



1<sup>st</sup> INTERNATIONAL MEETING AGRISCIENCE & PRACTICE,  
10<sup>th</sup> - 11<sup>th</sup> May 2018, Stip, Faculty of Agriculture  
Goce Delcev University – Stip, R. Macedonia



# APPLICATION OF PHYTOHORMONES IN THE IMPROVEMENT OF AGRICULTURAL AND HORTICULTURAL SPECIES AT *IN VITRO* AND *IN VIVO* CONDITION

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**In plant biotechnology, the role of phytohormones and plant growth regulators PGRs is irreplaceable.**

## Abscisic acid (ABA)

### Auxins

Indole-3-butyric acid (IBA)

Indole-3-acetic acid (IAA)

1-Naphthaleneacetic acid (NAA)

Phenil-3-acetic acid (PAA)

### Cytokinins

Kinetin (KIN)

Zeatin (ZEA),

6-benzylaminopurine (BAP)

Diphenylurea (DFU)

Thidiazuron (TDZ)

### Other known hormones

Brassinosteroids

Jasmonates

Polyamines

### Gibberellins (GAs)

Giberellic acid ( $GA_3$ )

### Ethylene





**Powerful methods of plant biotechnology are unusable without the application of phytohormones and plant growth regulators**

**1950s First used of tissue culture by orchid industry**

**1970s - 1980s Rapid development of world plant biotechnology  
first commercial plant tissue laboratories  
mid 1980s – 20 millions plant per year micropropagated**

**1990s - Macedonian plant biotechnology (University laboratories)**

**1990s - 2000 PSI Institute of Southern Crops (ISC)  
Plant Biotechnology Laboratory**

**2007 Faculty of Agriculture, Goce Delcev University, Stip**

**Department of Plant Biotechnology, FA, GDU**



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# APPLICATION OF PHYTOHORMONES IN THE IMPROVEMENT OF AGRICULTURAL AND HORTICULTURAL SPECIES AT *IN VITRO* CONDITION

## 1990s - MICROPROPAGATION OF AGRICULTURE SPECIES

Species	Explant	Medium + PGRs mg·l <sup>-1</sup>	Results
<i>Capsicum annuum</i> L.	apical buds	MS + 5.0 BAP + 0.5 NAA MS + 10.0 BAP + 0.5 IAA MS + 1.0 ZEA	callus shoots
	anthers	CP + 0,01 KIN + 0,01 2,4D R <sub>1</sub> + 0,01 KIN	embryos
	hypocotyls 1/3 cotyledons	MS + 10.0 BAP + 0.5 NAA MS + 30.0 BAP + 1.0 IAA MS + 5.0 ZEA MS + 2.5 2iP	callus
<i>Lycopersicon esculentum</i> Mill.	apical buds	MS + 4.5 BAP + 0.3 IBA MS + 6.0 BAP + 0.4 IBAA MS + 4.5 KIN + 0.3 IAA	shoots
	hypocotyls 1/3 cotyledons	MS + 1.5 BAP + 0.1 IBA MS + 3.0 KIN + 0.1 IAA MS + 6.0 BAP + 0.4 IBA	callus
<i>Cucumis sativus</i> L.	apical buds	MS + 11.0 KIN + 3.5 IBA	shoots
	hypocotyls	MS + 2.0 KIN	callus
	1/3 cotyledons	MS + 6.5 BA+10.0 2,4 D	callus



# 1990s - MICROPROPAGATION OF AGRICULTURE SPECIES



MS + 2.0mg/l BAP + 2.5mg/l 2.4 D  
MS + 2.5mg/l BAP + 1.5mg/l NAA  
MS + 2.0mg/l 2iP + 0.5mg/l IAA  
MS + 0.5mg/l KIN + 1mg/l IAA



*LYCOPERSICON ESCULENTUM MILL.  
VAR. CERASIFORME (DUNAL)*



1/4



Species	Family	Medium + PGRs mg/l
Целер, <i>Apium graveolens</i> L.	Apiaceae	
Морков, <i>Daucus carota</i> spp. <i>sativus</i> L.	Apiaceae	MS + 3 KIN + 3 BAP
Магданос, <i>Petroselinum sativum</i>	Apiaceae	MS + 0.4 NAA + 2 KIN
Цвекло, <i>Beta vulgaris</i> ssp. <i>esculenta</i> L.	Amaranthaceae	NN + 0.8 IAA + 4 KIN
Брокула, <i>Brassica oleracea</i> var. <i>italica</i> ,	Brassicaceae	LS + 2 IAA + 2 IBA + 2KIN
Зелка, <i>Brassica oleracea</i> var. <i>capitata</i> L.	Brassicaceae	LS + 5 KIN
Ротквица, <i>Raphanus sativus</i> var. <i>radicola</i>	Brassicaceae	MS + 1 IAA
Модар патлиџан, <i>Solanum melongena</i> L.	Solanaceae	MS + 1 NAA
Тиквичка, <i>Cucurbita pepo</i> var. <i>cylindrical</i>	Cucurbitaceae	



2/2

морков

4/9  
ротквица

4/6

сокинка

4

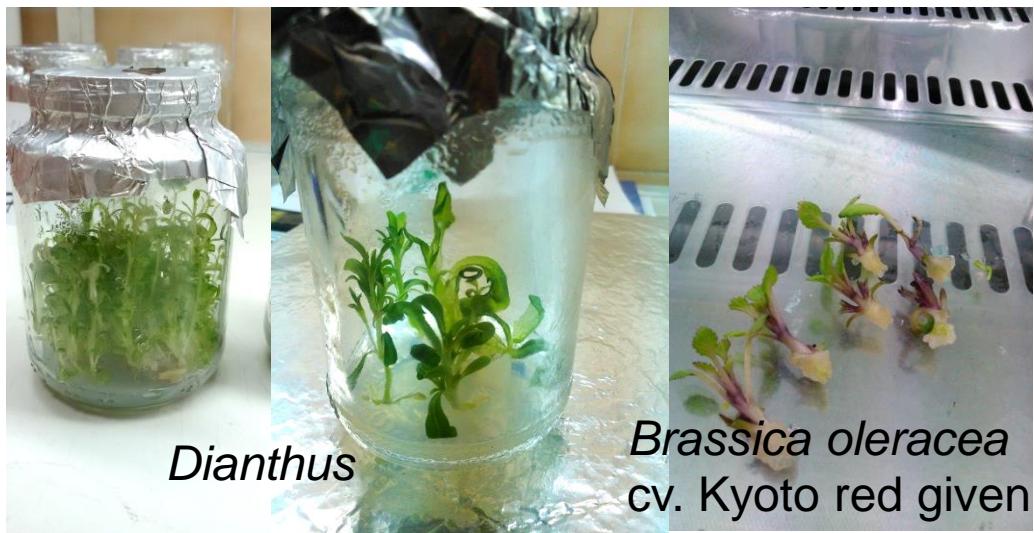
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2. MS + 0.4 mg/l NAA + 2 mg/l KIN

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## 2000s MICROPROPAGATION OF ORNAMENTAL SPECIES



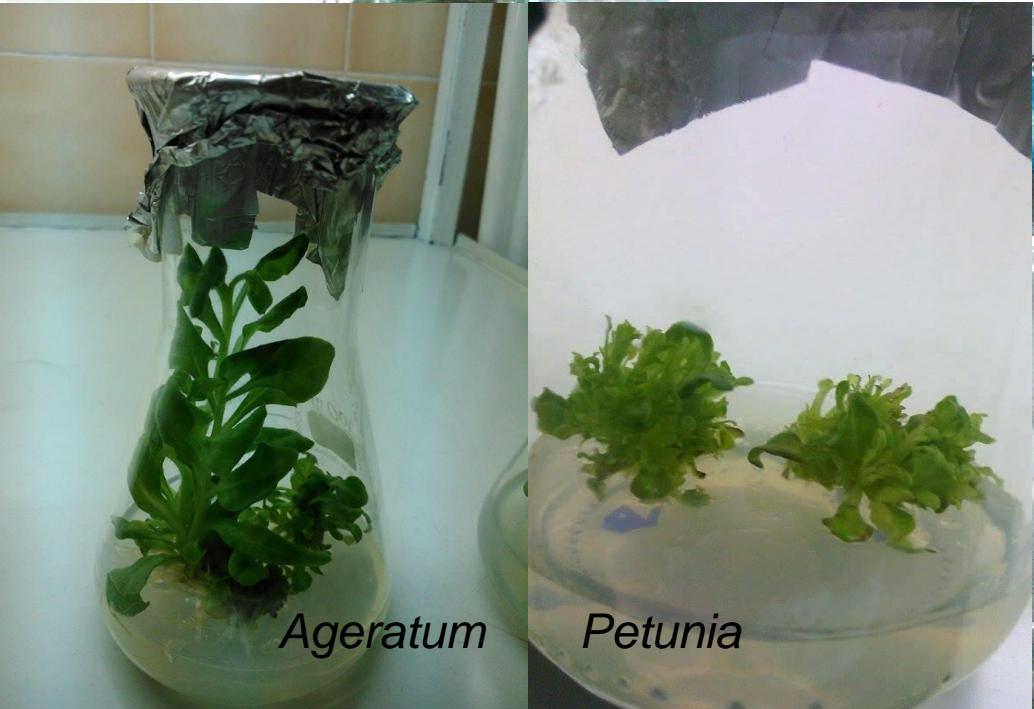
Dianthus

*Brassica oleracea*  
cv. Kyoto red given

MS + 2mg/l BA + 0.1mg/l IAA + 0.1mg/l GA<sub>3</sub>  
MS + 2mg/l BA + 0.1mg/l NAA  
MS + 2mg/l BA  
MS + 5mg/l BA + 5mg/l NAA  
MS + 5mg/l BA  
MS + 3mg/l BA + 1.5mg/l NAA

### ROOTING:

MS + 0.5mg/l IAA + 2.5mg/l IBA.



Ageratum

Petunia



## 2000s - MICROPROPAGATION OF AROMATIC AND MEDICAL SPECIES

*Eruca sativa* L.

*Coriandrum sativum* L.

*Rosmarinus* sp.

*Origanum vulgare* L.

*Lavandula vera* L.

*Melissa officinalis* L.

*Matricaria chamomilla* L.

*Salvia officinalis* L.

*Hypericum perforatum* L.



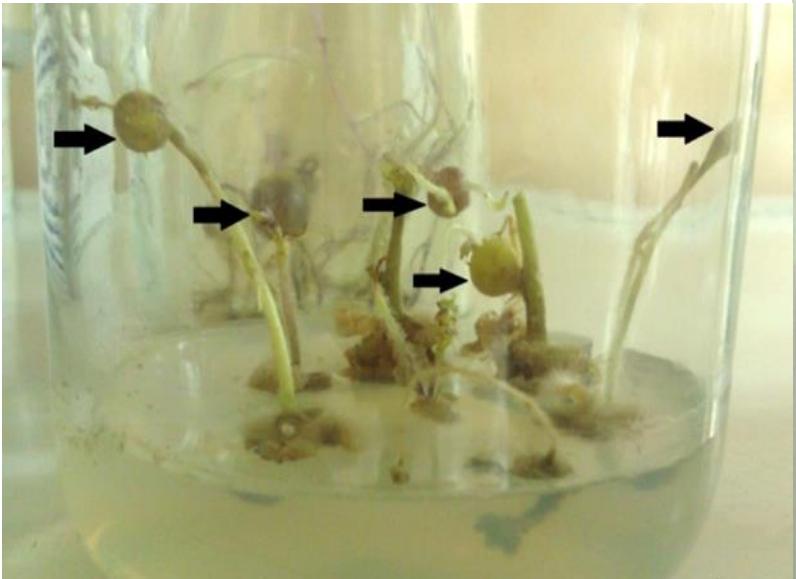
Shoots proliferation from hypocotyl *Melissa officinalis* L. explants in salad rocket (*Eruca sativa* L.)



*Coriandrum sativum* L.



# 2000s MICROTUBERISATION

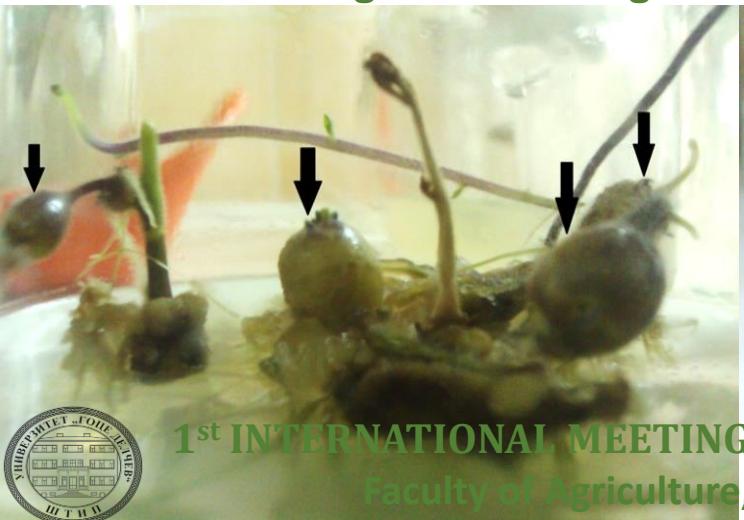


2 ppm  $\text{GA}_3$ ,  
12 ppm  $\text{GA}_3$   
22 ppm  $\text{GA}_3$

Culture of  
- sprouts  
- nodules  
- tubers

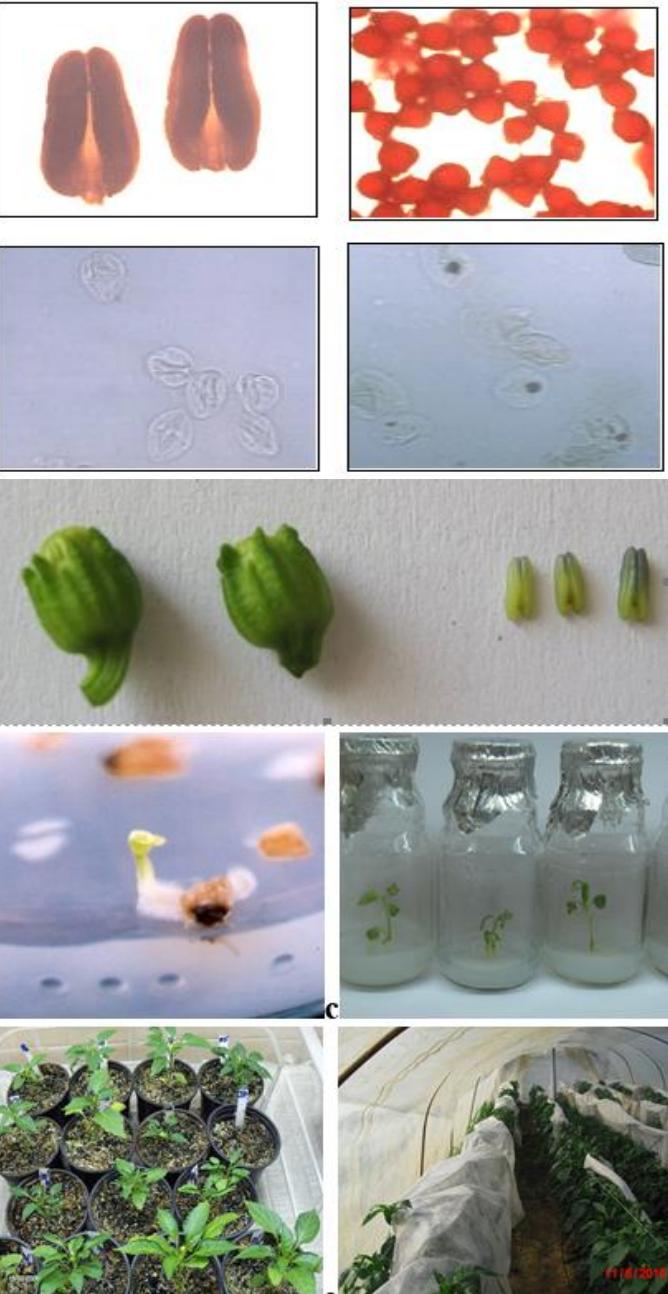


MS + 2mg/L BAP + 2mg/L IAA + 30 g/L sucrose  
MS + 1mg/L BAP + 0.5mg/L IAA + 40 g/L sucrose  
MS + 1mg/L BAP + 0.5mg/L IAA + 60 g/L sucrose  
MS + 4mg/L BAP + 2mg/L IAA + 60 g/L sucrose  
MS + 6mg/L BAP + 2mg/L IAA + 90 g/L sucrose



# 2000s – 2010 ANDROGENESIS *Capsicum annuum* L.

Pepper genotype	Total nr. anthers	Embryogenic anthers (%)	Number of embryos per 100 anthers	Embryogenic response
Féherözön	1502	17.39 a	32.60 bc	Excellent
Tura	300	17.05 a	17.05 ab	Good
Pritavit F1	330	9.23 abc	9.39 abc	Fair
California wonder	151	6.67 abc	5.67 c	Fair
Zlaten medal SR	1031	6.12 abc	8.97 bc	Fair
Majori	330	5.83 abc	6.73 c	Fair
Piran	823	5.03 abc	34.05 ab	Poor
Zlaten medal ŠT	723	4.29 bc	18.57 bc	Poor
Tomato shaped sweet	360	4.17 bc	4.54 c	Poor
Kurtovska kapija BG	620	2.90 bc	50.55 a	Poor
Kurtovska kapija SR	875	2.73 bc	10.20 bc	Poor
Slatko luta	140	2.43 bc	3.33 c	Poor
Feferona	79	0.00 c	0.00 c	No
Vezena luta	83	0.00 c	0.00 c	No
Sivrija	104	0.00 c	0.00 c	No
Rotund	109	0.00 c	0.00 c	No
Kurtovska kapija TU	236	0.00 c	0.00 c	No
Kurtovska kapija MK	122	0.00 c	0.00 c	No
Bonbona	270	0.00 c	0.00 c	No



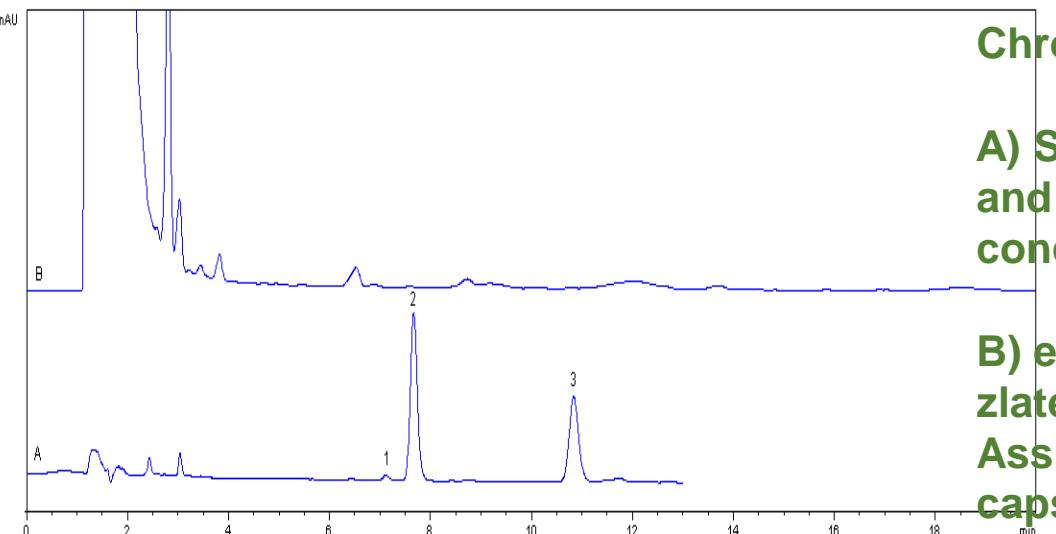
## Capsaicin - Inhibitory Factor for Somatic Embryogenesis in Pepper Anther Culture

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Chromatogram of

A) Standard solution, a mixture of capsaicin and dihydrocapsaicin (at equimolar concentrations 10 µg/mL),

B) ethanolic extract of pepper, genotype zlaten medal, obtained by Soxlet extraction.  
Assignment: 1. nordihydrocapsaicin, 2. capsaicin, 3. dihydrocapsaicin.

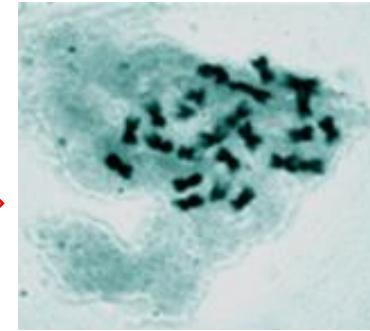
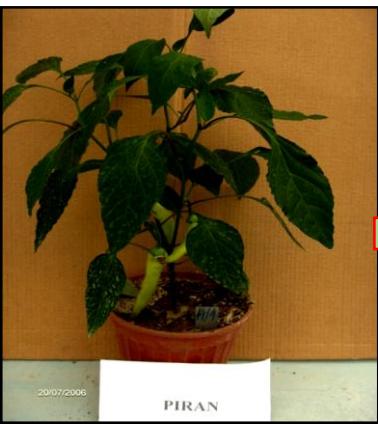
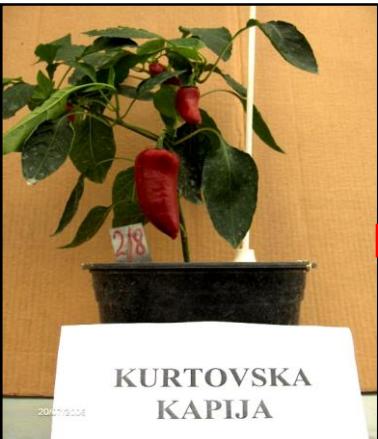


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# 2010s ANDROGENESIS *Capsicum annuum* L.



# 2010s APPLICATION OF PHYTOHORMONES IN THE IMPROVEMENT OF AGRICULTURAL AND HORTICULTURAL SPECIES AT *IN VIVO* CONDITION

*Rosmarinus officinalis* L.

*Salvia officinalis* L.

*Sambucus nigra* L.



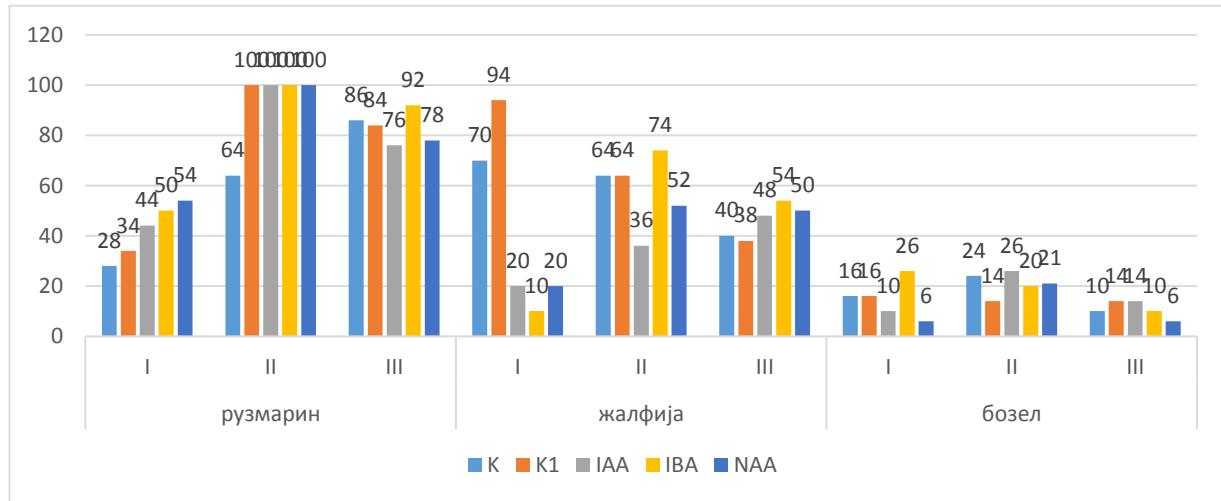
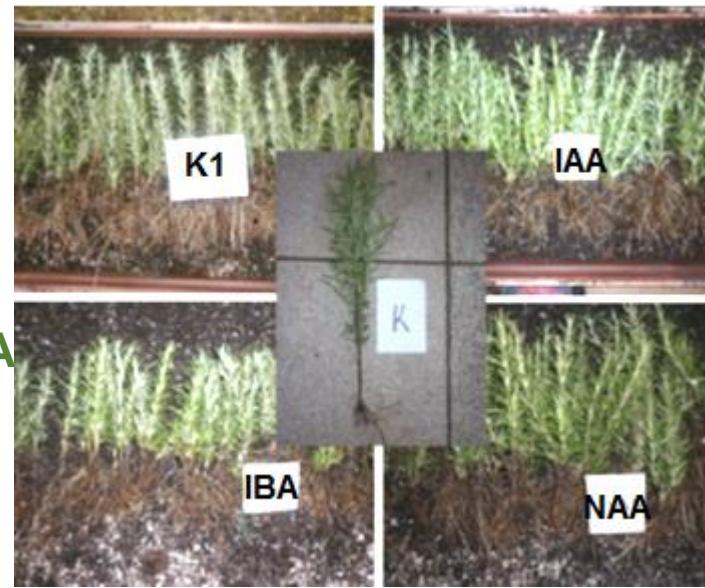
K - Control

K1 - Radicin 0,003% IBA

IAA - 5 ppm

IBA - 5 ppm

NAA - 5 ppm



# 2010 – PRESENT MISSION OF DEPARTMENT OF PLANT BIOTECHNOLOGY

## Project: Application of biotechnological methods for improvement of plant species

**Anthura – Kocani  
Orhids**

**SBW Romero Vitro  
Vinica  
2 000 species**





# **APPLICATION OF PHYTOHORMONES IN THE IMPROVEMENT OF AGRICULTURAL AND HORTICULTURAL SPECIES IN THE REPUBLIC OF MACEDONIA?**

## **IMPLEMENTATION OF PLANT BIOTECHNOLOGY IN THE REPUBLIC OF MACEDONIA?**



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**Science can amuse and fascinate us all,  
but it is engineering that changes the world.**

**Isaac Asimov (1920 - 1992)**



**Thank you!**

