AfricaYam II (2021-2022) Progress Report

IBRC   Ryohei Terauchi   17 Sept. 2022

2.1.1.1. Identify QTLs controlling key traits (tuber quality traits, YMV, YAD) using mapping population as well as GWAS panel

2.1.2.1. Elucidation of molecular mechanisms of flower sex expression in *D. rotundata*

2.1.2.2. Conduct RNA sequencing to complete gene models of Guinea yam and to understand gene regulatory network underlining important traits (flower sex, and plant growth habit, defense against pathogens)

2.1.2.3. Exploration of genomics of Guinea yam wild relatives for identification of useful alleles for crop improvement (Guinea yam Pan-Genome)
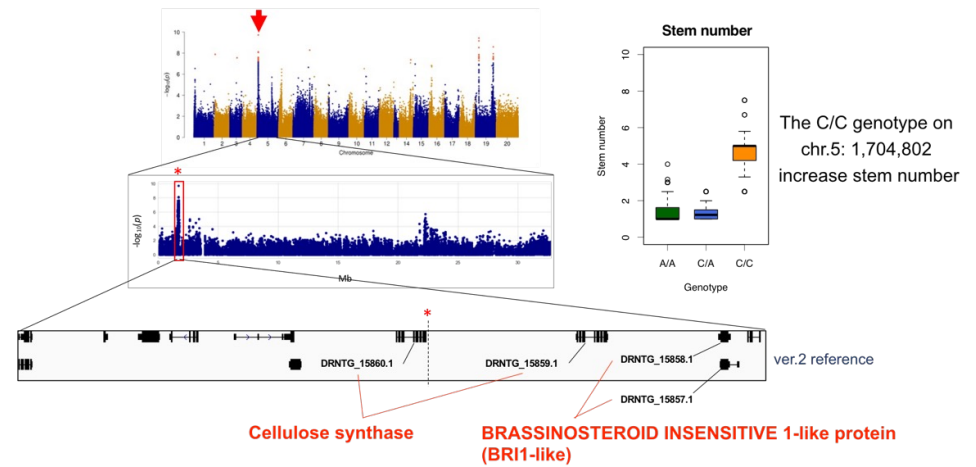
2.2.1.1. Develop a genomic prediction pipelines using state of the art machine learning approaches

Tuber color
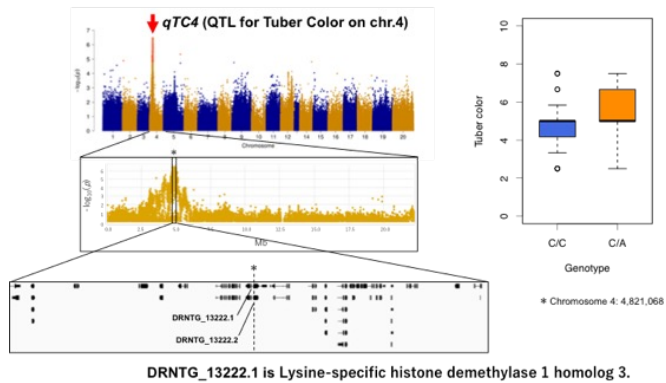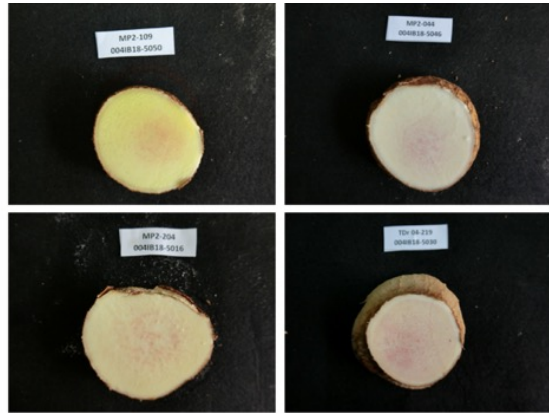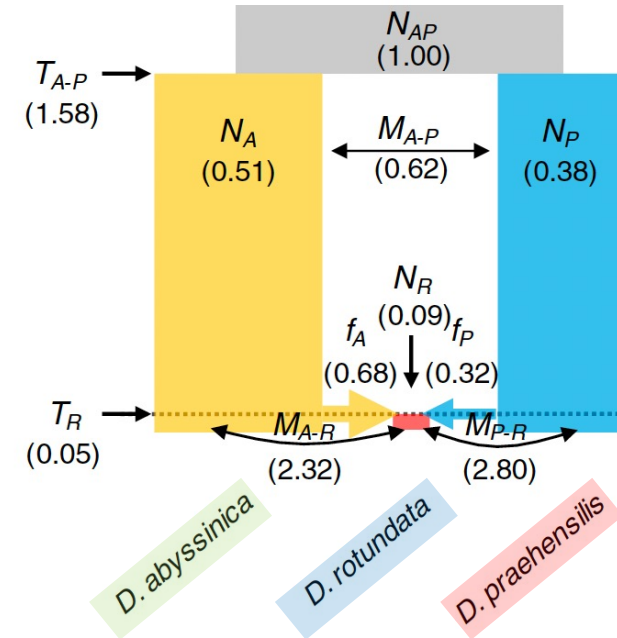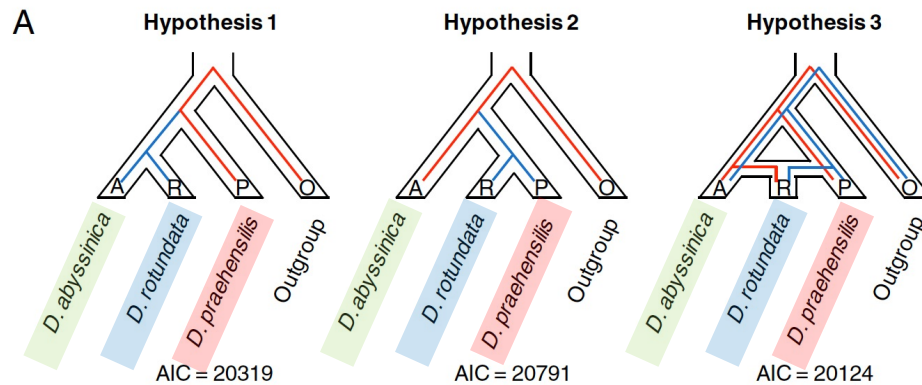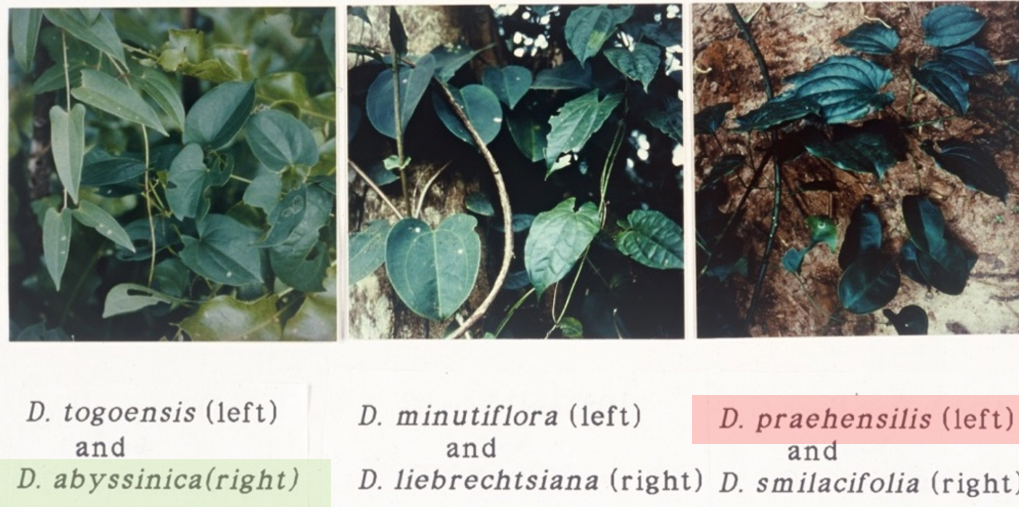

MP2 F1 mapping population (*n* = 156)

Stem number

GWAS of stem number (*n* = 333)

Genome analyses reveal the hybrid origin of the staple crop white Guinea yam (*Dioscorea rotundata*)

Yu Sugihara[a], Kwabena Darkwa[b], Hiroki Yaegashi[c], Satoshi Natsume[c], Motoki Shimizu[c], Akira Abe[c], Akiko Hirabuchi[c], Kazue Ito[c], Kaori Oikawa[c], Muluneh Tamiru-Oli[c,d], Atsushi Ohta[a], Ryo Matsumoto[b], Paterne Agre[b], David De Koeyer[b,e], Babil Pachakkil[f,g], Shinsuke Yamanaka[f], Satoru Muranaka[f], Hiroko Takagi[f], Ben White[h], Robert Asiedu[b], Hideki Innan[i], Asrat Asfaw[b,1], Patrick Adebola[b,1], and Ryohei Terauchi[a,c,1]

Sugihara et al. 2020 PNAS

D. togoensis (left) and *D. abyssinica* (right)

D. minutiflora (left) and *D. liebrechtsiana* (right)

D. praehensilis (left) and *D. smilacifolia* (right)

A

Hypothesis 1

A R P O

D. abyssinica *D. rotundata* *D. praehensilis* Outgroup

AIC = 20319

Hypothesis 2

A R P O

D. abyssinica *D. rotundata* *D. praehensilis* Outgroup

AIC = 20791

Hypothesis 3

A R P O

D. abyssinica *D. rotundata* *D. praehensilis* Outgroup

AIC = 20124

N_{AP} (1.00)

T_{A-P} (1.58)

N_A (0.51)

M_{A-P} (0.62)

N_P (0.38)

N_R (0.09)

f_A (0.68) f_P (0.32)

T_R (0.05)

M_{A-R} (2.32)

M_{P-R} (2.80)

D. abyssinica

D. rotundata

D. praehensilis
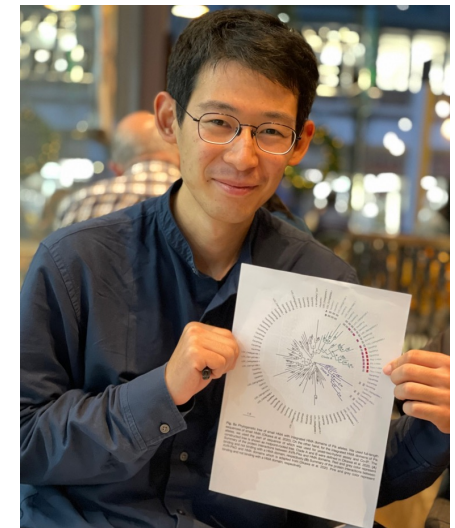
2.1.2.1. Elucidation of molecular mechanisms of flower sex expression in *D. rotundata*

Aoi Kudoh

Yu Sugihara

Dioecy of *Dioscorea*

Population Genomics of Yams: Evolution and Domestication of *Dioscorea* Species

Yu Sugihara, Aoi Kudoh, Muluneh Tamiru Oli, Hiroki Takagi,
Satoshi Natsume, Motoki Shimizu, Akira Abe, Robert Asiedu, Asrat Asfaw,
Patrick Adebola, and Ryohei Terauchi

Table 2 Reported sex-determination systems in *Dioscorea*

Species	Sex-determination system	Methods	Reference
D. alata L.	XY	QTL detection	Cormier et al. (2019a)
D. bulbifera L.	XY	Cytological observations	Ramachandran (1962)
D. deltoidea Wall.	ZW	Cytological observations	Bhat and Bindroo (1980)
D. discolor	[a]	Cytological observations	Smith (1937)
D. fargesii Franch.	[a]	Cytological observations	Smith (1937)
D. gracillima Miq.	XY	Cytological observations	Nakajima (1937)
D. japonica Thunb.	XY	Cytological observations	Nakajima (1942)
D. macroura Harms.	[a]	Cytological observations	Smith (1937)
D. pentaphylla L.	XY	Cytological observations	Ramachandran (1962)
D. reticulata C. Gay	XO	Cytological observations	Smith (1937)
D. rotundata	ZW	QTL detection	Tamiru et al. (2017)
D. sinuata Vell.	XO	Cytological observations	Meurman (1925)
D. spinosa Roxb.	XY	Cytological observations	Ramachandran (1962)
D. tokoro Makino	XY	AFLP analysis	Terauchi and Kahl (1999)
D. tomentosa Koenig	XY	Cytological observations	Ramachandran (1962)

[a]Heterogametic sex

Dioecy in *Dioscorea*
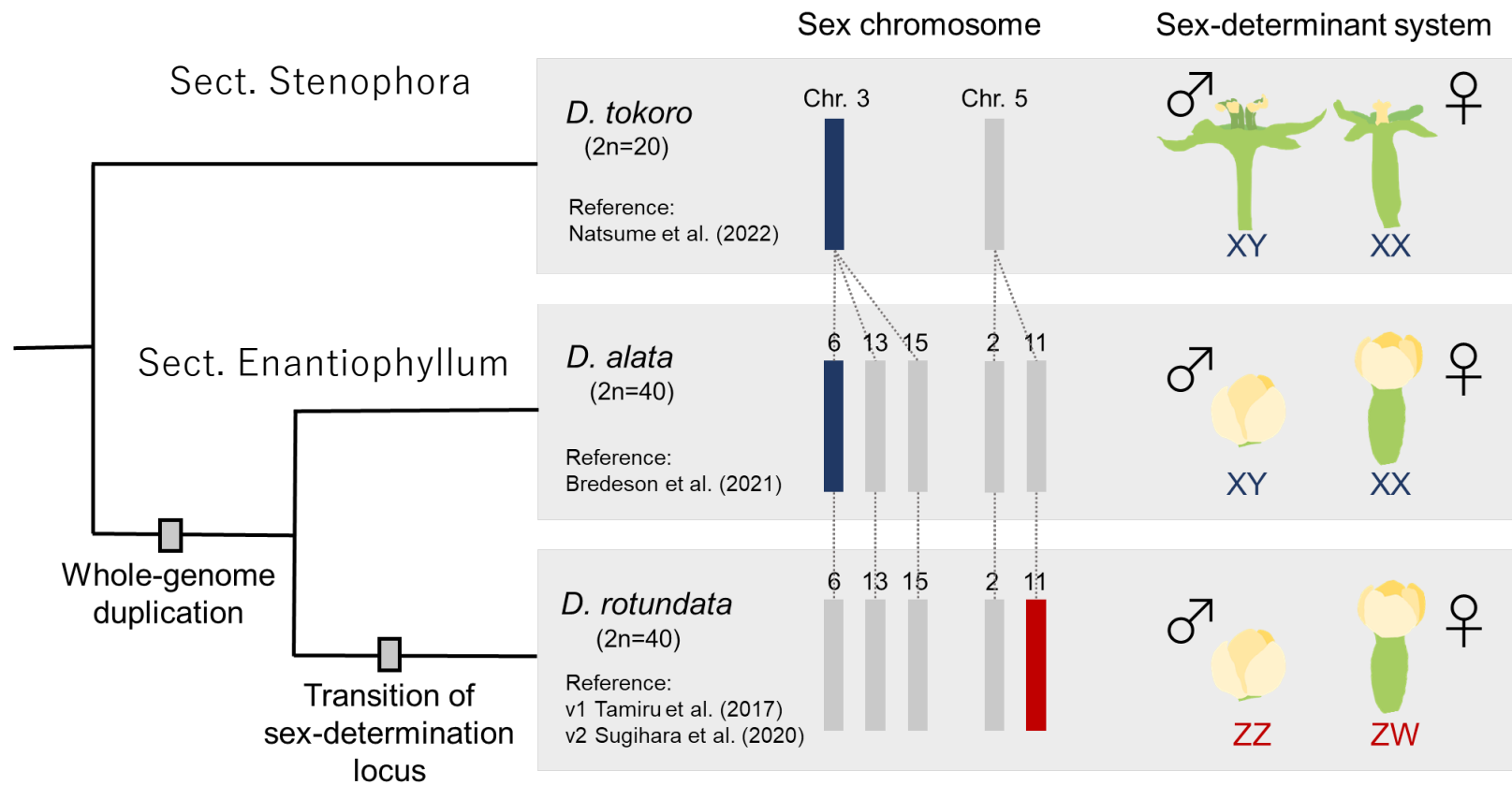
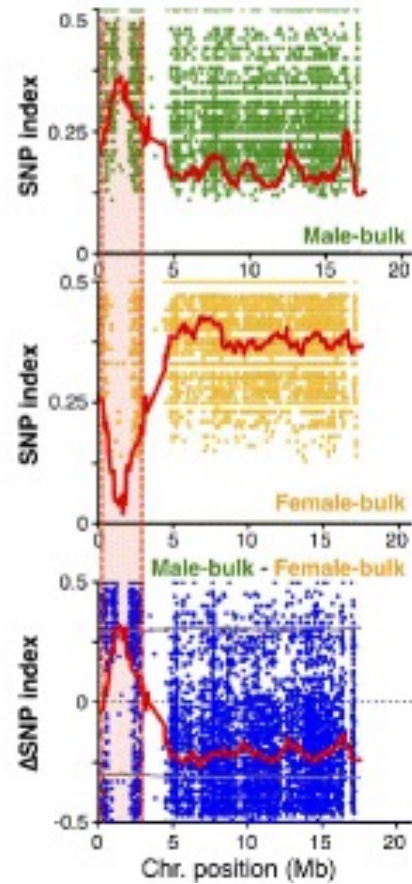# Variable sex expression in *Dioscorea rotundata*

Female **ZW**

Male **ZZ**

(Photos by JIRCAS)

b chromosome 11

(Tamiru et al. 2017)
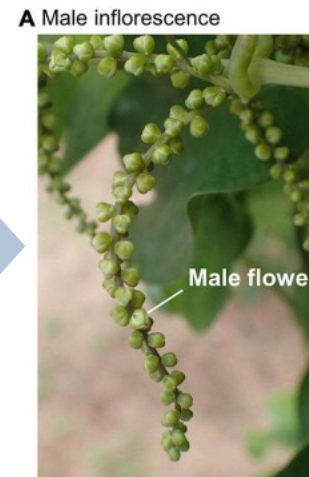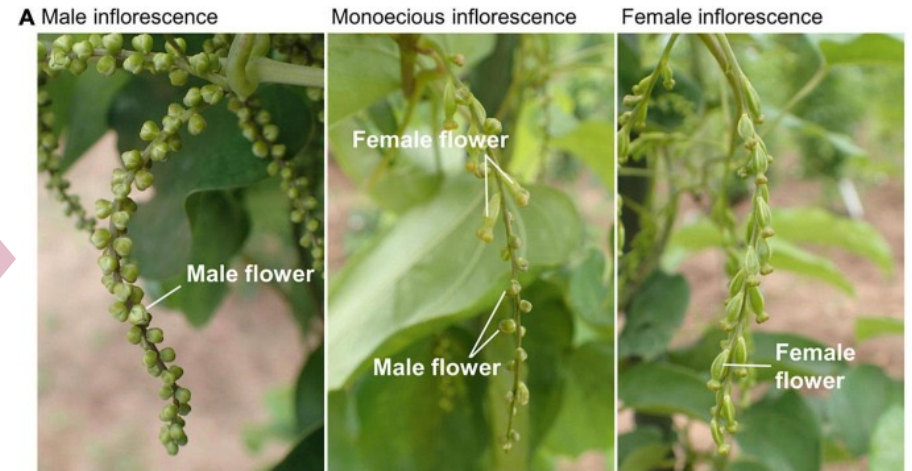
However, Female (*ZW*) shows variability of sex phenotypes.

(Iseki et al. 2022)

Overview of the hypothesis and the experimental approach

Male **ZZ**

A Male inflorescence

Male flower

Female **ZW**

Female inflorescence

Female flower

Monoecious inflorescence

Female flower

Male flower

Male inflorescence

Male flower

(Figure2 in Iseki et al. 2022)

Z
Z

Z
W

Gene or miRNAs for Female sex-determinant

Gene for Male sex-determinant

Gene or miRNAs for Female sex-determinant

Gene for Male sex-determinant

Male flower

Female flower

Male flower

Select genes on the genomic region related sex-determination
(**GWAS on MP2 population**)

High expression genes & miRNAs in female flowers
(**RNA-seq, small RNA-seq**)

Construct female specific genome region
(**Construct genome reference ver. 3 with longer contigs using Nanopore sequence**)

Identify candidate gene for female sex-determinant

RNA-seq & small RNA-seq for 3 development stages of flowers

RNA-seq

Female flowers 🌷
Stage 1 → Stage 2 → Stage 3

Male flowers 🌼
Stage 1 → Stage 2 → Stage 3

Non-reproductive tissues
11 parts (Ex. leaves, stems, roots, tubers)
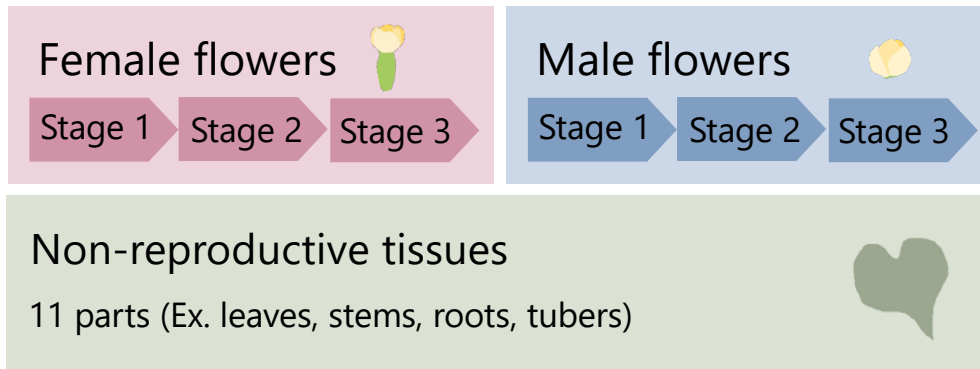
RNA-seq data from Tamiru et al. 2017

Muluneh Tamiru[1†], Satoshi Natsume[1†], Hiroki Takagi[1†], Benjamen White[2†], Hiroki Yaegashi[1†], Motoki Shimizu[1†], Kentaro Yoshida[3], Aiko Uemura[1], Kaori Oikawa[1], Akira Abe[1], Naoya Urasaki[4], Hideo Matsumura[5], Pachakkil Babil[6], Shinsuke Yamanaka[7], Ryo Matsumoto[7], Satoru Muranaka[7], Gezahegn Girma[8], Antonio Lopez-Montes[8], Melaku Gedil[8], Ranjana Bhattacharjee[8], Michael Abberton[8], P. Lava Kumar[8], Ismail Rabbi[8], Mai Tsujimura[9], Toru Terachi[9], Wilfried Haerty[2], Manuel Corpas[2], Sophien Kamoun[10], Günter Kahl[11*], Hiroko Takagi[7*], Robert Asiedu[8*] and Ryohei Terauchi[1,12*]

small RNA-seq

Female flowers 🌷
Stage 1 → Stage 2 → Stage 3

Male flowers 🌼
Stage 1 → Stage 2 → Stage 3

Flower samples were collected at IITA field

Small RNA extraction
1. Extracted RNA (Ambion Plant RNA Isolation Aid)
2. Isolated small RNA (mirVanaTM miRNA Isolation Kit)

Library prep
NEBNext Multiple Small RNA Library Prep Set for Illumina

Sequencing
Illumina's NovaSeq

Male | Female
Stage 1 | Stage 1
Stage 2 | Stage 2
Stage 3 | Stage 3

(Photos by JIRCAS)

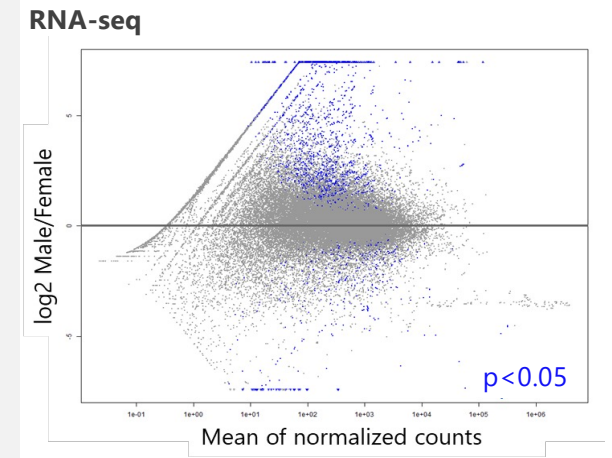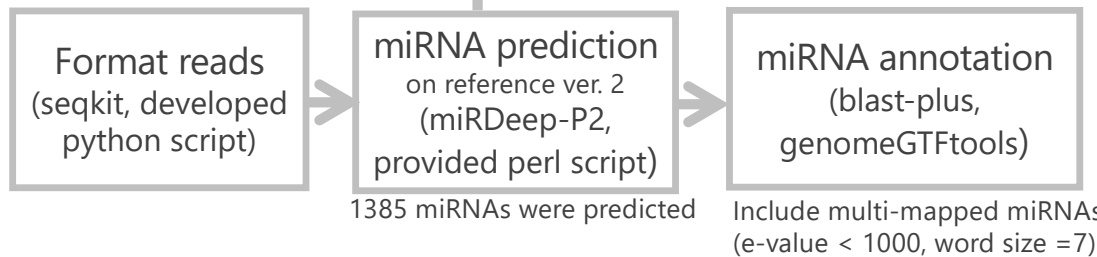# RNA-seq & small RNA-seq reveal high expression genes in female flowers

RNA-seq

```
Filtering          →   Alignment          →   Count            →   Differential
(Phred)                to reference ver. 2     mapped reads         expression
                       (hisat2)                (featureCounts)      analysis
                                                                    (DESeq2)
```

♀ vs ♂

small RNA-seq

```
Filtering          →   Alignment          →   Count            →   Differential
(FaQCs, seqkit)        to reference ver. 2     mapped reads         expression
19≤read length≤25      (bowtie)                (provided perl       analysis
      ↓                     ↑                   script)             (DESeq2)
Format reads       →   miRNA prediction   →   miRNA annotation
(seqkit, developed     on reference ver. 2     (blast-plus,
python script)         (miRDeep-P2,            genomeGTFtools)
                       provided perl script)
```

1385 miRNAs were predicted

Include multi-mapped miRNAs
(e-value < 1000, word size =7)

♀ vs ♂

RNA-seq

log2 Male/Female vs Mean of normalized counts

p<0.05

558 genes have higher expression in female flowers

small RNA-seq

log2 Male/Female vs Mean of normalized counts

p<0.05
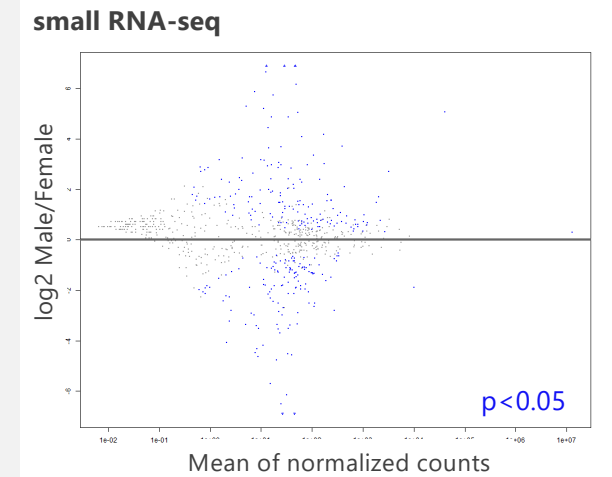
130 miRNAs have higher expression in female flowers

GWAS for sex phonotype in MP2 populations shows the region associated with sex in chromosome 11

Bonferroni correction
2,746 SNP associated with sex
(2,480 SNP on chromosome 11)

FDR correction
3,433 SNP associated with sex
(3,096 SNP on chromosome 11)

Identified genomic region associated with sex phenotype
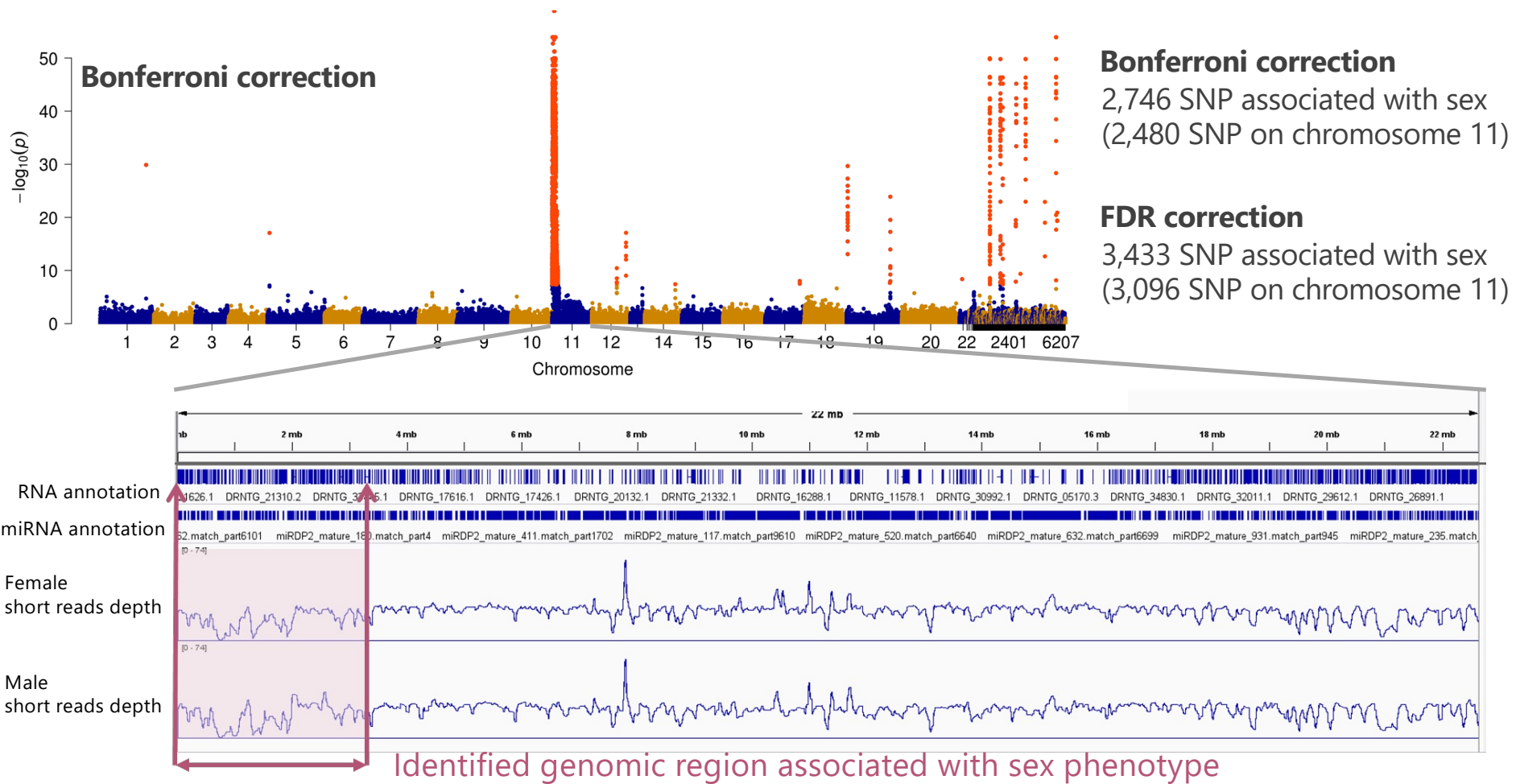
Identify the candidate genes for sex-determination

High expression genes in female flowers in **RNA-seq** ♀

558 genes

Identify genes or miRNAs on the chr11 genomic region associated with sex
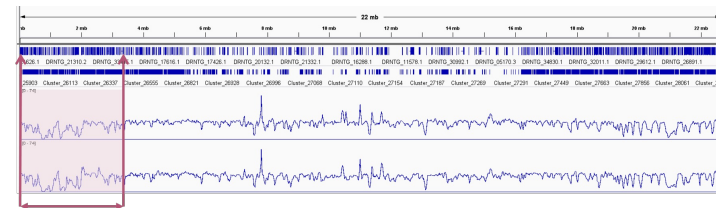
3 genes

Genes mapped with female reads only (short read alignment of MP2 parents)

0 genes

High expression miRNAs in female flowers in **small RNA-seq** ♀

130 miRNAs

49 miRNAs *Include multi mapped miRNAs

Next step

Construct *W*-region contig on *D. rotundata* reference genome ver. 3

Construction of reference genome ver. 3

Nanopore reads were provided for **ver. 3** assemblies

	PromethION 1 flowcell	MinION 2 flowcell	MinION Q20+Kit 1 flowcell
	Ver.2 assembly	For this report	For this report
Read filter setting	>q7, >l5k	>q8, >l5k	>q8, >l5k
Mean read length	9,162	11,818	8,926
Number of reads	1,766,523	1,090,710	672,249

The **ver. 3** assemblies has longer contigs

Feature	Ver.2	Ver.3
Number of contigs	6,513	1,276
Total contig size (Mbp)	579.41	517.55
Logest contig (Mbp)	2.28	6.38
N50 (Mbp)	0.15	0.98
Total 'N' bp	953	0
Complete BUSCOs (%)	90.1	94.1
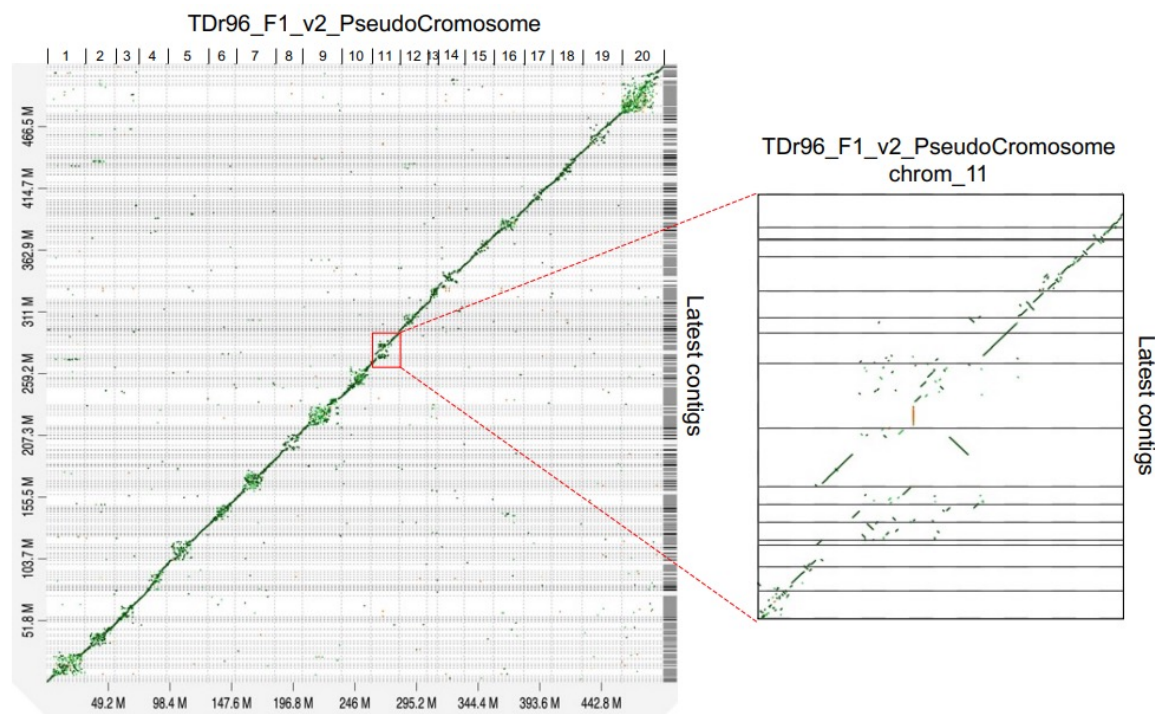
The longer contigs in ver. 3 improved the sequences in chromosome 11 (sex chromosome).

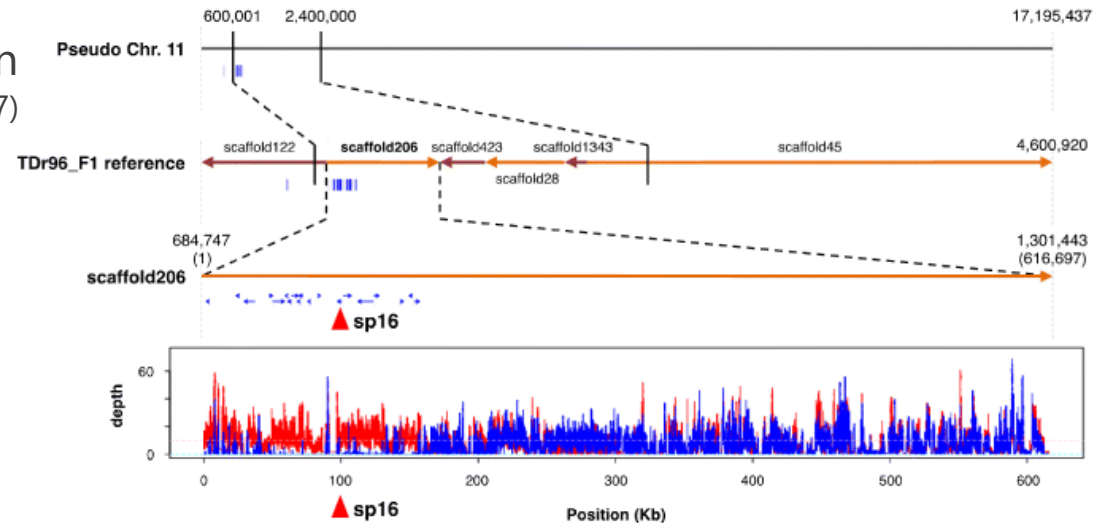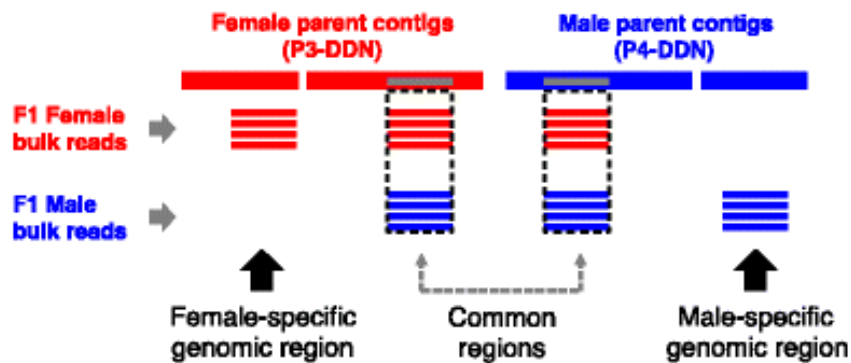# "Contig 216" with *W*-linked marker was constructed in reference ver. 3

Reference ver. 1

A molecular marker sp16 on W-linked region
(Tamiru et al. 2017)

Reference ver. 3

A contig 216 (~0.6 Mb)
With sp16 constructed
by Nanopore sequencing
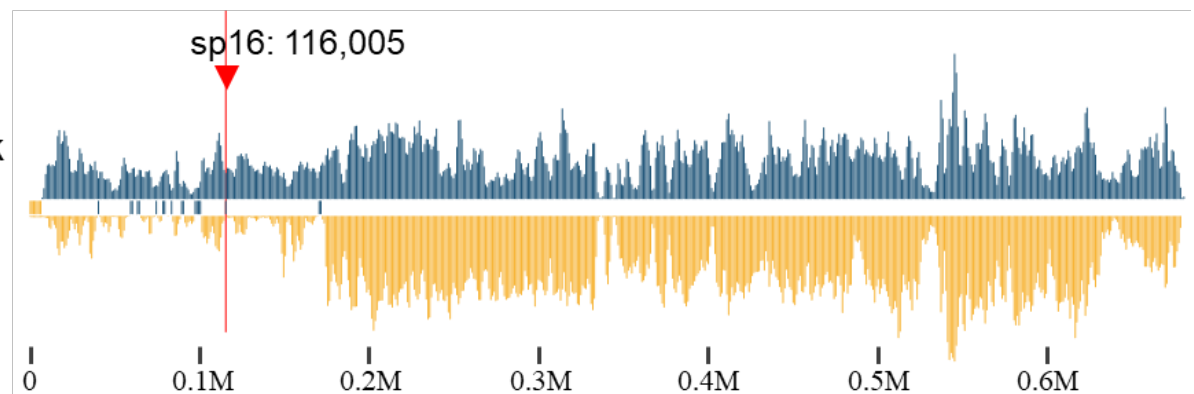
"Contig 216" with sp16 marker has female specific regions

A "Contig 216" with sp16 marker

Female_bulk
Male_bulk

sp16

0   0.1   0.2   0.3   0.4   0.5   0.6 Mb

Female & Male bulk sequence were mapped to reference ver. 3

▼

Checked the region covered by only female bulk reads.

B

Sex specific region
Gene loci

sp16

0   0.02   0.04   0.06   0.08   0.1   0.12   0.14   0.16   0.18   0.2 Mb

Genomic regions covered by only female bulk reads.

■ Region covered by only female reads
□ Region covered by female & male reads
■ Region not covered by female & male reads

1) High expression in female flowers
2) Mapped on the genomic region associated with sex phenotype

49 miRNAs*

Select miRNAs mapped on female specific region →

Final candidate miRNAs for female sex determinant

13 miRNAs*

*Include multi mapped miRNAs

Candidate miRNA for female sex determination

A strong candidate miRNA_708

(Rojas et al. 2020 Fig. 1a)

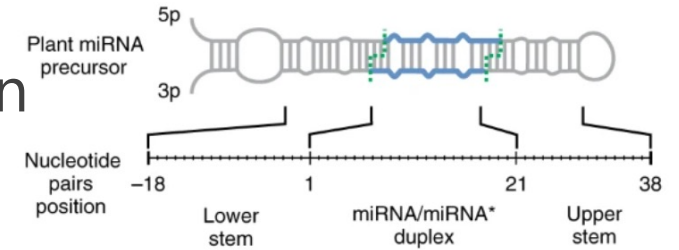| miRNA	DESeq2		Per.Ident with female specific region				Conservation of precursor and primary sequence on chrom_11	Number of hit target genes (psRNATarget2: mismatch $\leq$ 2)
	log2 male/female	padj	per.ident	length	mismatch	gap-open		
miRDP2_mature_1251	-1.47	3.8E-07	95.8	24	1	0	No	2 genes
miRDP2_mature_338	-2.90	4.9E-07	95.7	23	1	0	No	2 genes
miRDP2_mature_82	-2.44	1.7E-06	91.3	23	2	0	No	16 genes
miRDP2_mature_1044	-1.20	4.9E-05	94.7	19	1	0	Yes	24 genes
miRDP2_mature_1231	-2.06	0.00015	95.5	22	1	0	No	3 genes
miRDP2_mature_1291	-0.74	0.00017	95.0	20	1	0	No	0 genes
miRDP2_mature_93	-4.48	0.00028	94.7	19	1	0	No	1 genes
miRDP2_mature_708	-1.82	0.00033	100.0	24	0	0	Yes	1 gene (on chrom_20)
miRDP2_mature_1039	-0.83	0.00045	94.7	19	1	0	No	23 genes
miRDP2_mature_1210	-0.72	0.00142	100.0	16	0	0	No	0 genes
miRDP2_mature_925	-3.36	0.00186	94.7	19	1	0	No	0 genes
miRDP2_mature_138	-1.57	0.00308	95.8	24	1	0	No	6 genes
miRDP2_mature_783	-0.95	0.00682	91.3	23	1	1	No	0 genes

miRNA_708 targets a gene for polygalacturonase 1 beta-like protein 3

miRNA (24 bp)

```
miRNA     24  GGGGGGUGUGAGGGCAGCUGUGCA  1
              :...  ::.:::::::::::::
Target   455  CUUUAAACGUUCCCGUCGACACGU  478
```

Target gene (2,232 bp)

Target gene

PREDICTED: *Dioscorea cayenensis* subsp. *rotundata* polygalacturonase 1 beta-like protein 3 (LOC120251752), mRNA

- Query cover = 84 %
- E value = 0.0
- Per. Ident = 100 %

Polygalacturonase may work for pollen development

Reported as male flower specific genes in dioecious species, genus of *Salix*.

Plant Cell Physiol. 41(1): 16–26 (2000)
JSPP © 2000

Male Flower-Specific Expression of Genes for Polygalacturonase, Pectin Methylesterase and β-1,3-Glucanase in a Dioecious Willow (*Salix gilgiana* Seemen)

Norihiro Futamura[1], Hitoshi Mori[2], Hiroshi Kouchi[3] and Kenji Shinohara[1, 4]

[1] *Molecular and Cell Biology Section, Bio-Resources Technology Division, Forestry and Forest Products Research Institute (FFPRI), Inashiki, Ibaraki, 305-8687 Japan*
[2] *Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi, 464-8601 Japan*
[3] *National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305-8602 Japan*

Male sterile line of cotton mutated in *GhNSP* (*GhNSP* encodes a polygalacturonase protein)

Plant Biotechnology Journal

Plant Biotechnology Journal (2022) **20**, pp. 1054–1068

doi: 10.1111/pbi.13785

Degradation of de-esterified pctin/homogalacturonan by the polygalacturonase GhNSP is necessary for pollen exine formation and male fertility in cotton

Yuanlong Wu[1], Xiao Li[2], Yanlong Li[1], Huanhuan Ma[1], Huabin Chi[1], Yizan Ma[1], Jing Yang[3], Sai Xie[1], Rui Zhang[1], Linying Liu[1], Xiaojun Su[1], Rongjie Lv[1], Aamir Hamid Khan[1], Jie Kong[3], Xiaoping Guo[2], Keith Lindsey[4], Ling Min[1,*] and Xianlong Zhang[1]
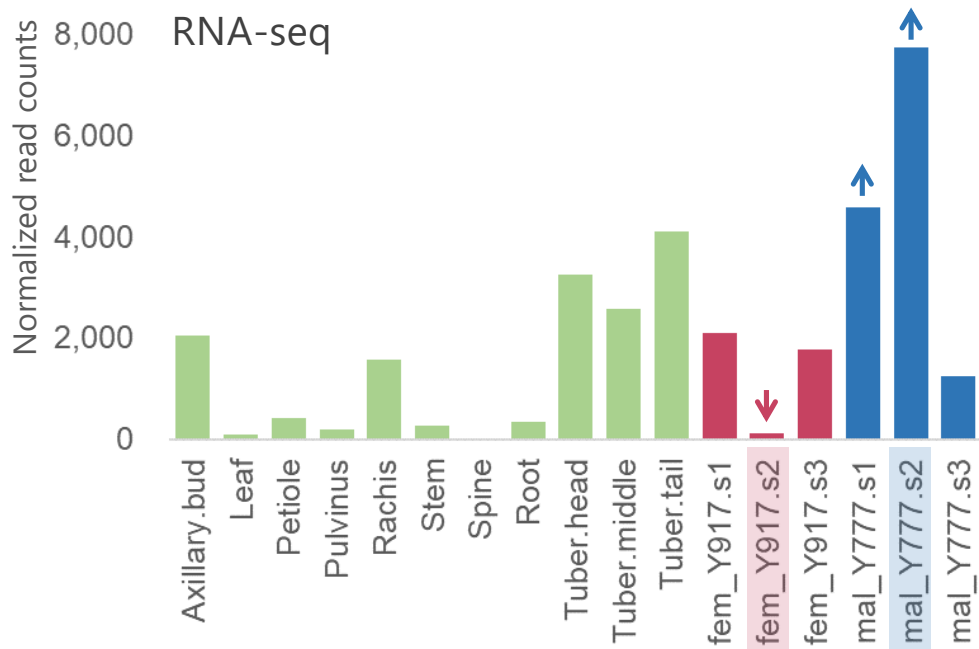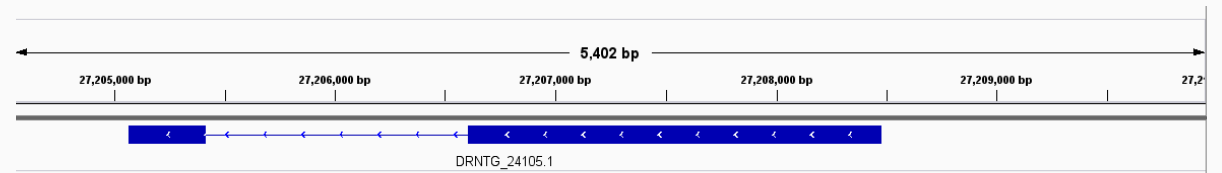
Target gene expressed in the stage 1&2 of male flowers
but suppressed expression in the stage 2 of female flowers
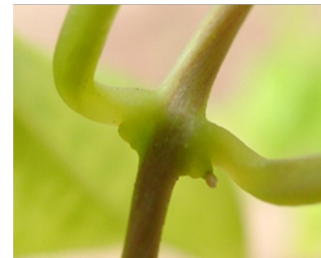
Target gene: DRNTG_24105.1 (chrom_20)

```
Score = 3469 bits (1878),  Expect = 0.0
Identities = 1878/1878 (100%), Gaps = 0/1878 (0%)
```
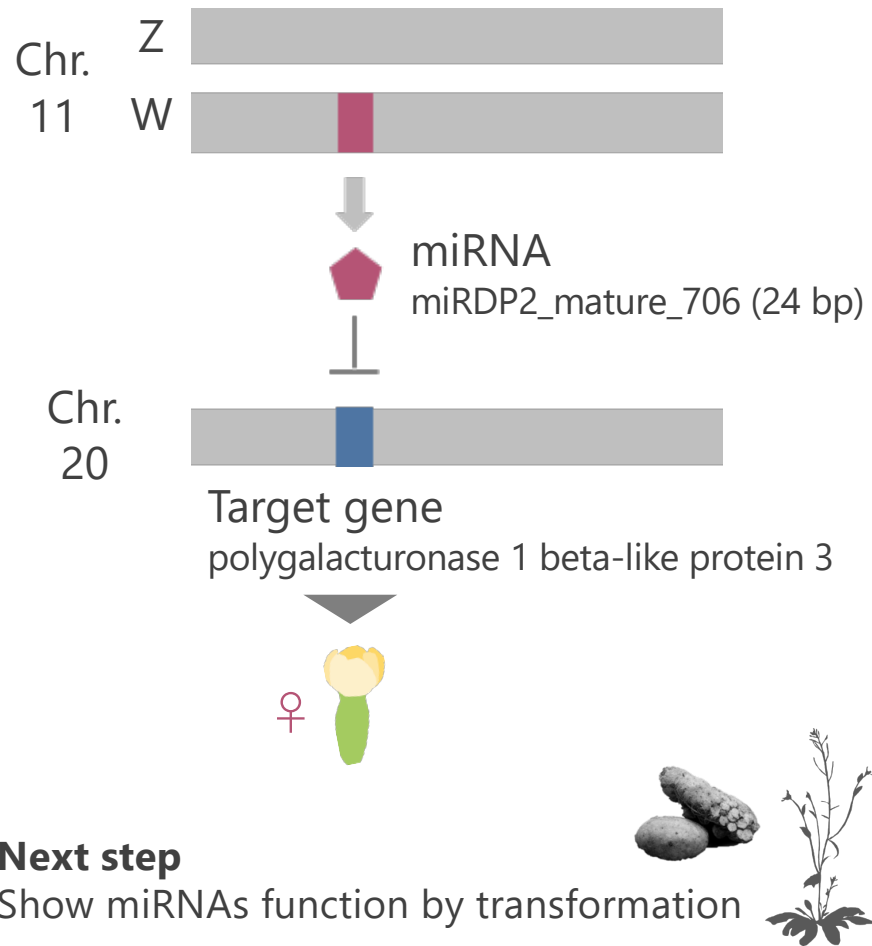

(Photos by JIRCAS)

Hypothesis

Chr. 11

Z

W

miRNA
miRDP2_mature_706 (24 bp)

Chr. 20

Target gene
polygalacturonase 1 beta-like protein 3

♀

Next step
Show miRNAs function by transformation

Possibility of self-fertilization of
a monoecious plant

Z

W

Z

W

♀ × ♂

Quick fixation of desired alleles
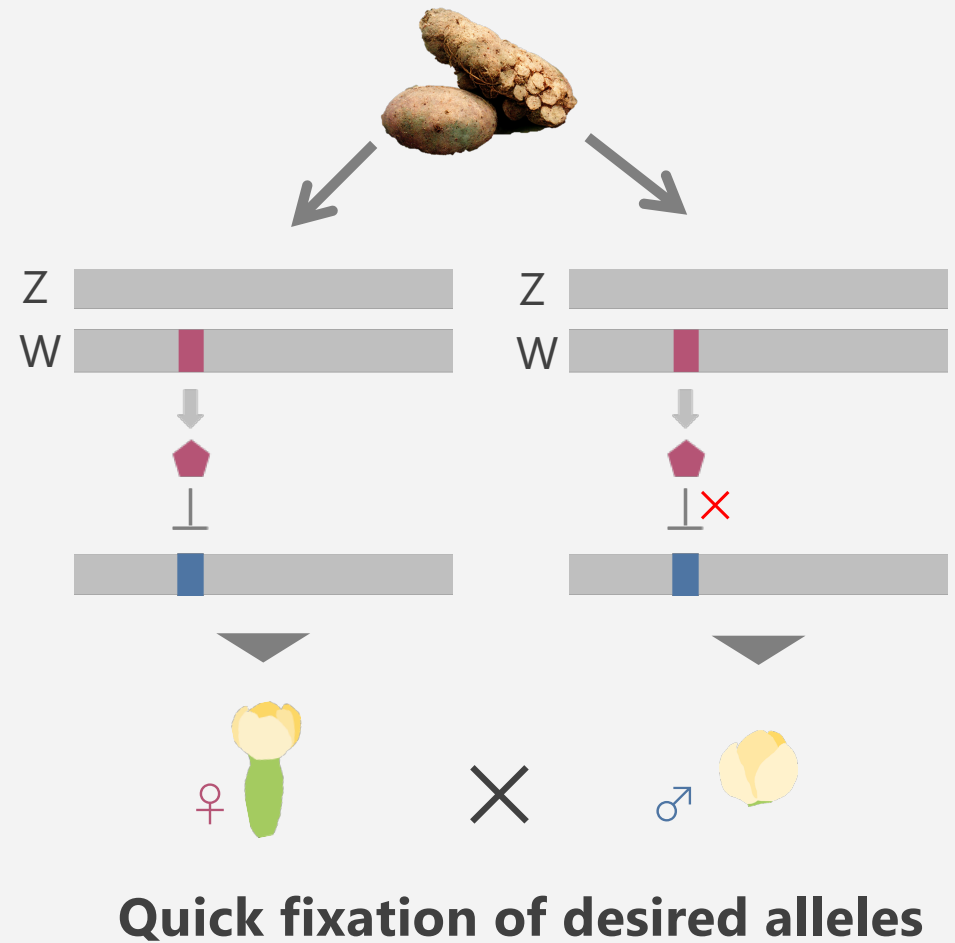
Prospects

1. Manipulation of Guinea yam sex to accelerate cross breeding

2. Focusing disease resistance: Pan-NLRome of Guinea yam

3. Utilizing alleles of Guinea yam wild relatives

Special thanks to all AfricaYam community