires

- a.oliveira@cgiar.org

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