Production of Rosmarinic Acid from *in-vitro* culture of Basil (*Ocimum bacilicum* L.) and Lemon balm (*Melissa officinalis* L.)

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Outlines

- Intoduction
- Rosmarinic acid & its medicinal uses.
- Basil & Lemon balm.
- Plant tissue culture.
- Results.
- Conclussions.

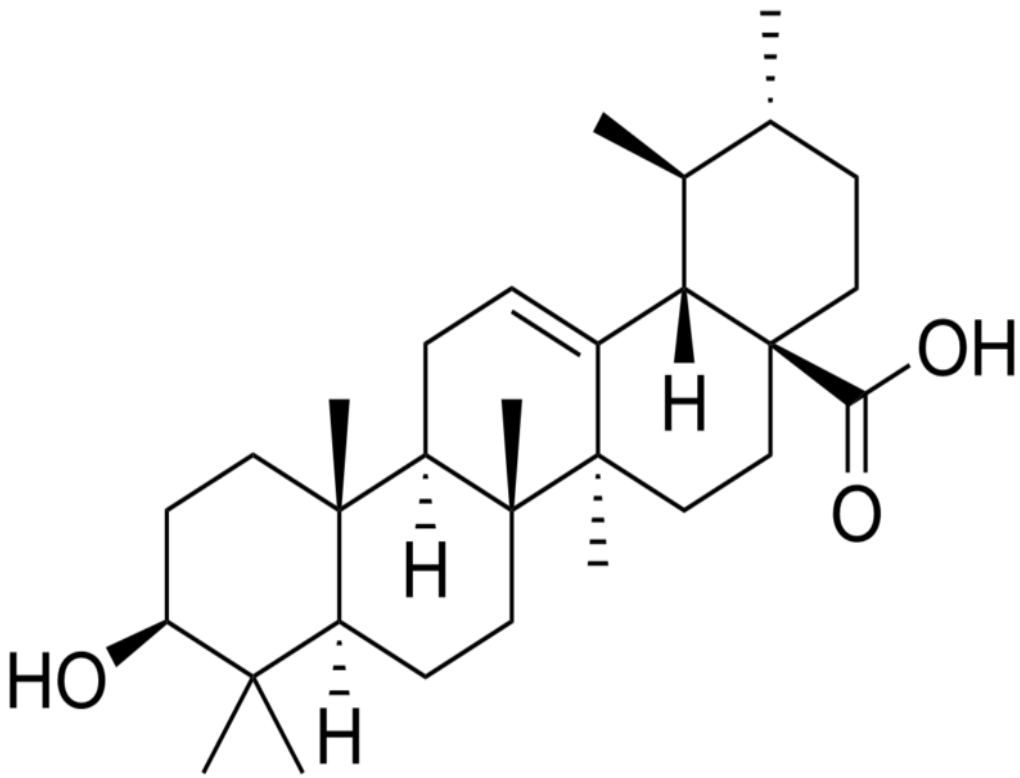
Secondary Metabolites

- Studies on plant secondary metabolites have been increasing over the last 50 years.
- Many higher plants are a major source of natural products used as:

- Pharmaceuticals and Agrochemicals.
- Flavor and fragrance ingredients.
- Food additives and Pesticides.

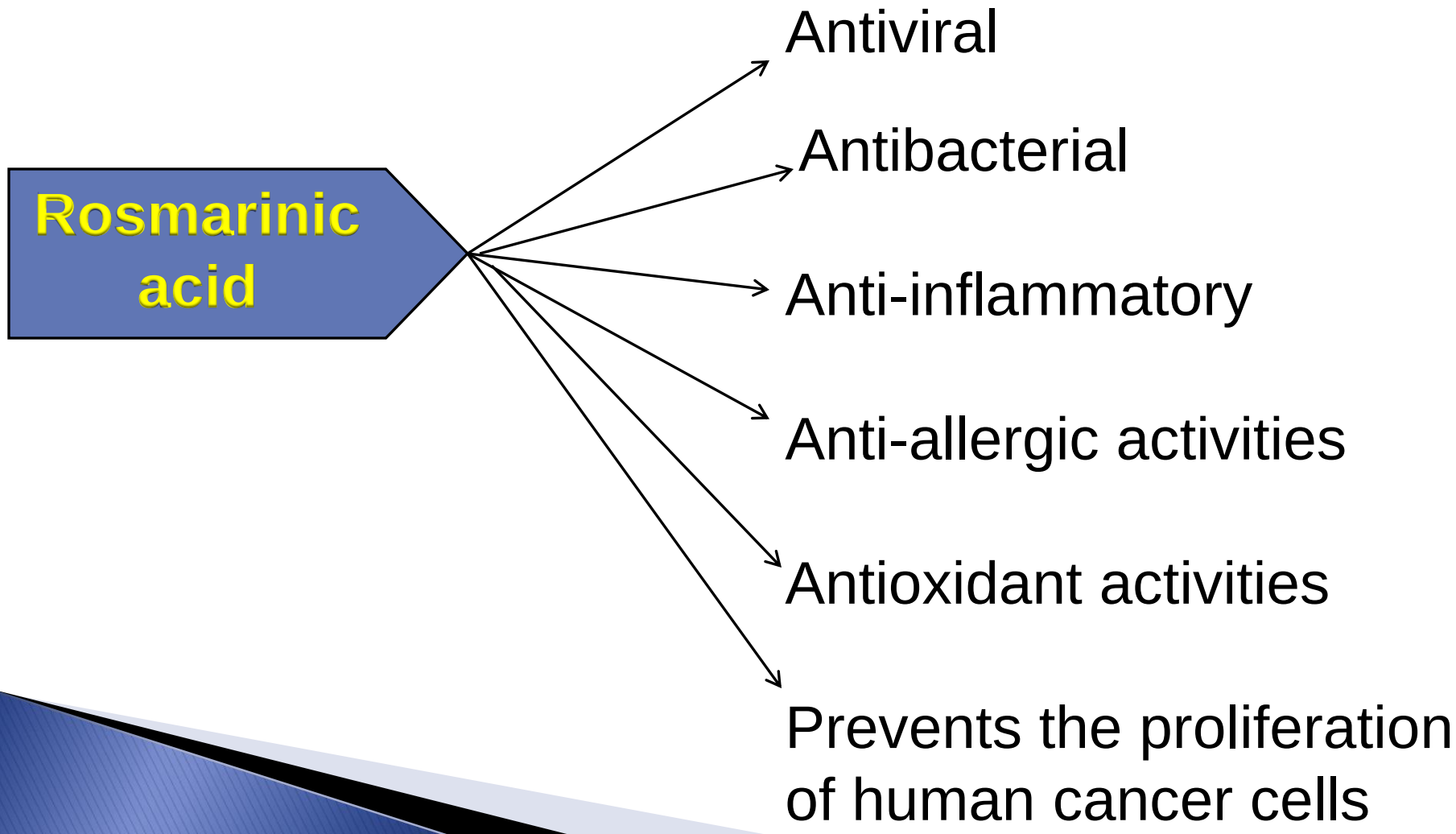
Rosmarinic acid

- First isolated from *Rosmarinus officinalis* by (Scarpati and Oriente, 1958)
- it is an ester of caffeic acid and (3, 4-dihydroxyphenyl) lactic acid.



Rosmarinic acid

Medicinal uses



Basil

Ocimum bacilicum is a member of the Lamiaceae (mint) family. The common name is sweet basil.



medicinal properties of *O.bacilicum*

- Anti bacterial (gram positive & gram negative bacteria).
- Anti-inflammatory
- Strong antioxidant activity
- Anti proliferative effect
- Anti cancer
- Antiviral
- Antifungal activity







Lemon balm

Melissa officinalis is a member of the Lamiaceae family. The common name is lemon balm.



medicinal properties of *M.officinalis*



- Antioxidant
- Antibacterial
- Antifungal agent
- Anti cancer
- Antiviral properties (against HIV-1 and the influenza virus).
- useful in the treatment of Alzheimer's disease.









Plant Tissue Culture

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs (explants) under sterile conditions on a nutrient culture medium of known composition.



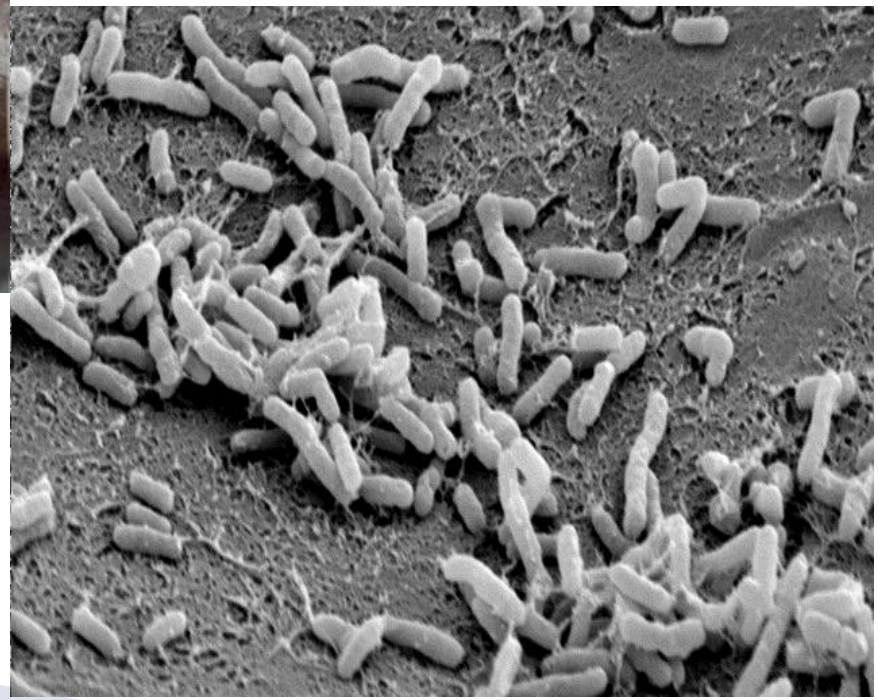
Plant tissue culture

- Over the past three decades, plant cell cultures techniques have proved to be a valuable tool for study biosynthesis and production of secondary natural products.
- One of the plant tissue culture protocols that is considered as an attractive alternative for the production of many valuable natural products is hairy root culture.

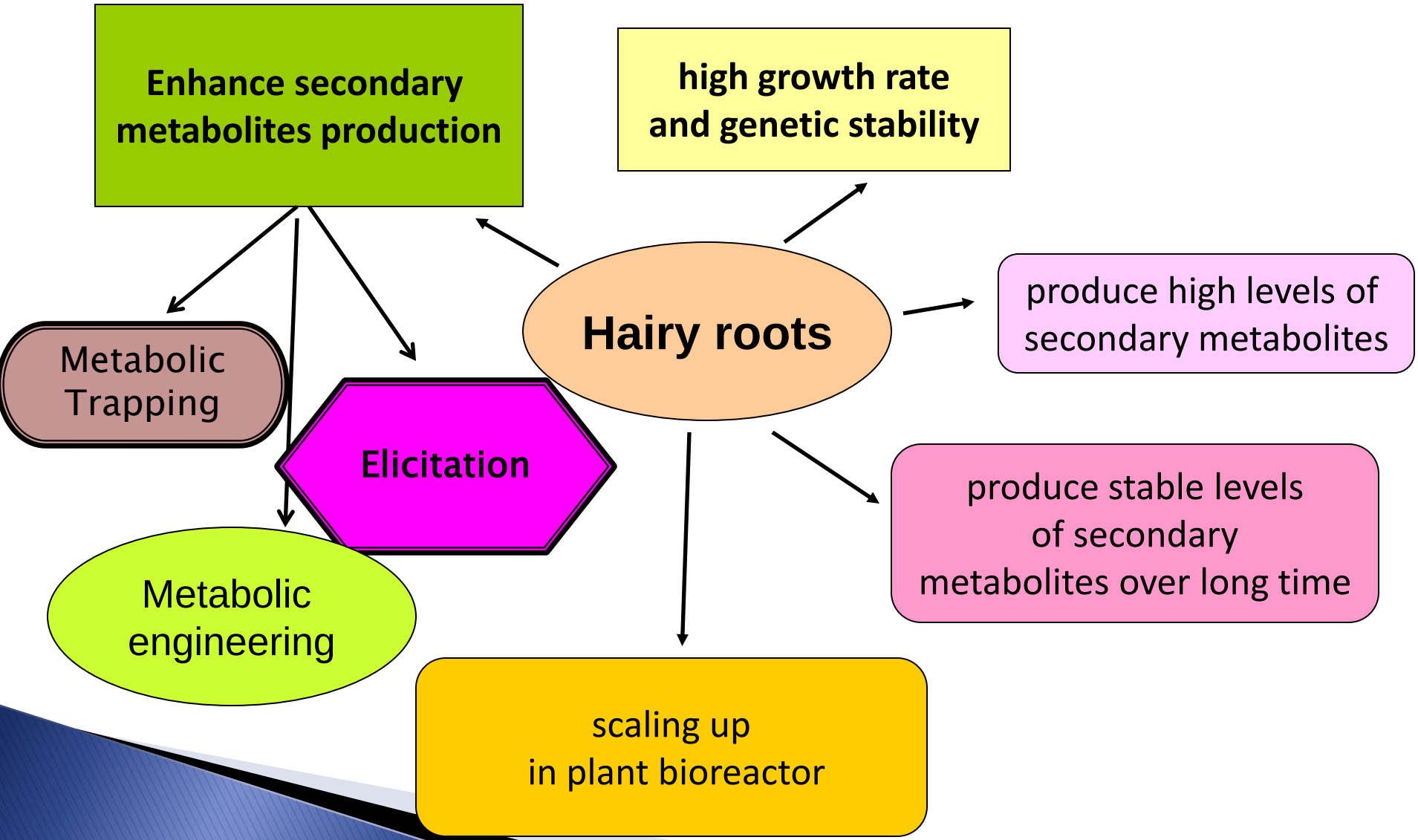
Hairy root culture



Hairy root is a transgenic phenotype resulted from the infection of suitable plant tissues with the gram-negative soil pathogen *Agrobacterium rhizogenes* .



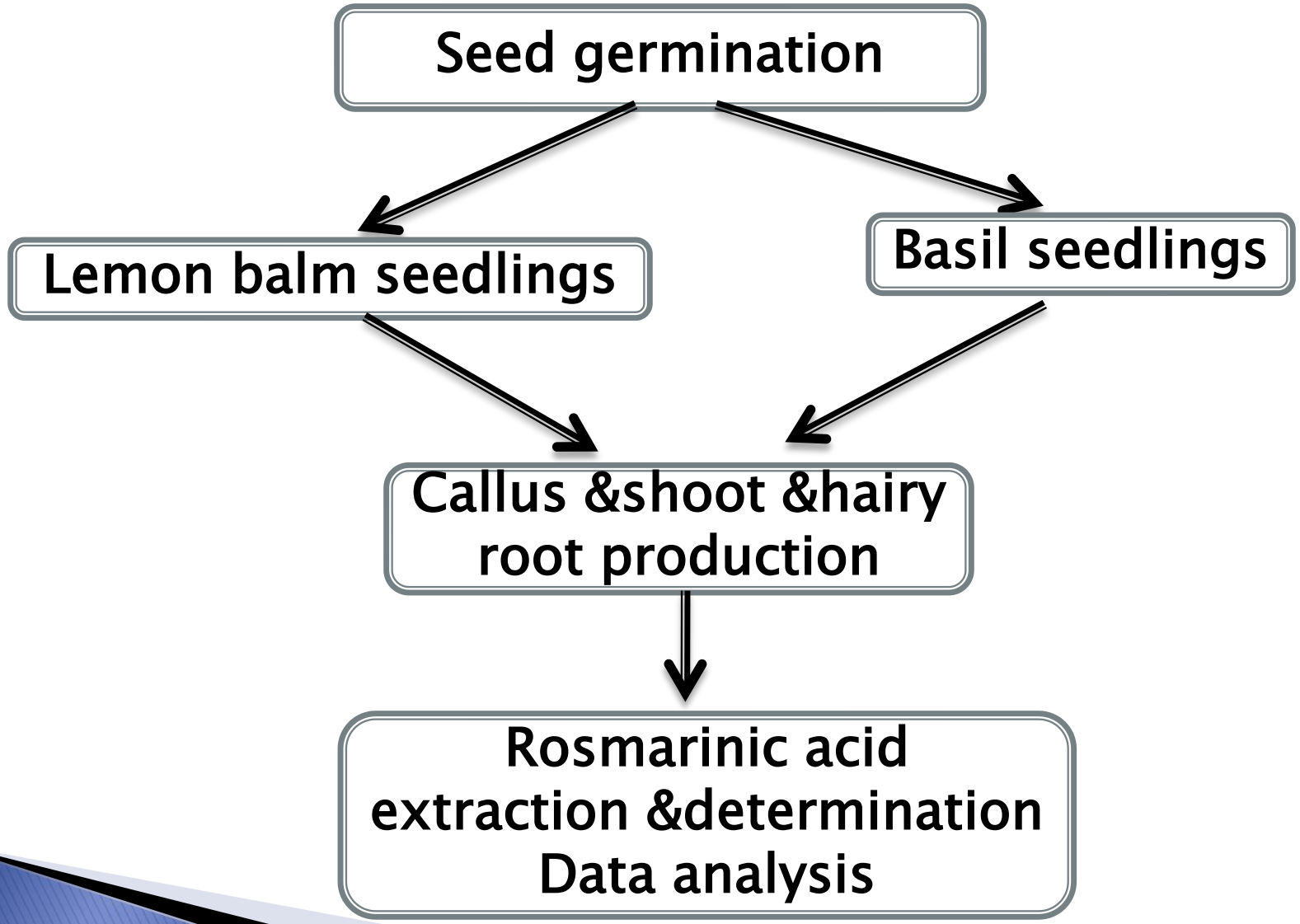
Advantages of hairy root cultures



Aim of The work

study the efficiency of using plant tissue culture techniques for the production of rosmarinic acid from basil and lemon balm.

Materials and Methods



Results

Seed germination:

Ms medium was used for germination of *O.bacilicum* and *M.officinalis* seeds under aseptic condition



Ocimum bacilicum
Germination percentages 63%



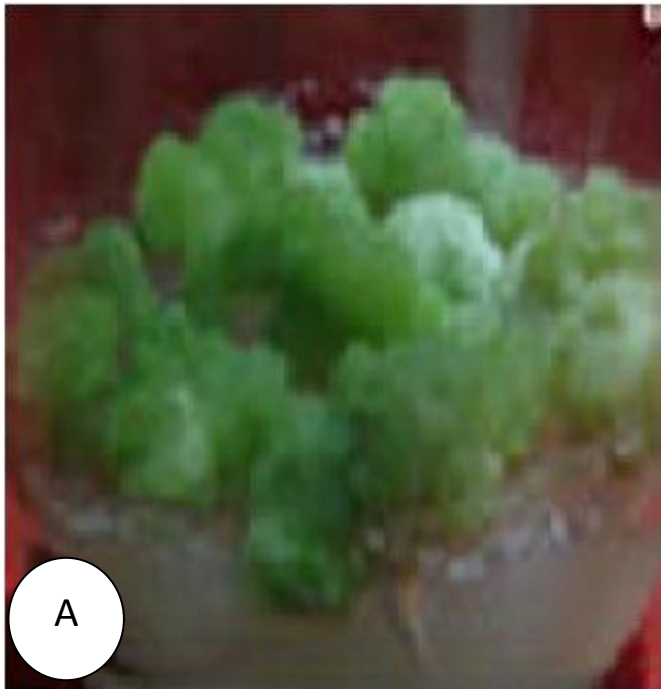
Melissa officinalis
Germination percentages 84%

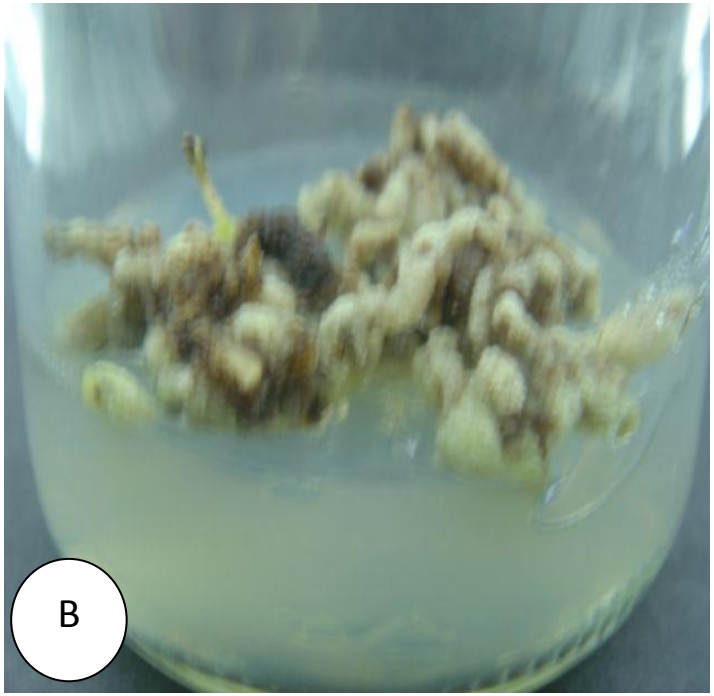
Callus production in lemon balm :

Media type	Leaf	Stem	Root
MS1(basal MS)	------	------	------
MS2(MS+1mg/l BA+1mg/l NAA)	11.75±1.85	8.52±1.04	6.23±0.85
MS3(MS+3mg/l BA+1mg/l NAA)	8.54±1.22	4.24±0.25	3.17±0.22
MS4(MS+5mg/l BA+1mg/l NAA)	4.35±0.43	3.42±0.33	2.21±0.25

Callus production in basil:

Media type	Leaf	Stem	Root
MS1(basal MS)	------	------	------
MS2(MS+1mg/l BA+1mg/l NAA)	6.55±0.64	4.90±0.54	5.74±0.67
MS3(MS+3mg/l BA+1mg/l NAA)	7.33±0.82	2.28±0.74	4.82±0.42
MS4(MS+5mg/l BA+1mg/l NAA)	10.45±1.01	5.88±0.83	4.21±0.32



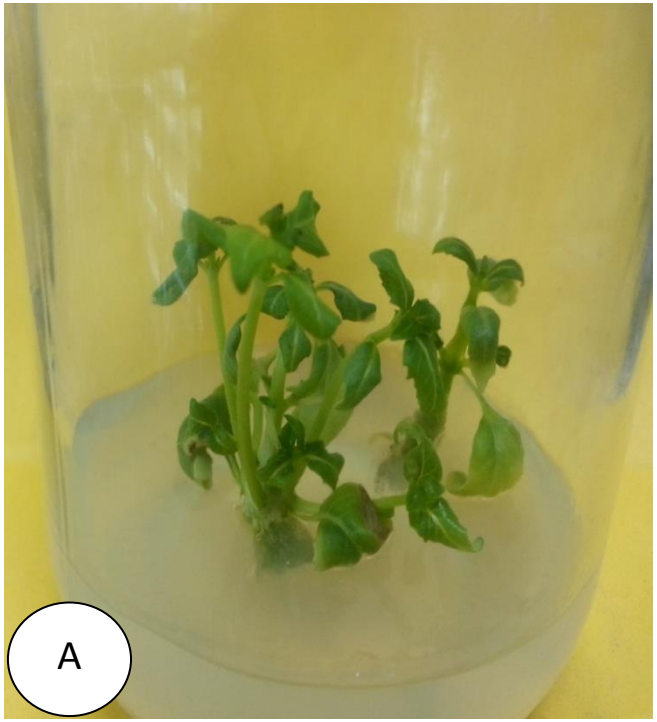


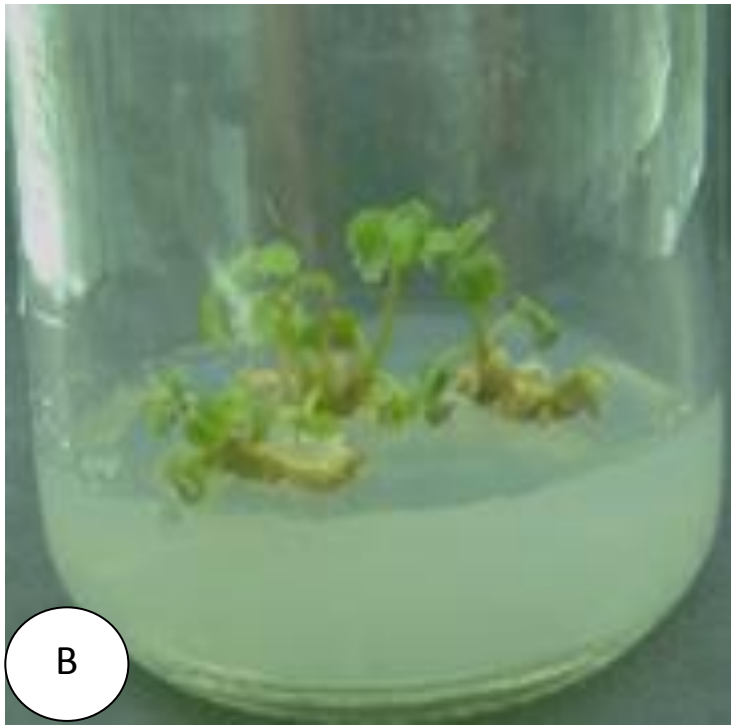
A- Basil callus culture derived from leaf explants grow on MS+1mg/l BA and 1mg/l NAA (MS2).

B- Lemon balm callus cultures derived from leaf explants grow on MS+ 5mg/l BA + 1mg/l NAA (MS4).

Shoot production in lemon balm :

Media type	No. of shoots/explant		
	Leaf	Stem	Root
M1 (basal MS)	0	0	0
M2 (MS+1mg/l KIN+1mg/l 2,4-D)	0	12±1.5	0
M3(MS+ 3mg/l KIN+1mg/l 2,4-D)	0	7±0.8	0
M4(MS+5mg/l KIN+ 1mg/l 2,4-D)	0	3±0.4	0





Shoot culture culture of basil and lemon balm.

A-Basil shoot culture derived from shoot tip explants grow on MS+ 0.4mg/l thiamine HCl + 1mg/l BA.

B-Lemon balm shoot culture derived from stem explants grow on MS+1mg/l of both Kin and 2,4-D (M2).

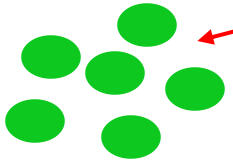
Hairy root culture

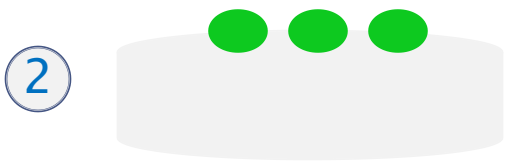
Method of transformation. Procedure 1

Leaf discs, stem discs, or seedling segments

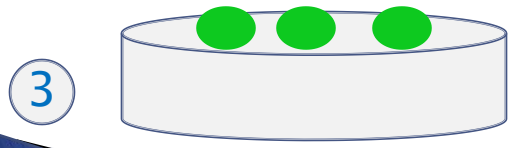
A. rhizogenes

1 Inoculation



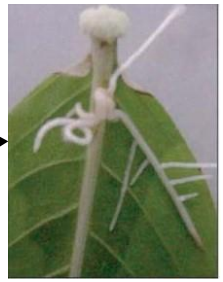


2 Co-cultivation (1-5 days)



3 Selection on antibiotic containing medium

4



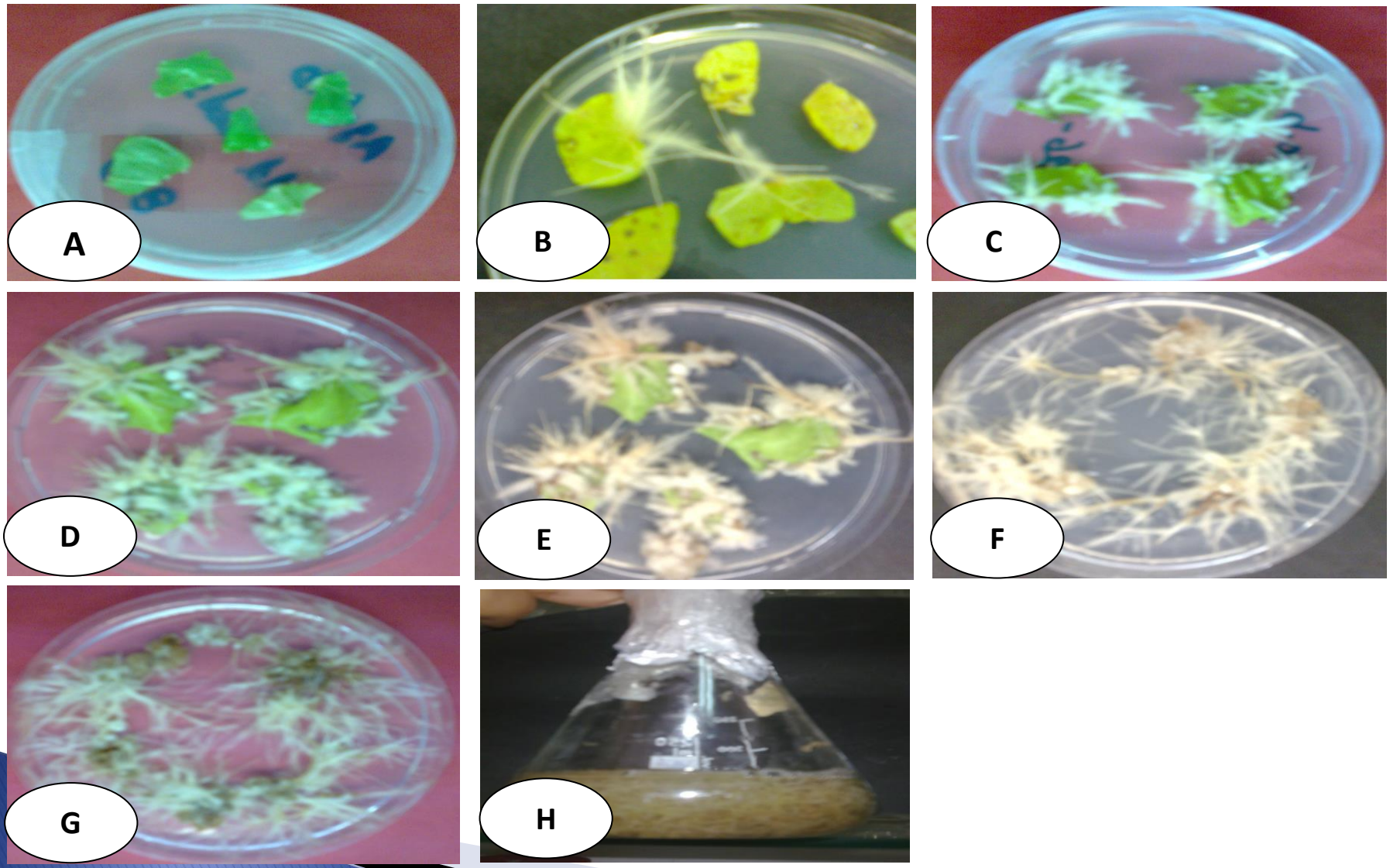
Hairy root formation

5



Cultivation in liquid medium

Agrobacterium **mediated transformation of basil and hairy root culture production using procedure (1).**



Effect of explant type on transformation frequency and hairy root production in basil infected with R 1000 strain.

Explant type	Incubation period	Total number of infected explants	Number of explants Producing roots	Number of hairy roots per explant	Root length (cm)	Transformation frequency (%)
Leaf	1hr	143	110	branched	1.0 ± 0.2	77
Stem	1hr	47	0	0	0	0

Data collected after 15 days on selection plates

Effect of explant type on transformation frequency and hairy root production in basil infected with A4 strain.

Explant type	Incubation period	Total number of infected explants	Number of explants Producing roots	Number of hairy roots per explant	Root length (cm)	Transformation frequency (%)
Leaf	**1hr**	**154**	**134**	**8.0 ± 0.2**	**1.5 ± 0.5**	**87**
Stem	**1hr**	**52**	**0**	**0**	**0**	**0**

Data collected after 15 days on selection plates

Agroinfiltration. Procedure 2

In the method a suspension of Agrobacterium is injected into a plant tissues, where it transfers the desired gene to plant cells.





Agrobacterium **mediated transformation of lemon balm and hairy root culture production using procedure (2). Arrows showed hairy roots produced after stem injection with** ***Agrobacterium*** **R1000 strain.**

Effect of explant type on transformation frequency and hairy root production in lemon balm infected with R 1000 strain.

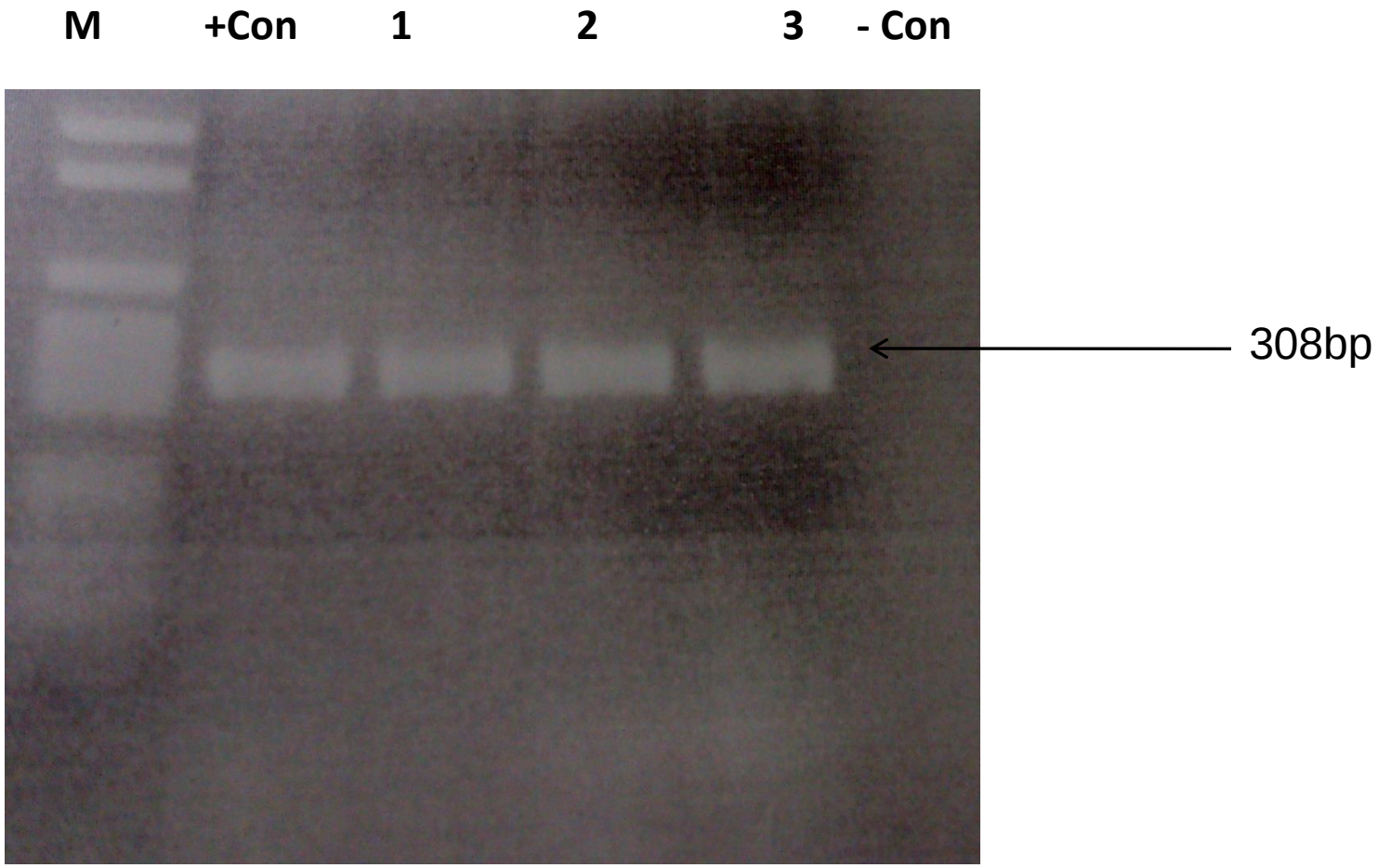
Explant type	Incubation period	Total number of infected explants	Number of explants Producing roots	Number of hairy roots per explant	Root length (cm)	Transformation frequency (%)
Leaf	injection	56	0	0	0	0
Stem	injection	40	15	7.0 ± 0.2	2.0 ± 0.5	37.5

Data collected after 30 days after infection

Effect of explant type on transformation frequency and hairy root production in lemon balm infected with A4 strain.

Explant type	Incubation period	Total number of infected explants	Number of explants Producing roots	Number of hairy roots per explant	Root length (cm)	Transformation frequency (%)
Leaf	injection	48	0	0	0	0
Stem	injection	36	12	2.0 ± 0.1	1.5 ± 0.5	33.3

Data collected after 30 days after infection



PCR amplification bands of WT and transformed hairy roots.

Rosmarinic acid levels (ug/mg DW) in control, callus, shoot and hairy root cultures of basil and lemon balm.

	Basil	Lemon balm
Control	9.42±1.27	12.82±0.83
Callus	13.20±1.21	28.12±0.39
Shoot	25.30±0.24	38±25±0.73
Hairy Root	68.52±1.83	42.77±0.98

Each treatment is average of three replicates ± SE

Conclusions

Plant tissue culture techniques specially Agrobacterium Mediated transformation can be used successfully to produce natural products such as rosmarinic acid.



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Thank You for Attention