Appendix 5-3-1~6

Dispatched short-term experts (Component 4: Tree Breeding)

【Artificial Crossing】

Appendix 5-3-1

Report of short term expert (Artificial Crossing)

Expertise	Name	Term
Artificial Crossing	Dr. So Hanaoka	2017.12.6～12.15
Artificial Crossing	Dr. Michiya Matsushita	2017.12.6～12.15

1. Itinerary

Date	Activity
6 Dec. (Wed.)	Move to Haneda
7 Dec. (Thu.)	Move to Dubai, arrival at Nairobi　Move to Kibwezi
8 Dec. (Fri.)	Survey on Kibwezi Melia seed orchard and PTSs in Kibwezi, Voi and Kasigau Move to Kitui
9 Dec. (Sat.)	Study on artificial crossing method of Melia
10 Dec. (Sun.)	Study on artificial crossing method of Melia
11 Dec. (Mon.)	Study on artificial crossing method of Melia in Tiva seed orchard with Cps Move to Nairobi
12 Dec. (Tue.)	Analysis of data collected in sites observation
13 Dec. (Wed.)	Discussion on Melia artificial crossing with CPs in KEFRI
14 Dec. (Thu)	Report to Kenya JICA Office Move to Dubai
15 Dec. (Fri.)	Move to Narita Move to Hokkaido and Mito

2. Result of major activities

2.1 Observation of progeny test sites in Tiva, Kibwezi, Kasigau and Voi

2 or 3 years have passed since Melia progeny test sites were established, and the good survival and growth of the sits were observed. The highest growth was identified in Tiva through more than 10cm DBH and 7m height. Growth difference between the sites is very clear depending on the environmental condition such as precipitation and soil condition as we expected. It was found that the height growth of trees showed hitting a peak in Voi and Kasigau where it had low precipitation.

Stem vending of trees was observed in Voi and Kasigau. A trait of stem straightness could be taken in the assessment.

It was found that some individuals planted in 2014 were putting fruits in Tiva and Kibwezi PTSs.

2.2 Observation of Melia seed orchards in Tiva and Kibwezi

Melia seed orchards have been established in 2013 and the first flowering and seed production started in 2015. Seed collections were carried out for 3 times in 2016, and almost trees, about 3000 trees in Tiva seed orchard, took good flowering and fruiting in 2017 according to Mr. Kariuki. It was found that some trees with better growth had fruits in abundance and the branches were hanging down because of the heavy weight.

2.3 Consideration of setting crossing booth inside a greenhouse in Kitui center nursery

We discussed setting of crossing booth for study on Melia artificial crossing inside a greenhouse. As some grafted Melia seedlings took fruits in the nursery, a new crossing method was considered, which included the following measure; several grafted Melia seedlings for only selected clone could be putted in the greenhouse for taking natural crossing inside closed space. This study will be started from 2018.

2.4 Study on Melia artificial crossing method in Tiva seed orchard

It would have been usually a season for flowering of Melia. It, however, had already passed this year, and unfortunately no flowers were observed in Tiva seed orchard. Only one flowering tree was identified through investigation of 3000 trees, so the study on artificial crossing for self-pollination was carried out on the tree. Basic techniques for actual artificial crossing on Melia, which included how to remove opened petal, isolate the bud and cover it with paper bag, was guided to Mr. Kariuki.

2.5 Discussion on study of production of Melia superior 2nd generation

As the study on production of Melia 2nd generation, the outline of study approach was introduced and some merits and demerits on the methods were discussed with CPs. The work plan is as follows;

a. Study on basic artificial crossing measure using crossing bags in which the different clones are crossing
b. Study on crossing inside of a greenhouse
c. Evaluation of genetic performance on candidate plus trees in accordance with PTSs assessment, and study on crossing style through DNA analysis

3. Issues in the future

a. Artificial crossing

   Experiment of artificial crossing should be carried out in suitable period when flowering duration is not so long and limited.

b. Sharing data and information concerned

   All data of PTSs assessment should be shared in all concerned so that project activities will be implemented smoothly or efficiently.

c. Tree breeding strategy of Kenya

   Tree breeding strategy of Kenya should be required in the future. to select important traits of Melia

d. Skill up of statistical analysis for CPs

"R" is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS, and it has disseminated to researches all of the world. This software, however, has not been used in African countries including Kenya, so that only a few researchers have used expensive licensed software for statistical analysis in KEFRI. To disseminate basic and user-friendly statistical software to CPs, we suggested to make a textbook of "R" and statistical analysis and guide the method of analysis. The guidance will upgrade step by step from basic class to advanced class. It can contribute to improvement of the research capacity and skill on KEFRI staff.

Photos

Melia PTS in Kasigau

Melia PTS in Tiva

Melia Seed Orchard in Kibwezi

Grafted Melia seedlings in Kitui Center nursery, on which fruiting was found.
A greenhouse for study on indoor crossing to produce Melia 2nd generation (right).

Putting a bug over flowers for artificial crossing in Kitui (Tiva)

Putting a bug over flowers for artificial crossing in Kitui (Tiva)

Guidance of data analysis to Cps

Appendix 5-3-2

Report of short term expert (Artificial crossing and Project management)

Expertise	Name	Term
Artificial crossing	Dr. Michinari Matsushita	13 - 20 May 2018
Project management	Mr. Shizuo Kamizore	13 - 20 May 2018

1. Itinerary

Date	Activity
13 May (Sun.)	Move from Hitachi to Haneda, move to Dubai
14 May (Mon.)	Arrival at Nairobi Meeting with Chief Advisor
15 May (Tue.)	Move to Kibwezi, Observation of Melia seed orchard, PTS and Acacia seed stand Move to Voi
16 May (Wed.)	Attendance to Promotion Seminar on Melia Woodlot in Kenya Move to Kilifj
17 May (Thu.)	Meeting with KOMAZA staff Observation of Melia farmer's plantation conducted by KOMAZA
18 May (Fri.)	Meeting with Chief Advisor Move from Kilifi to Nairobi, move to Dubai
19 May (Sat.)	Dubai to Haneda
20 May (Sun.)	Move from Haneda to Hitachi

2. Results of major activities

2.1 Promotion Seminar on Melia Woodlot in Kenya

Promotion Seminar on Melia Woodlot in Kenya was held in Voi, Taita Tabeta county, which has been designated as a pilot county of CADEP component 2. Short term experts from FTBC attended the seminar.

Participants were approximately 30, and farmers in Taita Taveta and Kilifi counties, private sector (Komaza and Gatsby Africa), NGO (Wildlife of Africa), officials of Taita Tabeta county, officials of KFS participated in the seminar. Dr. Muturi, Dr. Ndufa and Mr Kyalo participated as lecturers to explain tree breeding activities and seedling production of Melia and so on.

Mr. Takano, Chief Advisor of CADEP project, introduced the ouit line of CADEP project activities. Dr. Muturi, manager of component 4, explained a concept of tree breeding and advantage of improved Melia. A participant asked the amount of seed production of improved Melia and how to get the seeds. A NGO suggested that establishment of a small scale nursery for school might be an effective method to extend improved Melia in Kenya.

2.2 Observation of Kibwezi site

1) Melia seed orchard

It seems that weeding has just finished as the best timing, because a rainy season has just terminated at this week. New buds of Melia tree have got slightly lignified and grown healthily.

2) Melia progeny test site

Almost trees planted in 2015 have got for height growth and have had appropriate crowns that have suitable branches.

3) Acacia seed stand

It was found that some trees could get flowering. It was not identified that the varietal differences could exist, so seed production should be checked in the next PTS assessment on Jul.

2.3 Observation of Melia plantation conducted by KOMAZA

1) Outline of KOMAZA

KOMAZA is a company that has promoted tree plantations by local farmers using funds collected from private and governmental investors. KOMAZA considers having own seeds production resource for Melia because a demand to Melia is increasing in recent years. Mr. Takano got the information of KOMAZA and arranged observation of the Melia plantation site with CPs of CADEP, so FTBC joined the observation in this time.

KOMAZA has been established in Kilifi county by an American CEO and spent 10 years. The predecessor organization of KOMAZA was a NGO concerned with health for local people in Kenya. KOMAZA has conviction that tree plantation by local farmers should be supported, and it should be promoted even though celling seedlings could become more profitable than plantation. A Japanese staff for finance has been employed since the last year, and our visit to KOMAZA in this time was arranged by him.

3,800ha plantation by 14,000 farmers have been carried out through KOMAZA fund, and the main planting species are Eucalyptus species (60%), which is a hybrid between *E. camaldulensis* and *E. grandis* called as Eucaly-CG in Kenya, and *Melia volkensii* (40%). KOMAZA produced 14,000 seedlings of Melia for increasing demand in this year. Since productivity of seedlings was less than 50 %., improvement of nursery and seed resource should be considered. Recently, Melia seeds have been procured from a superior farmer who has planted Melia extensively. KOMAZA, however, predicts that more than 400kg of Melia seeds will be required to support the plantation in the near future and considers establishment of its own Melia seed orchard for sustainable seed production. As Melia plantation, KOMAZA designates suitable farmers for plantation in accordance with a soil map of Kilifi, which might be made by local government, and finally 5,000 farmers have been selected from 15,000 participants this year for Melia plantation. As a plantation system, KOMAZA distributes Melia seedlings to farmers for free, and the farmers plant and take care the seedlings in their own lands. As harvesting trees, KOMAZA has responsibility to purchase the trees and has a plan to establish wood processing facilities to add significant value to harvested woods.

2) Observation of Melia plantaion

We observed two plantation sites in Kilifi. One had poor growth with DBH:6-12cm, height:3-6m, another had good growth with DBH:10-16cm, height:7-10m, in which spacing for planting was

5m by 5m. Eucalyptus trees were planted adjacently.
KOMAZA has conducted training program on tree planting and management for farmers, and managing officers of KOMAZA have managed all plantation sites by using smart-phones, in which all data such as GPS data, date of planting and bud thinning and so on.

3) Discussions

Dr. Matsushita explained some advantages of Melia improved seed and seedling using the results of growth and seed production from PTS data analysis.

KOMAZA asked an appropriate management for Melia seedling production and how to purchase improved Melia seeds, and Mr. Ndiati handed out "Guidelines to On-Farm *Melia volkensii* Growing in the Dryland Areas of Kenya", in which appropriate methods for seedling production, plantation and management on Melia are described, and Dr. Ndufa explained that KOMAZA could purchase Melia improved seeds from KEFRI but the available amount of seed for purchase was limited because total seed production from Melia seed orchard was about only 100kg last year.

Concerning establishment of Melia seed orchard using plus trees, KOMAZA asked how much area for the orchard would be needed and how much amount of seeds would be produced. Dr. Matsushita explained that required or expected traits, growth, seed production or drought tolerance, should be decided at first, and then plus tree selection and the number of mother trees in an orchard would be considered according to priority of traits.

KEFRI, furthermore, suggested that an agreement for securing traceability of Melia improved seeds and seedlings should be signed between KEFRI and KOMAZA if KOMAZA will establish a Melia seed orchard.

3. Subjects for project management

・It seems that KOMAZA has managed the farmers' plantation soundly more than expected, and ability of the staffs would have better skills for tree plantation. KOMAZA has a plan to employ foresters as the staffs who is dedicated to the tree plantation.
・Workshop for corroboration between each component of CADEP will be held on Jun. 26 with the participants of all component managers and staffs to enhance each activity through the corroboration. FTBC will dispatch some short term experts to attend the workshop and introduce the result of tree breeding activities.
・The JCC meeting will be held on Jul. 19 in Nairobi. Successors of a chief advisor and forest extension expert will join the meeting, and FTBC will also join the meeting.

4. Contact persons

Mr. Tevis Howard, CEO, Komaza,
Mr. Dan Mcgovern, Field Operations, Komaza
Mr. Charles Gitahi, Nursery, Komaza
Mr. Tomonobu Kumahira, Finance, Komaza
Dr. Gabriel Muturi, Manager of Component 4, KEFRI
Mr. Peter Nduati, Manager of Component 2, KFS
Dr. Omondii, Researcher for DNA analysis, KEFRI
Mr. Kenichi Takano, Chief Advisor, CADEP
Ms. Naoimi Matsue, Forestg Extension, CADEP

Photo

Photo 1. Promotion Seminar on Melia Wood lots in Voi (Presentation from Mr. Takano)

Photo 2. Promotion Seminar on Melia Wood lots in Voi (Presentation from Dr. Muturi)

Photo 3. Melia Seed Orchard in Kibwezi (Weeding just finished)

Photo 4. Melia PTS in Kibwezi (planted in Dec. 2015)

Photo 5. Acacia seed stand in Kibwezi (planted Dec. 2015, just finished for weeding)

Photo 6. Acacia seed stand in Kibwezi (planted Dec. 2015)

Photo 7. Flowering (?) of Acacia (white flowers?, planted Dec. 2015, Kibwezi)

Photo 8. Flowering (?) of Acacia (white flowers?, planted Dec. 2015, Kibwezi)

Photo 9. Melia plantation by farmer (H:3-6m, planted Nov. 2013, by KOMAZA)

Photo 10. Melia plantation by farmer (H:3-6, planted Nov. 2013, by KOMAZA)

Photo 11. Melia plantation by farmer ((H:7-10m, planted Nov. 2013, by KOMAZA)

Photo 12. Melia plantation by farmer (H:7-10m, planted Nov. 2013, by KOMAZA)

Photo 13. Collecting scions for Eucalyptus cutting (KOMAZA nursery)

Photo 14. Nursery beds for Eucalyptus cutting seedlings (KOMAZA nursery)

Photo 15. Demonstration of sawing Melia seeds (KOMAZA nursery)

Photo 16. Remaining Melia seedlings (KOMAZA nursery)

Appendix 5-3-3

Report of short term expert (Project Management and Artificial Crossing)

Expertise	Name	Term
Project Management	Mr. Shizuo Kamizore	2018.6.23～6.30
Artificial Crossing	Dr. Michiya Matsushita	2018.6.23～6.30

1. Itinerary

Date	Activity
23 Jun. (Sat.)	Move to Haneda
24 Jun. (Sun.)	Move to Dubai, arrival at Nairobi
25 Jun. (Mon.)	Meeting with KEFRI CPs Making material for workshop
26 Jun. (Tue.)	Attendance to CADEP component interaction workshop
27 Jun. (Wed.)	Move to Kitui Study on artificial crossing method of Melia with CPs in Tiva seed orchard
28 Jun. (Thu.)	Observation of Better Globe Forestry Melia plantation site Move to Nairobi
29 Jun. (Fri.)	Meeting with Japanese experts Move to Dubai
30 Jun. (Sat.)	Move to Narita Move to Hitachi and Mito

2. Results of major activities

2.1 CADEP component interaction workshop (refer to Annex for agenda)

CADEP component interaction workshop was held on 26th Jun, Nairobi to enhance a relationship between CADEP each component and promote each activity effectively. In component 4, Mr. Kariuki explained the outline of component activities, and Dr. Matsushita made a presentation on the result of tree breeding research, which focused on the growth and seed production, based on data analysis of 2nd year PTSs assessment of Melia.

The outline of activity on each component was presented from CPs in the morning session, and a group discussion was held in the afternoon session. The themes of discussion were how to contribute completion of 10% forest cover in Kenya for each component and how to exert a synergy effect on 5 components of CADEP. The themes were actively discussed in 3 groups to which all participants were divided. With regard to component 4, following suggestions and comments were indicated; 1) Improved seedlings with high quality for growth will be supplied to dray land through understanding tree breeding activities, however, the seeds and seedlings are not supplied with a sufficient amount. 2) Pest and disease control must be required for limited tree species. 3) Collaboration between component 2 and 4 through information sharing is significant. Melia plantation guide book can be effective tool to promote it. 4) Component 1 will support component 2 for establishment of local seed orchards or nurseries on institutional measures.

2.2 Study on Melia artificial crossing

Experiment on Melia artificial crossing was carried out with Mr. Karituki in Tiva seed orchard.

The artificial crossing adopted an ordinary method for fruit cultivation that pollen, collected from Melia flower and reserved in a dry bin, was inoculated to isolated pistil of another clone. Mr. Kariuki will observe the experiment.

2.3 Study on Melia cutting

We checked the result of Melia cutting carried out on last April. No rooting and no callus formation were unfortunately observed on all scions of Melia. A CP mentioned that it could be caused by longer term rainy season and cold temperature. It was found that Melia cutting could be more difficult than expected because callus formation was identified in spite of using appropriate scions for cutting in this time. All conditions including selection of scions, soil and so on should be considered again in accordance with the condition for succeed result of Japanese Melia cutting.

2.4 Observation of Melia plantation site by private sector (Better Globe Forestry)

We observed a large scale Melia plantation site in Kitui county.

Better Globe Forestry (hereinafter BGF), which is a company to plant trees using fund collected from mainly European countries, has started tree plantation since 2007 near Kiambere dam lake.

BGF had planted neem trees in the area, but it was not successful for the plantation. BGF, therefore, changed plantation species from neem tree to Melia, and have implemented about 300ha Melia plantation since 2007. The superior plantation site in 2009 takes the growth of DBH:16-22, H: 12-14m. The spacing of plantation is 4 by 4m, and the expected log size is diameter:40-50cm and height:5-6m. BGF aims to export Melia to European countries as material for furniture making as well as mahogany wood. 2,500 farmers have contracted with BGF for Melia plantation, and BGF will buy the logs from the farmers. BGF has furthermore a plan to build a saw-mill near future.

Seeds to produce seedlings have been collected from superior specific mother trees that are selected from the Melia plantation sites. A staff of BGF was interested in establishment of Melia seed orchard to get high quality seeds, and also Melia clone propagation. BGF sells their Melia seeds to KOMAZA, which can show that circulation of Melia seed and seedling could become brisk business in private sectors concerned.

➢ Discussions

Bud pruning on tree has not be carried out in BGF Melia plantation, and branch pruning has been done as alternated. It can be found that big nots are visible and the size of crowns are quite different each other. Vent of tree is noticeable and it could be caused by seed resources that depends on seed collection from natural trees by farmers.

BGF purchased improved Melia seeds from KEFRI, and requires sustained purchase of the Melia seeds. If BGF will establish the own seed orchard, an agreement for traceability of Melia improved seeds and seedlings should be secured between KEFRI and KOMAZA as well as KIMAZA. Cooperation between all staffs, experts and CPs, of component 2 and 4 should be required to achieve it.

3. Subjects for management

・ Joint Coordinating Committee(JCC) meeting is planned to be held on 19th Jul., and FTBC will dispatch 2 short term experts to attend the meeting. Project management is subject to discuss with Japanese experts and JICA staffs.

・ Election and appointment of new Director General of KEFRI has been conducted in the Ministry. Two counterpart personnel are nominated for the DG, but the total number of candidates would be unknown.

5. Contact persons

Mr. Jan Vandenabeele, Executive Director, Better Globe Forestry
Dr. Gabriel Muturi, Component manager of CADEP, KEFRI
Dr. James Ndufa, Director of KEFRI Kitui regional station, KEFRI
Mr. Jason Kariuki, Assistant Component manager of CADEP, KEFRI
Mr. Peter Nduati, Component manager of CADEP, KFS
Ms. Yoko Okonogi, JICA Kenya Office
Mr. Kazuhisa Kato, Executive Director, Japan Overseas Forestry Consultants Association
Mr. Ito, PASCO
Mr. Kenichi Takano, Chief Advisor, CADEP
Ms. Yuki Honjo, Coordinator, CADEP
Ms. Naomi Matsue, Forestry Extension, CADEP

Photo

Photo 1. Workshop
（Mr. Kariuki explained compo. 4 activities）

Photo 2. Group discussion in the workshop
(2 themes were discussed in 3 groups)

Photo 3. Collection of pollen from Melia anthotaxy (Melia seed orchard in Tiva)

Photo 4. Dry bin to reserve pollen

Phot 5. Guidance of pollen collection

Photo 6. Experiment of Melia artificial crossing
(inoculation of pollen to pistil)

Photo 7. Experiment of Melia artificial crossing (Covering pistil with paper bag)

Photo 8. Melia cutting study in Kitui centre (all unsuccessful, implemented on Apr. 2018)

Photo 9. Melia plantation site (BGF, planted in 2014)

Photo 10. Melia plantation site (BGF, superior growth planted in 2009)

Photo 11. Cross section of Melia planted tree (BGF, planted in 2007, D:22cm)

Photo 12. Melia plantation site and nursery under the trees (BGF, planted in 2007)

Photo 13. Collected fruits from plus trees
(Just smaller than those of Melia seed orchards)

Photo 14. Workers in BGF nursery
(Isolation of seeds from Melia nuts with getting 1 or 2 seeds from a nut, 3 to 5 seeds from a nut produced in Melia seed orchard)

Ministry of Environment and Forestry

KENYA Forest Service

Interaction Workshop for Capacity Development Project for Sustainable Forest Management (CADEP-SFM)

Date: Tuesday, 26th June 2018
Venue: Crowne Plaza Hotel, Nairobi

Time	Item	Facilitator	Responsible
8.30 - 9.00	Registration	Secretariat	
9:00 - 9.10	Opening remarks	Hewson Kabugi	
9:10 - 9.30	Objectives of workshop	Kenichi Takano Chief Advisor	
9:30 - 11:00	Presentation of each component (1) Component 1: Policy Support (2) Component 2: Pilot County (3) Component 3: REDD+ Readiness	Component Mangers, etc	
11.00 - 11.20	Tea Break	All	
11.20 - 12:20	Presentation of each component (4) Component 4: Tree Breeding (5) Component 5: Regional Cooperation	Component Managers, etc	
12:20 - 13:00	Plenary session	Peter Nduati	
13:00 - 14:00	Lunch	All	
14:00 - 15:20	Group discussions	Peter Nduati	
15:20 - 16:00	Group presentation		
16:00 – 16:20	Plenary session	Peter Nduati	
16:20 - 16:30	Way forward	Hewson Kabugi	
16:30-	Tea and closing	All	

Appendix 5-3-3-1

Jun. 2018 @Kenya

Joint Meeting for CADEP

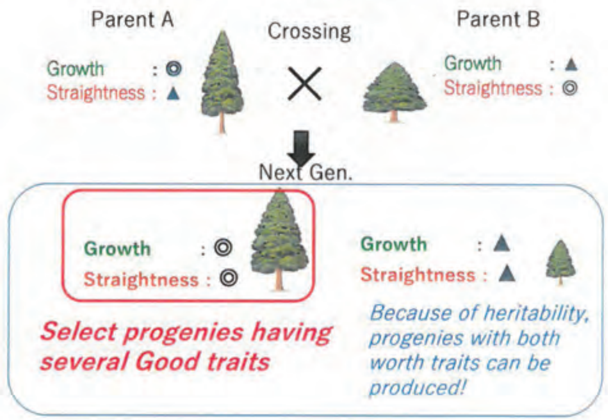
Component 4: Tree Breeding

-Current results of Breeding of *Melia volkensii* -

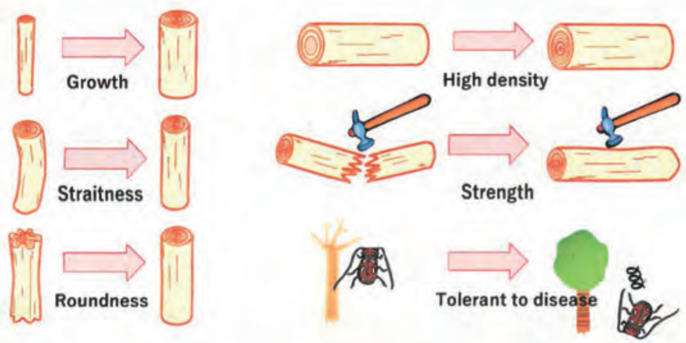
Dr. Michinari Matsushita (Forestry and Forest Product Research Iinstitute, FTBC,

Why breeding is important?

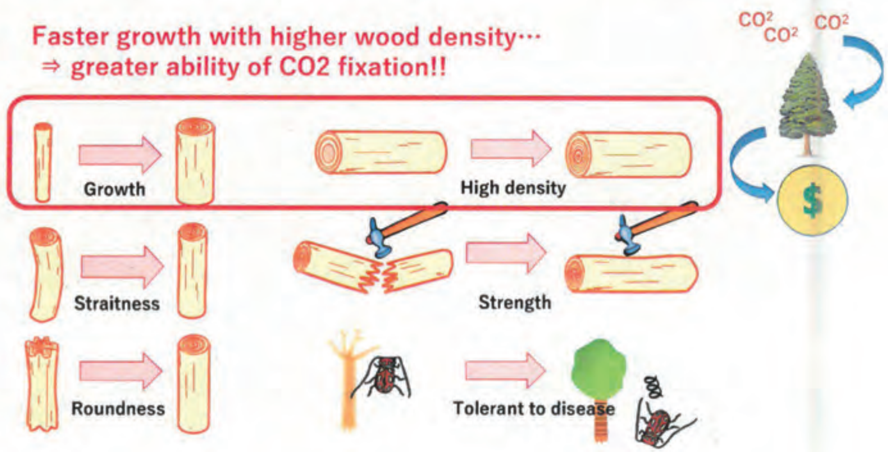
Breeding can produce next generations having several "Good traits"

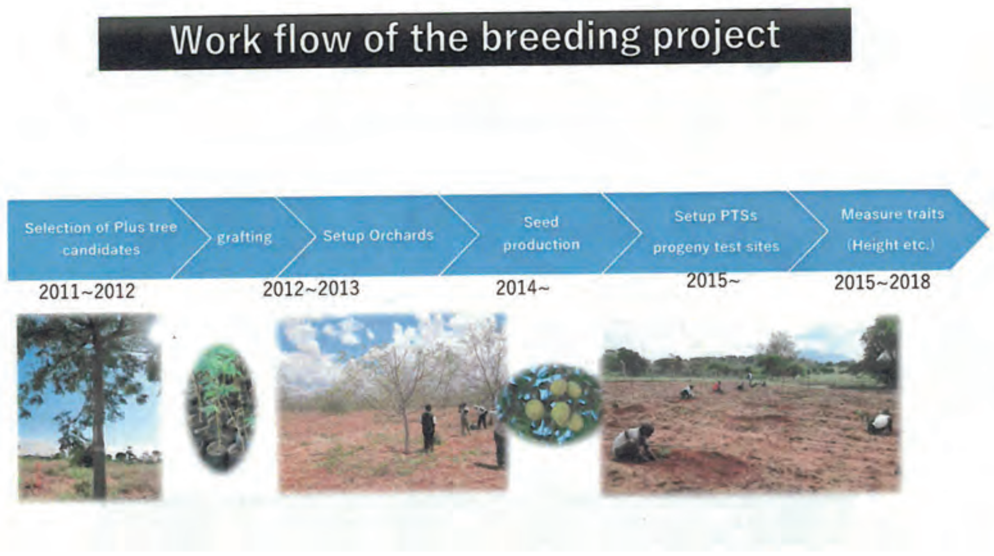


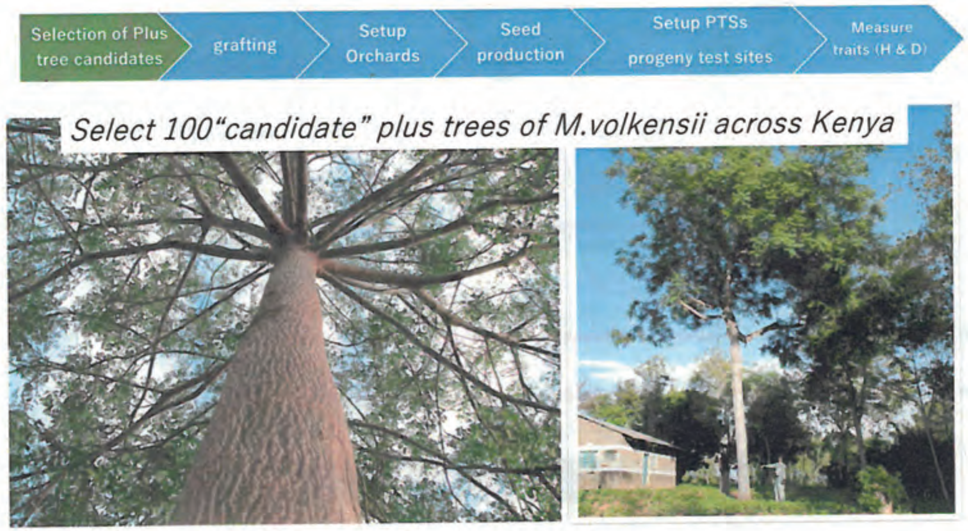
Which traits can be improved?

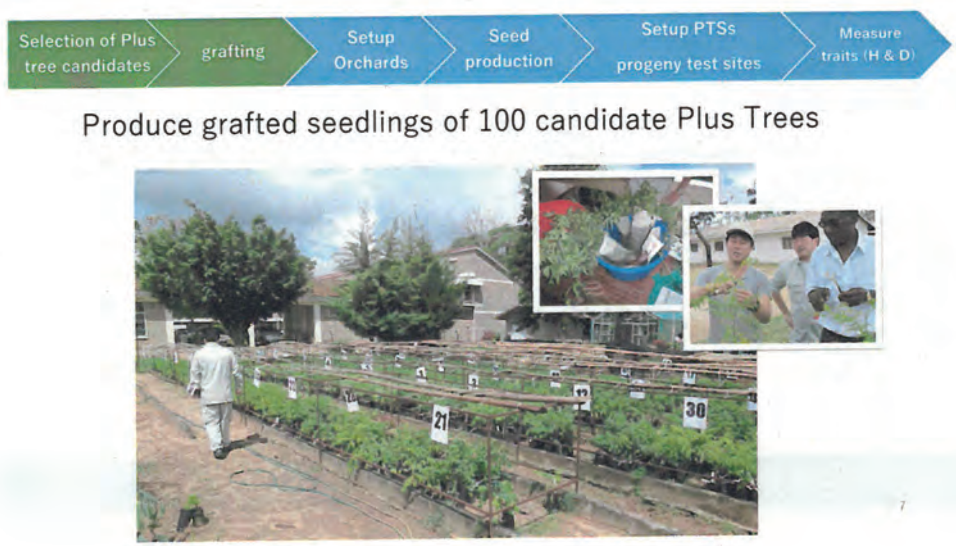


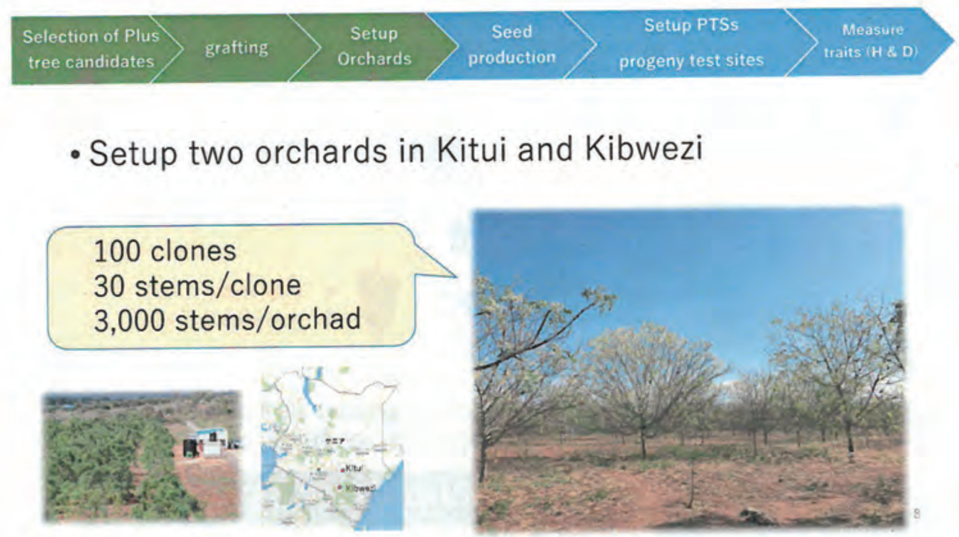
Breeding can change CO^2 to Economic success!

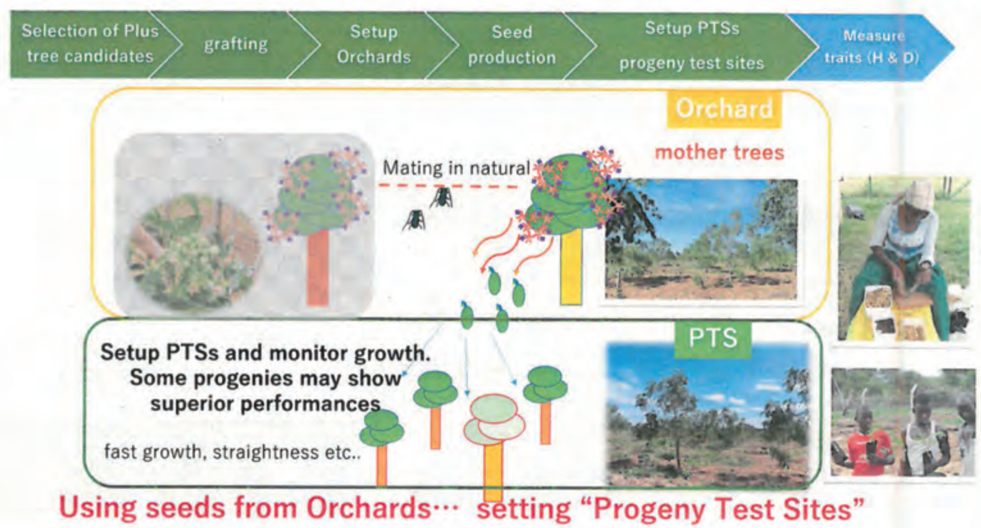


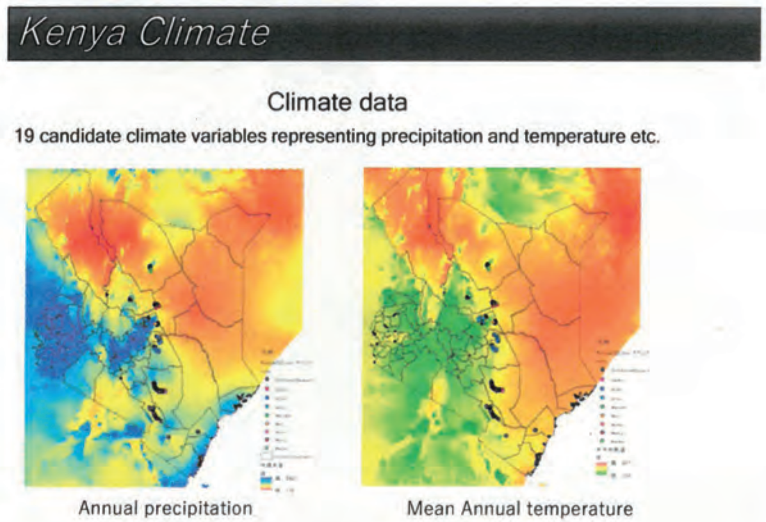

Work flow of the breeding project
Selection of Plus tree candidates
grafting
Setup Orchards
Seed production
Setup PTSs progeny test sites
Measure traits (Height etc.)
2011~2012
2012~2013
2014~
2015~
2015~2018

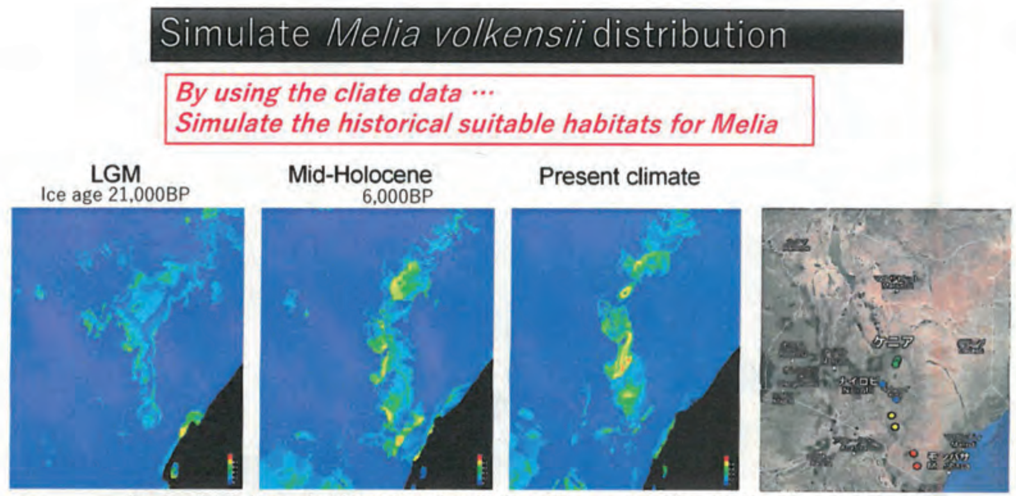

Selection of Plus tree candidates
grafting
Setup Orchards
Seed production
Setup PTSs progeny test sites
Measure traits (H & D)
Select 100"candidate" plus trees of M.volkensii across Kenya


Selection of Plus tree candidates
grafting
Setup Orchards
Seed production
Setup PTSs progeny test sites
Measure traits (H & D)
Produce grafted seedlings of 100 candidate Plus Trees
21
30
7


Selection of Plus tree candidates
grafting
Setup Orchards
Seed production
Setup PTSs progeny test sites
Measure traits (H & D)
• Setup two orchards in Kitui and Kibwezi
100 clones
30 stems/clone
3,000 stems/orchad
8





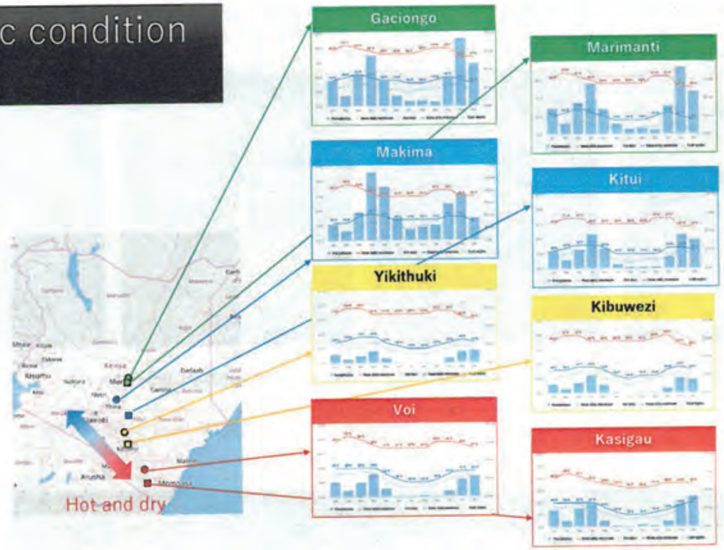


Species distribution probability of *M. volkensii* in East Africa predicted from the Maxent analysis, for Last glacial maximum (LGM) (21,000 BP), Mid Holocene (6000BP) and Present (0 BP). Higher distribution probability is shown as warm color, while lower probability is as cold color. Note that the area shown as black color is not land area (Indian sea).

4 main-PTSs & 4 sub-PTSs were setup

Based on the climate data & genetic background (history) of *Melia*, we setup eight main/sub PTSs

Climatic condition of PTS



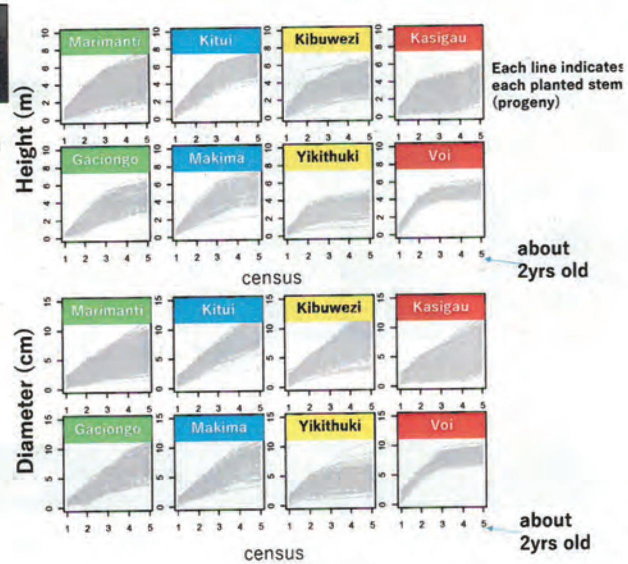
Checking the growth of *Melia* in Progeny test sites

1.5 years old after planting,
there is a significant growth variation among families
→ Select "good" families/progenies,
genetic improvement in next generation.

Feb. 2017

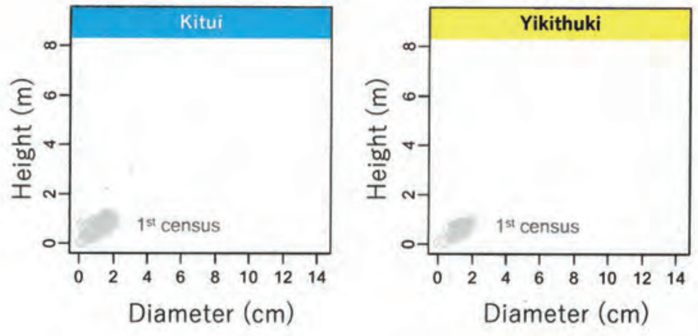
Checking the growth trend of progenies

Large between-sites variation in growth trends of progenies



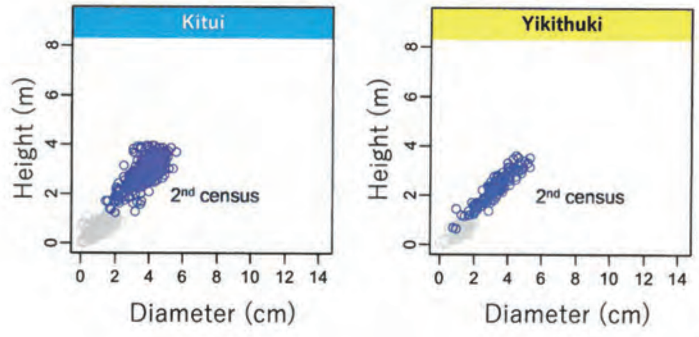
Checking the Height ~ Diameter relationship

About 1 months after planting



Each circle indicates each planted stem.

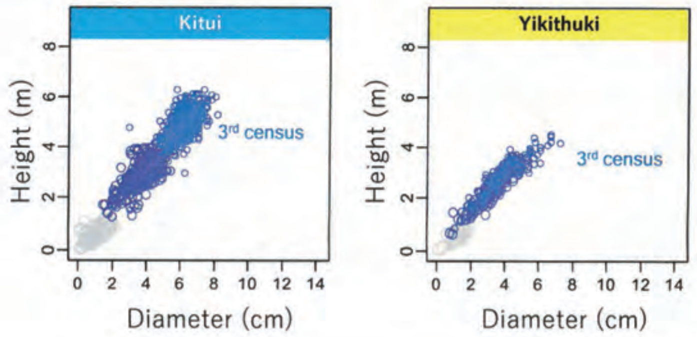
Checking the Height ~ Diameter relationship



Each circle indicates each planted stem.

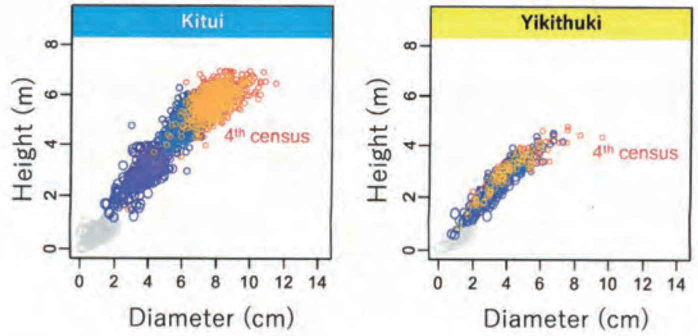
Checking the Height ~ Diameter relationship

About 1 years after planting



Each circle indicates each planted stem.

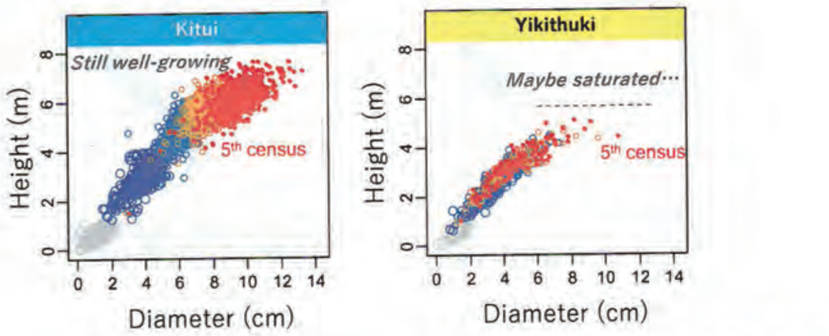
Checking the Height ~ Diameter relationship



Each circle indicates each planted stem.

Checking the Height ~ Diameter relationship

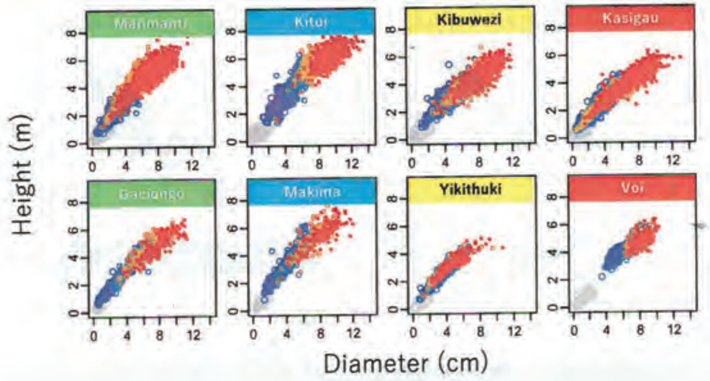
About 2 years after planting



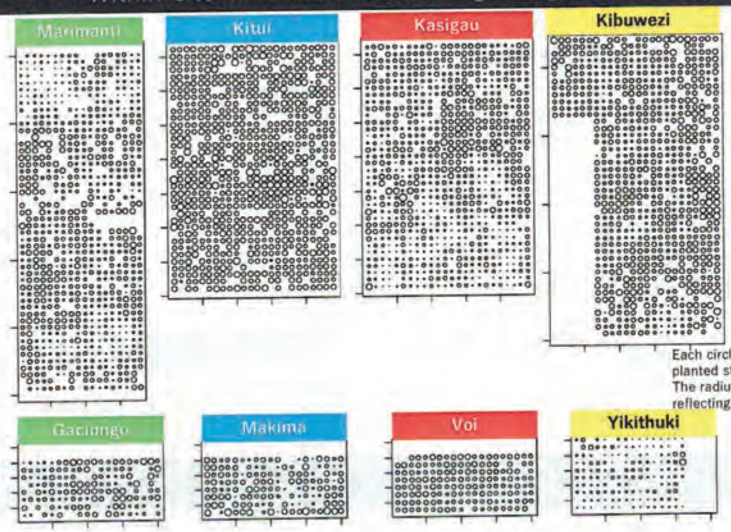
Each circle indicates each planted stem.

Checking the Height ~ Diameter relationship

There is a large growth variation between sites

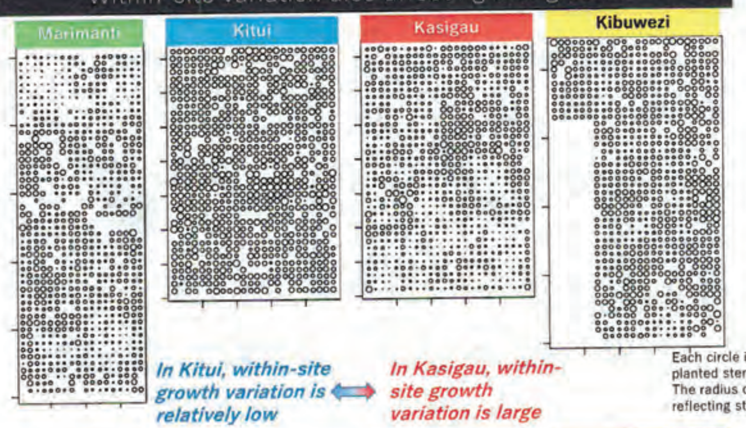


Within-site variation also affecting tree growth



Each circle indicates each planted stem.
The radius of circle is reflecting stem diameter.

Within-site variation also affecting tree growth

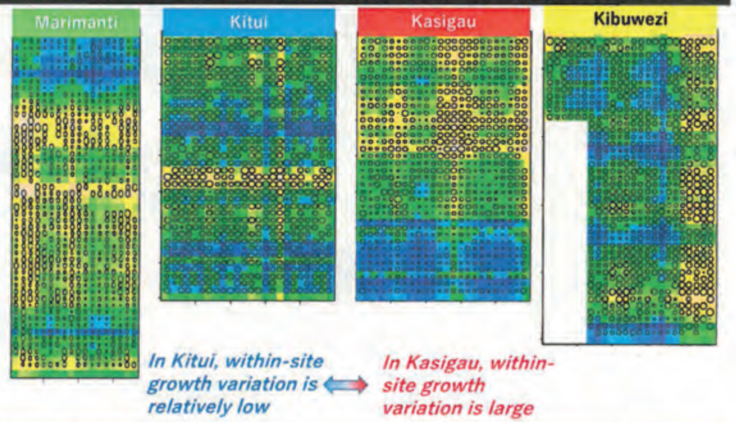


In Kitui, within-site growth variation is relatively low ⟺ *In Kasigau, within-site growth variation is large*

Each circle indicates each planted stem.
The radius of circle is reflecting stem diameter.

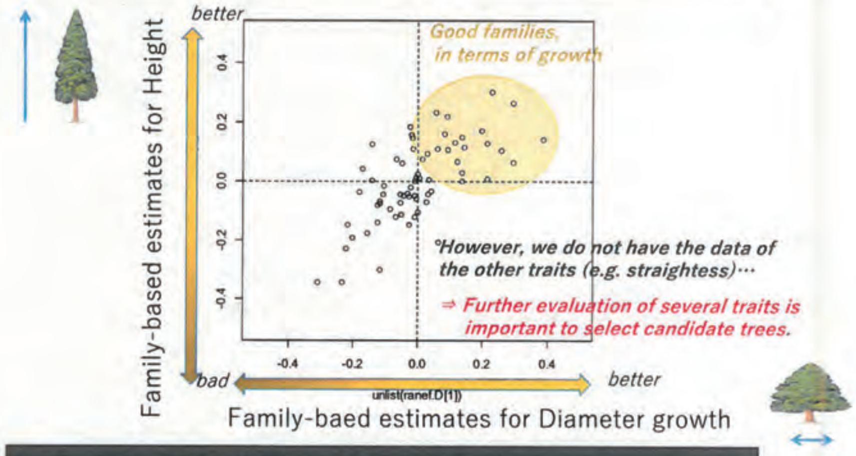
When evaluating the performances of families/progenies, we should treat carefully the within- and between-site environmental variation affecting growth.

Within-site variation also affecting tree growth

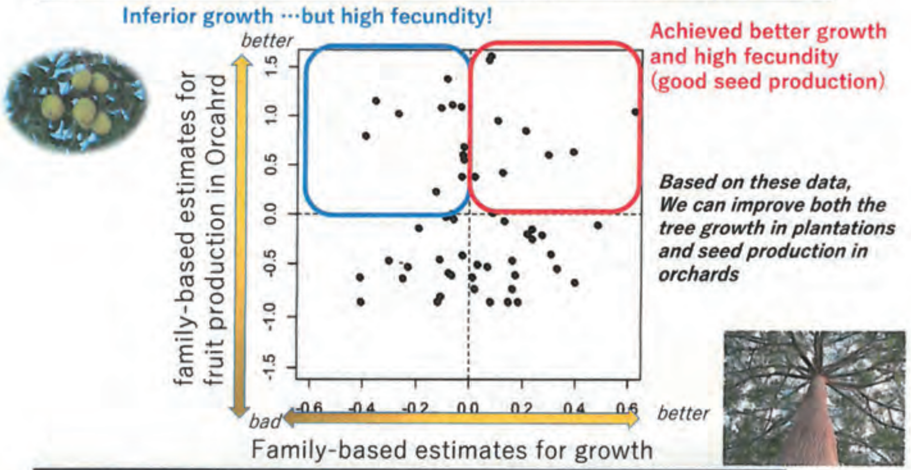


When evaluating the performances of families/progenies, we should treat carefully the within- and between-site environmental variation affecting growth.

Estimate the genetic performance in growth

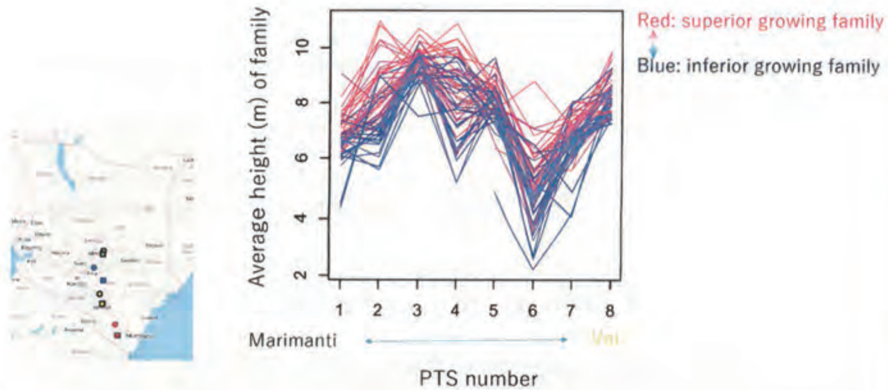


Estimate the genetic performance in Seed Production

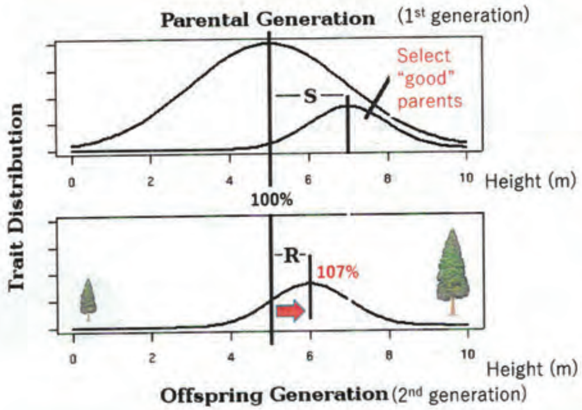


Growth performance across sites

Based on the statistical analysis…,
The progenies of "good" families always show faster growth.



Genetic improvement from 1st to 2nd generation



Percentage (%) of improvement per generation.

Height
107%

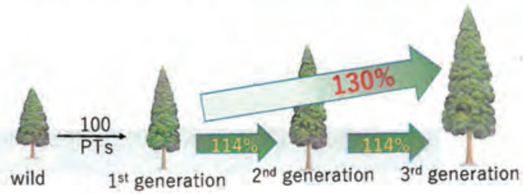
Diameter
104%

Stem volume
114%

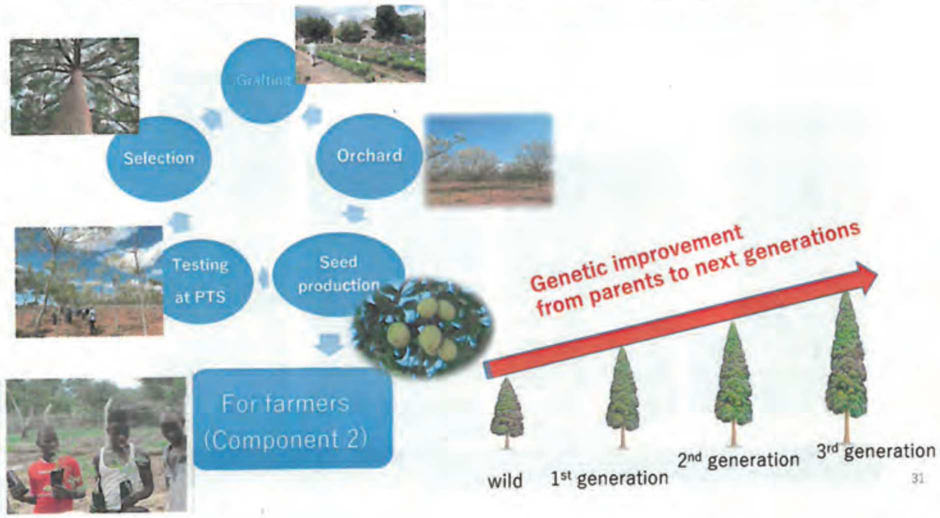
indicating…
improvement of
CO_2 fixation ability!

Summary

- **There was a significant growth variation among families, although there were large between- and within-site growth variation.**
- **Based on the breeding analysis, both performances of growth and seed production will be improved.**
- **The progenies of "good" families always show faster growth across sites.**
- **Genetic improvement per generation (from 1st to 2nd generation): volume 114%**



General cycle of tree breeding



Thank you for your listening

Appendix 5-3-4

Artificial Crossing/second generation

Field	Name	Term
Artificial Crossing	Dr. Michinari Matsushita	2018.12.9～12.15
Artificial Crossing	Dr. So Hanaoka	2018.12.9～12.16

1. Schedule

Date		Contents	Stay
9 Dec. (Sun.)		Move (to Haneda)	
10 Dec. (Mon.)		Move (Haneda - Doha – Nairobi) Move to Kitui	Kitui
11 Dec. (Tue.)		Observation of Melia seed orchard and PTS in Tiva Study on artificial crossing	Kitui
12 Dec. (Wed.)		Seminar on PC programme for statistical data analysis	Kitui
13 Dec. (Thu..)	AM PM	Seminar on PC programme for statistical data analysis Move to Nairobi	Nairobi
14 Dec. (Fri.)	AM PM	Meeting with CADEP experts Move to airport, move (Nairobi – Doha)	
15 Dec. (Sat.)		Move (Doha – Narita – Hitachi, for Matsushita)	Tokyo
16 Dec. (Sun.)		Move (Haneda – Sapporo, for Hanaoka)	

2. Results of major activities

2.1 Observation of Melia PTS in Tiva

On PTS assessment, it was confirmed that vent of stem and fruit production have been already taken as assessment matters since this year. These matters have been introduced in PTS investigation in Japan as well as growth, and it seems that KEFRI CPs are acquiring skills of evaluation method and purpose of PTS assessment.

2.2 Observation of Melia seed orchard in Tiva

(Melia seed orchard)

The Melia seed orchard was established in 2013 and started seed production in 2015. 3 times seed productions were implemented in 2016 and almost trees in the orchard could produce a lot of seeds. The amount of improved seed production from the orchard has been increasing year by year, however, it was found that several mother trees were not vigorous due to wood rot and grafting incompatibility. We discussed a future roadmap of tree breeding in Kenya with Mr. Kariuki, an assistant component manager, through considering the next generation and renewal of seed orchard on Melia, and suggested that 1 block of seed orchard would be improved by exchanging inferior clones to superior clones gradually as the result of discussion.

(Acacia seed stand)

It was found that a family A56 had superior traits for growth and straightness of stem, and variation between families was also identified. Evaluation of the families on Acacia should be conducted by data analysis as well as Melia.

2.3 Study on artificial crossing and mating system of Melia

KEFRI CPs have acquired the basic skills for artificial crossing of Melia such as collection of pollen, screening of pollen and actual artificial crossing technique through technical guidance from FTBC. Several young fruits were found on branches that artificial crossing was carried out on Oct. – Nov. 2018. Success rate of tested artificial crossing is not so high, however, this is a significant result for Meliaceae family because there could be no successful result for artificial crossing in this family, which includes mahogany known as expensive wood. DNA analysis will be carried out to verify the crossing.

As for study on mating system, sampling of material for DNA analysis will be continued for study on crossing system of Melia, which is conducted by Dr. Omondii.

2.4 Seminar on PC programme for statistical data analysis

Free data analysis software "R", which has been used in researchers worldwide for statistical analysis, making chart, programming and simulation etc., was introduced on this seminar as a programme to analyze the PTS assessment data efficiently. Free and general-purpose software for data analysis is required to strengthen the ability of researchers in developing countries. This seminar was planned in 2017 in accordance with our suggestion that a manual of usage of "R" programme and data analysis was needed for capacity building of KEFRI researchers, and the seminar and guidance were held in 2 days in Kitui regional centre with 8 participants.

(Contents of seminar)

The aim of seminar is to acquire skills how to install and use "R" and understand appropriate data control for statistical analysis, so this seminar can be regarded as "beginner" to "intermediate" level. On the first day, we introduced install method and basic operation of "R" on lecture and practice, and explained a method of data analysis and basic construction that are very useful in almost research activities. On the second day, a practical data analysis using actual PTS assessment data was carried out to strengthen data control ability on PTS assessment. An appropriate data format to use PTS assessment was introduced in the practice, and several analysis methods to analyze PTS assessment data were also introduced.

It was clear that the participants including Dr. Muturi took the lecture and practice in the seminar with great enthusiasm. In the next time on 2019, REML will be presented for more practical statistic data analysis on tree breeding as “higher” level.

3. Subjects

(Artificial Crossing and melting system)

It is a significant result that fruits production by artificial crossing was found on several branches. This is the first step to establish Melia artificial crossing technique. Flowering period for pollination is limited, so it is required to process artificial crossing at appropriate time. As for study on melting system of Melia, collecting seeds from more than 10 clones in seed orchard is needed to conduct DNA analysis for the study, and the analysis will start from Feb. 2019 when 30 seeds per 1 clone as samples will germinate successfully.

(Capacity building of statistical data analysis for tree breeding)

The seminar can be regarded as “beginner” to “intermediate” level, and REML method and concept of tree breeding will be introduced for more practical statistic data analysis on tree breeding as “higher” level on the next time 2019.

(Prospect of capacity building for tree breeding)

CPs research ability for investigation and data analysis of PTS is steadily advancing. Data on several traits of Melia such as stem straightness and wood property has been accumulated as well as data of the growth. Capacity building for data analysis based on PTS assessment will be conducted through lecture and practice in 2019.

Assessment of PTS at 5 years after planting on 2020 is considered as an evaluation time for selecting superior clones for second generation according to harvesting age 15 years on Melia. Therefore, a concept on a cycle of tree breeding can be introduced to KEFRI through the data analysis on 2020 and selection of superior clones, which is definitely based on selection of 1st generation, establishment of seed orchards and PTSs, and assessment of PTSs.

4. Contact persons

Dr. Muturi, Component Manager, KEFRI

Mr. Luvanda, Director, Kitui Regional Centre, KEFRI

Mr. Kariuki, Tree Breeding, KEFRI

Mr. Keiichi Takahata, Chief Advisor, CADEP

Ms. Yuki Honjo, Coordinator/Regional Cooperation, CADEP

Photo

Photo 1 PTS in Tiva

Photo 2 PTS in Tiva

Photo 3 Melia seed orchard in Tiva

Photo 4 Melia seed orchard in Tiva

Photo 5 Acacia seed stand in Tiva

Photo 6 Acacia seed stand

Photo 7 Observation of processing on artificial crossing

Photo 8 Observation of processing on artificial crossing

Photo 9 Observation of processing on artificial crossing

Photo 10 Seminar on statistical data analysis

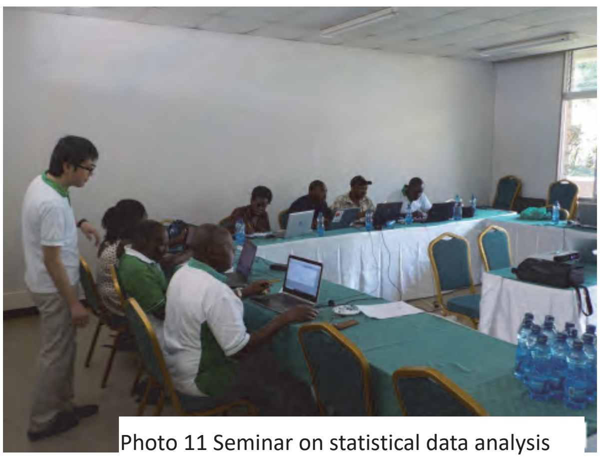


Photo 11 Seminar on statistical data analysis

Photo 12 Seminar on statistical data analysis

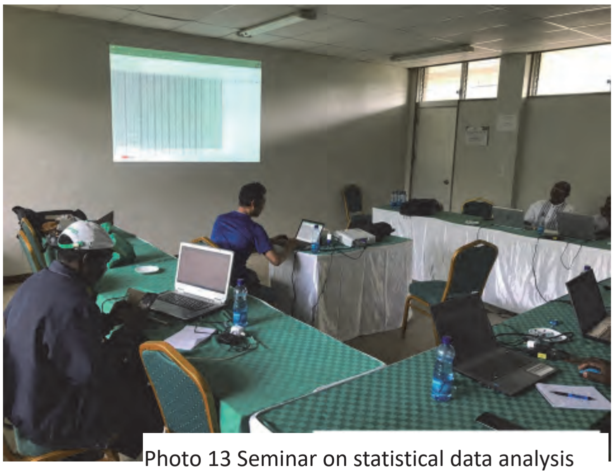


Photo 13 Seminar on statistical data analysis

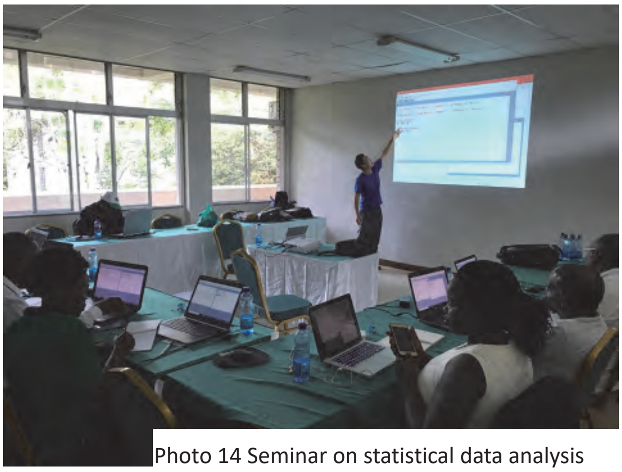


Photo 14 Seminar on statistical data analysis

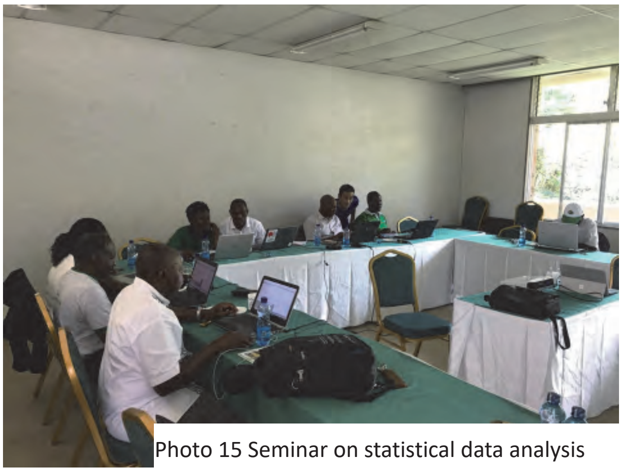


Photo 15 Seminar on statistical data analysis

Photo 16 Participants to the Seminar

Appendix 5-3-4-1

R training course for beginner

2018.12.11 in Kitui

So HANAOKA
Michinari MATSUSHITA

Forest Tree Breeding Center,
Forestry and Forest products Research Institute

Programs

- First Day:
  9:00~ Introduction
  9:30~ Basic operation of R
  Calculation, vector, data frame

  13:00~ Lunch time

  14:00~ Practical training
  Data handling, summary statistics, draw figure

What is R?

- R is free software environment for statistical computing and graphics.

What can R do??

What can R do?

- Example1: Caluculation

```
> 100+200
[1] 300
>
> 20*3
[1] 60
>
> 50/2
[1] 25
>
> (20+3)*2
[1] 46
>
```

You can use R as calculator.

What can R do?

- Example2: Data handling.

```
> head(data)
  sample height  dbh
1      1   12.1 13.7
2      2   13.4 13.8
3      3   13.5 14.4
4      4   13.5 14.6
5      5   14.0 15.0
6      6   14.2 15.1
>
> data$hd_ratio<- data$height / (data$dbh/100)
> head(data)
  sample height  dbh hd_ratio
1      1   12.1 13.7 88.32117
2      2   13.4 13.8 97.10145
3      3   13.5 14.4 93.75000
4      4   13.5 14.6 92.46575
5      5   14.0 15.0 93.33333
6      6   14.2 15.1 94.03974
>
```

The height – dbh ratio was calculated and the results were added to table

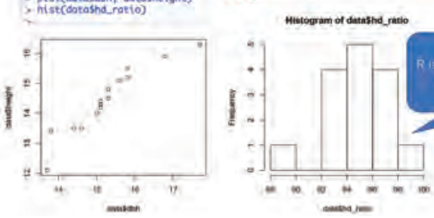
Batch-processing is easy.
Data handling is fast.

What can R do?

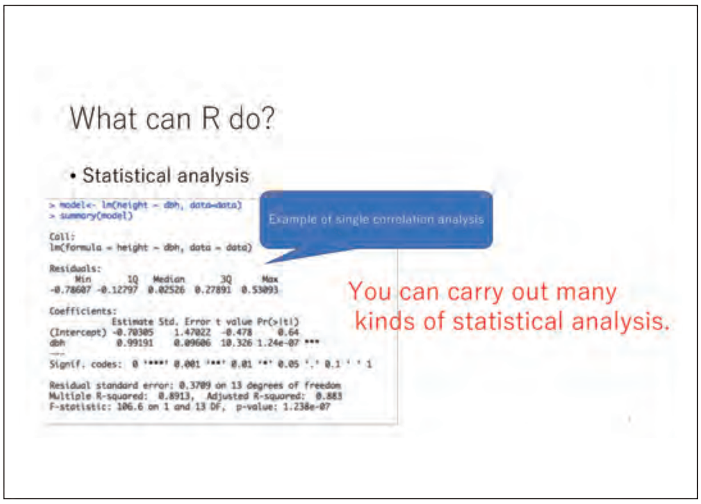
- Data visualization

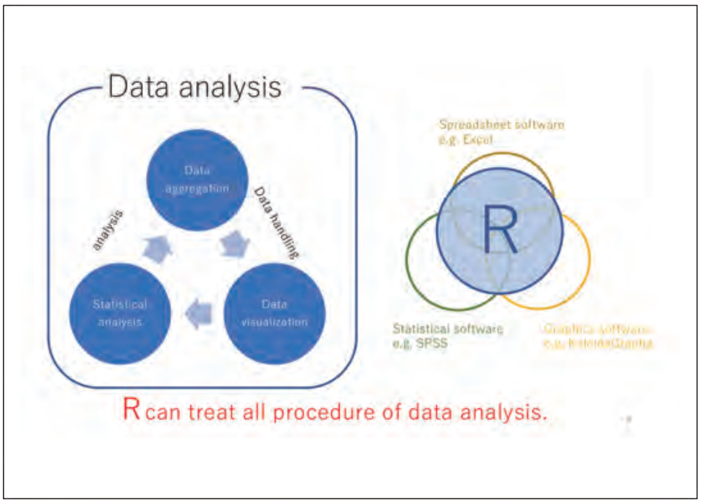
You can draw high-quality figures.

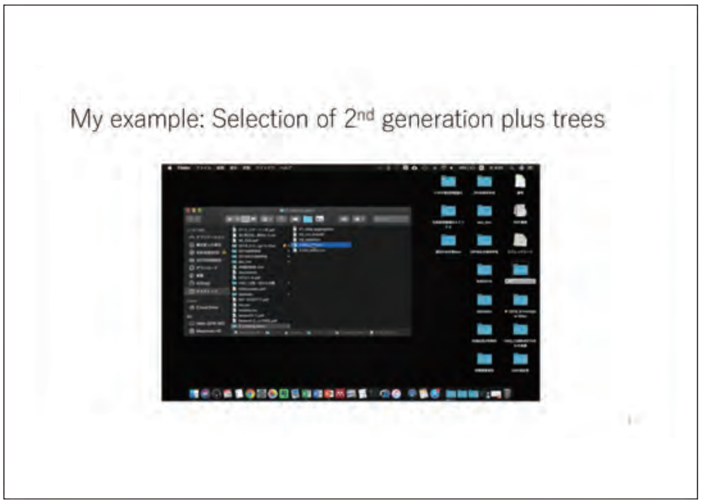
```
> par(mfrow=c(1,2))
> plot(data$dbh, data$height)
> hist(data$hd_ratio)
```

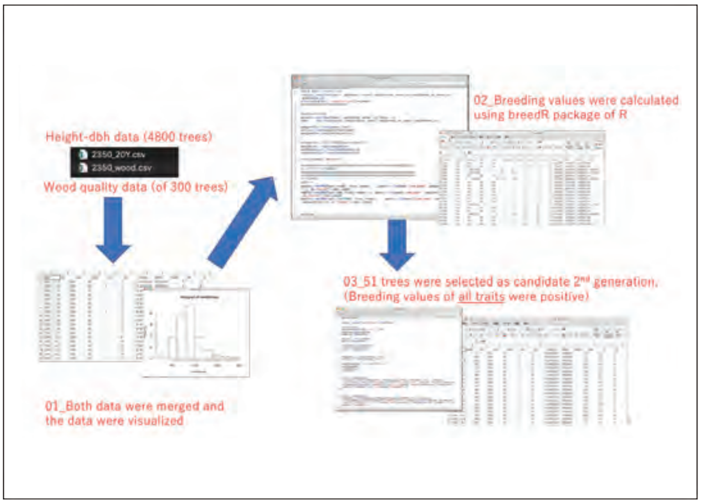


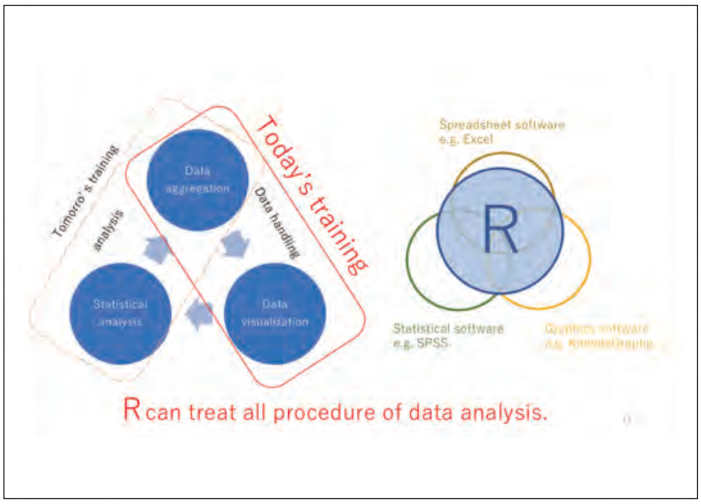
R is useful tool to visualize your data. Enough quality to write paper

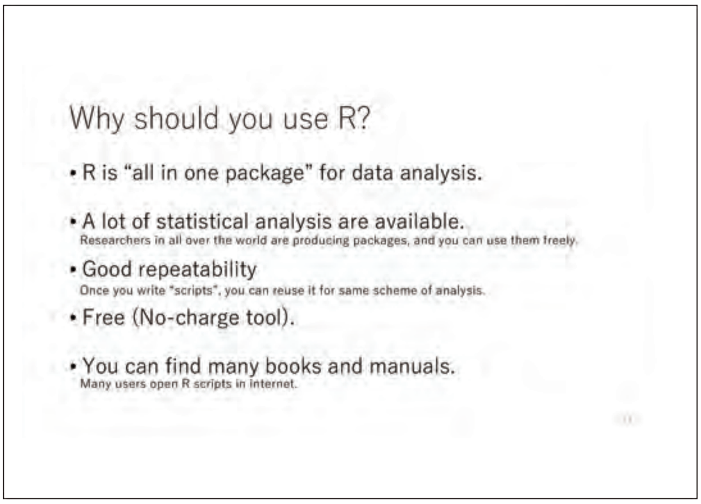

What can R do?
• Statistical analysis
> model<- lm(height ~ dbh, data=data)
> summary(model)
Call:
lm(formula = height ~ dbh, data = data)
Residuals:
Min 1Q Median 3Q Max
-0.78607 -0.12797 0.02526 0.27891 0.53093
Coefficients:
Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.70305 1.47022 -0.478 0.64
dbh 0.99191 0.09606 10.326 1.24e-07 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.3709 on 13 degrees of freedom
Multiple R-squared: 0.8913, Adjusted R-squared: 0.883
F-statistic: 106.6 on 1 and 13 DF, p-value: 1.238e-07
Example of single correlation analysis
You can carry out many kinds of statistical analysis.


Data analysis
Data aggregation
Data handling
Data visualization
Statistical analysis
analysis
Spreadsheet software
e.g. Excel
R
Statistical software
e.g. SPSS
R can treat all procedure of data analysis.


My example: Selection of 2nd generation plus trees


Height-dbh data (4800 trees)
2350_20Y.csv
2350_wood.csv
Wood quality data (of 300 trees)
01_Both data were merged and the data were visualized
02_Breeding values were calculated using breedR package of R
03_51 trees were selected as candidate 2nd generation.
(Breeding values of all traits were positive)


Tomorro's training
Today's training
Data aggregation
Data handling
analysis
Statistical analysis
Data visualization
Spreadsheet software
e.g. Excel
R
Statistical software
e.g. SPSS
R can treat all procedure of data analysis.


Why should you use R?
• R is "all in one package" for data analysis.
• A lot of statistical analysis are available.
Researchers in all over the world are producing packages, and you can use them freely.
• Good repeatability
Once you write "scripts", you can reuse it for same scheme of analysis.
• Free (No-charge tool).
• You can find many books and manuals.
Many users open R scripts in internet.

What is disadvantage of R?

- You have to learn R language.

It may be hard to learn R language by yourself.

Today's goals.

Today's goals are…

- to learn basic operation of R
- to learn R language
- to learn frequently used "functions"

Let's start training!

Please open R.

Double click this icon.

Let's use R!

This window is called "Console"

Let's calculate 1 + 2

```
> 1 + 2
[1] 3
>
```

Write 1 + 2 in console, then push enter key.

There are spaces, but R ignore these spaces.

```
> 1 + 2
[1] 3
>
```

Answer is shown here

"[1]" means "there is 1 answer".

Frequently used symbols for calculation

symbol	mean	Example	
+	addition	1 + 2	
^, **	power	2^3	(=2³)
-	substraction	5-3	
*	multiplication	2*4	(=2 × 4)
/	division	6/3	(=6 ÷ 3)

Most of them are same as excel.

$1 + 2 \times 3$

```
> 1+2*3
[1] 7
>
> 1+       #write 1+ then push enter key
+ 2*       #write 2* then push enter key
+ 3        #write 3  then  push enter key
[1] 7
>
```

If enter key was pushed in the middle of the formula, R does not show answer.

This "+" means that "this function is incomplete".

Sentences written after "#" are ignored.

Exercises

Please calculate using R!

$$\frac{100+30}{13}$$

$$3^4 + 2^3$$

$$\frac{(2^2 - 1) \times 4}{3}$$

Answers

Please calculate using R!

$$\frac{100+30}{13}$$

$$3^4 + 2^3$$

$$\frac{(2^2 - 1) \times 4}{3}$$

```
> (100+30)/13
[1] 10
>
> 3^4 + 2^3
[1] 89
>
> ((2^2 -1) * 4 )/ 3
[1] 4
>
```

Text editor

- Text editor (R editor) is useful to write and leave scripts.

File → New script

```
################
#use text editor
1 + 2

1+
2*
3
```

1)Write script in text editor

2)Activate row(s)

3) Right click and select "Run lines or selection" or "Ctl + R" is recommended.

Note: in the case of Mac OS, "command + enter"

Variable

If you use "<-", you can assign numeric and/or formula to "letter".

```
> x<- 1 + 3    #This means "x is 1 + 3"
>
> x
[1] 4
>
> x^2
[1] 16
>
> x*2
[1] 8
```

In this case, "x" is called variable
Note: R can recognize capital and small letter.

Calculation is possible using variable

```
> x<- 2
> x
[1] 2
>
> y<- 3
>
> x + y
[1] 5
> x * y
[1] 6
> x - y
[1] -1
>
```

x is overwritten here!

Vector

You can substitute multiple numeric to "letter" using "c()".

```
> x1<- c(1,2,3,4,5)
> x2<- c(1,3,5,7,9)
>
> x1 + x2
[1]  2  5  8 11 14
> x1 * x2
[1]  1  6 15 28 45
```

In this case,
x1 and x2 are called "vector"

Calculation is possible for vector

Frequently used functions for vector

Function	Mean	Example
mean()	Return mean	mean(x1)
sd()	Return standard deviation	sd(x1)
median()	Return median	median(x1)
min()	Return minimum value	max(x1)
max()	Return maximum value	max(x1)
sum()	Return summation	sum(x1)
range()	Return range	range(x1)

```
> mean(x1)
[1] 3
> sd(x1)
[1] 1.581139
> median(x1)
[1] 3
> min(x1)
[1] 1
> max(x1)
[1] 5
> sum(x1)
[1] 15
> range(x1)
[1] 1 5
```

If missing values (NA) are included, argument (na.rm=T) should be added.

```
> x3<- c(2,3,NA,5,6,7,9,NA,10)
> mean(x3)
[1] NA
> mean(x3, na.rm=T)
[1] 6
> median(x3)
[1] NA
> median(x3, na.rm=T)
[1] 6
>
```

Exercises

1. Please make two vectors named "x" including {1,2,3,4,5} and "y" including {2,4,NA,8,10}
2. Please calculate mean, median, summation of "x" and "y".
3. Please calculate x + y, x * y,

Answer

```
> x<- c(1,2,3,4,5)
> y<- c(2,4,NA,8,10)
>
> mean(x); mean(y)
[1] 3
[1] NA
> median(x); median(y)
[1] 3
[1] NA
> sum(x); sum(y)
[1] 15
[1] NA
>
> x+y; x*y
[1]  3  6 NA 12 15
[1]  2  8 NA 32 50
>
```

Data frame

- You can make "data frame" (table format)

```
> xydata<- data.frame(x1 = x, y1 = y )
> xydata
  x1 y1
1  1  2
2  2  4
3  3  6
4  4  8
5  5 10
>
```

Row names can be defined here

Access to data frame

```
> xydata[1,]
  x1 y1
1  1  2
> xydata[,1]
[1] 1 2 3 4 5
> xydata[,"x1"]
[1] 1 2 3 4 5
> xydata[,"y1"]
[1]  2  4  6  8 10
>
> xydata[1,2]
[1] 2
>
> xydata$x1
[1] 1 2 3 4 5
> xydata$y1
[1]  2  4  6  8 10
```

Access to "first" row in "xydata".

Access to "first" column in "xydata".

Access to column named "x1" in "xydata".

Access to row 1, column 2 in "xydata".

Access to row named "x1" in "xydata".

Access to row named "y1" in "xydata".

Calculation in data frame

```
> mean(xydata$x1)
[1] 3
> median(xydata$y1)
[1] 6
> xydata$x1 + xydata$y1
[1]  3  6  9 12 15
```

Mean of column "x1"

Median of column "y1"

x1 + y1

Calculation using "apply" function

"apply" function returns a vector obtained
by applying a function to margins of an array or matrix.

```
> apply(xydata, 1, mean)
[1] 1.5 3.0 4.5 6.0 7.5
> apply(xydata, 2, mean)
x1 y1
 3  6
```

Row direction

Function you want to apply.

Column direction

paste function can concatenate vectors after converting to character

You can add column "xy"

```
> xydata$xy <- paste(xydata$x, xydata$y, sep = "_")
> xydata
  x1 y1   xy
1  1  2  1_2
2  2  4  2_4
3  3  6  3_6
4  4  8  4_8
5  5 10 5_10
```

This function may be useful when original ID is created in data

Column's label can be changed.

```
> colnames(xydata)<- c("x", "y", "xy")
> xydata
  x  y   xy
1 1  2  1_2
2 2  4  2_4
3 3  6  3_6
4 4  8  4_8
5 5 10 5_10
>
> names(xydata)[2] <- "y1"
> xydata
  x y1   xy
1 1  2  1_2
2 2  4  2_4
3 3  6  3_6
4 4  8  4_8
5 5 10 5_10
>
> names(xydata)[ which(names(xydata) == "y1")] <- "y"
> xydata
  x  y   xy
1 1  2  1_2
2 2  4  2_4
3 3  6  3_6
4 4  8  4_8
5 5 10 5_10
```

All column's label were changed

A label in column 2 was changed to "y1"

A label named "y1" was changed to "y"

Let's start practical training

- Please create "New Folder" on the desktop
- "File" → "Change directory" → select "New Folder"
  R can specify directory to save and read files.
- Example data set "iris" is used in this training course.

How to save(export) and read (import) data?

```
data(iris)     #open example data
help(iris)
write.csv(iris, "irisdata.csv", row.names = F)     #save example data
irisdata<- read.csv("irisdata.csv")     #read data file
head(irisdata)   #check first six rows
```

Data frame

File name is shown here

File name you want to read

Data format

- R can not open Excel (.xlsx) files.
- csv format (comma-separated format) is recommended to treat data in R.

	A	B	C	D	E
1	Sepal.Length	Sepal.Width	Petal.Length	Petal.Width	Species
2	5.1	3.5	1.4	0.2	setosa
3	4.9	3	1.4	0.2	setosa
4	4.7	3.2	1.3	0.2	setosa
5	4.6	3.1	1.5	0.2	setosa
6	5	3.6	1.4	0.2	setosa
7	5.4	3.9	1.7	0.4	setosa
8	4.6	3.4	1.4	0.3	setosa
9	5	3.4	1.5	0.2	setosa
10	4.4	2.9	1.4	0.2	setosa
11	4.9	3.1	1.5	0.1	setosa
12	5.4	3.7	1.5	0.2	setosa

Note:
Excel does not show comma

iris.csv

```
"Sepal.Length","Sepal.Width","Petal.Length","Petal.Width","Species"
5.1,3.5,1.4,0.2,"setosa"
4.9,3,1.4,0.2,"setosa"
4.7,3.2,1.3,0.2,"setosa"
4.6,3.1,1.5,0.2,"setosa"
5,3.6,1.4,0.2,"setosa"
5.4,3.9,1.7,0.4,"setosa"
4.6,3.4,1.4,0.3,"setosa"
5,3.4,1.5,0.2,"setosa"
4.4,2.9,1.4,0.2,"setosa"
4.9,3.1,1.5,0.1,"setosa"
5.4,3.7,1.5,0.2,"setosa"
4.8,3.4,1.6,0.2,"setosa"
4.8,3,1.4,0.1,"setosa"
```

Check data

```
> head(irisdata)   #check first six rows
  Sepal.Length Sepal.Width Petal.Length Petal.Width Species
1          5.1         3.5          1.4         0.2  setosa
2          4.9         3.0          1.4         0.2  setosa
3          4.7         3.2          1.3         0.2  setosa
4          4.6         3.1          1.5         0.2  setosa
5          5.0         3.6          1.4         0.2  setosa
6          5.4         3.9          1.7         0.4  setosa
>
```

head() shows first six rows.
head(df, n) shows first n rows.
Note: df is data frame

Five components are included.
- Sepal length
- Sepal width
- Petal length
- Petal width
- Species

- Check data structure
  How many rows?
  How many columns?

```
> nrow(irisdata)        # num. of row
[1] 150
> ncol(irisdata)        # num. of column
[1] 5
> dim(irisdata)         # num. of row and column
[1] 150    5
```

Summary staitistics

```
> summary(irisdata)    #summary statistics
  Sepal.Length    Sepal.Width     Petal.Length    Petal.Width          Species
 Min.   :4.300   Min.   :2.000   Min.   :1.000   Min.   :0.100   setosa    :50
 1st Qu.:5.100   1st Qu.:2.800   1st Qu.:1.600   1st Qu.:0.300   versicolor:50
 Median :5.800   Median :3.000   Median :4.350   Median :1.300   virginica :50
 Mean   :5.843   Mean   :3.057   Mean   :3.758   Mean   :1.199
 3rd Qu.:6.400   3rd Qu.:3.300   3rd Qu.:5.100   3rd Qu.:1.800
 Max.   :7.900   Max.   :4.400   Max.   :6.900   Max.   :2.500
>
```

Minimum, maximum, 1st and 3rd Quartile, mean, median are automatically calculated.

1st quartile: a value of 25% from top
3rd quartile: a value of 75% from bottom

Ratio of sepal length and sepal width are calculated and the results are binded to table

Original data frame / Added row(vector) / formula

```
> irisdata$ratio_sepal<- irisdata$Sepal.Length / irisdata$Sepal.Width
> head(irisdata)
  Sepal.Length Sepal.Width Petal.Length Petal.Width Species ratio_sepal
1          5.1         3.5          1.4         0.2  setosa    1.457143
2          4.9         3.0          1.4         0.2  setosa    1.633333
3          4.7         3.2          1.3         0.2  setosa    1.468750
4          4.6         3.1          1.5         0.2  setosa    1.483871
5          5.0         3.6          1.4         0.2  setosa    1.388889
6          5.4         3.9          1.7         0.4  setosa    1.384615
```

This row was added

Exercise

- Please calculate and combine "ratio_petal" (petal length/petal width).
- Check first six rows.

```
> irisdata$ratio_petal<- irisdata$Petal.Length / irisdata$Petal.Width
> head(irisdata)
  Sepal.Length Sepal.Width Petal.Length Petal.Width Species ratio_sepal ratio_petal
1          5.1         3.5          1.4         0.2  setosa    1.457143        7.00
2          4.9         3.0          1.4         0.2  setosa    1.633333        7.00
3          4.7         3.2          1.3         0.2  setosa    1.468750        6.50
4          4.6         3.1          1.5         0.2  setosa    1.483871        7.50
5          5.0         3.6          1.4         0.2  setosa    1.388889        7.00
6          5.4         3.9          1.7         0.4  setosa    1.384615        4.25
```

Check mean value of each species.

- tapply function is available.

Data you want to calculate / List of factors / Function you want to apply

```
> tapply(irisdata$Sepal.Length, irisdata$Species, mean)
   setosa versicolor  virginica
    5.006      5.936      6.588
> tapply(irisdata$Sepal.Length, irisdata$Species, sd)
   setosa versicolor  virginica
0.3524897  0.5161711  0.6358796
```

How to extract data?

subset() function is used to extract data which satisfy conditional expression

Conditional expression

```
> setosadata<- subset(irisdata, irisdata$Species == "setosa")
> summary(setosadata)
  Sepal.Length    Sepal.Width     Petal.Length    Petal.Width          Species
 Min.   :4.300   Min.   :2.300   Min.   :1.000   Min.   :0.100   setosa    :50
 1st Qu.:4.800   1st Qu.:3.200   1st Qu.:1.400   1st Qu.:0.200   versicolor: 0
 Median :5.000   Median :3.400   Median :1.500   Median :0.200   virginica : 0
 Mean   :5.006   Mean   :3.428   Mean   :1.462   Mean   :0.246
 3rd Qu.:5.200   3rd Qu.:3.675   3rd Qu.:1.575   3rd Qu.:0.300
 Max.   :5.800   Max.   :4.400   Max.   :1.900   Max.   :0.600
  ratio_sepal     ratio_petal
 Min.   :1.268   Min.   : 2.667
 1st Qu.:1.386   1st Qu.: 4.688
 Median :1.463   Median : 7.000
 Mean   :1.470   Mean   : 6.908
 3rd Qu.:1.541   3rd Qu.: 7.500
 Max.   :1.957   Max.   :15.000
```

Only setosa is extracted.

Multiple patterns

```
#remove setosa
# "!=" means "is not equal to"
nosetosa<- subset(irisdata, irisdata$Species != "setosa")
summary(nosetosa)

# "|" mens "or"
nosetosa2<- subset(irisdata, irisdata$Species == "versicolor" | irisdata$Species == "virginica")
summary(nosetosa2)
```

symbol	mean
==	equal
!=	is not equal
&	and
|	or

Multiple condition

```
#small sepal is removed from data
irisdata2<- subset(irisdata, irisdata$Sepal.Length > 5.1)
dim(irisdata2)

#multiple condition
irisdata3<- subset(irisdata, irisdata$Sepal.Length > 5.1 & irisdata$Petal.Length < 5.1)

dim(irisdata3)
irisdata3  #check all data
tapply(irisdata3$Species, irisdata3$Species, length) # count each species
```

Sort

```
#check data
head(setosadata)

#"order()" function return ranking of each data
order(setosadata$Sepal.Length)

#You can sort data following ranking
setosa2<- setosadata[order(setosadata$Sepal.Length) , ]

setosa3<- setosadata[order(setosadata$Sepal.Length, decreasing = T) , ]

head(setosa2)   ascending order
head(setosa3)   descending order
```

Sort: multiple condition

```
setosa4<- setosadata[order(setosadata$Sepal.Length, setosadata$Petal.Length, decreasing = T) , ]
setosa4
```

	Sepal.Length	Sepal.Width	Petal.Length	Petal.Width	Species	ratio_sepal	ratio_petal
15	5.8	4.0	1.2	0.2	setosa	1.450000	6.000000
19	5.7	3.8	1.7	0.3	setosa	1.500000	5.666667
16	5.7	4.4	1.5	0.4	setosa	1.295455	3.750000
34	5.5	4.2	1.4	0.2	setosa	1.309524	7.000000
37	5.5	3.5	1.3	0.2	setosa	1.571429	6.500000
6	5.4	3.9	1.7	0.4	setosa	1.384615	4.250000
21	5.4	3.4	1.7	0.2	setosa	1.588235	8.500000
11	5.4	3.7	1.5	0.2	setosa	1.459459	7.500000
32	5.4	3.4	1.5	0.4	setosa	1.588235	3.750000
17	5.4	3.9	1.3	0.4	setosa	1.384615	3.250000
49	5.3	3.7	1.5	0.2	setosa	1.432432	7.500000
28	5.2	3.5	1.5	0.2	setosa	1.485714	7.500000
33	5.2	4.1	1.5	0.1	setosa	1.268293	15.000000

Merge function

Sample ID was added to data frame.
n : m create numbers from n to m.
In this case, maximum number was
Set as number of rows.

Preparation of example data

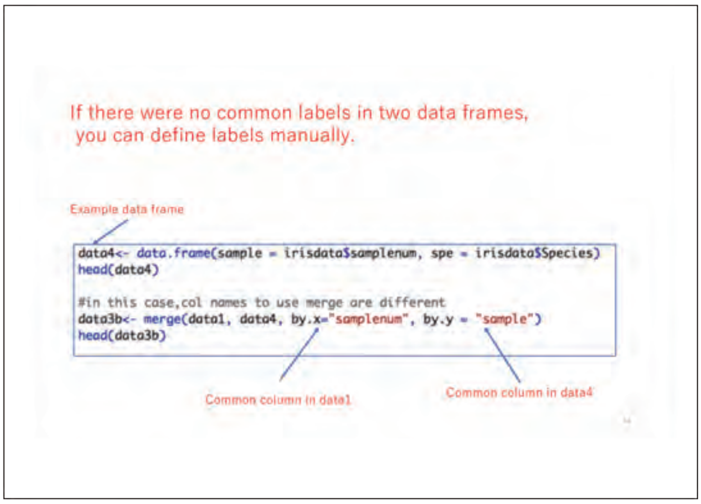
```
head(irisdata)
irisdata$samplenum<- 1:nrow(irisdata)
head(irisdata)

data1<- data.frame(samplenum = irisdata$samplenum, sepal_length = irisdata$Sepal.Length,
                   petal_length = irisdata$Petal.Length)
data2<- data.frame(samplenum = irisdata$samplenum, species = irisdata$Species)

head(data1)
head(data2)
```

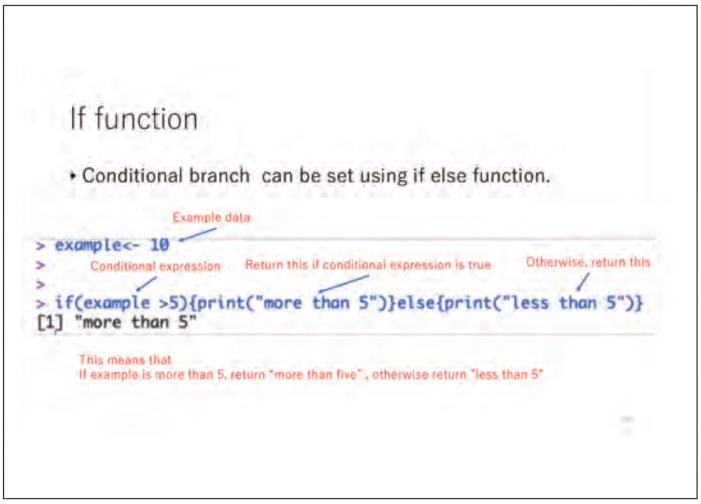
You can merge data1 and data2 easily.

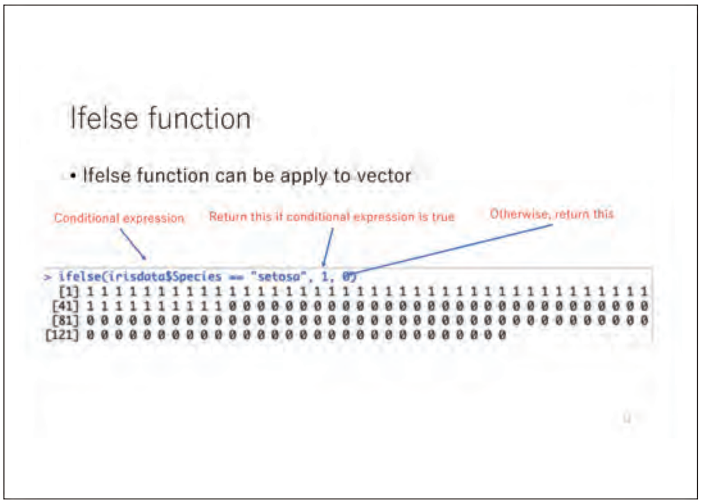
Common column is recognized automatically and
two data frames (data1 and data2) were merged.
In this example, "samplenum" was included in both data.

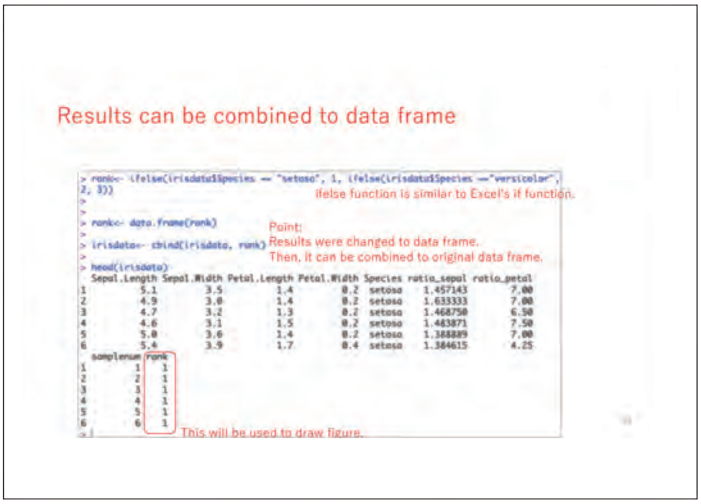
```
> data3<- merge(data1, data2, all = T)
> head(data3)
  samplenum sepal_length petal_length species
1         1          5.1          1.4  setosa
2         2          4.9          1.4  setosa
3         3          4.7          1.3  setosa
4         4          4.6          1.5  setosa
5         5          5.0          1.4  setosa
6         6          5.4          1.7  setosa
> tail(data3)
    samplenum sepal_length petal_length   species
145       145          6.7          5.7 virginica
146       146          6.7          5.2 virginica
147       147          6.3          5.0 virginica
148       148          6.5          5.2 virginica
149       149          6.2          5.4 virginica
150       150          5.9          5.1 virginica
```

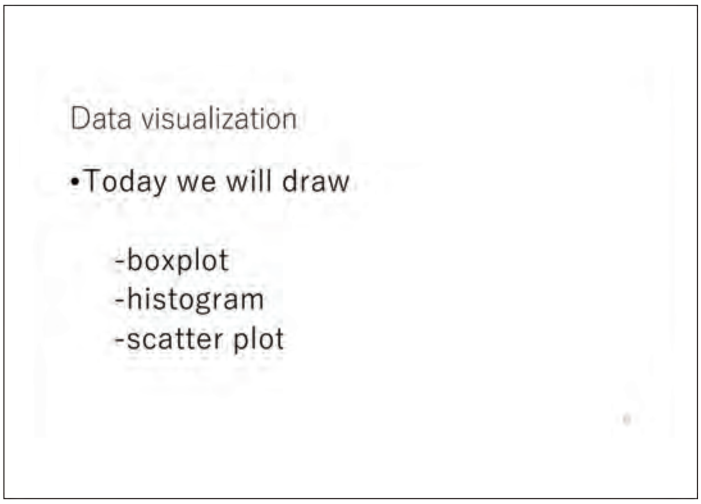
tail() shows last 6 rows.

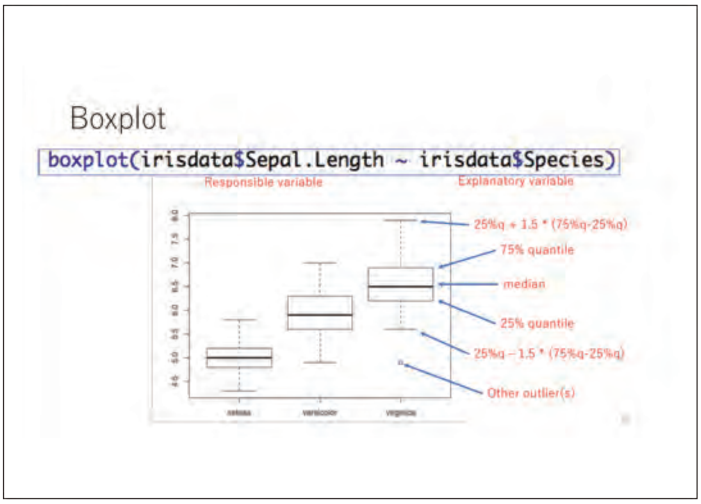

If there were no common labels in two data frames,
you can define labels manually.
Example data frame
data4<- data.frame(sample = irisdata$samplenum, spe = irisdata$Species)
head(data4)
#in this case,col names to use merge are different
data3b<- merge(data1, data4, by.x="samplenum", by.y = "sample")
head(data3b)
Common column in data1
Common column in data4


If function
Conditional branch can be set using if else function.
Example data
> example<- 10
>
>
Conditional expression
Return this if conditional expression is true
Otherwise, return this
> if(example >5){print("more than 5")}else{print("less than 5")}
[1] "more than 5"
This means that
If example is more than 5, return "more than five", otherwise return "less than 5"


Ifelse function
Ifelse function can be apply to vector
Conditional expression
Return this if conditional expression is true
Otherwise, return this
> ifelse(irisdata$Species == "setosa", 1, 0)
[1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
[41] 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
[81] 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
[121] 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0


Results can be combined to data frame
> rank<- ifelse(irisdata$Species == "setosa", 1, ifelse(irisdata$Species =="versicolor", 2, 3))
ifelse function is similar to Excel's if function.
> rank<- data.frame(rank)
> irisdata<- cbind(irisdata, rank)
Point:
Results were changed to data frame.
Then, it can be combined to original data frame.
> head(irisdata)
Sepal.Length Sepal.Width Petal.Length Petal.Width Species ratio_sepal ratio_petal
1 5.1 3.5 1.4 0.2 setosa 1.457143 7.00
2 4.9 3.0 1.4 0.2 setosa 1.633333 7.00
3 4.7 3.2 1.3 0.2 setosa 1.468750 6.50
4 4.6 3.1 1.5 0.2 setosa 1.483871 7.50
5 5.0 3.6 1.4 0.2 setosa 1.388889 7.00
6 5.4 3.9 1.7 0.4 setosa 1.384615 4.25
samplenum rank
1 1 1
2 2 1
3 3 1
4 4 1
5 5 1
6 6 1
This will be used to draw figure.


Data visualization
Today we will draw
-boxplot
-histogram
-scatter plot


Boxplot
boxplot(irisdata$Sepal.Length ~ irisdata$Species)
Responsible variable
Explanatory variable
25%q + 1.5 * (75%q-25%q)
75% quantile
median
25% quantile
25%q – 1.5 * (75%q-25%q)
Other outlier(s)

Check other traits

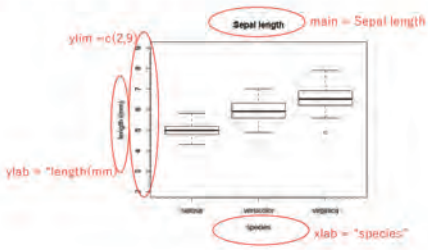
```
boxplot(irisdata$Sepal.Width ~ irisdata$Species)
boxplot(irisdata$Petal.Length ~ irisdata$Species)
boxplot(irisdata$Petal.Width ~ irisdata$Species)
```

arguments

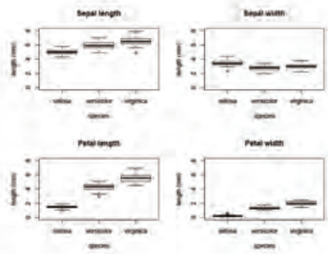
- Please check help file of boxplot (?boxplot)

You can set many arguments

```
#argument
boxplot(irisdata$Sepal.Length ~ irisdata$Species, main = "Sepal length", ylim =c(2,9),xlab =
 "species", ylab = "length (mm)")
```



Layout



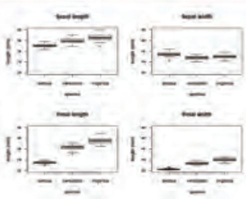
If you want to make n x m layout

par(mfrow=c(n.m)) is used

n: num. of rows
m: num. of. columns

```
xl<- "species"
yl<- "length (mm)"
ylm<- c(0,8)

par(mfrow=c(2,2))
boxplot(irisdata$Sepal.Length ~ irisdata$Species, main = "Sepal length", ylim =ylm, xlab = xl, ylab = yl)
boxplot(irisdata$Sepal.Width ~ irisdata$Species, main = "Sepal width", ylim =ylm, xlab = xl, ylab = yl)
boxplot(irisdata$Petal.Length ~ irisdata$Species, main = "Petal length" , ylim =ylm, xlab = xl, ylab = yl)
boxplot(irisdata$Petal.Width ~ irisdata$Species, main = "Petal width" , ylim =ylm, xlab = xl, ylab = yl)
```

Common arguments were defined here



Please extract data in each species

```
dseto<- subset(irisdata, irisdata$Species == "setosa")
dvers<-  subset(irisdata, irisdata$Species == "versicolor")
dvirg<-  subset(irisdata, irisdata$Species == "virginica")

head(dseto); head(dvers); head(dvirg)
```

histogram

- hist() function is used.

$A \leq B < C$

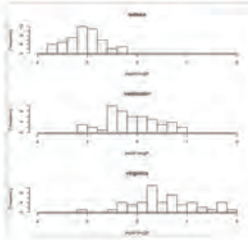
```
hist(dseto$Sepal.Length)
hist(dseto$Sepal.Length, right = T)
```

$A < B \leq C$

Please check difference!

```
par(mfrow=c(3,1))
hist(dseto$Sepal.Length, seq(4,8,0.2), right = T, main = "setosa", xlab = "sepal length")
hist(dvers$Sepal.Length, seq(4,8,0.2), right = T, main = "versicolor", xlab = "sepal length")
hist(dvirg$Sepal.Length, seq(4,8,0.2), right = T, main = "virginica", xlab = "sepal length")
```

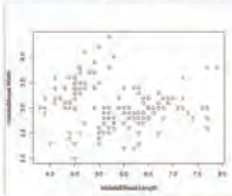
Range of x-axis is from4 to 8
Range of each class is 0.2



Scatter plot

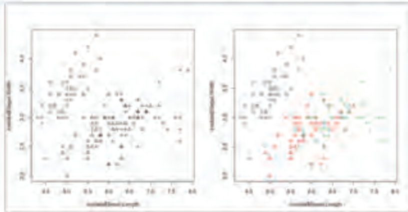
- plot() is used.

```
plot(irisdata$Sepal.Length , irisdata$Sepal.Width )
```



arguments: pch= change symbol, col= change color

```
par(mfrow=c(1,2))
plot(irisdata$Sepal.Length , irisdata$Sepal.Width, pch = irisdata$speciesnum)

plot(irisdata$Sepal.Length , irisdata$Sepal.Width, pch = irisdata$speciesnum, col = irisdata$speciesnum)
```



```
#Let's check pch and color!
xx<- 1:20
yy<- rep(1,20)
plot(xx,yy, col=xx, pch=xx)
```

Legend

- Legend can be added to figure.

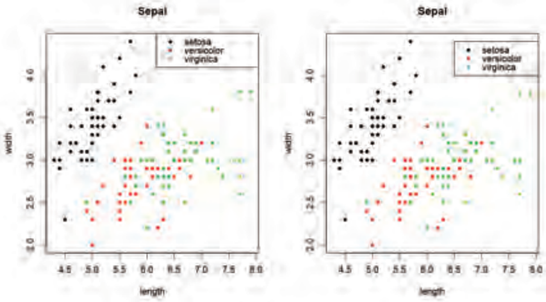
```
par(mfrow =c(1,2))

plot(irisdata$Sepal.Length , irisdata$Sepal.Width, col = irisdata$speciesnum, pch = 16, xlab="length", ylab = "width",
main = "Sepal")
legend("topright", legend = c("setosa","versicolor","virginica"), col = c(1,2,3), pch = 16)

#position of legend is defined as (x,y) coordinate
plot(irisdata$Sepal.Length , irisdata$Sepal.Width, col = irisdata$speciesnum, pch = 16, xlab="length", ylab = "width",
main = "Sepal")
legend(6.5,4.4, legend = c("setosa","versicolor","virginica"), col = c(1,2,3), pch = 16)
```

Position of legend's top-left
(x,y) = (6.5, 4.4)

legends

Color
1, 2, 3

Symbol
All are 16



Reviews of today's training

- Today we did
  - calculations using R
  - vector and data frame operations
  - creation of figures

Reviews of today's training

- Data are treated as vector and/or data frame.
- Calculation is possible for vector and data frame.
  Calculated results can be combined for data frame.
- R can draw many kinds of figures.
  You can edit them if you add arguments in scripts.

Thank you very much

Appendix 5-3-4-2

R training course
-medium level class-

2018.12.13 in Kitui

Michinari MATSUSHITA
So HANAOKA

Forest Tree Breeding Cetenr,
Forestry and Forest products Research Institute

- Second day [Medium-level class = practical course]
  - Let's start practice…
    using the "real" PTS data of Kenya!
  - Today's topics …
    0) INTRODUCTION
    1) learn what is better way to manage data of PTS (e.g. Excel seat).
    2) draw informative figures to show the breeding results.
    3) learn the way (coding) to run basic statistical analyses in R.

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INTRODUCTION: Remember…
presentation of Breeding results for CADEP meeting Jun.2018

Informative figures are important to show the impact of Breeding

Jun. 2018 @Kenya

Joint Meeting for CADEP

Component 4: Tree Breeding

-Current results of Breeding of *Melia volkensii*-

Dr. Michinari Matsushita (Forestry and Forest Product Research Iinstitute, FTBC, Japan)

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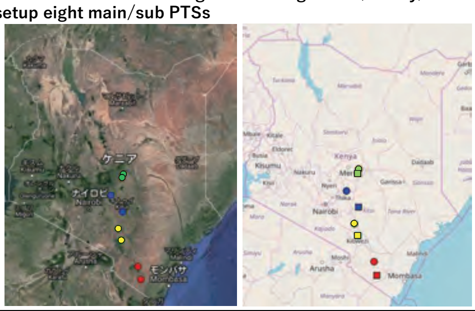
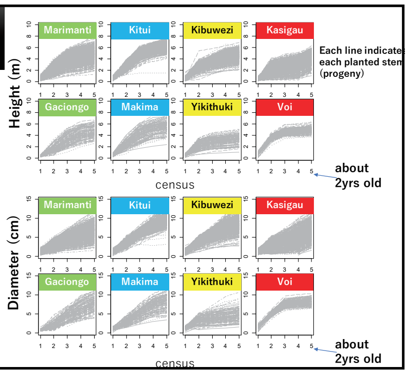
4 main-PTSs & 4 sub-PTSs were setup

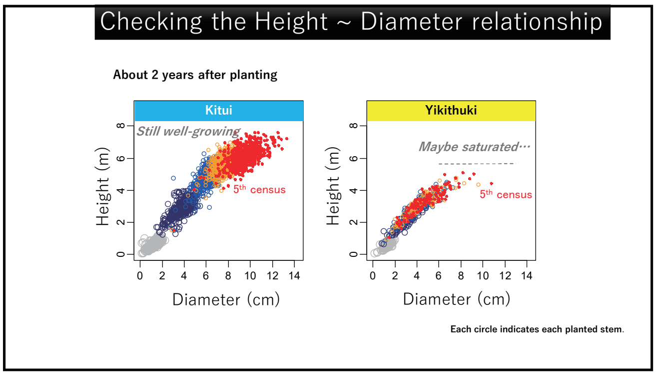
Based on the climate data & genetic background (history) of *Melia*, we setup eight main/sub PTSs

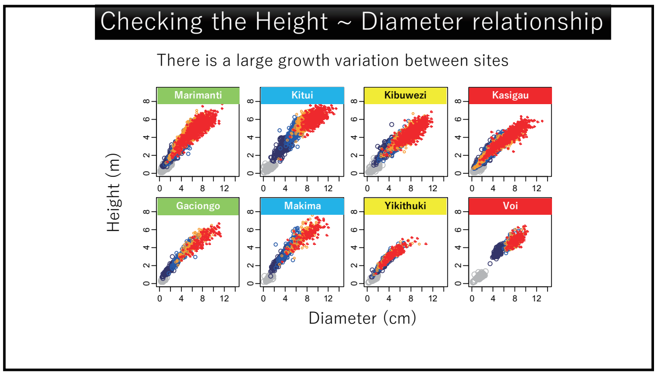


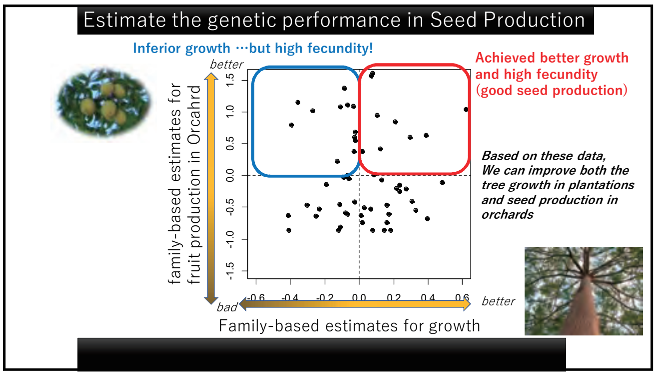
Checking the growth trend of progenies

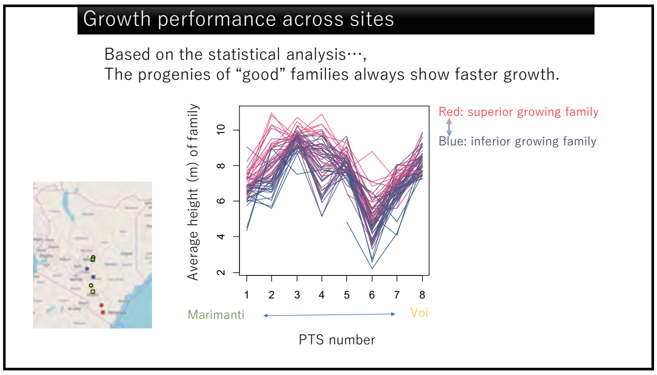
Large between-sites variation in growth trends of progenies

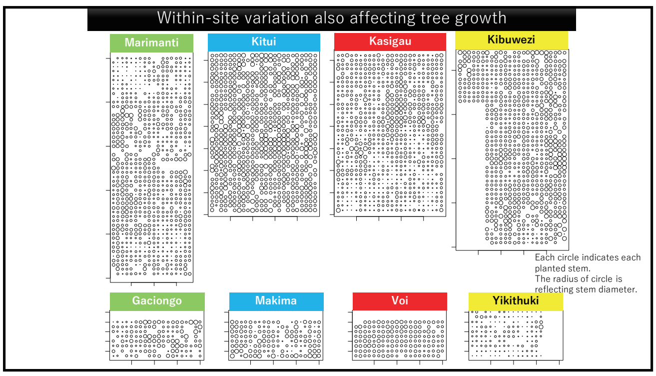


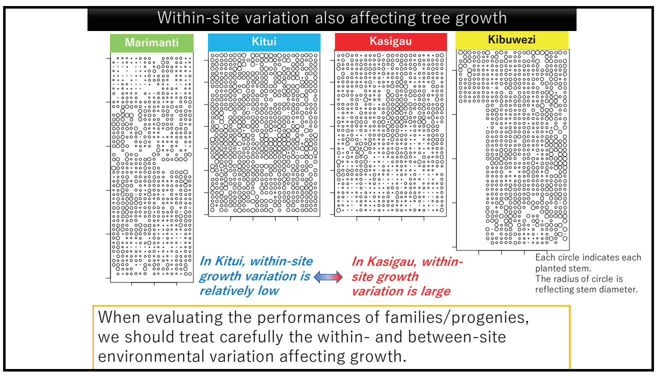

Checking the Height ~ Diameter relationship
About 2 years after planting
Kitui
Still well-growing
5th census
Yikithuki
Maybe saturated…
5th census
Height (m)
Diameter (cm)
Each circle indicates each planted stem.


Checking the Height ~ Diameter relationship
There is a large growth variation between sites
Marimanti
Kitui
Kibuwezi
Kasigau
Gaciongo
Makima
Yikithuki
Voi
Height (m)
Diameter (cm)

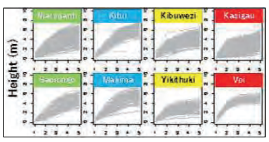

Estimate the genetic performance in Seed Production
Inferior growth …but high fecundity!
Achieved better growth
and high fecundity
(good seed production)
Based on these data,
We can improve both the
tree growth in plantations
and seed production in
orchards
better
bad
better
family-based estimates for
fruit production in Orcahrd
Family-based estimates for growth

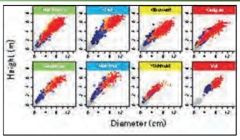

Growth performance across sites
Based on the statistical analysis…,
The progenies of "good" families always show faster growth.
Red: superior growing family
Blue: inferior growing family
Average height (m) of family
Marimanti
Voi
PTS number

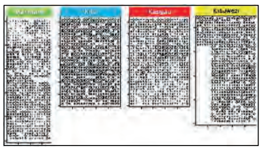

Within-site variation also affecting tree growth
Marimanti
Kitui
Kasigau
Kibuwezi
Each circle indicates each planted stem.
The radius of circle is reflecting stem diameter.
Gaciongo
Makima
Voi
Yikithuki


Within-site variation also affecting tree growth
Marimanti
Kitui
Kasigau
Kibuwezi
In Kitui, within-site growth variation is relatively low
In Kasigau, within-site growth variation is large
Each circle indicates each planted stem.
The radius of circle is reflecting stem diameter.
When evaluating the performances of families/progenies,
we should treat carefully the within- and between-site
environmental variation affecting growth.

Let's learn the R coding to draw figures







You can draw informative figures easily and freely by using R!

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• Second day [Medium-level class = practical course]

・Let's start practice…
using the "real" PTS data of Kenya!

・ Today's topics …

0) INTRODUCTION
1) learn what is better way to manage data of PTS (e.g. Excel seat).
2) draw informative figures to show the breeding results.
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But… first you need import data into R.

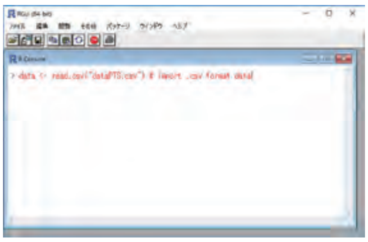
Remember . . .
How to read (import) data ?

Answer!
use the functions…

read.csv("dataPTS1.csv")

Or

read.delim("clipboard")



First you must copy the target region of data in Excel,
If you want to use the function read.delim()

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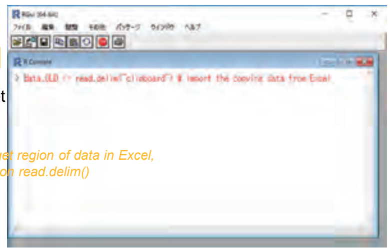
But… first you need import data into R.

Lets use the function

read.delim("clipboard")

We treat example dataset of Kitui PTS



First you must copy the target region of data in Excel,
If you want to use the function read.delim()

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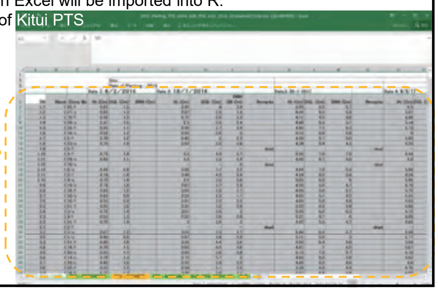
Before using the function "read.delim()"…
first you copy the target region of data in Excel.

The data region you copy in Excel will be imported into R.
We treat example dataset of Kitui PTS



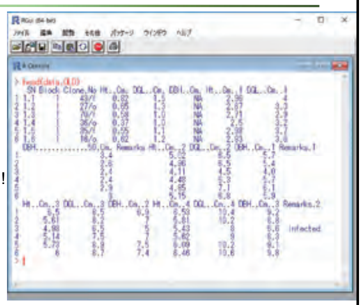
Check the data format ... By using the function "head()"

Lets use the function

head(data.OLD)

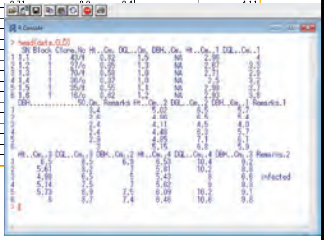
To check the data format.

But…
the column name is not good!!



Compare the original data form in Excel and the imported form in R.

			Date 1: 8／2／2016			Date 2: 18／7／2016				Date3: 20-1-2
SN	Block	Clone No	Ht (Cm)	DGL (Cm)	DBH (Cm)	Ht (Cm)	DGL (Cm)	DBH (50 Cm)	Remarks	Ht (Cm)
1.1	1	43/f	0.82	1.5		2.96	4	3.4		5.02
1.2	1	27/o	0.65	1.3		2.67	3.3	2.6		4.96
1.3	1	70/f	0.58	1.0						
1.4	1	36/o	0.37	1.0						
1.5	1	35/f	0.55	1.1						
1.6	1	16/o	0.62	1.2						
1.7	1	2/f	0.70	1.2						
1.8	1	23/o	0.76	1.4						
1.9	1	5/f								
1.10	1	9/f	0.75	1.4						
1.11	1	29/o	0.45	1.1						
1.12	1	10/o								
2.12	1	8/o	0.44	0.9						
2.11	1	2/f	0.74	1.4						
2.10	1	4/o	0.75	1.4						
2.9	1	19/o	0.74	1.8						
2.8	1	34/f	0.65	1.2						
2.7	1	59/o	0.85	0.9						



" space (brank)", "parences ()" in Excel are automatically convert to "period ." in R.

The important rule of data formats of PTS and Orchards

…for more convenient analyses latter.

1) In data title (column name),
   Do not use … **"space (brank)", "parences ()", "minus -", "plus +", " × ", "slash/ or back slash¥"** .

for example …

Bad		*Good*
DBH cm	⇒	DBH_cm
DBH(cm)	⇒	DBH.cm
DBH × Height	⇒	DBH.x.Height
Height / DBH	⇒	Height.per.DBH

"Under bar _ " is OK

Trunk shape parameter… indicating slenderness

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Old data form

			Date 1: 8／2／2016			Date 2: 18／7／2016				Date3: 20-1-2
SN	Block	Clone No	Ht (Cm)	DGL (Cm)	DBH (Cm)	Ht (Cm)	DGL (Cm)	DBH (50 Cm)	Remarks	Ht (Cm)
1.1	1	43/f	0.82	1.5		2.96	4	3.4		5.02
1.2	1	27/o	0.65	1.3		2.67	3.3	2.6		4.96
1.3	1	70/f	0.58	1.0		2.71	2.9	2.4		4.11
1.4	1	36/o	0.37	1.0		2.5	3.2	2.4		4.48
1.5	1	35/f	0.55	1.1		2.98	3.7	2.9		4.85
1.6	1	16/o	0.62	1.2		2.93	3.8	3		5.15
1.7	1	2/f	0.70	1.2		2.45	3	2.3		4.58
1.8	1	23/o	0.76	1.4		2.62	3.5	2.8		4.34
1.9	1	5/f				–	–	–	dead	
1.10	1	9/f	0.75	1.4		3.3	4.5	3.7		5.56
1.11	1	29/o	0.45	1.1		2.3	3.3	2.9		4.60
1.12	1	10/o				–	–	2	dead	
2.12	1	8/o	0.44	0.9		2.68	3.1	2.2		4.84
2.11	1	2/f	0.74	1.4		3.44	4.2	3.4		4.24
2.10	1	4/o	0.75	1.4		2.6	3.5	2.6		5.05
2.9	1	19/o	0.74	1.8		2.87	3.7	2.9		4.26
2.8	1	34/f	0.65	1.2		3.88	3.5	2.7		4.89
2.7	1	59/o	0.85	0.9		2.22	2.3	1.7		4.05

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The important rule of data formats of PTS and Orchards

…for more convenient analyses latter.

2) In data,
   also Do not use … **"space (brank)", "parences ()", "minus -", "plus +", " × ", "slash/ or back slash¥"** .

SN	Block	Clone No	Ht (Cm)	DGL (Cm)	DBH (Cm)	Ht (Cm)	DGL (Cm)	DBH (50 Cm)	Remarks	Ht (Cm)
1.1	1	43/f	0.82	1.5		2.96	4	3.4		5.02
1.2	1	27/o	0.65	1.3		2.67	3.3	2.6		4.96
1.3	1	70/f	0.58	1.0		2.71	2.9	2.4		4.11
1.4	1	36/o	0.37	1.0		2.5	3.2	2.4		4.48
1.5	1	35/f	0.55	1.1		2.98	3.7	2.9		4.85
1.6	1	16/o	0.62	1.2		2.93	3.8	3		5.15
1.7	1	2/f	0.70	1.2		2.45	3	2.3		4.58
1.8	1	23/o	0.76	1.4		2.62	3.5	2.8		4.34
1.9	1	5/f				–	–	–	dead	
1.10	1	9/f	0.75	1.4		3.3	4.5	3.7		5.56
1.11	1	29/o	0.45	1.1		2.3	3.3	2.9		4.60
1.12	1	10/o				–	–	2	dead	

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New data form in Excel (arranged by Matsushita)

Position	Block	CloneNo	Orchard	Ht.01	DG.01	Ht.02	DG.02	Ht.03	DG.0	Ht.04	DG.04	Ht.05	DG.05	Site	Col	Row
1.10	1	43	f	0.82	1.50	2.96	4.0	5.02	6.5	6.50	8.5	6.53	10.4	1	1	1
1.20	1	27	o	0.65	1.30	2.67	3.3	4.96	6.5	5.61	8.2	5.81	10.2	1	1	2
1.30	1	70	f	0.58	1.00	2.71	2.9	4.11	4.5	4.98	6.5	5.43	8	1	1	3
1.4	1	36	o	0.37	1.00	2.50	3.2	4.48	6.3	5.14	7.5	5.62	9	1	1	4
1.5	1	35	f	0.55	1.10	2.98	3.7	4.85	7.1	5.73	8.9	6.09	10.2	1	1	5
1.6	1	16	o	0.62	1.20	2.93	3.8	5.15	6.8	6.00	8.7	6.46	10.6	1	1	6
1.7	1	2	f	0.70	1.20	2.45	3.0	4.58	5.7	5.85	7.3	6.32	9.1	1	1	7
1.8	1	23	o	0.76	1.40	2.62	3.5	4.34	5.4	5.25	7.0	5.71	8.5	1	1	8
1.9	1	5	f											1	1	9
1.10	1	9	f	0.75	1.40	2.80	4.5	5.56	7.6	6.44	10.0	6.92	12.1	1	1	10
1.11	1	29	o	0.45	1.10	2.30	3.3	4.60	6.7	5.90	8.7	6.49	10.9	1	1	11
1.12	1	10	o											1	1	12
2.12	1	8	o	0.44	0.90	2.68	3.1	4.84	7.6	5.96	9.8	6.44	11.6	1	2	12
2.11	1	2	f	0.74	1.40	3.44	4.2	4.24	6.5	4.24	7.0	4.31	8	1	2	11
2.10	1	4	o	0.75	1.40	2.60	3.5	5.05	6.5	5.96	7.7	6.73	9.9	1	2	10
2.9	1	19	o	0.74	1.80	2.87	3.7	4.26	5.6	4.76	7.1	5.31	8.3	1	2	9

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Compare New and Old data form in Excel

Position	Block	CloneNo	Orchard	Ht.01	DG.01	Ht.02	DG.02	Ht.03	DG.0	Ht.04	DG.04	Ht.05	DG.05	Site	Col	Row
1.10	1	43	f	0.82	1.50	2.96	4.0	5.02	6.5	6.50	8.5	6.53	10.4	1	1	1
1.20	1	27	o	0.65	1.30	2.67	3.3	4.96	6.5	5.61	8.2	5.81	10.2	1	1	2
1.30	1	70	f	0.58	1.00	2.71	2.9	4.11	4.5	4.98	6.5	5.43	8	1	1	3
		36	o	0.37	1.00	2.50	3.2	4.48	6.3	5.14	7.5	5.62	9	1	1	4
		35	f	0.55	1.10	2.98	3.7	4.85	7.1	5.73	8.9	6.09	10.2	1	1	5
		16	o	0.62	1.20	2.93	3.8	5.15	6.8	6.00	8.7	6.46	10.6	1	1	6
		2	f	0.70	1.20	2.45	3.0	4.58	5.7	5.85	7.3	6.32	9.1	1	1	7
		23	o	0.76	1.40	2.62	3.5	4.34	5.4	5.25	7.0	5.71	8.5	1	1	8
		5	f											1	1	9
		9	f	0.75	1.40	2.80	4.5	5.56	7.6	6.44	10.0	6.92	12.1	1	1	10
		29	o	0.45	1.10	2.30	3.3	4.60	6.7	5.90	8.7	6.49	10.9	1	1	11
		10	o											1	1	12
		8	o	0.44	0.90	2.68	3.1	4.84	7.6	5.96	9.8	6.44	11.6	1	2	12
		2	f	0.74	1.40	3.44	4.2	4.24	6.5	4.24	7.0	4.31	8	1	2	11
		4	o	0.75	1.40	2.60	3.5	5.05	6.5	5.96	7.7	6.73	9.9	1	2	10
		19	o	0.74	1.80	2.87	3.7	4.26	5.6	4.76	7.1	5.31	8.3	1	2	9

Block	Clone No
1	43/f
1	27/o
1	70/f
1	36/o
1	35/f
1	16/o
1	2/f
1	23/o
1	5/f
1	9/f
1	29/o
1	10/o
1	8/o
1	2/f
1	4/o
1	19/o
1	34/f
1	59/o

**1) Separate the information of clone numbers and origin
this is because we want to treat a lot of clones as "integers"**

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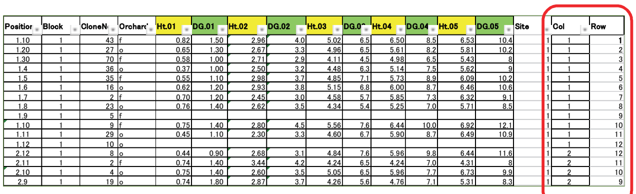
Compare New and Old data form in Excel



2) Separate the planting position as "Col" and "Row" .
If the information (Col and Row) are separated,
we will easily draw a figure of planting position map, using R function "plot"

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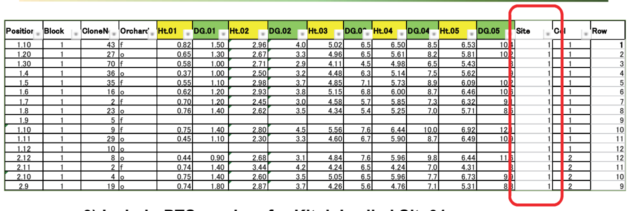
Compare New and Old data form in Excel



3) Include PTS number, for Kitui, I called Site01.
… I strongly recommend to set PTS numbers
to easily manage much more PTSs that will set in the future.

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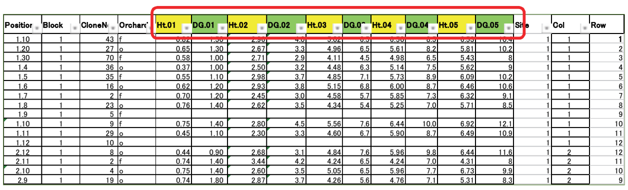
Compare New and Old data form in Excel



4) Combine and rename the each-census data of Height and Diameter
1st census Ht.01 and DG.01,
2nd census Ht.02 and DG.02, …

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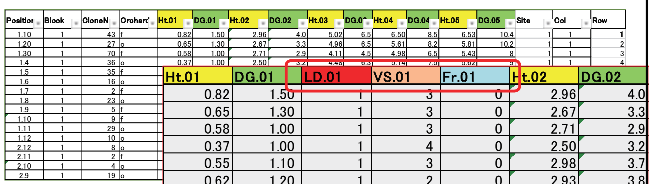
Compare New and Old data formats in Excel



Ht.01	DG.01	LD.01	VS.01	Fr.01	Ht.02	DG.02
0.82	1.50	1	3	0	2.96	4.0
0.65	1.30	1	3	0	2.67	3.3
0.58	1.00	1	3	0	2.71	2.9
0.37	1.00	1	4	0	2.50	3.2
0.55	1.10	1	3	0	2.98	3.7
0.62	1.20	1	2	0	2.93	3.8
0.70	1.20	1	1	0	2.45	3.0
0.76	1.40	1	4	0	2.62	3.5
		0				

4) Combine ar
1st census
2nd censu

You can also include status score data as similar format.
e.g. living (alive 1 or dead 0), vending stem (1 ~ 5), fruiting level at PTS (1~3)

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Then… Let's import NEW (arranged) data into R.

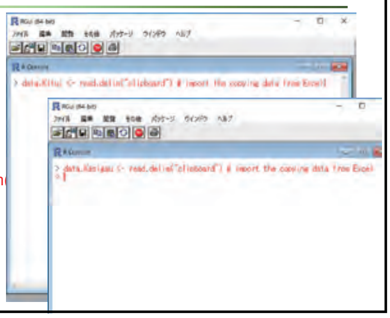
Lets use the function

read.delim("clipboard")

data.Kitui <- read.delim(".

data.Kasigau <- read.delim

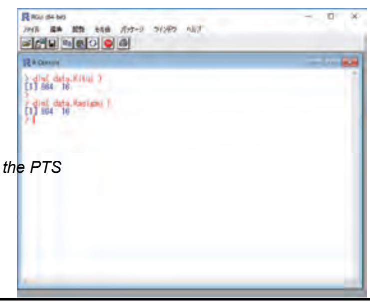
… and so on



Check the dimension of datasets

use the functions…
dim(data.Kitui)

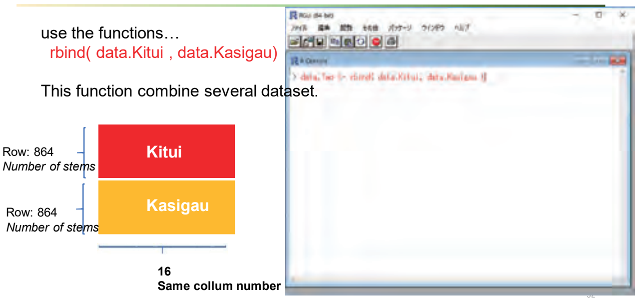
This returns
column = 16
row = 864
↑
Number of stems planted in the PTS



Combine two datasets

use the functions…
rbind(data.Kitui , data.Kasigau)

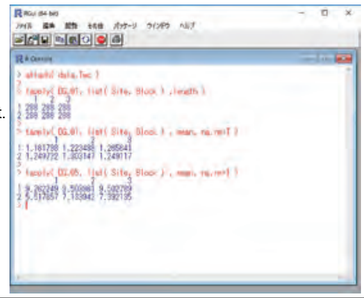
This function combine several dataset.



Check the mean values of trees in Two Sites x Blocks

use the functions…
attach(data.Two)

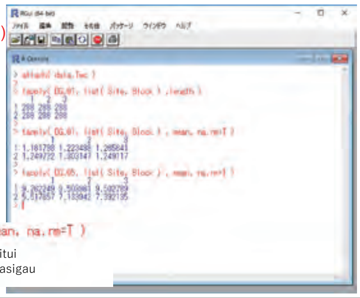
This function allows you
to access each value of dataset.



Check the mean values of trees in Two Sites x Blocks

use the functions…
tapply(DG.05, list(Site,Block) , mean)

This returns summarized values..
In this case,
mean values of DG.05 is returned,
separately for each Block x Site

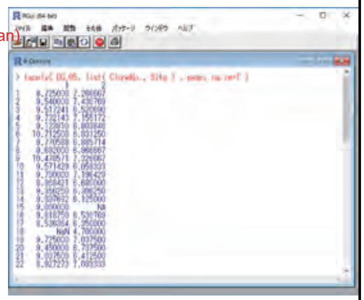


```
> tapply( DG.05, list( Site, Block ) , mean, na.rm=T )
         1        2        3
1 9.262249 9.503861 9.502789   ← Site 1: Kitui
2 5.517857 7.133942 7.392135   ← Site 2: Kasigau
>
```

Check the mean values of trees … Sites x Clones

use the functions…
tapply(DG.05, list(CloneNo.,Site), mean)

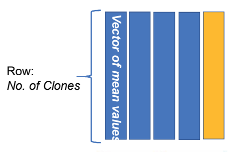
This returns summarized values..
In this case,
mean values of DG.05 is returned,
separately for each CloneNo. x Site

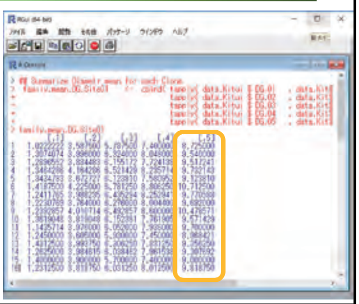


Summarize the clone-mean Height & Diameter values at each census

use the functions…
cbind(*A*, *B*, *C* ,,,)

This function “cbind” combine
several vectors .



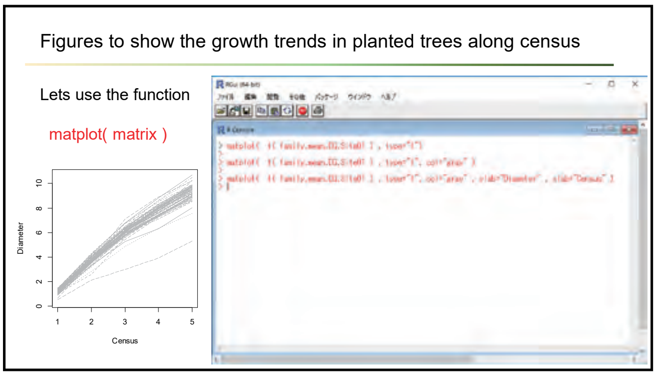


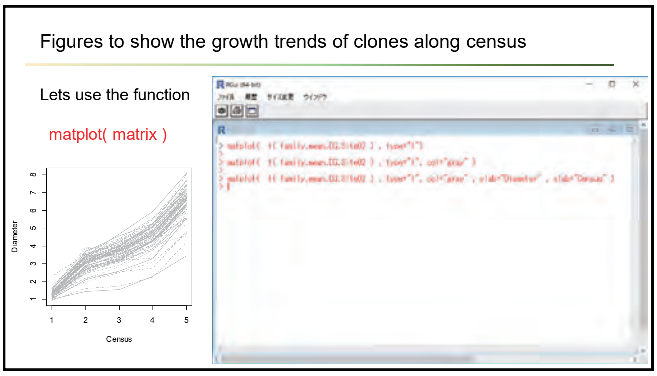
• Second day [Medium-level class = practical course]

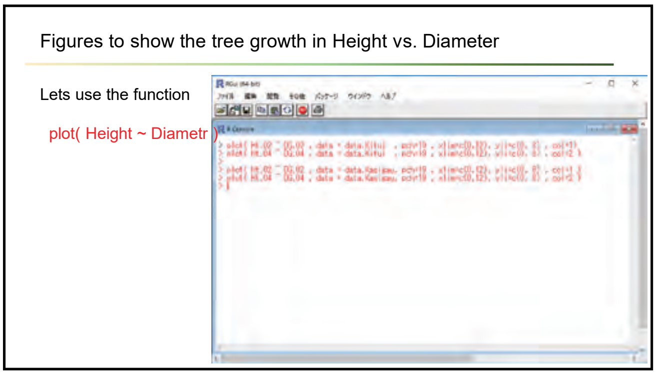
• Let’s start practice…
using the “real” PTS data of Kenya!

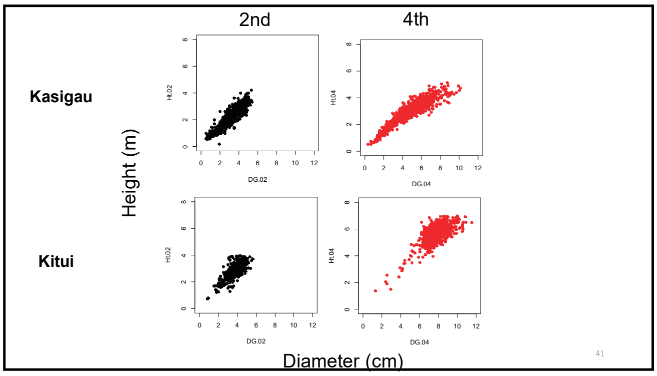
• Today’s topics …
0) INTRODUCTION
1) learn what is better way to manage data of PTS (e.g. Excel seat).
2) draw informative figures to show the breeding results.
3) learn the way (coding) to run basic statistical analyses in R.

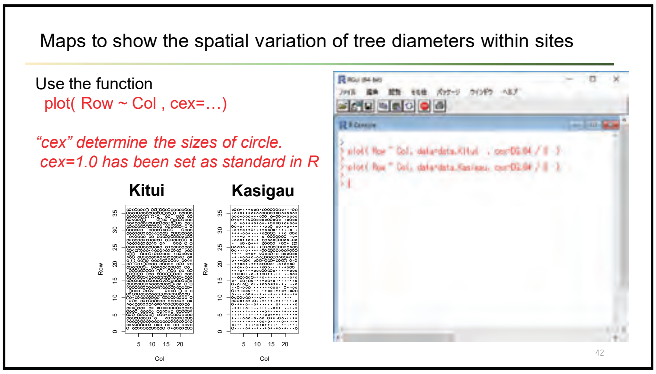
37

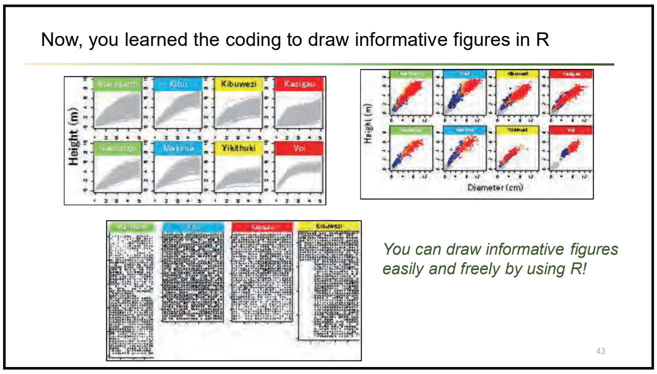

Figures to show the growth trends in planted trees along census
Lets use the function
matplot(matrix)
Diameter
Census


Figures to show the growth trends of clones along census
Lets use the function
matplot(matrix)
Diameter
Census


Figures to show the tree growth in Height vs. Diameter
Lets use the function
plot(Height ~ Diametr)


2nd
4th
Kasigau
Kitui
Height (m)
Diameter (cm)
41


Maps to show the spatial variation of tree diameters within sites
Use the function
plot(Row ~ Col , cex=…)
"cex" determine the sizes of circle.
cex=1.0 has been set as standard in R
Kitui
Kasigau
Row
Col
42


Now, you learned the coding to draw informative figures in R
Height (m)
Kitui
Kibuwezi
Kasigau
Makima
Yikithuki
Voi
Diameter (cm)
You can draw informative figures easily and freely by using R!
43

- Second day [Medium-level class = practical course]

  - Let's start practice…
    using the "real" PTS data of Kenya!

- Today's topics …
  0) INTRODUCTION
  1) learn what is better way to manage data of PTS (e.g. Excel seat).
  2) draw informative figures to show the breeding results.
  3) learn the way (coding) to run basic statistical analyses in R.
     3.1 Analysis of Variance (ANOVA)
     3.2 Linear Regression

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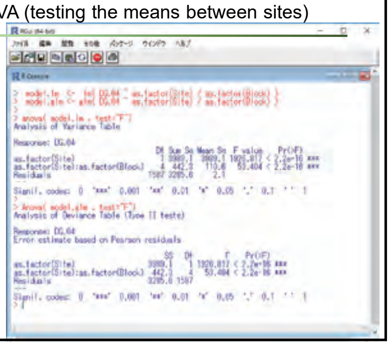
Analysis of Variance: ANOVA (testing the means between sites)

use the functions…
lm(y ~ x , data=…)

glm(y ~ x , data=…)

Above functions do….
Linear model
or
Generalized linear model

… including …
- ANOVA, ANCOVA,
- linear regression … etc,



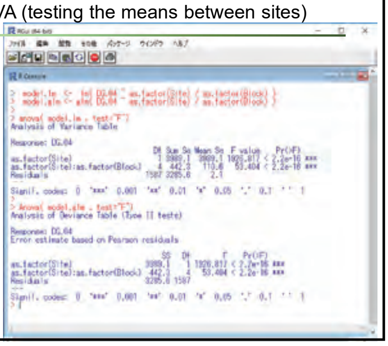
Analysis of Variance: ANOVA (testing the means between sites)

use the functions…
lm(*y* ~ *x* *data=…*)

glm(*y* ~ *x* *data=…*)

y indicates response variable
while
x indicates explanatory variables

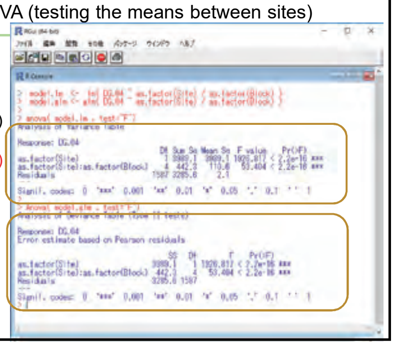
In this analysis,
We tested whether…
Are mean values of y(DG.04)
different among x(sites)?



Analysis of Variance: ANOVA (testing the means between sites)

According to ANOVA result,
You can find there was a
significant difference in DG.04
between sites (Kitui vs. Kasigau)

Use the function: anova(model)

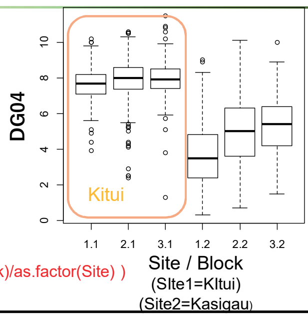


Analysis of Variance: ANOVA (testing the means between sites)

According to ANOVA result,
You can find… *there was a significant difference in DG04 between Kitui and Kasigau*

On average,
the tree diameters are greater in Kitui than in Kasigau.

You can draw this Boxplot.
boxplot(DG.04 ~ as.factor(Block)/as.factor(Site))



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- Second day [Medium-level class = practical course]

  - Let's start practice…
    using the "real" PTS data of Kenya!

- Today's topics …
  0) INTRODUCTION
  1) learn what is better way to manage data of PTS (e.g. Excel seat).
  2) draw informative figures to show the breeding results.
  3) learn the way (coding) to run basic statistical analyses in R.
     3.1 Analysis of Variance (ANOVA)
     3.2 Linear Regression

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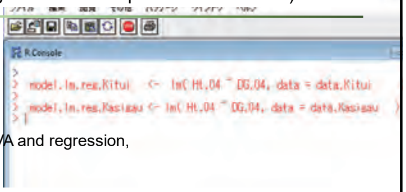
Linear regression (testing the relationship between Y and X)

use the functions…
 lm(*y ~ x , data=…)*

 glm(*y ~ x , data=…)*

You can conduct both ANOVA and regression,
only using same functions
 using similar formula.



Only the difference between them
is explanatory variable *X*.
 In ANOVA : factorial or categorical variables (e.g. Site A vs Site B)
 In regression: continuous variable (diameter, height, volume etc.)

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Summary of linear regression

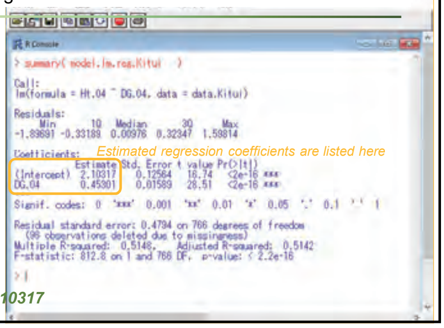
use the function…
 summary(*model*)

to get the result of
 linear regression.

Y ~ aX + b

b: intercept = *2.10317*
a: coef. for DG.04
 = 0.45301

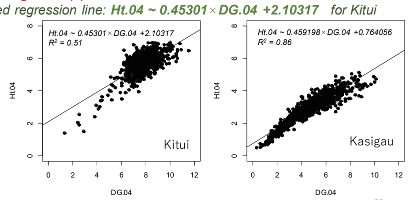
Y ~ 0.45301X + 2.10317
Height = 0.45301DBH +2.10317



Summary of linear regression

If you want to draw a figure,
use the function…
 plot(Ht.04 ~ DG.04 , data = data.Kitui , xlim=c(0,12), ylim=c(0, 8))
 abline(summary(model.lm.reg.Kitui))

 # this command draw the predicted regression line: ***Ht.04 ~ 0.45301 × DG.04 +2.10317*** *for Kitui*



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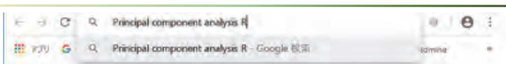
You can also a lot of statistical analyses, using R



For example,,,
If you want do "principal component analysis (PCA)" in R,
let's google it!

Google

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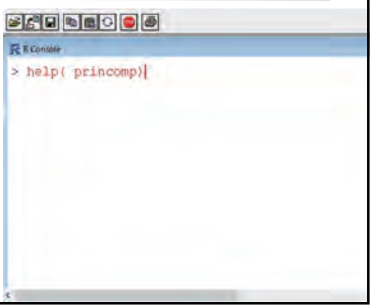
You can get many example R code from Internet!

If you want do "principal component analysis (PCA)" in R,
Google may teach you that
let's use the function "princomp()"

And then,
You can ask…
 how to use the function "princomp"?
 by using "help" in R console

 help(princomp)



By using the function "help()", you can find example code for PCA

Let's copy and paste the example code.
You can find the demonstration of PCA

```
Examples:

     require(graphics)

     ## The variances of the variables in the
     ## USArrests data vary by orders of magnitude, so scaling is appropriate
     (pc.cr <- princomp(USArrests))  # inappropriate
     princomp(USArrests, cor = TRUE) # =^= prcomp(USArrests, scale=TRUE)
     ## Similar, but different:
     ## The standard deviations differ by a factor of sqrt(49/50)

     summary(pc.cr <- princomp(USArrests, cor = TRUE))
     loadings(pc.cr)  # note that blank entries are small but not zero
     ## The signs of the columns are arbitrary
     plot(pc.cr) # shows a screeplot.
     biplot(pc.cr)
```

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Appendix 5-3-5

Report of short term expert（Artificial Crossing）

Expertise	Name	Term
Artificial Crossing	Dr. Michinari Matsushita	2019.8.18～8.25
Artificial Crossing	Dr. So Hanaoka	2019.8.18～8.25

Date	Activity
18 Aug. (Sat.)	Move to Haneda
19 Aug. (Sun.)	Move (Doha-Nairobi) Move to Kitui
20 Aug. (Mon.)	Seminar on PC programme for statistical data analysis
21 Aug. (Tue.)	Seminar on PC programme for statistical data analysis
22 Aug. (Wed.)	Meeting with C/P(mating system, artificial crossing and selection for second generation) Observation of Melia seed orchard and PTS in Tiva
23 Aug. (Thu.)	Move to Nairobi Meeting with Japanese expert Move to Doha
24 Aug. (Fri.)	Move to Narita (Dr.Matsushita)
25 Aug. (Sat.)	Move to Haneda Haneda to Hokkaido (Dr.Hanaoka)

１． Itinerary

２． Results of major activities

2-1. Observation of Melia PTS in Kitui(Tiva)

We confirmed the result of PTS assessment. KEFRI steadily surveyed not only tree height and DBH, but also state of trees such as vent of stem, flowering and fruit production. They are paying attention to keep their concentration for accurate assessment, doing at early morning or evening. We also confirmed the improvement of assessment system to keep the continuous assessment with low cost, low labor and high efficiency.

2-2. Observation of Melia seed orchard in Kitui(Tiva)

The Melia seed orchard was established in 2013 and started seed production in 2015. 3 times seed productions were implemented in 2016 and almost trees in the orchard could produce a lot of seeds in 2017. The amount of improved seed production from the orchard has been increasing year by year, as of Aug 2019, almost all trees are blooming and flowering entire the seed orchard. Therefore we confirmed these two seed orchard take an important role for sustainable forest management in East Africa amid the high ambitious for tree planting in farmers and private sector.

We discussed a future roadmap of tree breeding in Kenya with Mr. Kariuki, an assistant component manager, through considering the next generation and renewal of seed orchard on Melia. He explained KEFRI is planning signing MOU with BGF and KOMAZA, to enhance seed production capacity. We suggested that small or middle size seed orchard should be established, KEFRI should direct the establishment and management of those seed orchards and take a genetic resource management .

2-3. Study on artificial crossing and mating system of Melia

We have never found how to extent the germination rate in *Melia volkensii* and whether inbreeding depression would be occurred or not. If the repercussion by them is higher, their performance of seedlings from seed orchard might not be maximized. Therefore, we are proceeding the estimation of mating system using by DNA markers.

As for study on mating system, sampling of material for DNA analysis will be continued for study on crossing system of Melia, which is conducted by Dr. Omondii. The extraction of DNA will be started in Dec. 2019, and be analyzed for evaluating the mating system in Jun 2020 after the DNA testing.

We also made a technical guidance about the experimental designs including the number of families and samples for analyzing, and, introduced the genetic analysis technique using free data analysis software “R”.

2-4. Seminar on PC programme for statistical data analysis

Free data analysis software “R”, which has been used in researchers worldwide for statistical analysis, making chart, programming and simulation etc., was introduced on this seminar as a programme to analyze the PTS assessment data efficiently. Free and general-purpose software for data analysis is required to strengthen the ability of researchers in developing countries. This seminar was planned in 2017 in accordance with our suggestion that a manual of usage of “R” programme and data analysis was needed for capacity building of KEFRI researchers, and the seminar and guidance were held in 2 days in Kitui regional centre with 8 participants following the previous seminar on December 2018.

(Contents of the seminar）

This seminar can be regarded as “intermediate” level, which aimed at strengthening data aggregation technic, statistics analysis and drawing figures on forestry research activity.

On the first day, after reviewing the last time lecture, we explained basic technic for drawing figures and analysis method, which is applicable to the wide range of research. The second day, we implemented a practical guidance so that C/Ps would be able to do programming by themselves. Until this time, we could strengthened their capacity for data management, drawing figure and data analysis with basic – intermediate level. Next time we are going to do more practical one such as breeding scheme and breeding statistics analysis method.

2-5. Lecture and meeting for selecting *Melia* second generation

Given that the data for growth and flowering is accumulating well in Melia PTS which was established in 2014, we agreed with Mr.Kariuki about selecting Melia second generation between Dec 2019 and Feb 2020.

In this time we explained tree breeding system in Japan, mainly the management of PTSs (ex. number of new establish PTS a year, planting seedlings per PTS, research labor cost, Schedule and time span for next generation PTS, Areas, Blocks, and the no. of families/stems per PTS).

Upon that, KEFRI will consider a possible draft implementation guide for selecting second generation and PTS management guide.

3. Subjects

(Artificial crossing and mating system)

As for studying mating system, after sprouting and extracting DNA from leaves, we will conduct analysis by using DNA marker.

(Capacity building of statistical analysis for tree breeding)

We will introduce more practical statistic data analysis on tree breeding as “higher” level on the next time.

(Strengthen improved seed production and promotion in Kenya)

We discussed with Mr. Saito (Component 2) about the following theme.

1. The necessity of establishing new seed orchard
2. Optimization of private sector nursery such as BGF and KOMAZA
3. How to explain about the breeding effect to farmers

As for 3, we will disseminate the knowledge of tree breeding though guideline and brochure as soon as we quantified the effect of breeding.

(Prospects of capacity building for tree breeding)

CPs research ability for investigation and data analysis of PTS is steadily advancing. The pace of tree height growing is slowing down in PTS at 5 years after planting. We think it is the right time to evaluate of selecting superior clones for next generation. Capacity building for line evaluation method though selecting second generation would be conducted by the end of Japanese FY 2019.

Data on several traits of Melia such as stem straightness and wood property has been accumulated as well as data of the growth. We forecast that selecting second generation is going to be conducted between 2019 to 2020 by KEFRI in practice, they would be acquired the skills regarding the series of tree breeding cycle.

4. Contact persons

Dr. Muturi, Component manager, KEFRI
Mr. Kariuki, Tree Breeding, KEFRI
Dr. Omondi, KEFRI
Mr. Katsuro Saito, Deputy Chief Advisor, CADEP

Photos






Appendix 5-3-6

Report of short term expert（Artificial Crossing）

Expertise	Name	Term
Artificial Crossing	Dr. Michinari Matsushita	2019.12.7～12.13

１． Itinerary

Date	Activity
7 Dec. (Sat.)	Move to Narita
8 Dec. (Sun.)	Move (Doha-Nairobi) Move to Kitui
9 Dec. (Mon.)	Lecture for selecting *Melia* second generation by referring PTSs assessment data
10 Dec. (Tue.)	Lecture for selecting *Melia* second generation by referring PTSs assessment data
11 Dec. (Wed.)	Confirming and marking to the selected Melia tree at Tiva PTS Move to Nairobi Meeting with Japanese expert
12 Dec. (Thu.)	Making a report Move to Doha
13 Dec. (Fri.)	Move to Narita Move to Hitachi

２． Results of major activities

2-1. Observation of Melia PTS in Kenya

I confirmed the progress of PTS assessment. KEFRI steadily surveyed not only just tree height and DBH but also index evaluation such as straightness, the amount of flowering and fruit production. I suggested that healthiness should be added to the index because recently three diseases on tree canopy were found in seed orchard and plantation. They are doing an assessment in early morning or evening to keep their concentration as accurate as possible. We also confirmed that the assessment data was centrally stored and checked by multiple KEFRI staff, the assessment system was well managed to keep the continuous assessment with low cost, low labor and high efficiency.

2-2. Observation of Melia seed orchard in Kenya

The Melia seed orchard was established in 2013 with 100 families and 1,000 mother trees and started seed production in 2015. in times seed productions were implemented in 2016an and almost trees in the orchard could produce a lot of seeds in 2017. The total improved seed production from the two orchards in 2018 has been reached to 1,600kg (nut base). Then we confirmed these two seed orchards take an important role in sustainable forest management in

East Africa amid the high ambitious for tree planting in farmers and the private sector. This trend is prevailing from Kenya to neighboring countries.

We discussed a future roadmap of tree breeding in Kenya with Mr. Kariuki, an assistant component manager, through considering the next generation and renewal of seed orchard on Melia. He explained KEFRI is planning to sign MOU and MOA with BGF and KOMAZA(already signed) to compliment seed production capacity.

I suggested that small or middle size seed orchard should be established, KEFRI should direct the establishment and management of those seed orchards and take genetic resource management.

I also advised that KEFRI should take an initiative to introduce high valued clones for breeding which estimated high performance of growth, straightness, and fecundity in this time estimation with preference to seed orchards in the private sector.

I also gave advice to him that selected 2nd generation (later mention in 2-3) of Melia should be propagated, and clonal seed orchards of 2nd gen. should be set up to get a seed for establishing PTSs of 3rd gen. .

2-3. Selecting *Melia* second generation by referring PTS assessment data

contents：（see Annex）

I and Mr.Kariuki took a step toward actual selection for next-generation in Kitui center based on PTS assessment data with lecturing the latest breeding analysis method. Overall steps were following;

① Lecture for the general theory and computation of Breeding style
② Estimation of Heritability in Melia volkensii
③ Evaluation of 1st generation(mother generation) through Backward selection(for mother’s performance)
④ Evaluation of next-generation through Forwarding selection(for progenies’ performance)
⑤ Selection of 2nd gen. based on breeding values, considering the improvement of multiple traits
⑥ Scientific discussion for the future direction of Melia volkensii

① Lecture for the general theory and computation of Breeding style

I lectured the practical method of breeding statistical analysis using free data analysis software “R” for Mr.Kariuki to enforce his programming capacity to reproduce statistical analysis on his PC.

② Estimation of Heritablility in Melia volkensii

We made an estimation of heritability for 6 traits (Height, Diameter, Volume, Straightness, Fecundity, Healthiness by using 8 2015-PTSs assessment data.

As a consequence, around 0.1[0.06～0.28] heritability has been estimated in multiple traits as well as other tree species. It shows that highly enough level to be improved in some traits of Melia volkensii.

③ the evaluation of 1st generation (mother generation) through Backward selection (for mother’s performance)

The breeding value of 1st generation (mother generation) has been estimated by using the growth trends of progenies in PTS that enable to estimate "good mother", which is better seed productivity, growth and straightness. It became clear to select mother tree families for establishing clonal seed orchard (production group) based upon the breeding value.

④ ***the evaluation of next-generation through Forward selection(for progenies' performance)***

In general, there find a better or worse growth even in PTS by land.

We evaluated the progenies' performance by correctly accounting based on AR spatial correction.

⑤ ***Selection of 2nd gen. based on breeding values, considering improvement of multiple traits***

The selection for improving multiple traits in simultaneous based on each breeding value has been implemented based on step ④ under the following consideration.

First, considered as many as diverse mother lines could contribute to next-generation because the breeding group in Melia volensii has already been narrowed to 100 groups (3,800 in first gen. of Japanese cedar). In the process of setting up each PTS, 60-70 fruited mothers from seed orchard. Then bottom ¼ of which has been excluded so that the rest 50 families could be contributed to the next generation.

Second, we set the criteria to select 5 progenies per PTS in the top 4 performance mother groups as in step ③. The number of candidate progenies was gradually allocated from the highest rank. The order of prioritizing traits are; 1.volume, 2.straightness and healthiness, 3.fecundity, it was considered that selecting estimated breeding values in each trait were as high as possible and not under the average. Finally, we virtually selected 85 individual Melia, which was around 10% of the number of average trees in each PTS. We actually confirmed the selected individual tree in Tiva PTS, marked better ones with yellow paint.

After that, we discussed a future Melia breeding plan that setting up 100 individuals in each region (2nd gen, 400 in entire Kenya) is a manageable breeding group in Kenya.

⑥ ***Scientific discussion for the future direction of Melia volkensii***

At this time, I explained the overview of tree breeding in Japan (ex. number of candidate tree in a breeding group of each generation, subpopulation, PTS management (ex. number of new set up, number of planting per PTS(families/progeny seedlings), annual labor cost, how to consider the age for selecting, diversity and extent of selection, etc.). Upon that, I advised that KEFRI should manage a breeding cycle (establishing 2nd gen. seed orchard in each breeding region, establishing PTS for 3rd gen. by seedlings from the seed orchard).

This time's evaluation was about a third of average harvest age (15 years, 30-40cm diameter), average DBH in PTSs were over 10cm (over 20cm in only 4 years old at best). I thought this evaluation was generally reasonable, the current PTS should continue to assess to know the correlation between the estimation in the younger age and that in the harvest age.

３. Subjects

(Capacity building of statistical analysis for tree breeding)

I have finished strengthening their ability of the general statistic data analysis on tree breeding at this time, keep following up some subjects such as programming.

(Prospects of strengthening seed production and promotion in Kenya)
The progress of cooperation with Component 2(Pilot Implementation) are the following;

1. To address the increasing demand for improved Melia seed and insufficiency of provision, KEFRI and long term experts are discussing the possible new seed orchard in addition to Kitui and Kibwezi.
2. They shared the view that it was quite difficult for the county government to manage seed orchard, they would support a certified private sector on behalf of counties as a way to strengthening the seed production capacity.
3. While increasing expectation to improved Melia among the farmers, it is not necessarily clear to explain how much was improved Melia compare to conventional Melia seeds.

Regarding 2, MOU and MOA have been signed between KEFRI and KOMAZA, so I would be able to support KOMAZA to establish a regional seed orchard by providing seeds from great Melia mother trees which were identified by analyzing. As for 3, the knowledge for tree breeding through the analysis should be widely promoted by handing out a guideline and brochure.

(Prospects for tree breeding in Kenya)
The series of tree breeding, (looking for Melia plus tree candidate, setup of seed orchard and PTSs, assessment of PTSs), have been implemented steadily. Given that KEFRI selected the second generation of Melia in this time, I hope that we have transferred the series of breeding cycle to Kenya, that could be enforced their research and development capacity.

４. Contact persons
Mr. Kariuki, Tree Breeding, KEFRI
Mr. Keiichi Takahata, Chief Advisor, CADEP
Mr. Katsuro Saito, Deputy Chief Advisor, CADEP
Ms. Yuki Honjo, Coordinator, CADEP

Appendix 5-3-6-1

Progress of Breeding of *Melia volkensii*
~Report for Business trip 7~13th Dec 2019 ~

Dr. Michinari MATSUSHITA (FFPRI FTBC)

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1) Progress of breeding project of *Melia volkensii* .
~ From previous JICA Kenya Breeding project to CADEP-SFM ~

2) Business trip Activity (7~13th Dec 2019)
~ Capacity development for selecting *"good"* 2nd generation *Melia* ~

Progress of the breeding of *Melia volkensii*

Selection of Plus tree candidates (2011~2012) → grafting → Setup Orchards (2012~2013) → Seed production (2014~) → Setup PTSs progeny test sites (2015~) → Measure traits (Height etc.) (2015~2019)

Selection of Plus tree candidates → grafting → Setup Orchards → Seed production → Setup PTSs progeny test sites → Measure traits (H & D)

Select 100"candidate" plus trees of M.volkensii across Kenya

Selection of Plus tree candidates | grafting | Setup Orchards | Seed production | Setup PTSs progeny test sites | Measure traits (H & D)

Produce grafted seedlings of 100 candidate Plus Trees (1st generations)

Selection of Plus tree candidates | grafting | Setup Orchards | Seed production | Setup PTSs progeny test sites | Measure traits (H & D)

1.5yrs after setting up orchards,
mother trees started seed production

Melia Nuts Production In Kitui and Kibwezi
From KEFRI Report

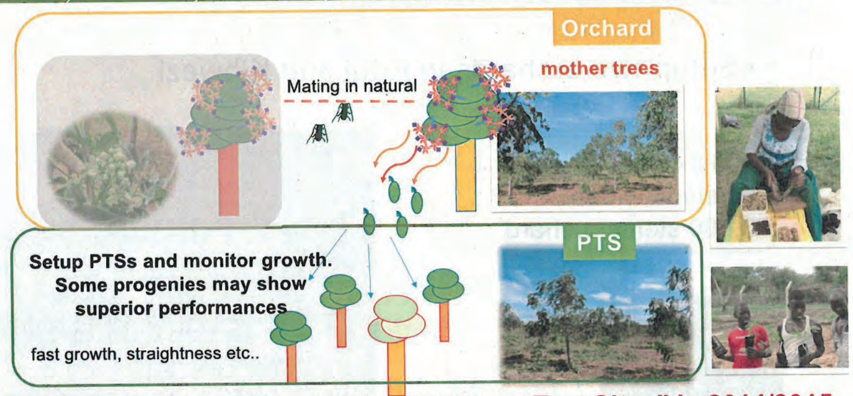
Total nuts production 1,600 kg !!

Selection of Plus tree candidates | grafting | Setup Orchards | Seed production | Setup PTSs progeny test sites | Measure traits (H & D)

- Setup two orchards in Kitui and Kibwezi

100 clones (1st gen.)
30 stems/clone
3,000 stems/orchard

Selection of Plus tree candidates | grafting | Setup Orchards | Seed production | Setup PTSs progeny test sites | Measure traits (H & D)

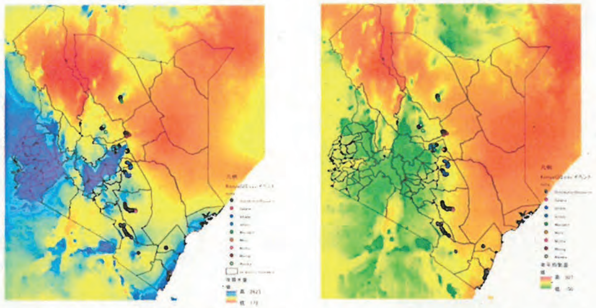


Using seeds from Orchards… setting "Progeny Test Sites" in 2014/2015.

Climate of Kenya

from World Climate database (WorldClime)

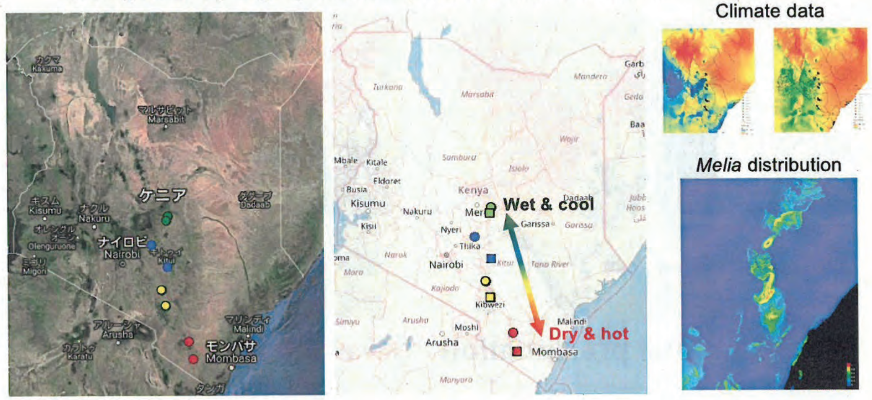
19 candidate climate variables representing precipitation and temperature etc.



Annual precipitation **Mean Annual temperature**

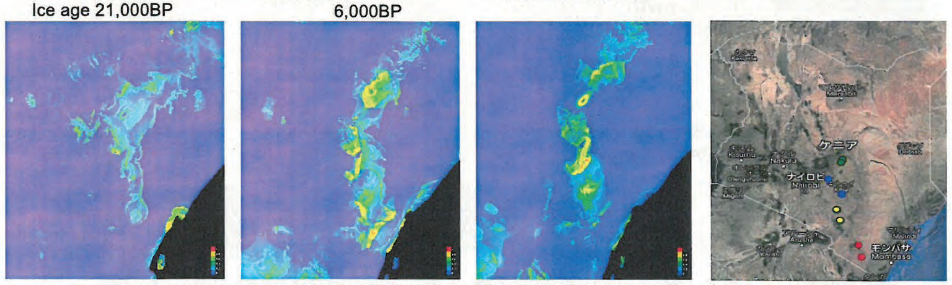
Setup of main- and sub-PTSs for *Melia*

According to climate data of Kenya and the distribution range of *Melia*, four eco-region were decided as the major breeding zones for *Melia*, and eight PTSs (Progeny Test Sites) were set in 2014/2015.



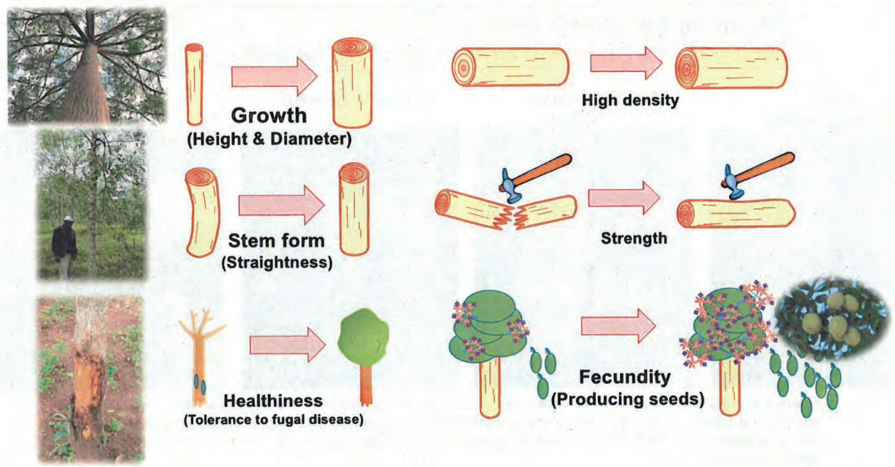
Simulation of *Melia volkensii* distribution range

By using the climate data …
Simulate the historical suitable habitats for Melia



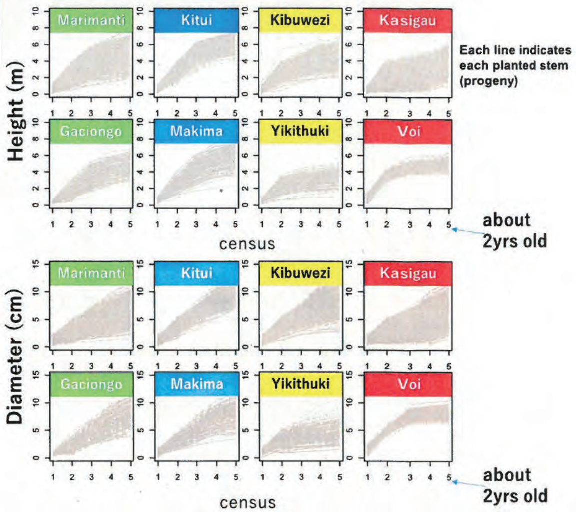
Species distribution probability of *M. volkensii* in East Africa predicted from the Maxent analysis, for Last glacial maximum (LGM) (21,000 BP), Mid Holocene (6000BP) and Present (0 BP). Higher distribution probability is shown as warm color, while lower probability is as cold color. Note that the area shown as black color is not land area (Indian sea).

In PTSs, several traits are measured.

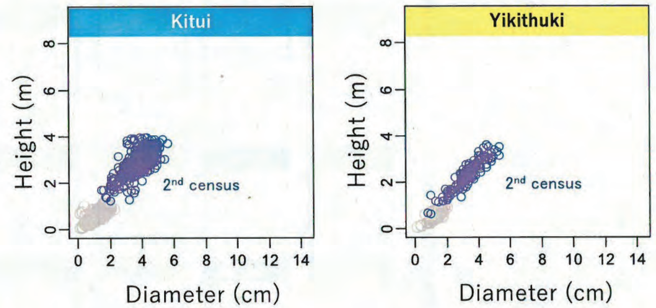


Checking growth trends of progenies in PTSs

Large between-sites variation in growth trends of progenies

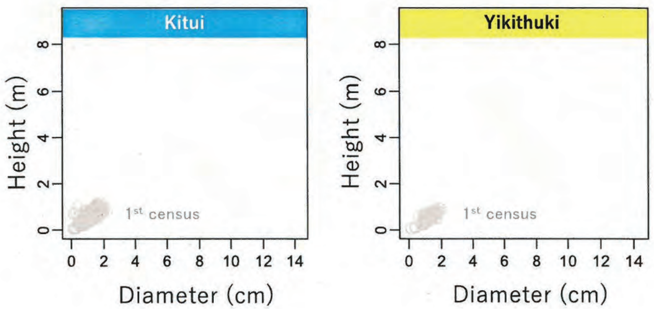


Checking the Height ~ Diameter relationship



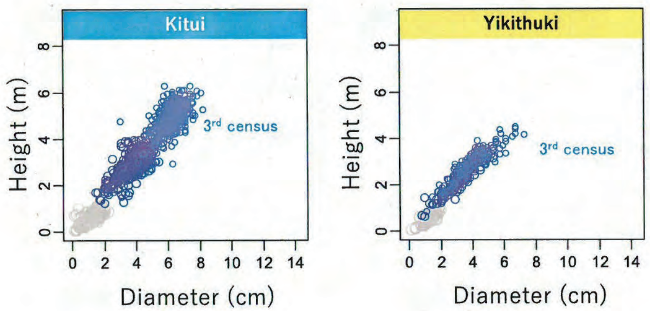
Checking the Height ~ Diameter relationship

About 1 months after planting

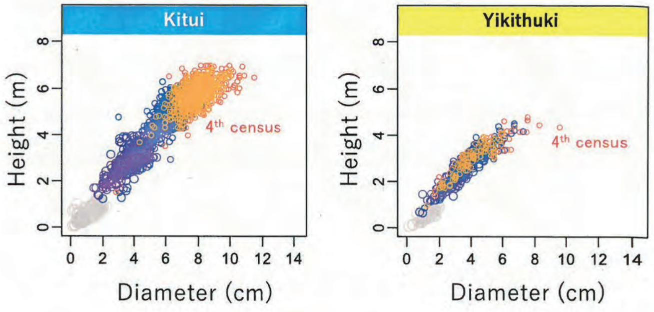


Checking the Height ~ Diameter relationship

About 1 years after planting



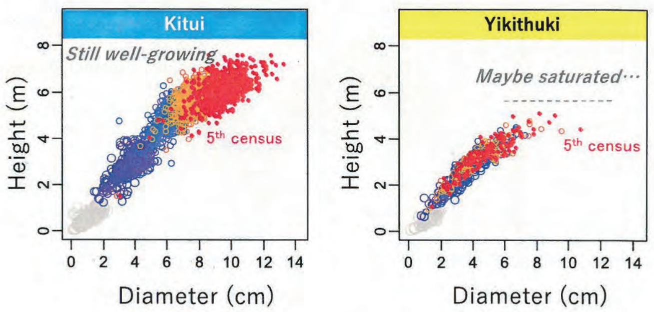
Checking the Height ~ Diameter relationship



Each circle indicates each planted stem.

Checking the Height ~ Diameter relationship

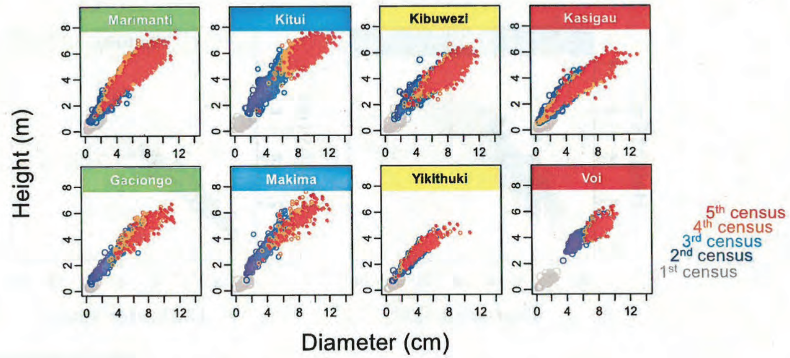
About 2 years after planting



Each circle indicates each planted stem.

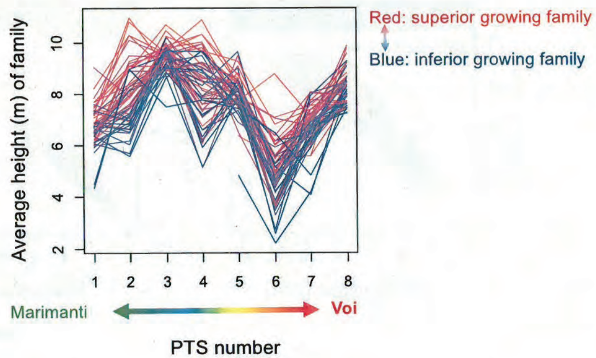
Checking the Height ~ Diameter relationship

There is a large growth variation between sites

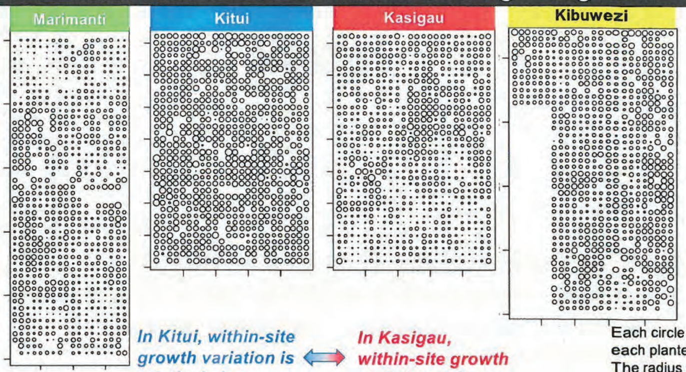


Growth performance across sites

Based on the result from PTS census…,
The progenies of "good" families almost show faster growth rates across sites.



Within-site environmental variation affecting tree growth



In Kitui, within-site growth variation is relatively low ⟺ *In Kasigau, within-site growth variation is large*

Each circle indicates each planted stem. The radius of circle is reflecting stem diameter.

When evaluating the performances of families/progenies, we should treat carefully the within- and between-site environmental variation that affects *Melia* growth.

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1) Progress of breeding project of *Melia volkens*
~ From previous JICA Kenya Breeding project to CADE

2) Business trip Activity (7~13th Dec 2019)

~ Capacity development for selecting *"good"* 2nd generation *Melia* ~

- **Lecture for the general theory and computation of Breeding analysis**
- **Estimation of Heritability**
- **Backward (for mother's performance) and Forward selection (for progenies' performance)**
- **Selection of 2nd gen. based on breeding values, considering improvement of multiple traits**
- **Scientific discussion for the future direction of *Melia* breeding (manageable breeding population size, the number of sub-populations, etc.)**

To analyse data correctly for the selection of next generation, a "free" useful statistical software "R" has been trained

1st Training: Dec 2018 2nd Training: Aug 2019

Heritability estimation for *Melia volkensii*, based on four-years-old census data.

To obtain general trends in the heritability estimation for *Melia*, combining the all census data across Kenya (4 main- & 4 sub-PTSs set in 2015).

Stem volume:	0.23 (0.05)
Tree height:	0.28 (0.06)
Trunk diameter:	0.16 (0.04)
Stem form:	0.16 (0.04)
Fecundity:	0.13 (0.04)
Healthiness:	0.07 (0.03)

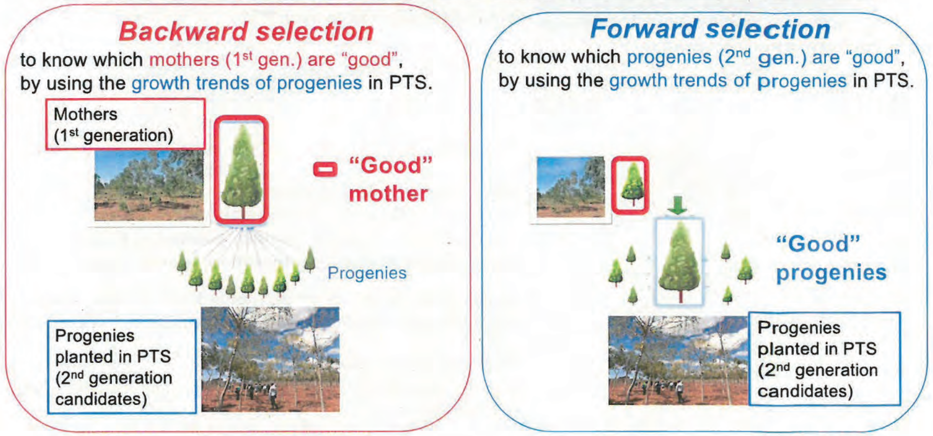
SEs are shown in ().

Site		#01	#02	#03	#04	#05	#06	#07	#08	All
N		748	794	769	754	153	137	138	133	3626
Diameter	Mean	12.9	9.0	10.0	11.0	11.6	10.4	8.6	10.3	10.6
	Max	21.5	17.4	17.6	20.5	15.3	19.2	16.5	16.2	21.5
	SD	2.1	2.3	2.3	2.1	1.2	3.3	2.1	2.1	2.6
Height	Mean	7.3	5.1	6.0	5.6	6.1	5.8	4.2	5.6	5.9
	Max	9.9	7.5	8.9	8.2	7.8	10.8	6.2	7.9	10.8
	SD	1.0	1.0	1.2	0.8	0.6	1.7	0.8	0.9	1.3

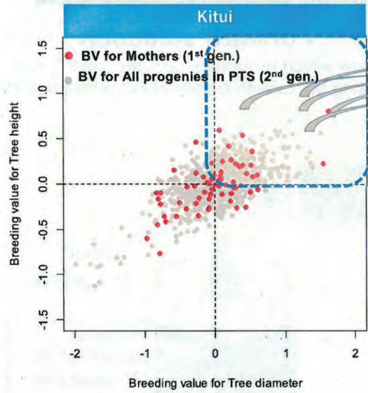
Although there were large among- and within-site environmental variation, It is confirmed that there were significant genetic variations among families and relatively moderate-level heritability in *Melia*.

In the breeding analysis, "BreedR" package of "R" were used to obtain Heritability and BLUP estimations.

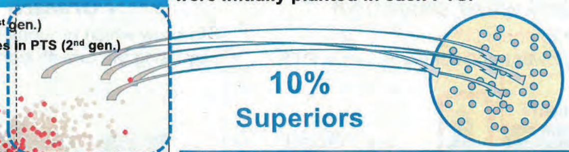
Breeding analysis provides a robust estimation of genetic performance not only for mothers (1st gen.) but also for progenies (2nd gen.), correctly accounting for environmental variation.



After getting the robust estimation of genetic performance by breeding analysis, top 10% progenies are selected as superior 2nd gen. *Melia*.



About 860 progenies (●) originated from 60~70 mothers (●) were initially planted in each PTS.



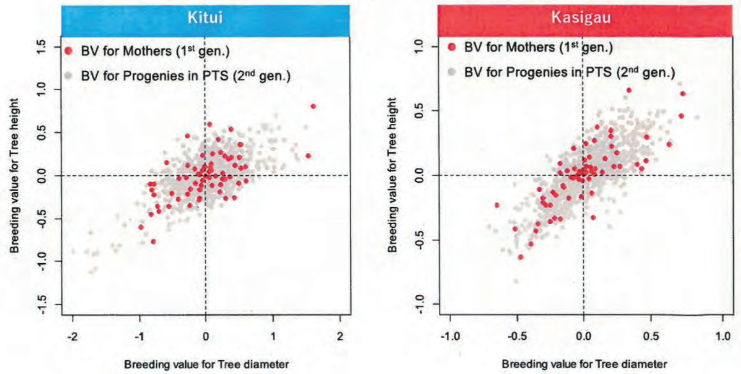
After our statistical analysis, about 10% (●, about 86 trees per site) out of the all progenies initially planted in PTS are selected as the 2nd generation "*good*" *Melia*.

Since the total no. of 1st gen. plus trees across Kenya are limited (only 100), selection strength is kept "weak".

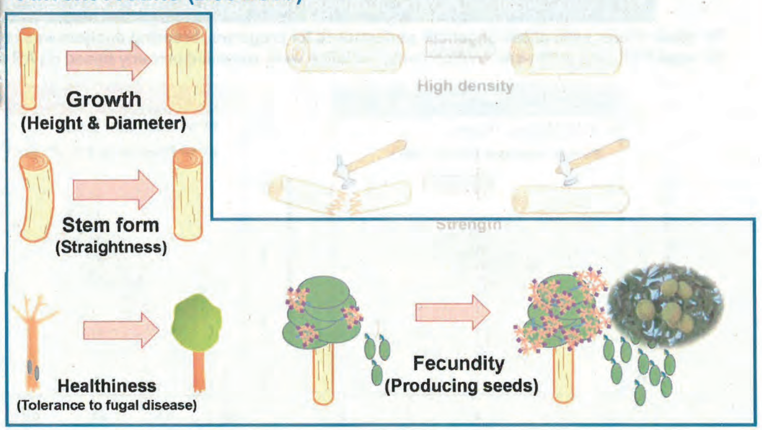
To keep genetic diversity, ¾ of 1st gen. *Melia* (about 50 out of 60~70 mothers) can contribute to the selected 2nd gen. progenies (●).

Breeding analysis provides a robust estimation of genetic performance not only for mothers (1st gen.) but also for progenies (2nd gen.), correctly accounting for environmental variation.

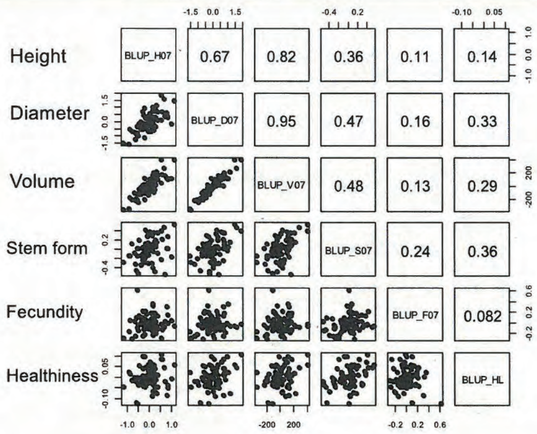
To obtain robust estimations of genetic performance for progenies, breeding analysis was conducted separately for each PTS, and within-site environmental variation were corrected properly based on AR spatial correction.



Several traits measured at PTS were analysed

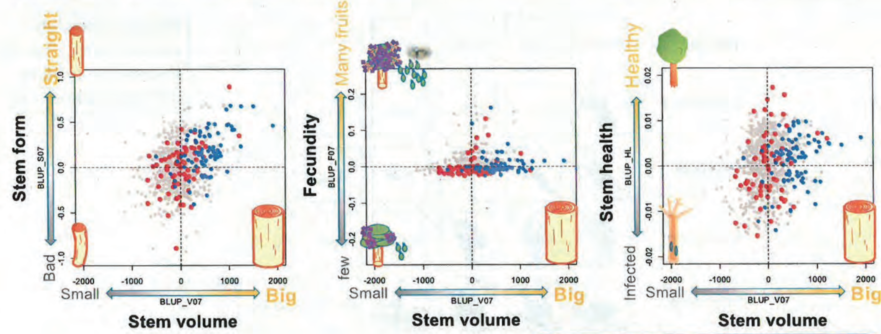


Based on the BV estimations for 1st generations (mothers), there were positive correlations among traits.



Positive correlations among traits allow us to improve multiple traits simultaneously.

Multiple traits (Volume, Stem form, Fecundity and Healthiness) were considered to improve ~ Selection result for Kitui PTS based on 4-years-old census~

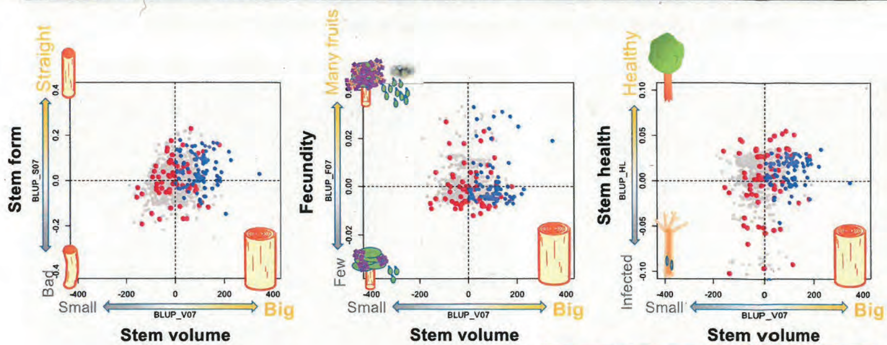


- Mothers (1st gen.)
- Selected Good Progenies in PTS (2nd gen. selected)
- All Progenies planted in PTS (2nd gen.)

Means at 4-yr-old: all and selected progenies

Tree height: 7.3 m → 8.2 m
Trunk diameter: 12.8cm → 15.2cm
Stem form index: 3.6 → 4.2

Multiple traits (Volume, Stem form, Fecundity and Healthiness) were considered to improve ~ Selection result for Kasigau PTS based on 4-years-old census~



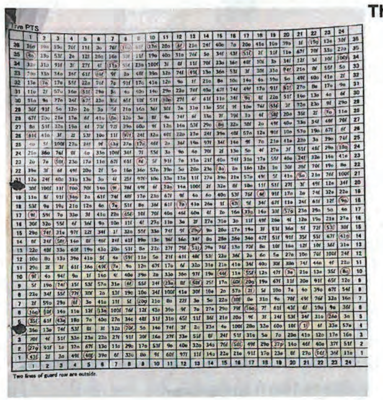
- Mothers (1st gen.)
- Selected Good Progenies in PTS (2nd gen. selected)
- All Progenies planted in PTS (2nd gen.)

Means at 4-yr-old: all and selected progenies

Tree height: 5.1 m → 6.1 m
Trunk diameter: 9.0cm → 11.8cm
Stem form index: 3.4 → 4.0

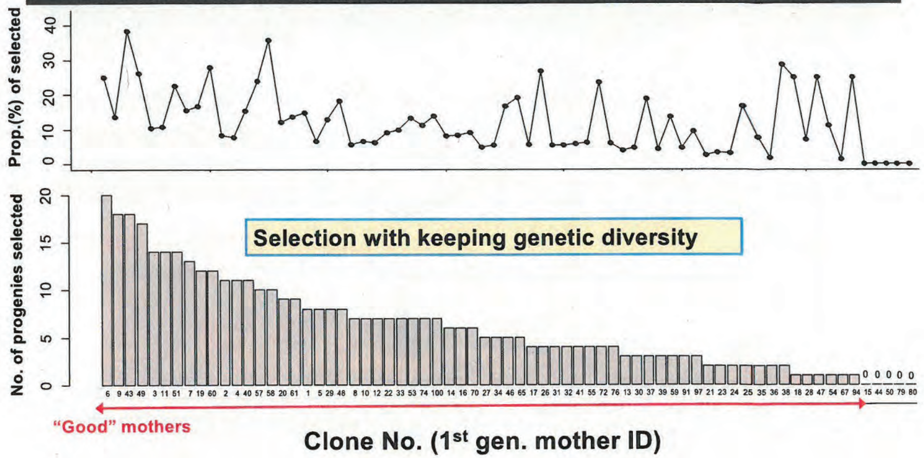
Several traits (Volume, Stem form, Fecundity and Healthiness) were considered to improve. ~ Selection result for Kitui PTS based on 4-years-old census~

Marking "good" 2nd gen .progenies selected in Kitui 2015-PTS

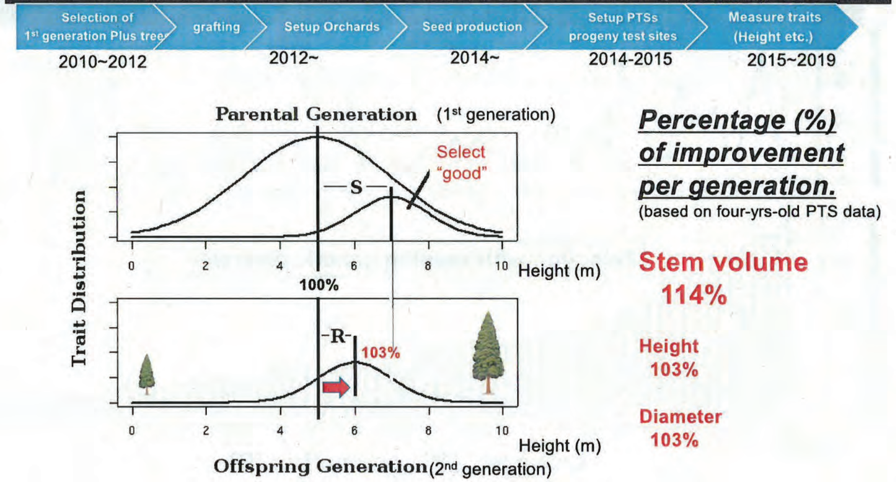


The largest tree within the PTS achieves 20cm diameter in only 4 yr

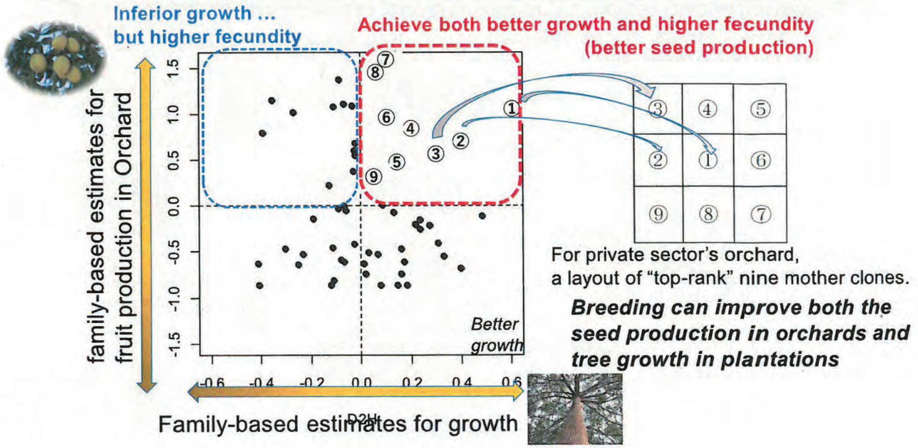
Out of 69 1st generation mothers for setting up PTSs across Kenya, 64 mothers are keeping contribution to the selected 2nd generations



Genetic improvement from 1st to 2nd generation (Just 10 yrs progress)

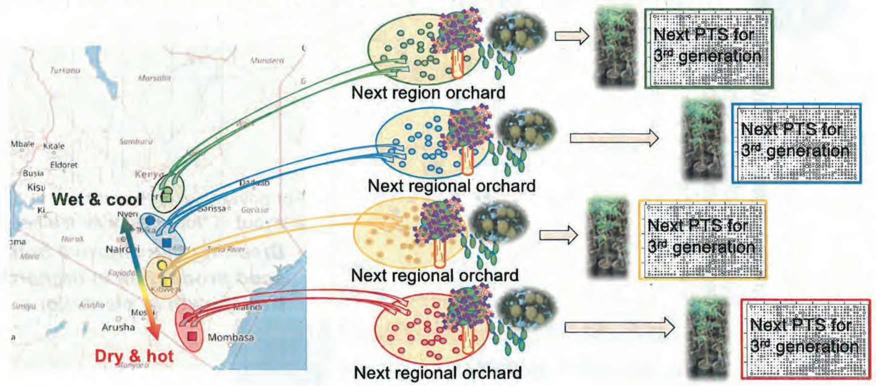


Based on the estimation both for Growth and Fecundity, the performance of current orchards can also be improved more.

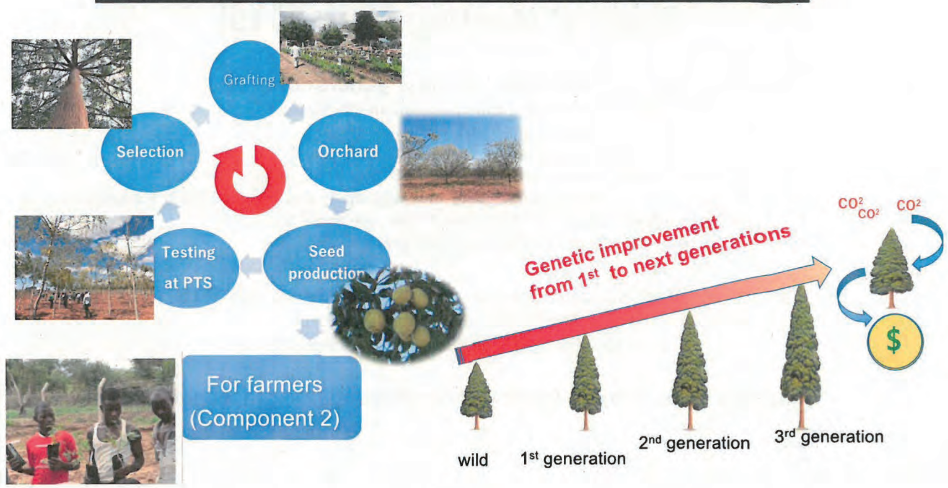


Future images for Breeding of *Melia volkensii*.
~ *from 2nd to 3rd generation* ~

In each breeding region, setup next regional orchards comprised by 100 "new mothers" those selected as 2nd gen. *Melia* in each region. Just an idea!



Cycle of tree breeding for *Melia*



Topic of Meeting (Aug 2019)

1) Schedule and time span for next generation PTS.

- In Japan (cedar), main logging span (commercial): 30~50years
  breeding span : 15 ~ 25yrs for 1st gen.
  breeding span : 12 ~ 20yrs for 2nd gen.
- In Japan (cedar), census period for each PTS: 5yrs (~1,5,10,15,20-yrs.old)
  each FTBC regional breeding region sets "new" PTSs: 2~4 sites/year.
- In Japan (cedar), the no. of PTS to test 1st gen. are about 30
  the no. of PTS to test 2nd gen. are about 25~.

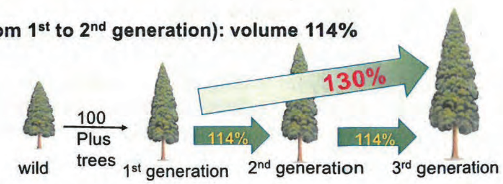
2) Areas, Blocks, and the no. of families/stems per PTS

- In Japan, 1440stems/PTS = 6 blocks x 240 stems/block
  about 48 families/PTS, and 30 stems(offsprings) / family

3) How many families (mother) and offspring (2nd gen. candidates)?

Current summary (var. Dec 2019)

- **There were significant genetic variations among families in several traits of *Melia*, although there were large between- and within-site variation.**
- **The progenies of "good" families almost show faster growth across sites.**
- **Based on breeding analysis accounting correctly for environmental variation, It is confirmed several traits (volume, stem form, fecundity and healthiness) can be improved.**
- **About 10% out of the all progenies initially planted were selected as 2nd gen.**
- **To keep genetic diversity, about ¾ of 1st gen. *Melia* (about 50 out of 60~70 mothers) contribute to the selected 2nd generation.**
- **Genetic improvement per generation (from 1st to 2nd generation): volume 114%**



Action plan (set at the meeting Aug 2019)

☑ **1) Setup general idea for Breeding (Schedule, Size, Number of PTSs etc.) in Kenya**
when : ~ Dec. 2019
who : Kariuki-san , Omondi-san , Muturi-san

☑ **2) Selecting the 2nd generation candidates**
when : ~ Feb .2020
who : Matsushita, Kariuki-san, Hanaoka-san, Miyashita-san

☐ **3) Confirm the 2nd generation *Melia*, accounting for Growth, Stem form, Fecundity, Healthiness and also Wood property**
when : ~August. 2020
who : Kariuki-san, Matsushita, Hanaoka-san, Omondi-san, Miyashita-san

☐ **4) Study for mating system of *Melia: checking selfing rates and inbreeding depression***
when : ~ Jun 2020 // Sep ~ Oct 2019, Feb 2020
who : Omondi-san, Hanaoka-san, Kariuki-san, Matsushita

Appendix 6 : Extension activities

Day/Month/Year	Occasion	Title	Contributor
30th October 2017	Workshop on technical development held by Hokkaido National Forest Regional Office in 2017	Introduction of Tree Breeding for *Melia volkensii* in Kenya	Dr. So Hanaoka
Jan.2018	Information Magazine of Tree Breeding	For scaling up" Tree breeding project in Kenya"	FTBC HP
13th Feb.2019	Poster presentation：Hokkaido Regional Workshop on research results held by FFPRI	Effort of tree breeding in Kenya	Dr. So Hanaoka
Sep.2019	IUFRO research meeting「21st International Nondestructive Testing and Evaluation of Wood Symposium」	Improvement of Seed Orchard according to the Progeny Test through Nondestructive Wood Property Testing on Tree Breeding Project in Kenya	Dr. Hisaya Miyashita
Oct.2019	IUFRO research meeting "The 4th IUFRO Seed Orchard Conference 2019"	Breeding for Drought Tolerance and Establishment of Clonal Seed Orchards of *Melia volkensii* in Drylands of Kenya	Dr. Hisaya Miyashita
Nov.2019	Information Magazine of Tree Breeding	Progress on Tree breeding project	FTBC HP
April.2020	Forest Genetics and Tree Breeding	International conference presentation "21st International Nondestructive Testing and Evaluation of Wood Symposium"	Dr. Hisaya Miyashita
April.2020	Forest Genetics and Tree Breeding	Report on International Academic Assembly "IUFRO Seed orchard Conference"	Dr. Hisaya Miyashita
April 2020	Information Magazine of Forestry Agency "Rinya"	Tree breeding project in Kenya	Forestry Agency HP
July.2020	Report for activities on the Tree Breeding Field	Root cutting propagation trial for *Melia volkensii*	FTBC HP
Aug.2020 (Japanese) Sep.2018 (English)	Brochures	FTBC Introduction Brochures	FTBC HP
Sep.2021	Poster presentation (web) : IUFRO World Day	Suggestion for genetic resources management of *Melia volkensii* in Kenya	Dr. So Hanaoka

TOPICS 02

ケニアにおける林木育種プロジェクト

■プロジェクトの概要

林木育種センターでは、気候変動適応策に資するため、国際的な技術協力や共同研究を通して林木育種技術の開発や技術移転を行っています。これらの取組を行っている国の一つであるケニアでは、国土の約八割が乾燥地・半乾燥地であり、平成二十二年時点で七％であった森林率を令和四年までに十％へ引き上げる政府目標を定めています。

林木育種センターでは、平成二十四年から平成二十八年まで国際協力機構（ＪＩＣＡ）の技術協力「気候変動への適応のための乾燥地耐性育種プロジェクト」を通じて、耐乾燥性を有する郷土樹種であるMelia volkensii（以下「メリア」）とAcacia tortilis（以下「アカシア」）を対象として、カウンターパートであるケニア森林研究所と協力して、乾燥地耐性育種に取り組んできました。

プロジェクトでは、メリアについてつぎ木を用いた二箇所のクローン採種園や八箇所の次代検定林を、また、アカシアについても二箇所の実生採種林をそれぞれ設置するなどを通じて、林木育種を進める上で必要な基盤を整備しました（図１）。

平成二十八年から、このプロジェクトはＪＩＣＡ技術協力「ケニア国持続的森林管理のための能力開発プロジェクト」の育種コンポーネントに引き継がれ、林木育種センターでは、引き続き、ケニア森林研究所とともに林木育種に取り組んでいます。

■メリア採種園等の改良

メリアは、銘木マホガニーや日本のセンダンの仲間の郷土樹種で、ケニアでは主に建材や家具材として利用されています。前プロジェクトでは、ケニア全土からメリア精英樹候補木を選び、つぎ木増殖した母樹を植栽して、優良な種子を生産するための採種園を造成しました（写真１）。本プロジェクトでは、次代検定調査結果を基に、※断幹及び整枝剪定や、間伐による劣勢個体の除去を行い、採種園の改良を進めています。


写真１　たわわに実るメリアの果実

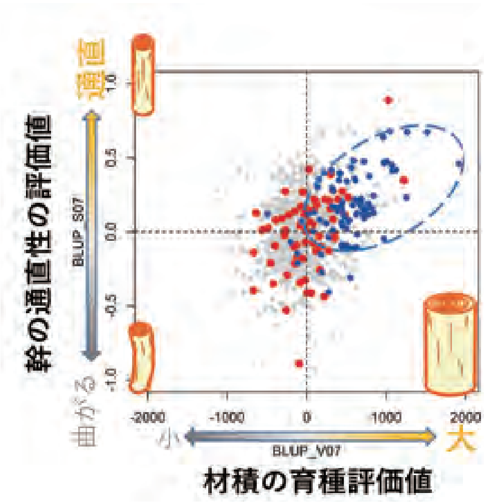
図１　採種園、検定林、採種林の位置図

■メリア第二世代の選抜

採種園産の苗木を用いて造成されたメリア次代検定林で植栽後４年間の樹高等を測定して統計解析を行った結果、成長の優劣等の系統による違いが明らかになりました（図２）。この結果を基に、当初、本プロジェクトでの達成は見込まれていなかった材積成長、幹の通直性、樹病への耐性等、複数の望ましい特性を兼ねる優良なメリア第二世代個体を、育種開始からわずか７年で選抜することができました（写真２）。

また、統計解析の結果、育種を進めると、世代あたり十％以上の成長の改良が見込まれると試算されました。

※断幹　樹高成長を抑制し、下枝を充実させ、その枝から果実（球果、種子）や穂木を採取しやすい樹形をつくるために、樹幹を任意の（作業しやすい）高さで切断すること。



●第１世代（親世代）
●選抜した第２世代

図２
材積成長の良さと幹の通直性の良さを兼ね備える優良なメリア第２世代（●）を選抜しました

写真２　選抜した優良な第２世代のメリアにペンキでマーキングをしました。評価の高い個体では植栽後４年で幹の直径が20cm に達しています

■増殖技術の研究開発

メリアのつぎ木増殖技術は、前プロジェクトでほぼ確立されましたが、クローン増殖により苗木を効率的に増やすことが出来るさし木技術については、まだ実用レベルには至っていません。このため、様々な育苗条件を変えた試験を進め、発根までを確認することが出来ました（写真３）。また、アカシアについても、つぎ木によるクローン増殖技術の開発に取り組んでいます。

写真３　発根したメリアのさし木

■アカシア採種林の改良

アカシアは、乾燥した地域でも成長に優れ、主に家畜飼料や薪炭材として利用されています。プロジェクトで造成したアカシアの採種林は、検定林の役割も持っています。優良な種子を得るため実生苗の検定を行い、劣勢個体を間伐することで、採種林の改良を図ることとしています（写真４）。

写真４
アカシア採種林
左の樹高が高い方が平成 27 年 12 月、右が平成 28 年４月に植栽。間伐により採種林を改良する予定です

■今後の取組

本プロジェクトを通じて改良される優良メリアの種子及び苗木は、在来のものに比べて成長が早く材質も優れているため、年々増加する需要に、種苗の供給が追いつかない程の人気となり、民間企業も強い関心を示すなど、ケニア国内では高く評価されています（写真５）。

写真５　農地に植えられた改良メリア

このため、より成長や材質に優れる次世代メリアを生み出すための育種を引き続き進めるとともに、プロジェクトを通じてケニア政府や郡政府、民間企業等による優良種苗の生産を支援し、東アフリカにおける持続可能な森林経営の実現に貢献していくこととしています。

Appendix 7 : Guidelines

P.O. Number	Title	Authors	Content
4-1-2	Genetic Performance and Plus Tree Traits Table for *Melia volkensii* in the Drylands of Kenya	Jason Kariuki Eitaro Fukatsu Michinari Matsushita James Ndufa Bernard Kamond	The plus tree table on *Melia volkensii* is a list of breeding values of important traits of Melia for forestry context, such as height and diameter growth, wood properties and seed productivity. The table will help researchers plan the breeding process of Melia including thinning and improvement of seed orchards, etc. It will make the Melia users (farmers and orchard manager) know more about the traits and properties of the strains they use and promote expectations for improved seeds of Melia.
4-1-4	Manual for Establishing and Managing *Melia volkensii* Seed Orchards in Kenya	Jason Kariuki Hisaya Miyashita James Ndufa Bernard Kamond	The Manual for establishing and managing Melia seed orchards is to guide how to manage the seed orchards. The contents are selection of candidate plus trees, establishment of clonal seed orchards, seed orchard management and collection of seed. It will make the orchard manager know more about effective management of the seed orchards.
4-1-5	Guideline on Clonal Propagation of *Melia volkensii*	Jason Kariuki Hisaya Miyashita Taiki Kobayashi James Ndufa Bernard Kamond	The Guideline on clone propagation of *Melia volkensii* is to guide how to make clones. The contents are Grafting and other clonal propagation Technique. It will make the researchers and orchard manager know more about clone propagation required in establishing seed orchards and foundation stock.
4-2-2	Breeding Strategies, Mating Systems and Future Perspective of Indigenous Tree Species Improvement in Kenya : A Case Study of *Melia volkensii*	Michinari Matsushita So Hanaoka Stephen Omondi Jason Kariuki Leonida Cherotich James Ndufa	The book of Breeding Strategies and Mating System is to guide how to conduct mating study. The contents are mating system, types of pollination and artificial pollination. It will make the researchers and orchard manager know more about mating system and artificial pollination for the next generation seed.
4-3	Guidelines for Establishment and Management of *Acacia tortilis* Seed Stands in Kenya	Jason Kariuki Hisaya Miyashita Taiki Kobayashi James Ndufa Dorothy Ochieng Josephine Wanjiku	The Guideline of establishment and Management of Acacia seed stands is to guide how to manage the seed stands. The contents are selection of candidate plus trees, establishment of seedling seed stands(progeny test), thinning and management of seed stands, seed production. It will make the orchard manager know more about effective management of the seed stands.