Advanced propagation methods in tea

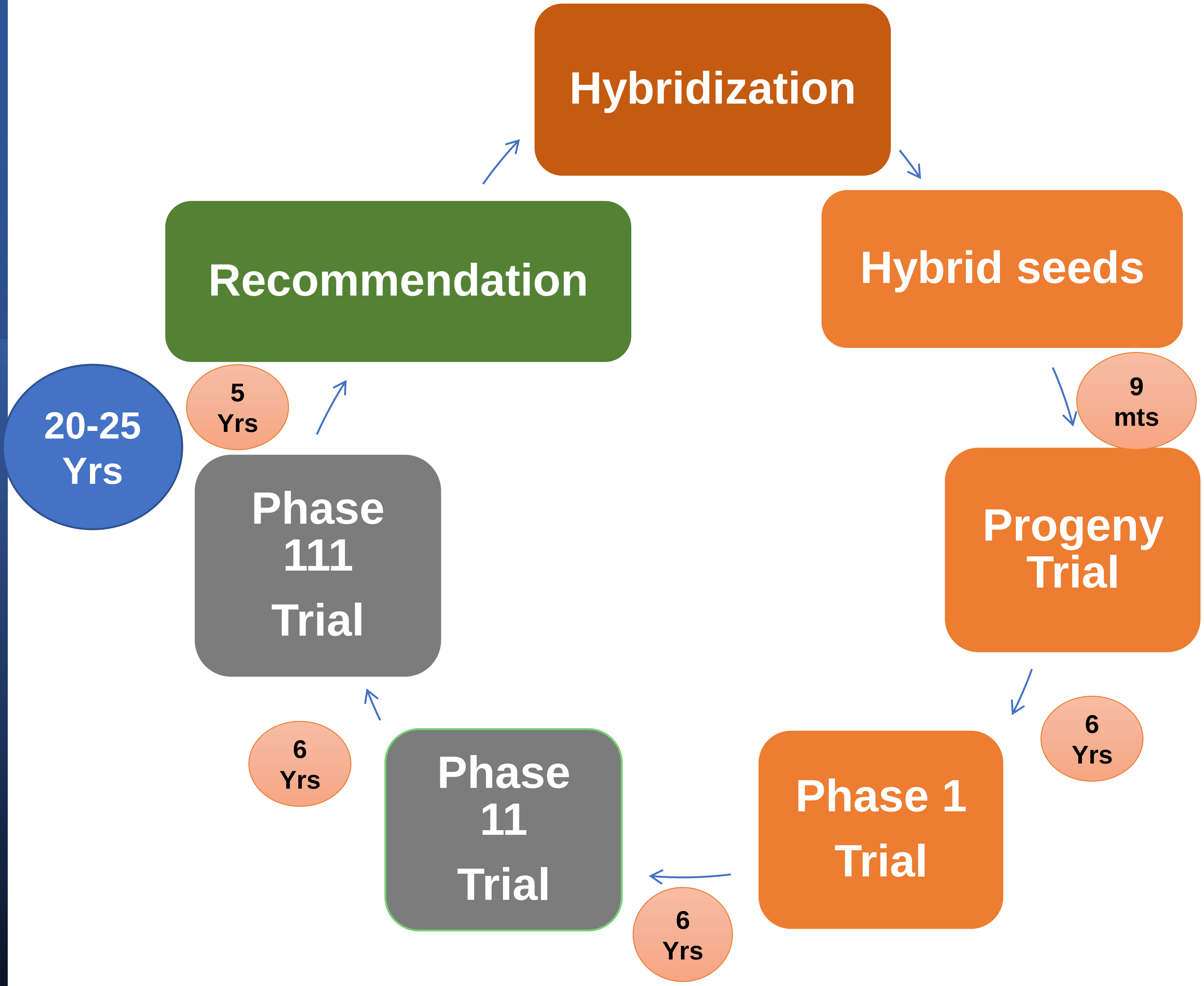
K.K.Ranaweera
Plant Breeding Division

INTRODUCTION

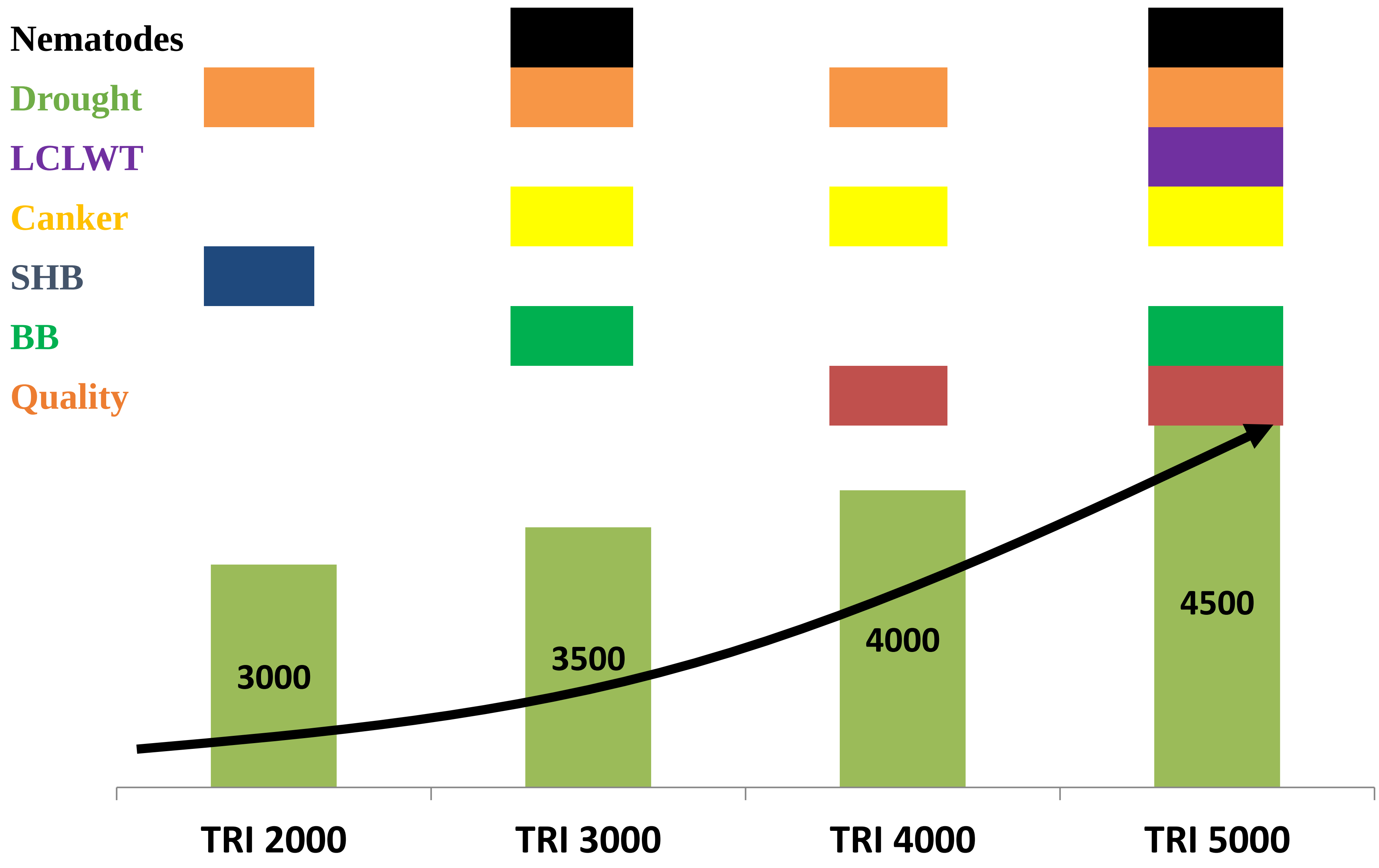
Grower’s Expectation From a New Cultivar

- ✓High yield
- ✓High quality
- ✓Resistance to pest & diseases
- ✓Drought tolerance

Conventional Tea breeding programme



Progress of cultivar development…..



New cultivars with wide array of attributes

TRI 5003

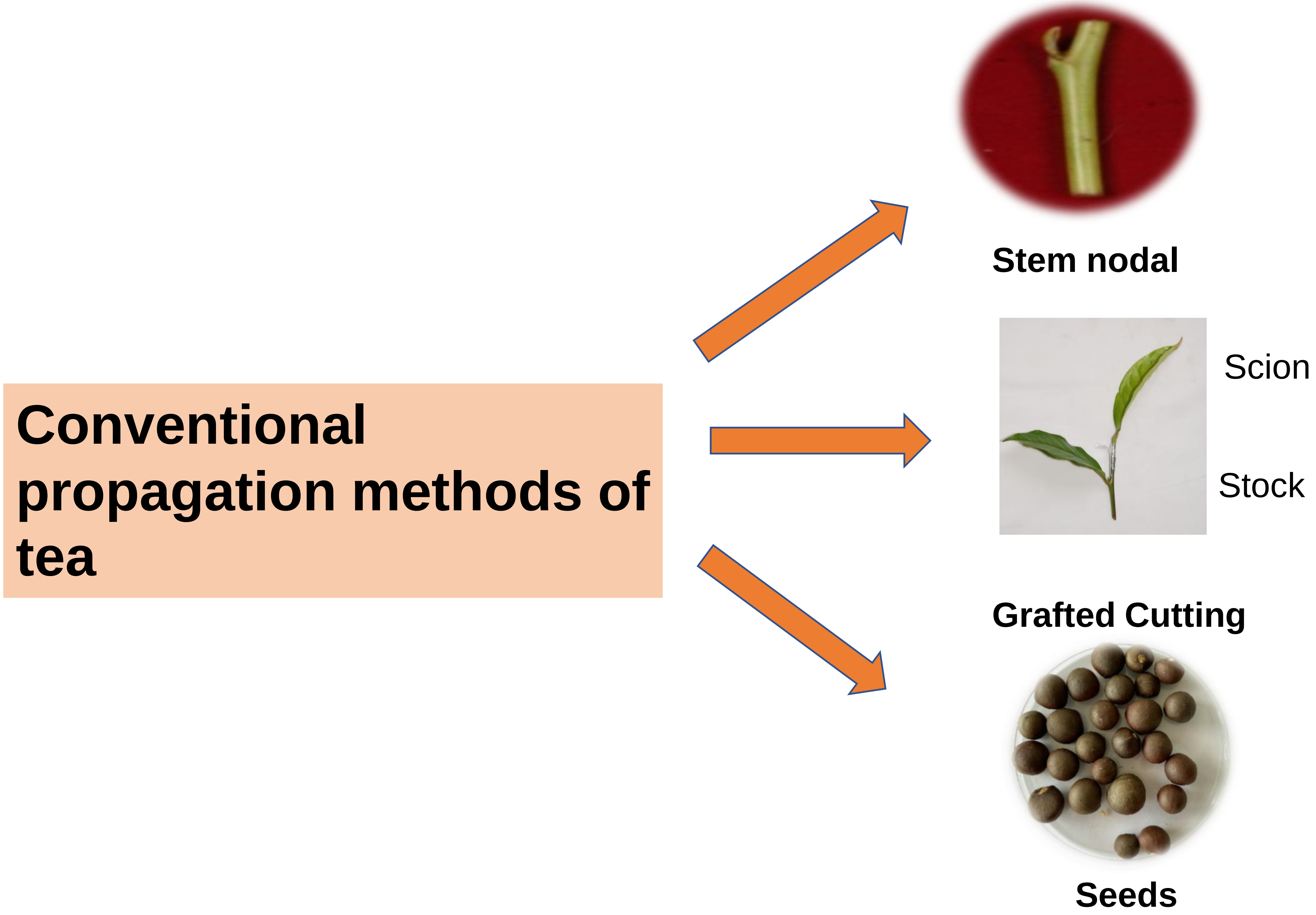
TRI 5004

TRI 5006

TRI 5002

TRI 5005

TRI 5001


Conventional propagation methods of tea
Stem nodal
Scion
Stock
Grafted Cutting
Seeds

Problem identification

Tea is mainly propagated by vegetative cuttings and seeds.

High demand

- Approximately 25%-30% Vegetative propagated (VP) tea fields and 40-50% seedling tea fields need to be infilled.
- Approximately 1532 milllion nursery plants required to infilling and replanting.

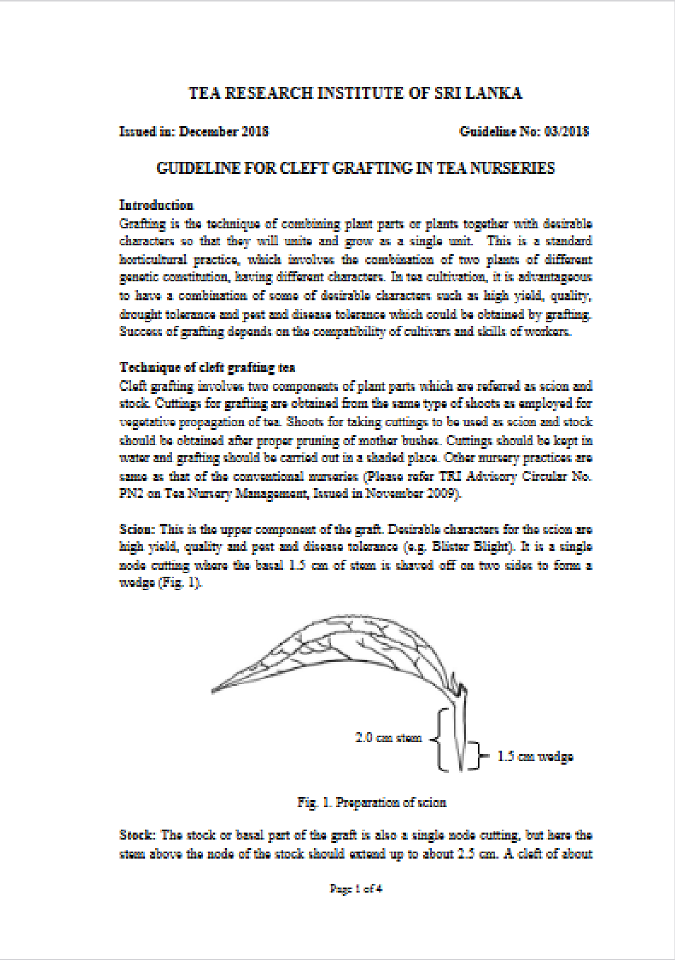
Limitations in conventional vegetative propagation and seeds

- Unfavorable weather condition and high incidence of pest and diseases
- Slow rate of propagation (Maximum 75 cuttings/bush/Year)
- Unavailability of suitable soil
- Poor survival rate at nursery and season dependent rooting ability of cuttings(Re supply cuttings several times, Actual COP high)
- Large land area requirement for maintaining mother bush sites and nurseries
- Increasing demand and the low productivity of exiting mother bush sites and seed gardens
- Propagated by seeds -higher heterogeneity

Limitations in conventional…Contd

- Unavailability of chemical for fumigation
- Unavailability of high tech nursery in tea sector
- Difficulties to maintain sand bed for seed germination due to high cost of sand.

Grafting with new cultivars



TEA RESEARCH INSTITUTE OF SRI LANKA

Issued in: December 2018 Guideline No: 03/2018

GUIDELINE FOR CLEFT GRAFTING IN TEA NURSERIES

Introduction

Grafting is the technique of combining plant parts or plants together with desirable characters so that they will unite and grow as a single unit. This is a standard horticultural practice, which involves the combination of two plants of different genetic constitution, having different characters. In tea cultivation, it is advantageous to have a combination of some of desirable characters such as high yield, quality, drought tolerance and pest and disease tolerance which could be obtained by grafting. Success of grafting depends on the compatibility of cultivars and skills of workers.

Technique of cleft grafting tea

Cleft grafting involves two components of plant parts which are referred as scion and stock. Cuttings for grafting are obtained from the same type of shoots as employed for vegetative propagation of tea. Shoots for taking cuttings to be used as scion and stock should be obtained after proper pruning of mother bushes. Cuttings should be kept in water and grafting should be carried out in a shaded place. Other nursery practices are same as that of the conventional nurseries (Please refer TRI Advisory Circular No. PN2 on Tea Nursery Management, Issued in November 2009).

Scion: This is the upper component of the graft. Desirable characters for the scion are high yield, quality and pest and disease tolerance (e.g. Blister Blight). It is a single node cutting where the basal 1.5 cm of stem is shaved off on two sides to form a wedge (Fig. 1).

2.0 cm stem
1.5 cm wedge

Fig. 1. Preparation of scion

Stock: The stock or basal part of the graft is also a single node cutting, but here the stem above the node of the stock should extend up to about 2.5 cm. A cleft of about

Page 1 of 4

Improvements to increase efficiency of the conventional propagation methods

For Quality

Region	Scion	Stock
Up country	TRI 777	TRI 2025
Up country	TRI 777	TRI 3019
Up country	TRI 777	TRI 3020
Up country	TRI 777	TRI 4052
Up country	TRI 777	TRI 4053
Up country	TRI 4067	TRI 3019
Up country	TRI 4079	TRI 2025
Up country	TRI 4079	TRI 4052

- Soil less media for plant propagation

  Plant propagation in coir pellets

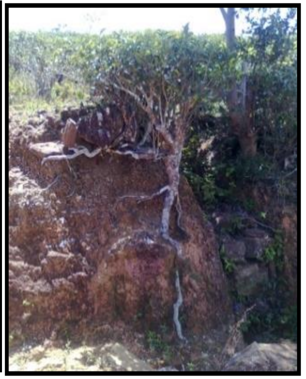






Seedling production through Improved seeds



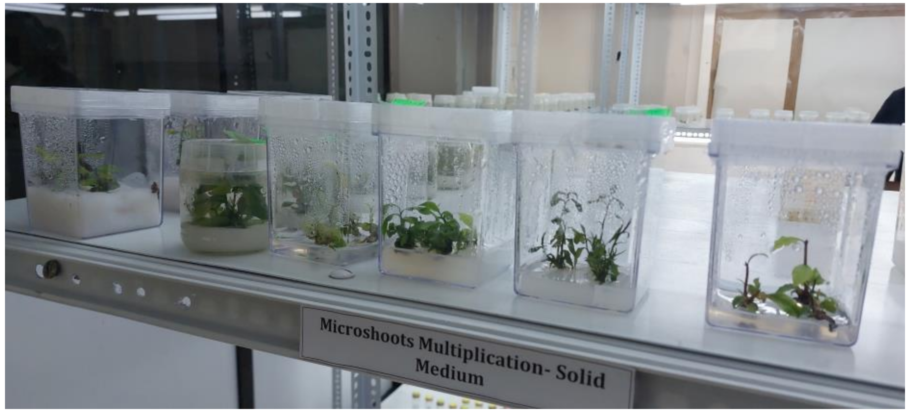



- Establishment of seedling tea field through improved tea seeds.

Tissue Culture

Advantages of Tea Tissue culture

- Mass propagation technique
- Year-round planting material production
- Excellent source for uniform seedling production through **Somatic Embryos**



- Growth performance of the TC plants in the laboratory

- Nursery performance of the TC plants compare with the conventional VP plants in nursery stage

TC Tea field in TRI

- Field performances of the TC plants

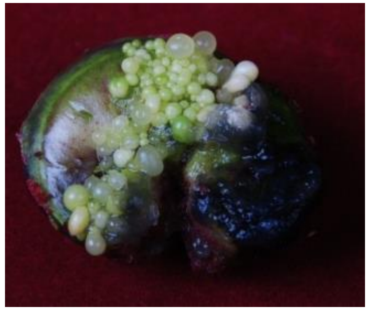
Limitations in the tissue culture protocol

1. High cost of production

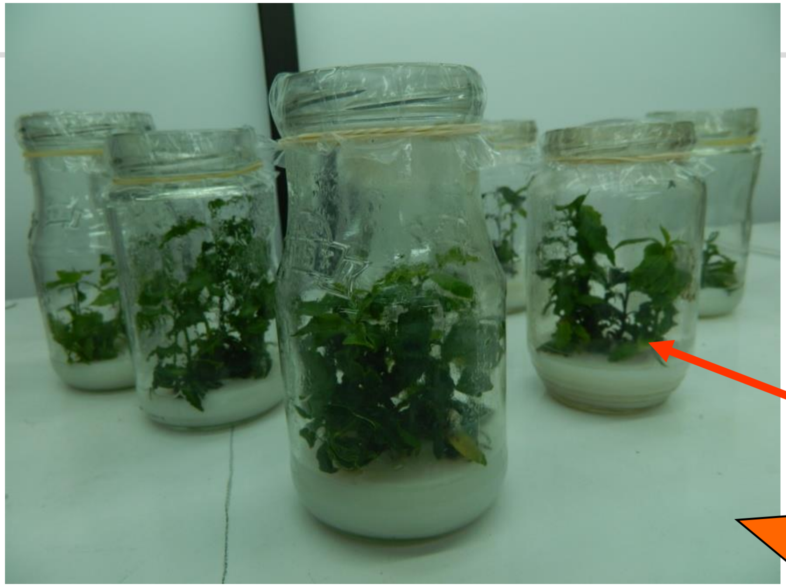
- Technical expertise
- Electricity (Energy)
- Laboratory chemicals
- Laboratory equipment

2. Low multiplication in nodal culture

3. Low success rate due to poor *in-vitro* rooting



- TRI has developed **cost effective micro propagation technique via embryo culture** and successfully applied for shortening the breeding cycle of tea



Sago cost
50Cents/ Plant

Agar cost
Rs 18/Plant

Kithul

Shoot multiplication on locally available low cost substitutes

Ex vitro Rooting & acclimatization of microshoots inside the propagator

RH >90%
Temperature
$30C^0 \pm 2$

Optimizing rooting substrate for *ex vitro* rooting of micro shoots

Transferring to the rooting medium

After 2 months

Transferring to the nursery bags

After 5 months

Transferring to the open environment

After 8 months

Field plantable plants

Harvesting stage in the field

Micropropagation in tea is an alternative method for planting material production, which has not reached up to a commercial level yet because of

Nodal culture limited by low multiplication rates of tea cultivars

- **Liquid culture technique** √
- **Photoautotrophic micropropagation** √

Optimization of liquid culture conditions to increase multiplication rate of new tea cultivars

- **Research location Tissue culture laboratory,** Tea Research Institute(TRI)- Talawakelle.

- **Plant materials-**

  Cultivar TRI 5001**,**TRI 5004,TRI 5002,

- **Explants**

➢ **Stem nodals**

➢ ***Cotyledonous SE***

➢ **Leaf Calli, Stem Calli**

a

b

c

Performances of the shoot growth with optimize fully liquid medium.

a) Higher multiplication rate (7 shoots/14 Weeks)

b) Higher relative shoot growth (90% Relative shoot growth /14 Weeks)

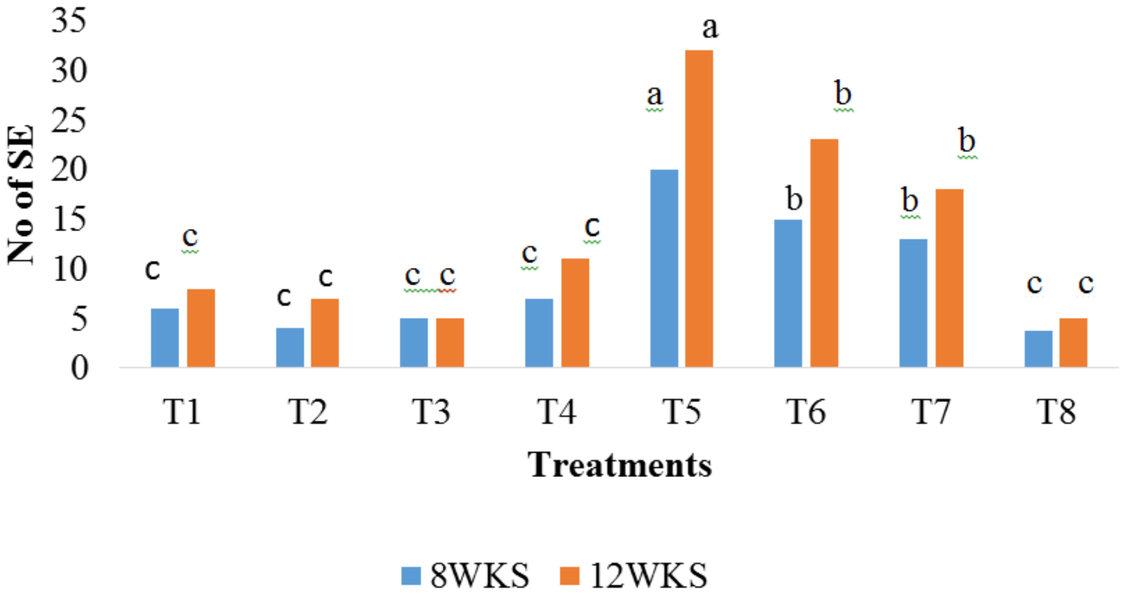
c) Growth performance in control (60% Relative shoot growth /14 Weeks)

(3 shoots/14 Weeks)

Somatic embryogenesis (Generation of embryos using a vegetative part of the plant) is one of the best micropropagation method, which can solve above mentioned limitations due to

- Resistance for the climatic condition due to well develop tap root
- Higher multiplication rate
- Reduced proliferation time
- Lower probability of genetic variation

Testing **liquid** media for somatic embryogenesis, multiplication and regeneration



A liquid medium was optimized to increase SE multiplication rate

Plant production through somatic embryogenesis

Acclimatization

Field planting

Optimization of light intensities and light qualities for plant growth and multiplication

- Research location – Upgraded Tissue Culture Laboratory, Tea Research Institute(TRI)- Sri Lanka

- MS liquid medium use for inoculation and subculturing

- Use uniform plant materials

- Control treatment with solid medium under 30 µmole m^{-2} s^{-1} PAR light Intensity.

0-1000 < µ mol m^{-2} s^{-1} Photosynthetically Active Radiation (PAR) light intensities can be maintained under the developed light system.

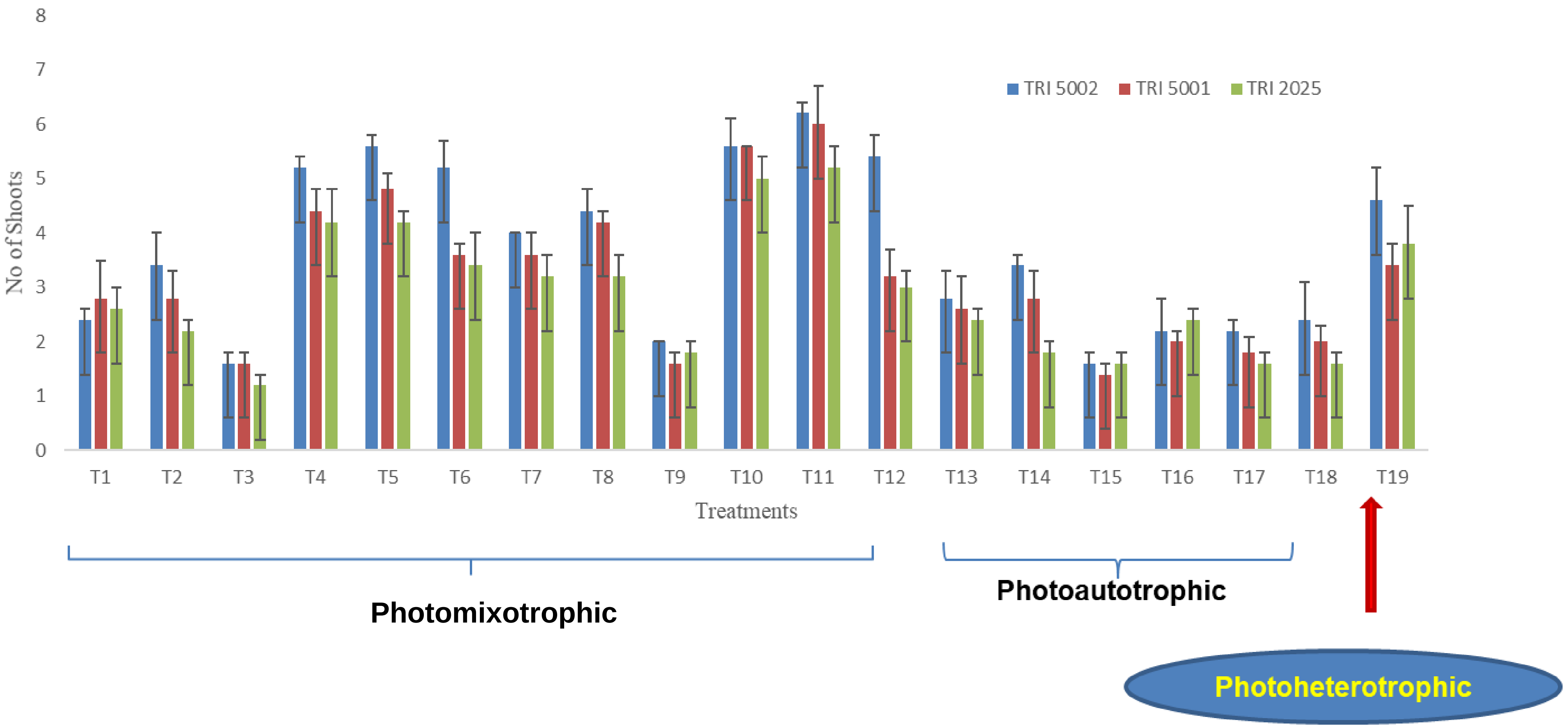
Photoautropic Micropropagation

Plants have a natural capacity to produce their own food (carbohydrates) by utilizing freely available inorganic substances by the process of photosynthesis.

Advantages

1. Promotion growth and photosynthesis of *in vitro* plants
2. Decrease in microbial contaminations
3. Shortening the *in vitro* multiplication cycle.
4. Higher survival rate.

Effect of light quality and quantity for microshoots proliferation and height growth under photoautotrophic and photomixotrophic conditions.



Performances of the microshoot multiplication of the optimized treatment vs control treatment

Higher shoot multiplication (6.2 Folds/Shoot) was observed in Photomixotrophic culture condition

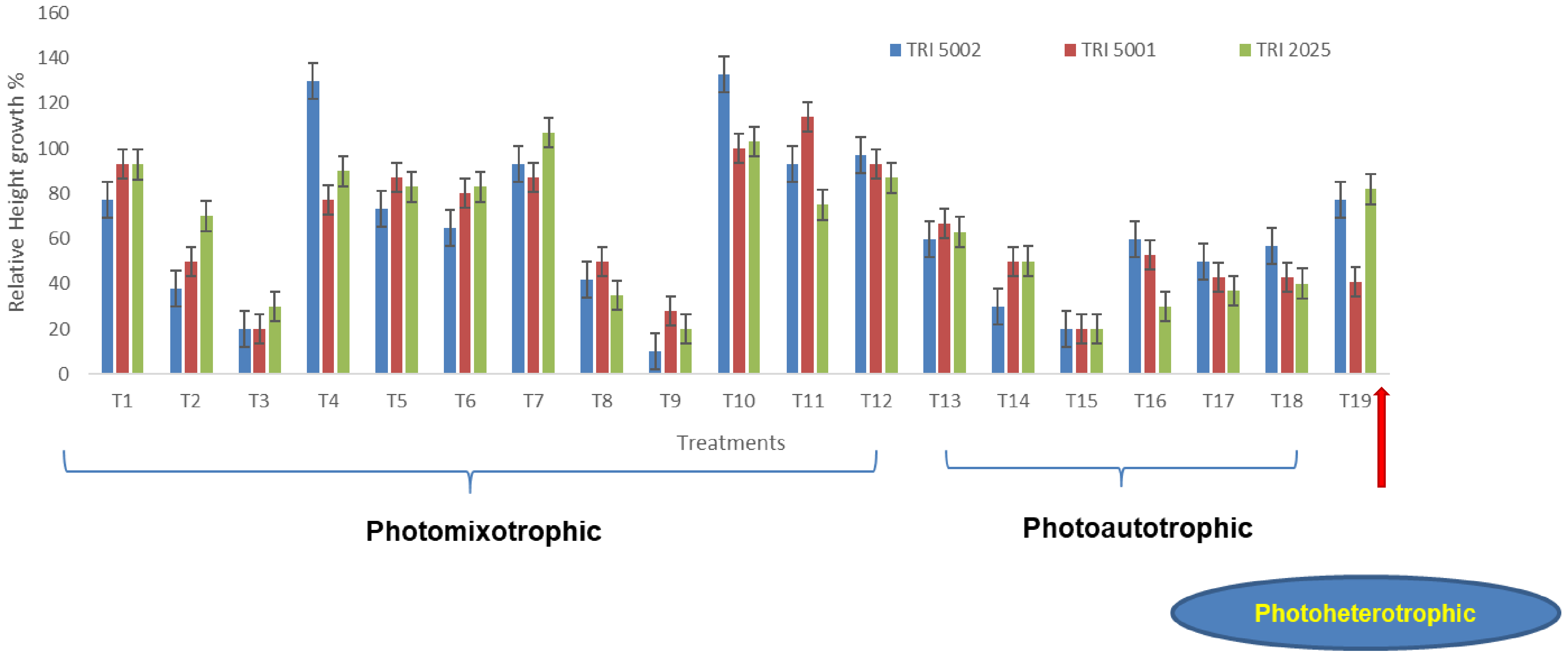
- MS Liquid
- 15g/L Sucrose
- 100 µmol m-^{2}s-^{1} Photosynthetic Active Radiation light intensity
- R:G:B 1:1:1 Ratio light quality

Shoot multiplication (4.6 Folds/Shoot) was observed in Photoheterotrophic control conditions

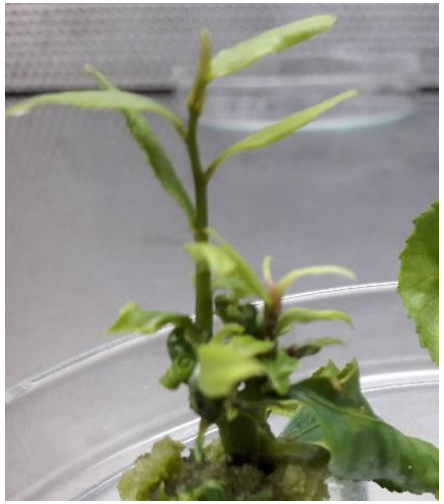
- MS Solid
- 30g/L Sucrose
- 30 µmol m-^{2}s-^{1} Photosynthetic Active Radiation light intensity
- R:G:B 1:1:1 Ratio light quality

Photoautotrophic without Sucrose - Multiplication rate was 2.2 Folds/Shoot

Relative height growth under different light levels and culture conditions



Performances of the relative shoot growth of the optimized treatment vs control treatment



Higher relative shoot growth (133%) was observed in Photomixotrophic culture condition

- MS Liquid
- 15g/L Sucrose
- 75 µmol $m^{-2}s^{-1}$ Photosynthetic Active radiation light intensity
- R:G:B 1:1:1 Light quality

Relative shoot growth(77%) was observed in Photoheterotrophic control treatment

- MS Solid
- 30g/L Sucrose
- 30 µmol $m^{-2}s^{-1}$ Photosynthetic Active Radiation light intensity
- R:G:B 1:1:1 Light quality

Cost of production of plants under Conventional Photoheterotrophic, Photo mixotrophic and Photoautotrophic micropropagation system.

Cost-benefit analysis of the different micropropagation protocols

Items for production of 1000 Plants	Conventional Photoheterotrophic micropropagation system	Photomixotrophic micropropagation system	Photoautotrophic micropropagation system
A) Labour	4617.6 (21.56%)	4617.7 (22.16%)	4617.6 (21.85%)
B) Supplies	4003 (18.69%)	2966.1(14.22%)	2933.74 (13.88%)
C) Materials	6123.7(28.59%)	6123(29.38%)	6123.68 (28.97%)
D)Equipment and Building	2124(9.9%)	1918.74(9.21%)	1918.74 (9.10%)
E) Building and installation	770.6(3.6%)	770.6(3.7%)	770.6 (3.6%)
F) Electricity	3607.9(16.84%)	4301.15(20.63%)	4301.15 (21.75%)
G)Maintenances of building and equipment	172(0.8%)	172 (0.7%)	172 (0.81%)
Total cost	20872.70	20348.12	20316.57
Unit cost Rs.	20.87	20.35	20.32

Potential of the production of microshoots through the different micropropagation systems

Micropropagation System	Relative height growth %	Multiplication Rate	No of sub cultures/ Year	Potential No. of micro shoots/Year	Average cost/Plant Rs.
Photomixotrophic micropropagation system	133	6.2	4	1296	3.90
Photoautotrophic micropropagation system	54	2	4	64	18.75
Photoheterotrophic micropropagation system	70	4	6	4096	6.99

Shoot multiplication through Bio-Reactor.

Increasing rate of in vitro planting material production using liquid culture technique in a prototype Bio reactor

Higher Multiplication Rate

Future Direction

Scale up the planting material production, combining with the optimized parameters (Liquid and light conditions) through a **Bio-Reactor**

Thank You