



Gibberellin *in vitro* research for vegetative propagation of aspen

Jonas Žiauka*, Sigutė Kuusienė

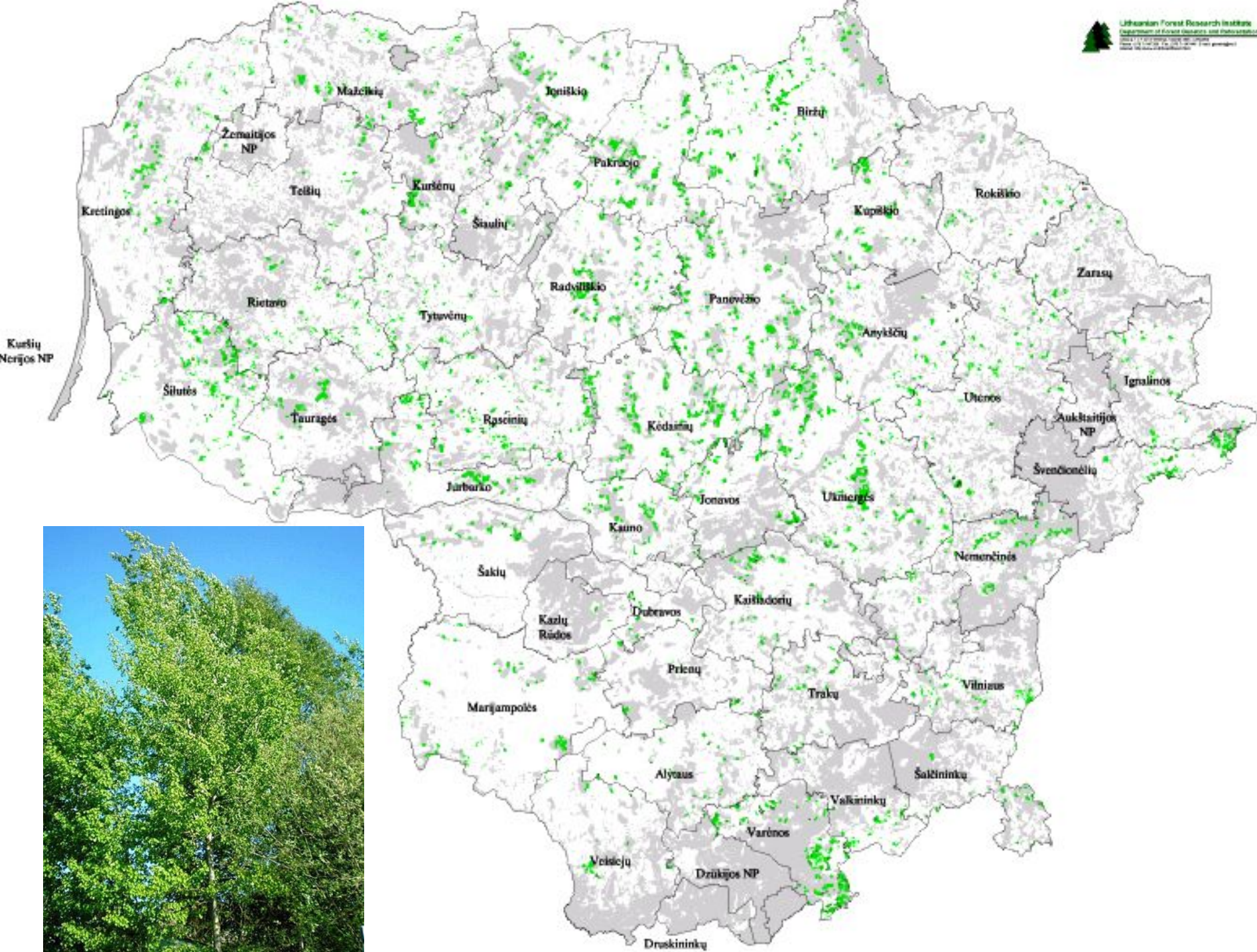
Lithuanian Forest Research Institute

Laboratory of Molecular Genetics and Biotechnology



Aspen in Lithuania

- *Populus tremula* L. – a native tree species in Lithuania
- Now aspen stands compose 3.3 % of Lithuanian forests
- Earlier considered as undesirable species in the industrial forest stands
- At the moment the recognition of aspen is increasing
- A suitable species for short-rotation forest plantations on the former agricultural lands
- An interest in perspective *Populus* hybrids is increasing
- A fast-growing hybrid between *Populus tremula* and North American *Populus tremuloides* is of special interest
- An effective method for vegetative propagation of selected aspen genotypes is required



Aspen hybrid production under laboratory conditions



1. Fertilization under controlled conditions



2. Cultivation of isolated ovaries *in vitro*



4. Testing of the offspring in the field



3. Primary testing of the offspring in *in vitro* culture

Plant hormones in vegetative propagation

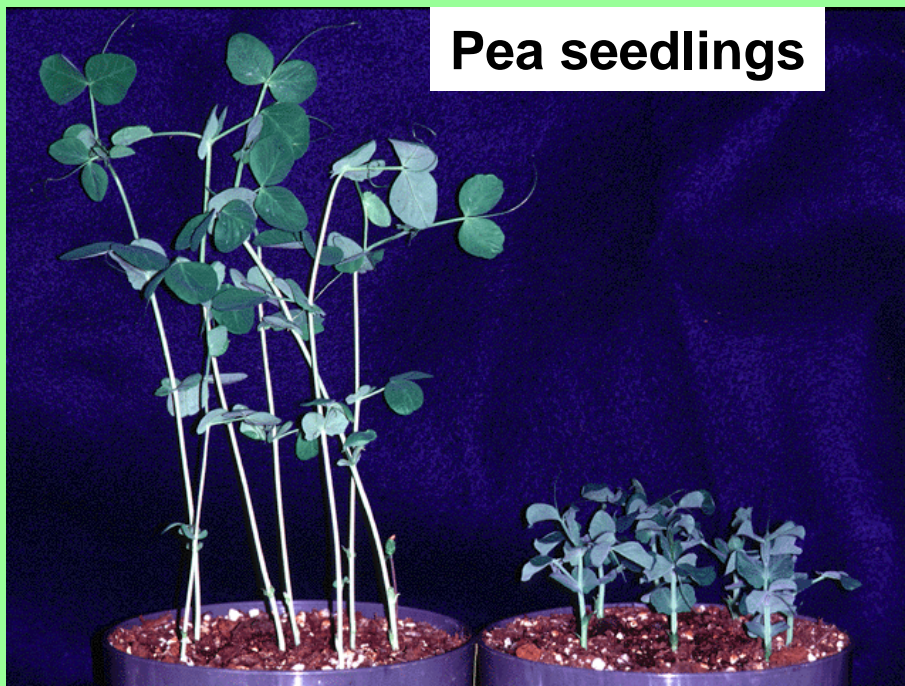
- **Chemicals** produced by plants
 - control growth and/or differentiation of plant cells
- Chemicals are **diffusible** but often influence same cells that produced them
- Sometimes referred to as **growth regulators**
- Effects of hormones on plant development are very **concentration dependent**
- Use of **exogenously applied** growth regulators is essential for improvement of plant vegetative propagation through *in vitro* system
- Plant hormones **cytokinins** (mostly for shoot multiplication) and **auxins** (mostly for rooting) are used most widely. The use of **gibberellins** in tissue culture is comparatively restricted

Gibberellin

A large number (>120) of gibberellins (GAs) is known but only few of them are biologically active, e.g. GA1, GA3, GA4, GA7

Bioactive gibberellins:

- promote seed germination
- promote stem elongation growth



Pea seedlings

GA3

Control



Aspen plantlets

Control

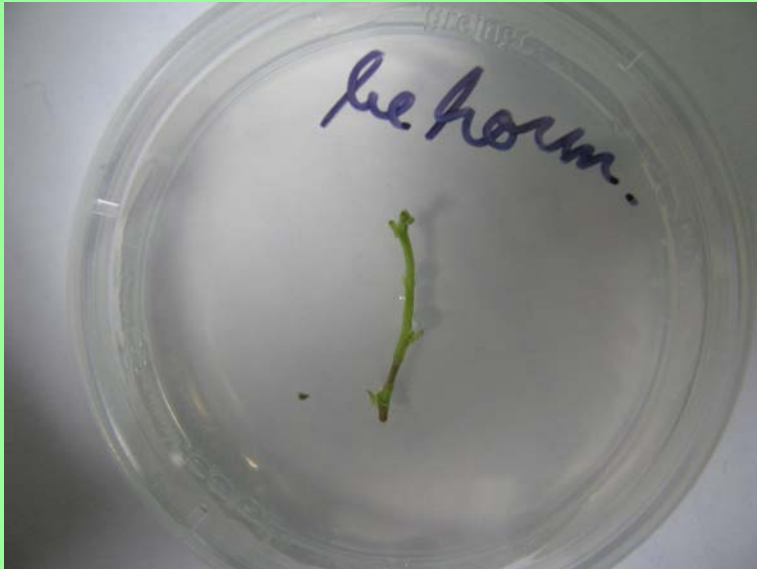
GA4/7

Plant material

Explants were prepared as cuttings (leaves and roots were removed) from *in vitro* developed aspen shoots (primary shoot culture *in vitro* was obtained from a mature *Populus tremula* tree DPL-38 (selected in Lithuania))

Two types of cuttings were used: non-decapitated (with intact apical shoot) and decapitated (apical bud removed)

Morphologic parameters were estimated after 5 weeks of cultivation in Petri dishes



Nutrient medium

Solidified Woody Plant Medium was supplemented with various growth regulators.

Growth regulators used during this research:

Gibberellins

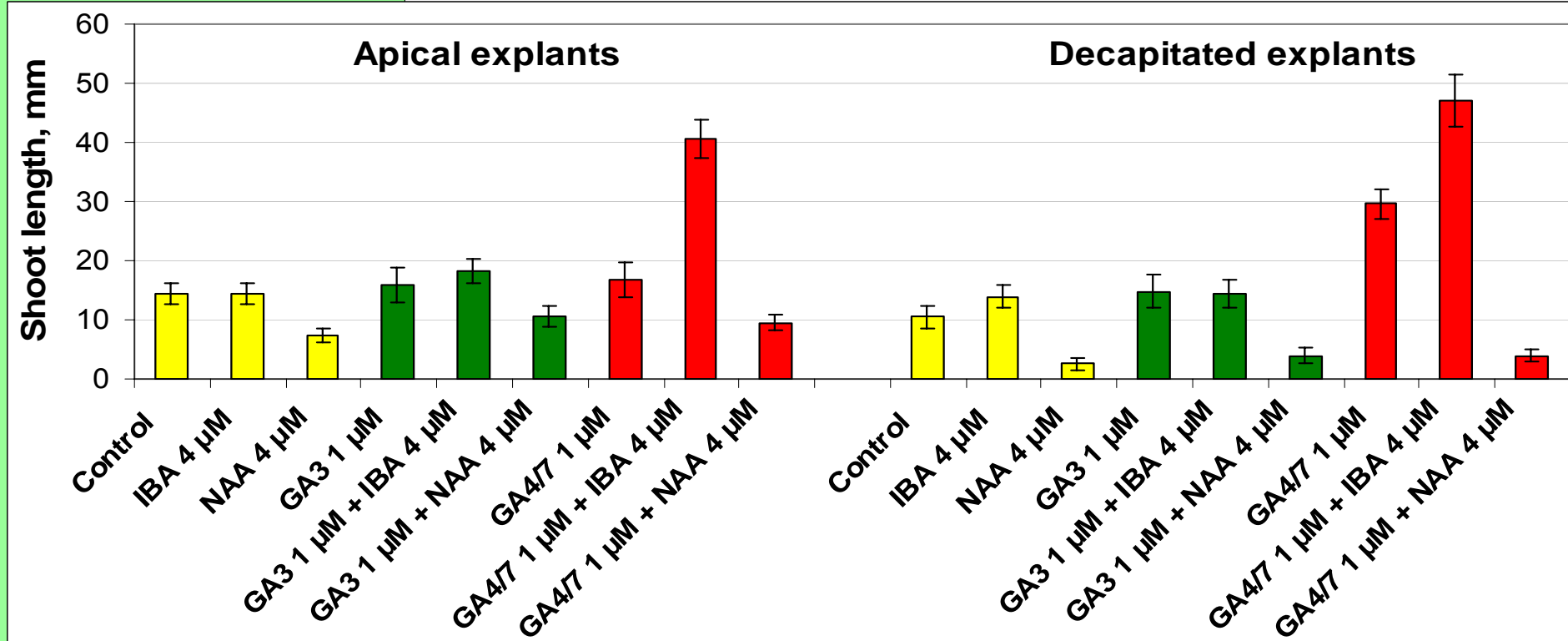
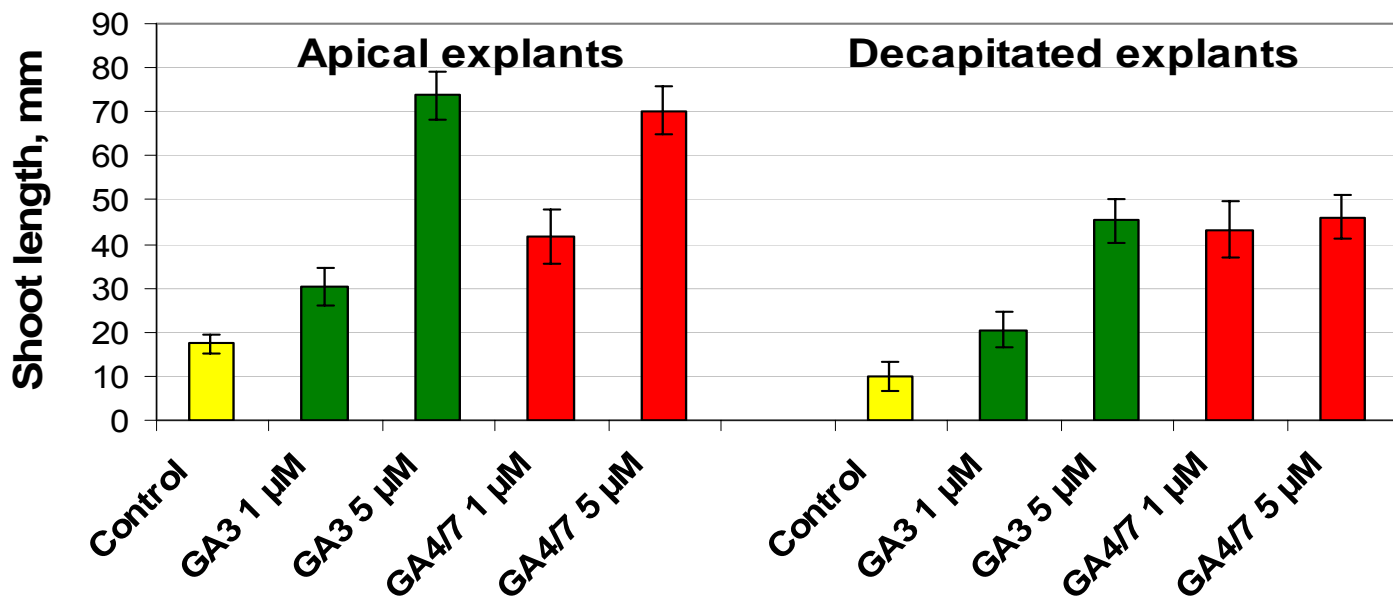
- GA3 in the concentrations of 1 μM (0.35 mg/l) and 5 μM (1.75 mg/l)
- GA4/7 in the concentrations of 1 μM (0.33 mg/l) and 5 μM (1.65 mg/l)

Auxins

- indole butyric acid (IBA) in the concentration of 4 μM (0.8 mg/l)
- naphthalene acetic acid (NAA) in the concentration of 4 μM (0.75 mg/l)

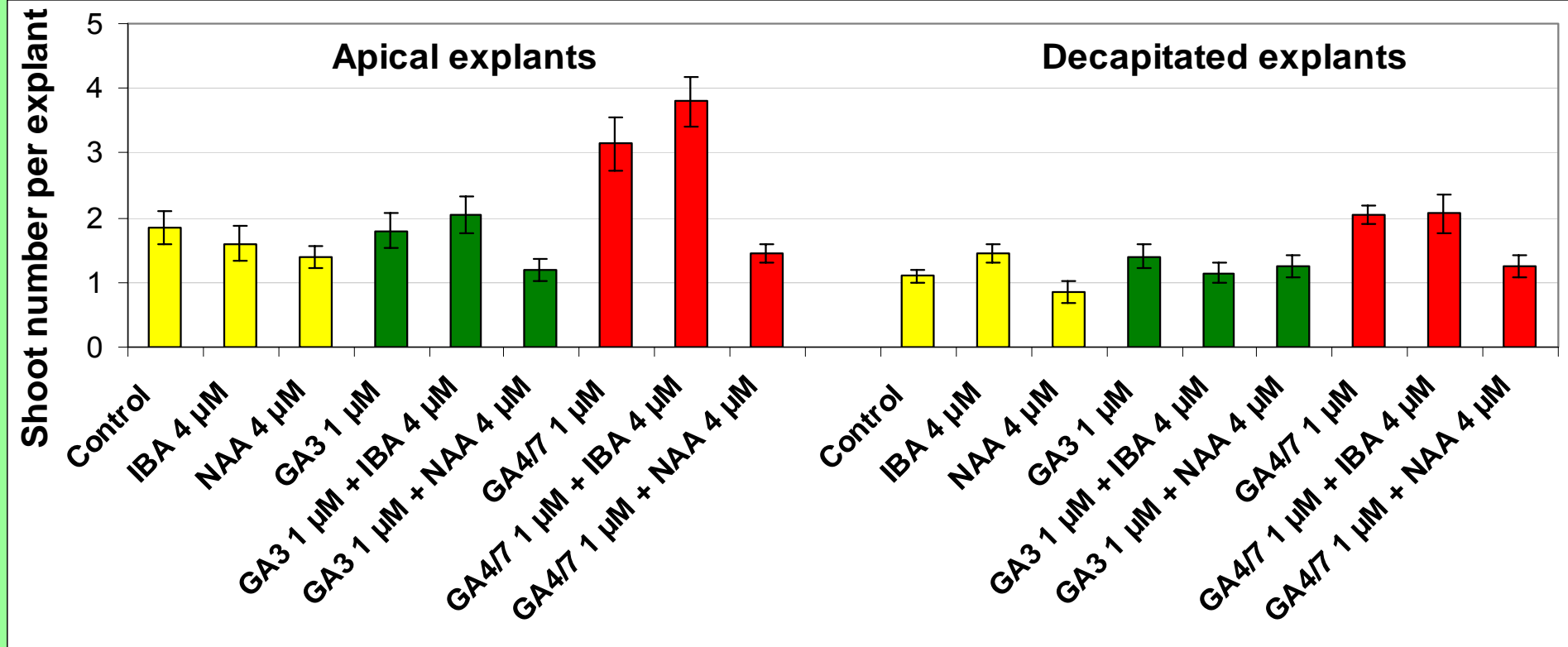
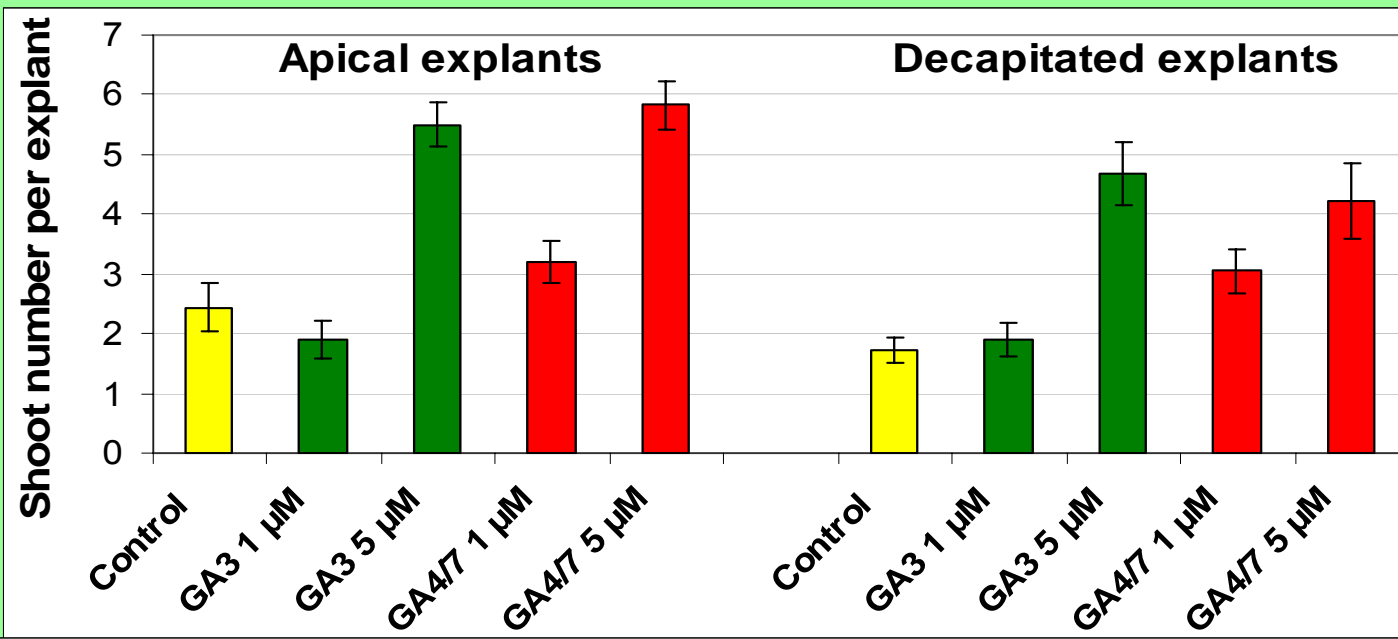
Results

Shoot growth



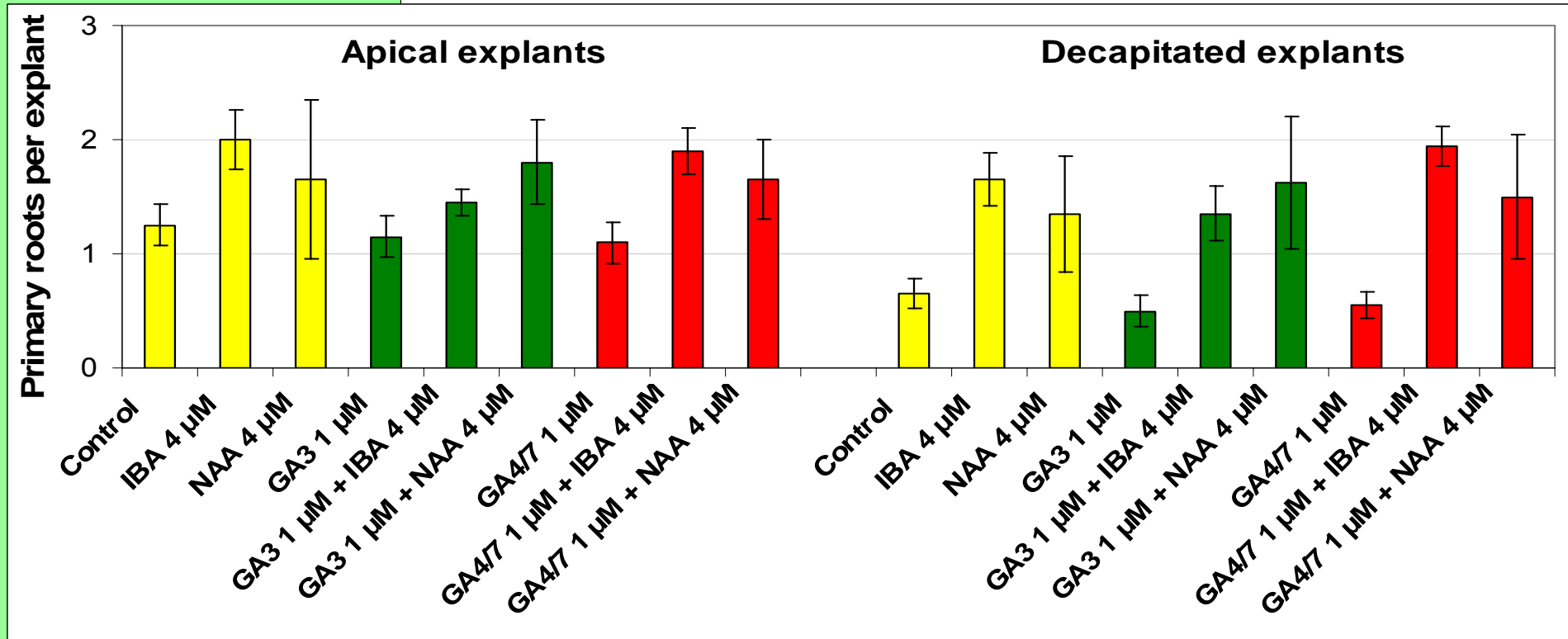
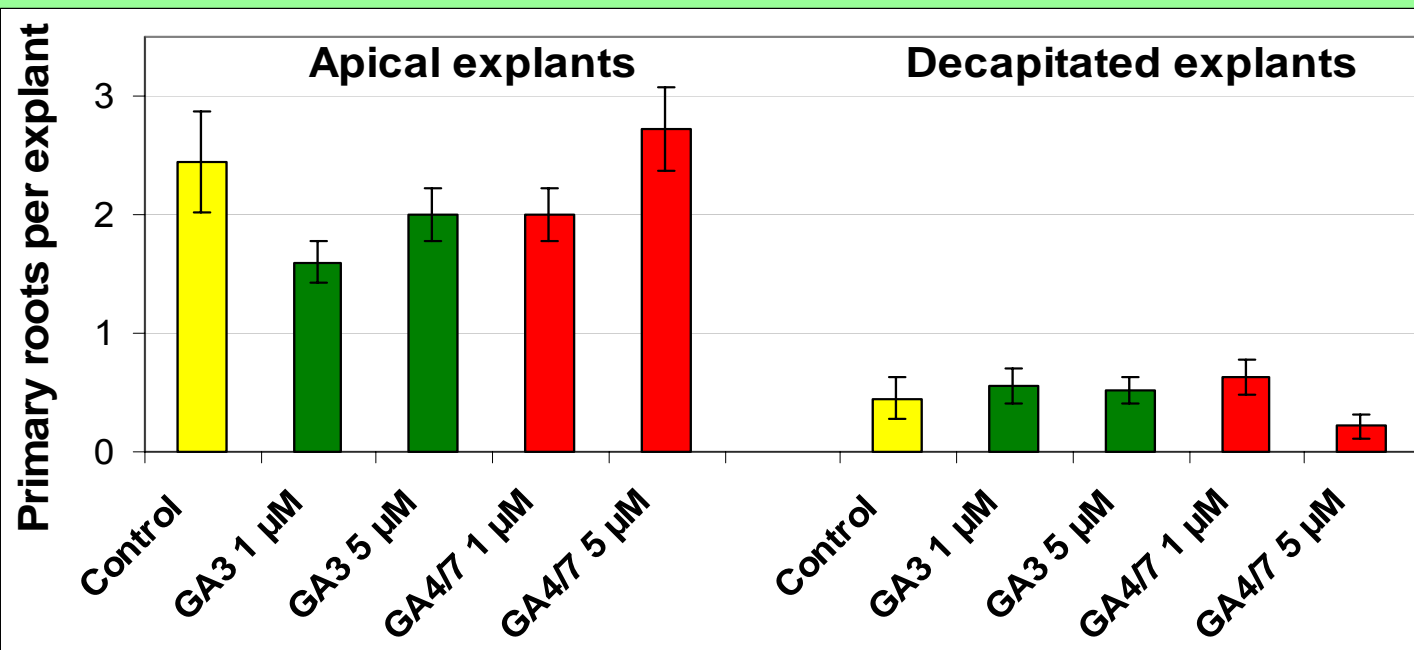
Results

Adventitious
shoot
development



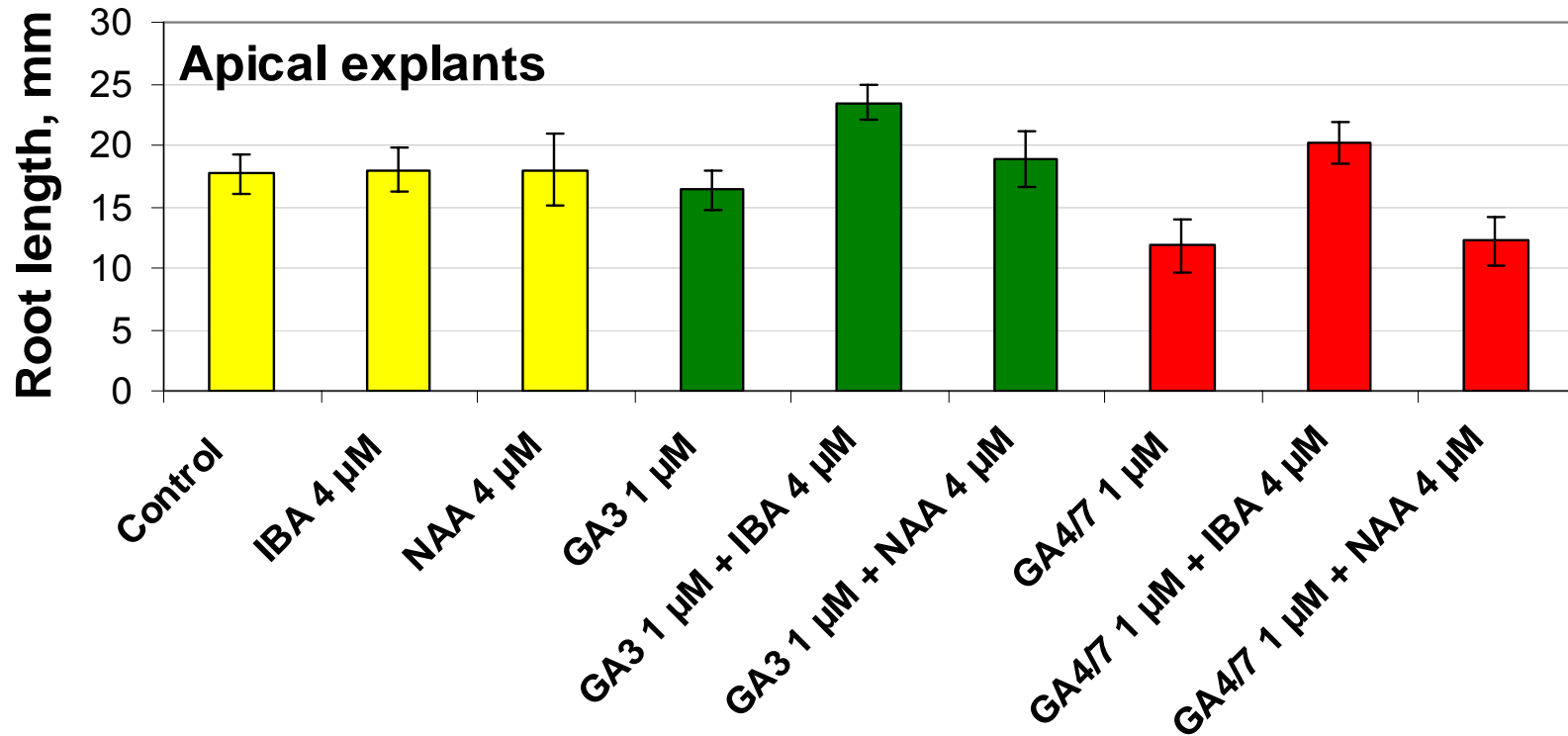
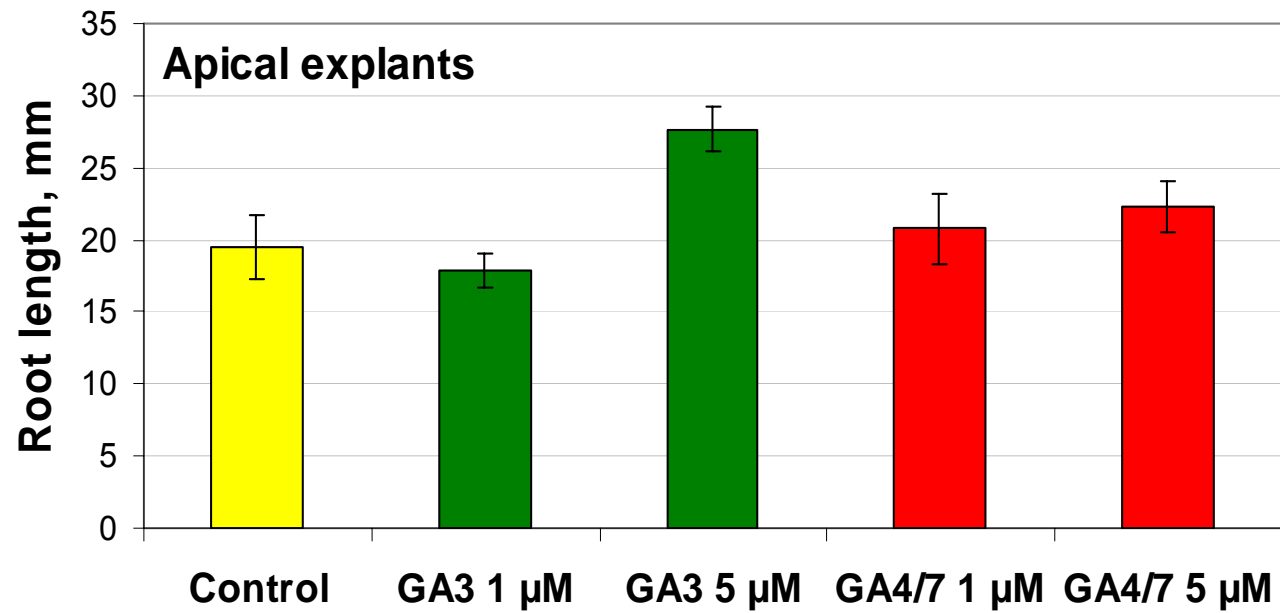
Results

Root formation



Results

Root growth



Conclusions

1. Both GA3 and GA4/7 in 5 μM concentration can be applied to the nutrient medium in order to obtain faster shoot growth or greater number of adventitious shoots in aspen *in vitro* culture
2. At 1 μM , GA4/7 is more effective than GA3 in the promotion of both shoot growth and adventitious shoot development, especially when applied in combination with auxin IBA
3. Application of auxin (IBA) in addition to lower concentration of gibberellin (GA4/7) is not necessary for adventitious shoot development but required for increased shoot growth
4. GA3, in contrast to GA4/7, seems to be more efficient not so much in shoot as in root elongation of aspen plantlets

THANK YOU FOR YOUR ATTENTION

