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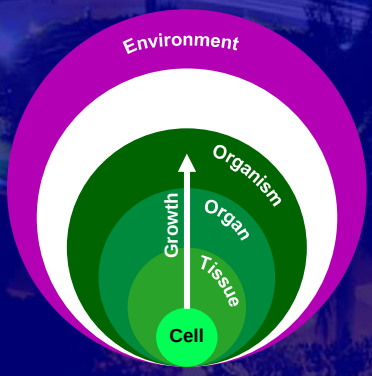
LECTURE 03:
PLANT GROWTH PARAMETERS



The most elementary processes of growth is cell growth and division that bring about final organ and whole-plant size and shape, and plant reproduction (Wuyts *et al*., 2015).

LEARNING OUTCOMES

After the completion of this lecture and mastering the lecture materials, students should be able

1. to explain parameters and indices of plant growth.
2. to explain parameters of plant growth to be measured in the analysis of plant growth.
3. to explain methods used to measure leaf area.
4. to measure leaf area of plants with the available methods.
5. to explain sample size and subsample.

LECTURE OUTLINE

1. INTRODUCTION
2. GROWTH PARAMETERS
   - 2.1 Plant Biomass
   - 2.2 Leaf Area and Number
   - 2.3 Plant Height
   - 2.4 Stem Diameter
   - 2.5 Cell Size
   - 2.6 Photosynthesis
   - 2.7 Reduced Carbon
3. LA MEASUREMENT
   - 3.1 Millimeter Paper Method
   - 3.2 Gravimetric Method
   - 3.3 Planimeter Method
   - 3.4 Length/Wide Methode
   - 3.5 Photographic Methode
4. SAMPLE SIZE
   - 3.1 Plant Sample Size
   - 3.2 Subsample
   - 3.3 Time of Observation
   - 3.4 Data Examination

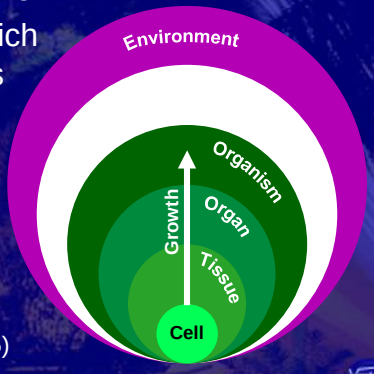
Chapter 3:
Sitompul, S.M. (2016).

1. INTRODUCTION

What to measure?

1. General View

- Plant growth can be regarded as a multi-level process, operating from the cellular to the whole-plant and plant community level.
- The choice of the level at which growth is measured depends heavily on the reason for measuring or the objective of research.



Wuyts *et al*. (2015)

- Measurement of growth across different levels (molecular–whole plant) aids in gaining a mechanistic understanding of growth, and in defining the spatial and temporal resolution of sampling strategies for molecular analyses in the model *Arabidopsis thaliana* and increasingly also in crop species.
- Plant biomass (W) is the main parameter used generally in the analysis of plant growth at plant or organ level that may be associated with the definition of plant growth – an irreversible increase in plant size accompanied by a *quantitative* change in biomass (weight).
- A great choice of measurement, however, is open to us when confronted by a higher plant.

2. First Steps

- Several questions need to be answered at the initial stage in the study scheme of plant growth, among others, are
  - What are parameters that have to be measured,
  - What are methods available for the measurement,
  - When are measurements to be undertaken,
  - How large are samples and subsamples that are reasonable on the basis of available laboratory facilities.
- Often these questions are not easy to answer especially for students peculiarly in general research with unspecific objectives. A consequence general to occur is that growth parameters to be observed are decided conventionally following what has been done previously. This brings about a difficulty in the presentation of results of studies.

2. GROWTH PARAMETERS

1. Term and Type of Parameters

- The description of growth across different levels constitutes an important aspect of research into growth regulatory processes.
- When the focus lies on a particular process at the molecular level, stable conditions with predictable plant, organ and cellular growth and development assist in the definition of the temporal and spatial resolution of sampling (sample where, when and how frequent?).
- A transcriptome analysis in either proliferating or expanding cells, for example, necessitates a precise delineation of the growth zone.

- The zones may consist of spatially distinct sections of cell proliferation and cell expansion at the tip of roots and at the base of monocot leaves.
- It is, however, necessary at the outset to clarify the term of plant growth parameters often used interchangeably with plant growth indices which are not intrinsic properties of *plants*, but rather mathematical constructs with functional significance.
- Plant growth parameters are direct properties of plants used to describe or as indicators of plant growth.
- Parameter is defined, among others, as any of a set of physical properties whose values determine the characteristics or behavior of something (
  - *parameters of the atmosphere*: *temperature, pressure & density*)

- **Number**: Number of parts in the various generally recognized main morphological categories of stem, leaves, roots and reproductive structures, or number of cells in a particular tissue or organ:
- **Linear dimension**: Heights of stems, lengths of roots: or areas and volumes – leaf area, stem volume, dimensions of cells.
- Weight: Fresh weight or dry weight of parts
- Angles: between parts or record particular shapes.
- The relationships of the different parts to each other;
  - how large is the plant before it begins to branch?
  - or whereabouts on the plant does the first flower appear?

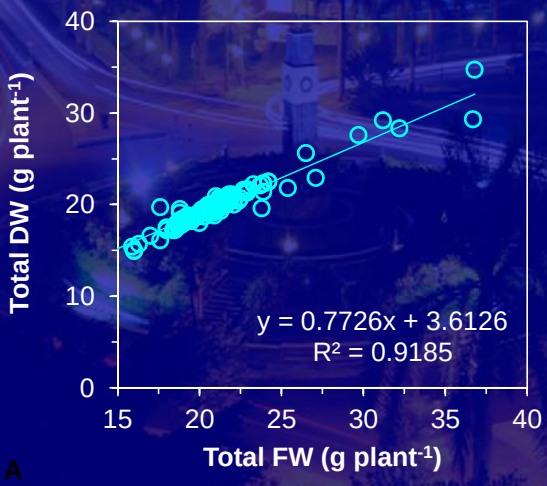
- Molecules and rate of process: Particular molecules or ions, or the rate of some process (e.g photosynthesis) can be analyzed at the sub-cellular level.

1. Plant Biomass (g plant^{-1} & g m^{-2})
   - Biomass of vegetative parts (leaves, stems & roots)
   - Biomass of generative parts or storage organs (seeds & tubers)
2. Leaf Area (cm^2 plant^{-1} & m^2 m^{-2})
3. Leaf number (number plant^{-1})
4. Plant height (cm)
5. Stem Diameter (cm)
6. Number of productive nodes (number plant^{-1})
7. Seed Number (number plant^{-1})
8. Seed Size [g/(100 seeds)$^{-1}$]
9. Light interception (fraction & mmol m^{-2} s^{-1})
10. Leaf Nitrogen (% & mg m^{-2} LA)

11. Time of emergence (days)
12. Time of flowering (days to 50% plants)
13. Cell Number (number cm^{-2})
14. Photosynthetic Pigment (mg g^{-1} & mg m^{-2}) leaves)
    - Khlorofil a, b & total, and Carotene
15. Photosynthesis (mmol CO_2 m^{-2} s^{-1})
16. Quanta Efficiency (mmol CO_2/mmol photon)
17. Transpiration Rate (E, mmol H_2O m^{-2} s^{-1})
18. Stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1})
19. Internal CO_2 concentration (Ci, mmol CO_2 mol^{-1})
20. Reduced carbon (sucrose, glucose, fructose, starch etc.; mg g^{-1} & mg m^{-2} LA)
21. Enzymic activities (e.g. RuBPase, *sucrose synthase*, mmol h^{-1} g^{-1} FW materials)

2. Plant Biomass

- The biomass of plant materials, the weight of plant materials without water, is the most often indicator used in plant growth analysis, and estimated with the dry weight (DW) of plant materials under study.
- Fresh weight (FW) can be used to describe plant growth for certain reasons and to represent the production of plant biomass particularly when it is linearly related to dry weight (DW) of plant materials under consideration.
- It should be remembered, however, that the water content of plant materials may vary between plants or plant parts due to a difference in plant age and environment that can be seen at variation of DW/FW ratio.



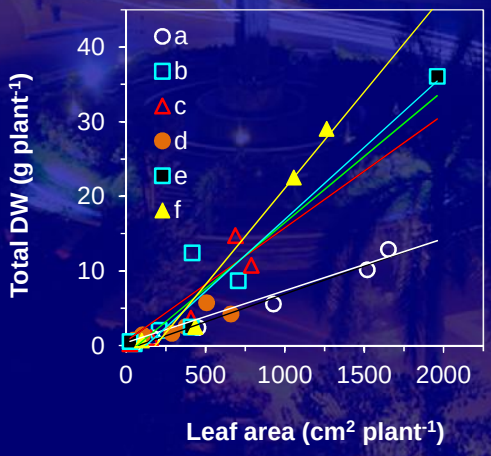
A

Relationship between total DW and total FW in soybean. Source: Sitompul (unpublished).

3. Leaf Area and Number

- Leaves are the major photosynthetic organ that produces photosynthate as the main component of plant biomass.
- For that reason, the measurement of leaf area is important either as an indicator of plant growth or as a supporting data used to explain the production of plant biomass.
- In the classical analysis of plant growth, leaf area is the second important parameter of plant growth after plant biomass.
- The number of leaf, the major leaf characteristic determining leaf area, is also often measured in the plant growth analysis.

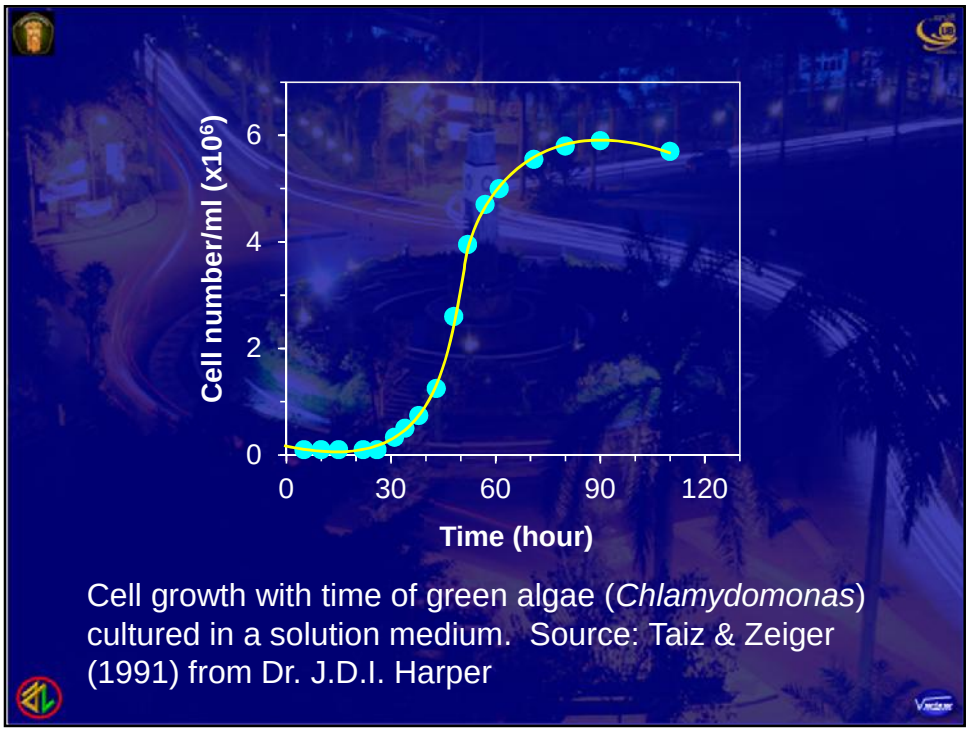
- The function of leaves as photosynthetic organ is demonstrated by the linear relationship between total plant dry weight and leaf area.



Total DW and Leaf are in soybean. Source: Sitompul (*unpublished*)

6. Cell Size

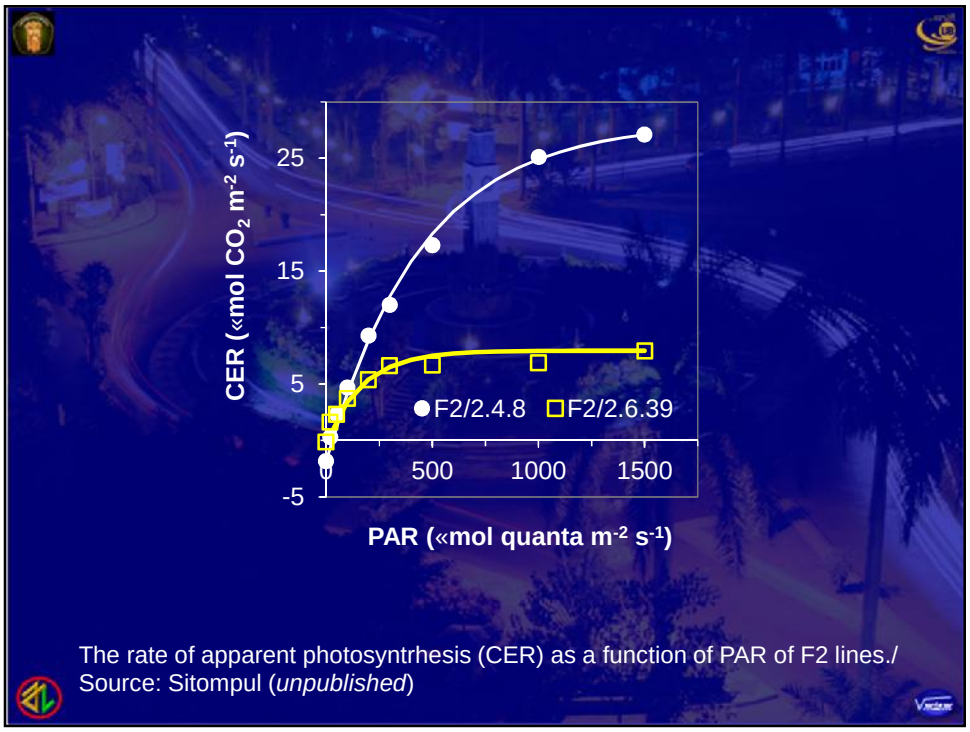
- It is necessary to assert that the irreversible increase of plant body is basically determined by number, size and content of cells.
- Therefore, plant growth can be measured at cellular level (types and quantity of molecules) which is a major interest in research in the last decades.
- Pigments, functioning as photosynthetic component for light interception and as plant defense, have received considerable attention in the last decades attributed to antioxidant
- It is almost impossible, however, to measure cell size such as cell number at plant or even organ level.
- In lower level organisms, the shape of increase in cell number follows generally a sigmoid pattern.



Cell growth with time of green algae (*Chlamydomonas*) cultured in a solution medium. Source: Taiz & Zeiger (1991) from Dr. J.D.I. Harper

7. Photosynthesis

- Photosynthesis, the process of CO_2 reduction to be carbohydrates then used to produce plant biomass, is estimated by CER (CO_2 exchange rate) which can be used to study plant growth.
- The measurement of CER is usually accompanied the data of stomatal conductance (r_s), internal CO_2 conecentration (Ci), an transpiration rate (E).
- The parameter of CER and r_s, sensitive to a change in environment particularly light and water, can be used to study genotypes tolerant to the condition of low light and limited water supply.
- Based on results of a study, the opportunity to develop high-yielding varieties of soybean with a high yield potential appears fairly high.



The rate of apparent photosyntrhesis (CER) as a function of PAR of F2 lines./ Source: Sitompul (*unpublished*)

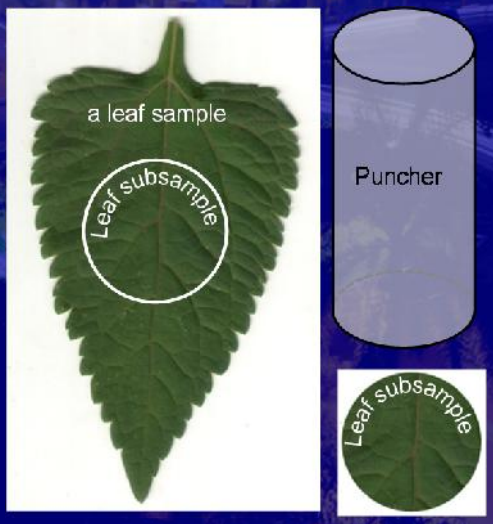
3. LEAF AREA MEASUREMENT

- Nowadays leaf are is generally measured with a leaf area meter device that works on the basis of a reduction in photocell output due to the presence of the leaf then giving an estimate of leaf area.
- However, this device is not always available and impractical for a large sample that an alternative method needs to be worked out.

1. Gravimetric Method

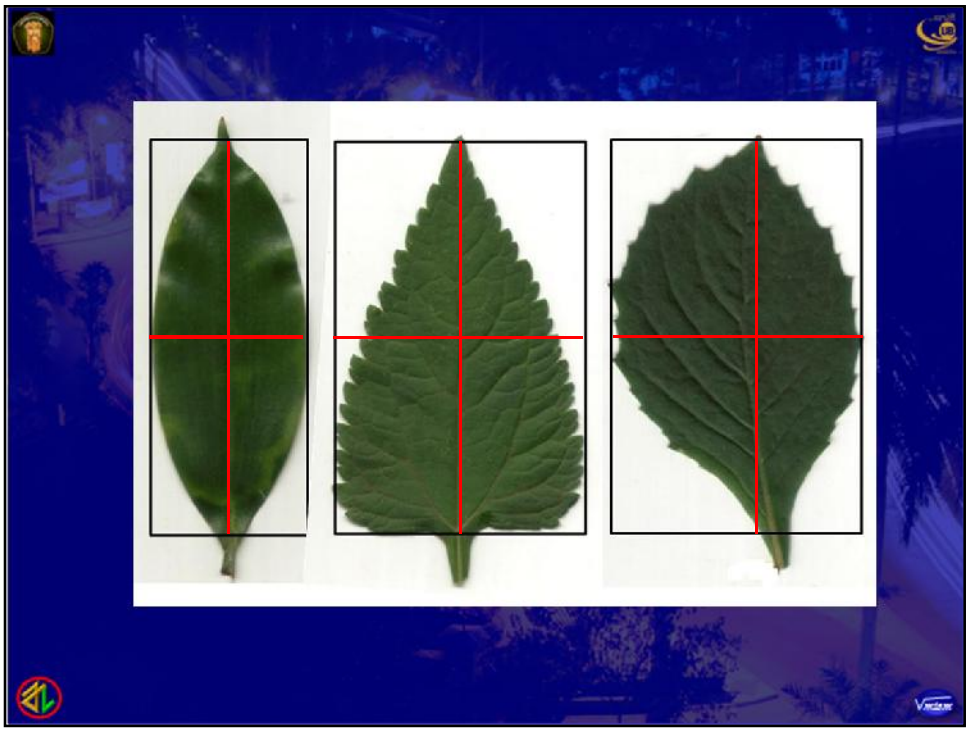
- The gravimetric method is the most efficient and most often used method particularly for a large sample.
- This method is simple, and requires no complicated equipment, but only a balance and an oven for drying samples.

- In principle, leaf area is estimated on the basis of weight comparison.



4. Length/Wide Methode

- Easy, accurate, inexpensive, and nondestructive methods to determine individual leaf area of plants are a useful tool in physiological and agronomic studies.
- For regular form of leaves, leaf area can be estimated from the length and width of the leaves which an alternative method for non-destructive samples.
- This method, however, is highly impractical for a large sample of leaves.
- To be able to use this method, a previous study is necessary to obtain calibration constants. If the form of leaves change with growth phase, then new constants should be determined in accordance with the change of leaf form.



4. SAMPLE SIZE

1. Plant Sample Size

- Sample size is often approached with standar error (SE, s/√n) known as MOE (*margin of error*).
- MOE, often expressed as E, is defined as a maximum difference between sample means (x') and actual (intrinsic) population means (μ) as shown below.

$$\mathbf{E} = \mathbf{Z}_{\alpha/2} \frac{\sigma}{\sqrt{\mathbf{n}}}$$

where $Z_{a/2}$ is a Z value at a certain level of confidence, s is standard deviation, and n is number of samples. The value of $Z_{a/2}$ = 1,96 dan 2,58 at the level of 95% and 99% (*p* = 0.05 & 0.01) confidence.

- The arrangement of the above equation results in an equation to estimate the number of minimum samples as follows.

$$\mathbf{n} = \left(\mathbf{Z}_{\alpha/2} \frac{\sigma}{\mathbf{E}} \right)^2$$

- In the estimation of sample size (n), Evans (1972) made an approach from *standard error* (SE) of means obtained from two populations with the number of observation samples (n) and standard deviation (SD, s) as shown below.

$$\mathbf{n} = 2\left(\frac{\mathbf{s} * \mathbf{t}}{\mathbf{d}} \right)^2$$

where n = sample number, s = SD, t = student t value for certain sample number and confidence (t = 2 for *p* = 0.05 & n = 60), and d = an expected difference.

- For instance, an old variety of soybean (A) is compared with a new variety (B) based on the number of pods. If var. B has at least 5 pods/plant more than that produced by var. A, var. B is regarded better than var. A.
- With a consideration that CV (coefficient of variation) of 20% is adequately good at the field level, s = 10 for an average number of 50 pods/plant, and the number of plant samples required is as follows.

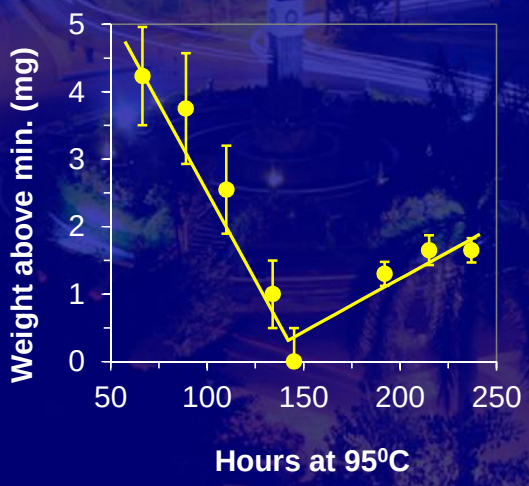
$$\mathbf{n} = 2\left(\frac{10 * 2}{5} \right)^2 = 32$$

- So the number of plant samples (32 plants) is adequately large. This increases with an increase in "s", and a decrease in "d".

2. Subsample

- Drying samples, aimed at getting rid of all water, is limited to subsamples that is small part representing samples (stems, leaves, roots and reproductive organs and storage organs).
- An alternative way that can be done is to take a plant or more as subsample which is than divided into its parts and cut into small pieces to make drying better and fast. This way also would increase efficiency use of facilities (oven) which are generally limited.
- The level of temperature usually used is 80^0C for a duration until no water in the samples (a constant dry weight of samples). To find a constant dry weight, a sample being dried needs to be weighed regularly which is not an easy work.

- In fact, a constant dry weight of plant materials may be never achieved as indicated by a result of study.



3. Time of Observation

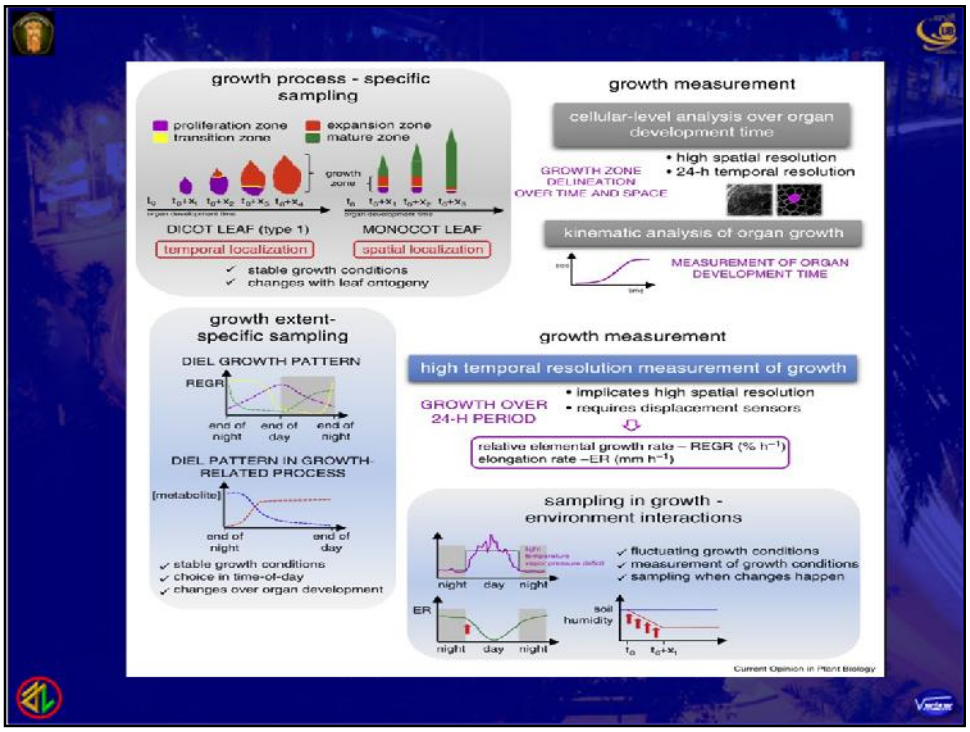
- Plant growth does not proceed in a constant fashion at all times, but follows certain rhythms changing with time.
- Accordingly it is important to make measurements at short intervals to obtain sufficiently complete growth kinetics.
- Such observation is rarely carried out with many reasons related to experiment size, economic, worker etc.
- The number of observations with time is usually limited that the determination of time for observations that may result in growth feature adequately representative is of great importance.

4. Data Examination

- Error in the measurement and recording (documenta-tion) of data may occur particularly when a large number of samples is involved.
- If a large number of samples is measured by a person, it would take a lot of time and energy to do the job so that mistakes are very likely due to tiredness.
- Besides, lost of plant biomass may also occur during the measurement resulting from wounds or sample handling for a long time.
- Increasing the number of persons for measurement (workers) is not always able to solve all problems as variation of measurement results may arise between individual workers.
- It is there important to do data examination before data analysis.

WHAT HAVE YOU GOT?

1. What is the main parameter of plant growth used in plant growth analysis
2. What is the difference between growth parameters and growth indices
3. Why is leaf area an important parameter of plant growth
4. How is leaf area measured when a leaf area meter is not available?
5. How is leaf area measured in intact, growing plants
6. How is the sample size enacted?
7. Why is the time of observation important to be carefully planned?
8. What is the main purpose of drying plant samples
9. What is the advantage of subsamples?
10. What is the importance of data examination?


growth process - specific sampling
proliferation zone
transition zone
expansion zone
mature zone
growth zone
DICOT LEAF (type 1)
MONOCOT LEAF
temporal localization
spatial localization
stable growth conditions
changes with leaf ontogeny
growth measurement
cellular-level analysis over organ development time
GROWTH ZONE DELINEATION OVER TIME AND SPACE
• high spatial resolution
• 24-h temporal resolution
kinematic analysis of organ growth
MEASUREMENT OF ORGAN DEVELOPMENT TIME
growth extent-specific sampling
DIEL GROWTH PATTERN
REGR
end of night
end of day
end of night
DIEL PATTERN IN GROWTH-RELATED PROCESS
[metabolite]
end of night
end of day
stable growth conditions
choice in time-of-day
changes over organ development
growth measurement
high temporal resolution measurement of growth
GROWTH OVER 24-H PERIOD
• implicates high spatial resolution
• requires displacement sensors
relative elemental growth rate – REGR (% h⁻¹)
elongation rate –ER (mm h⁻¹)
sampling in growth - environment interactions
night
day
night
ER
night
day
night
soil humidity
fluctuating growth conditions
measurement of growth conditions
sampling when changes happen
Current Opinion in Plant Biology