



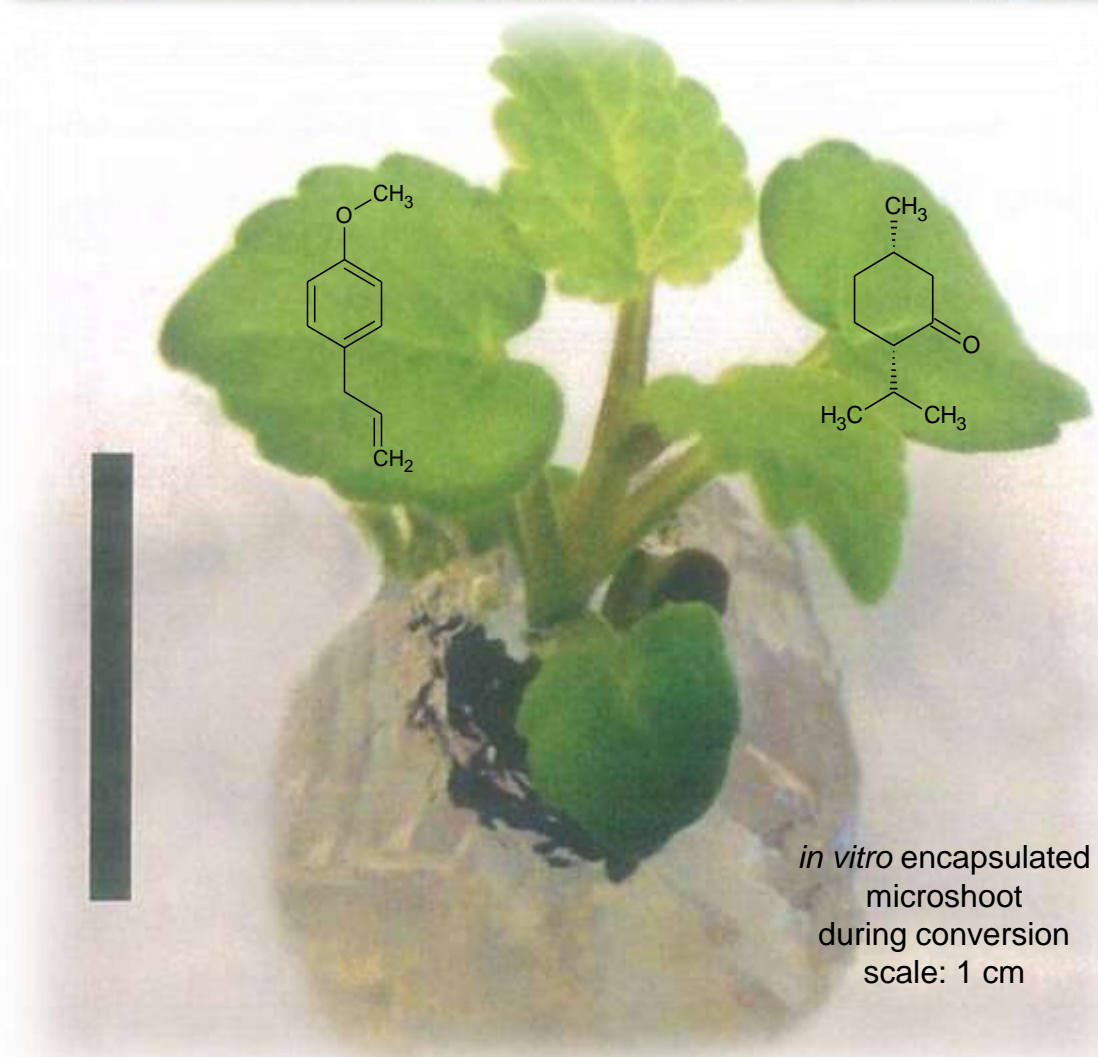
*Agastache rugosa* (Fisher & C.A.Meyer) O.Kuntze (Lamiaceae), is a traditional Far Eastern (China, Korea, Japan) medicinal plant. The aerial parts of the plant are used and known in Traditional Chinese Medicine.

The volatiles are stored in glandular trichomes in aerial parts and are composed mainly of phenylpropanoids (estragol, eugenol, chavicol), monoterpenes (*d*-limonene,

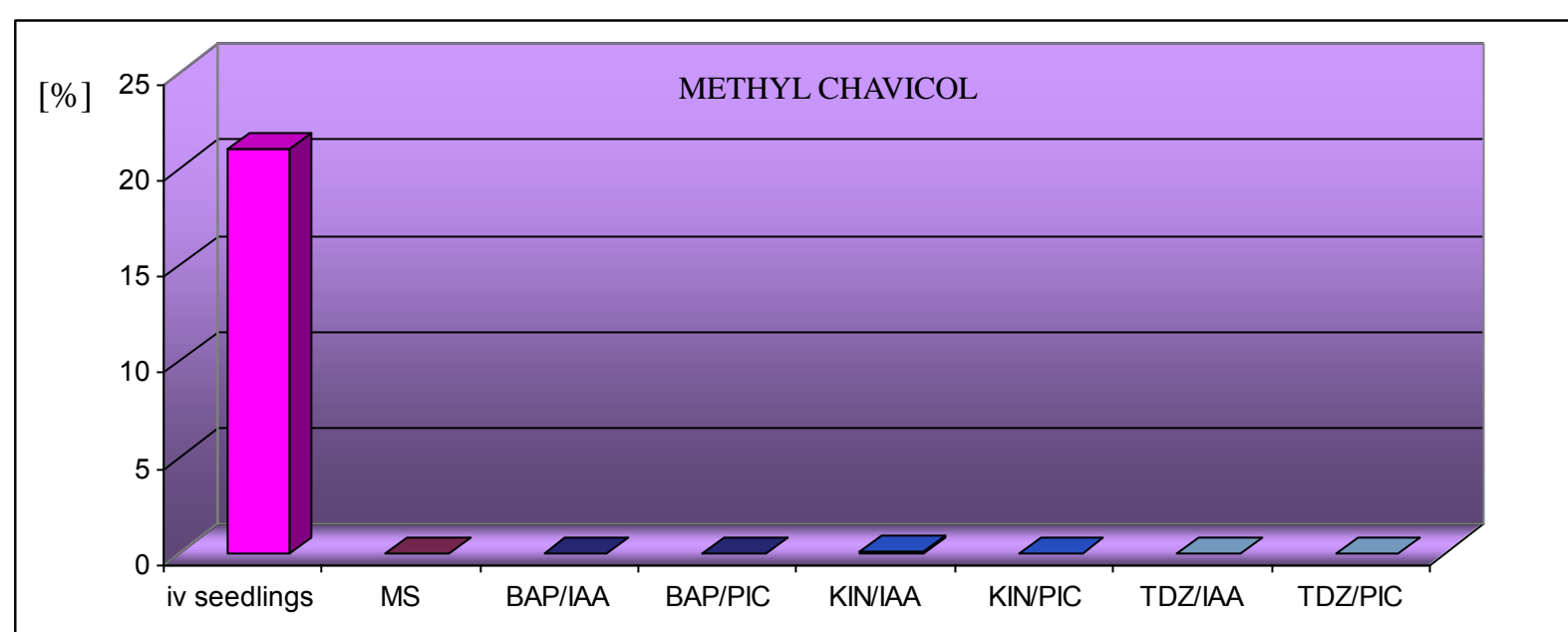
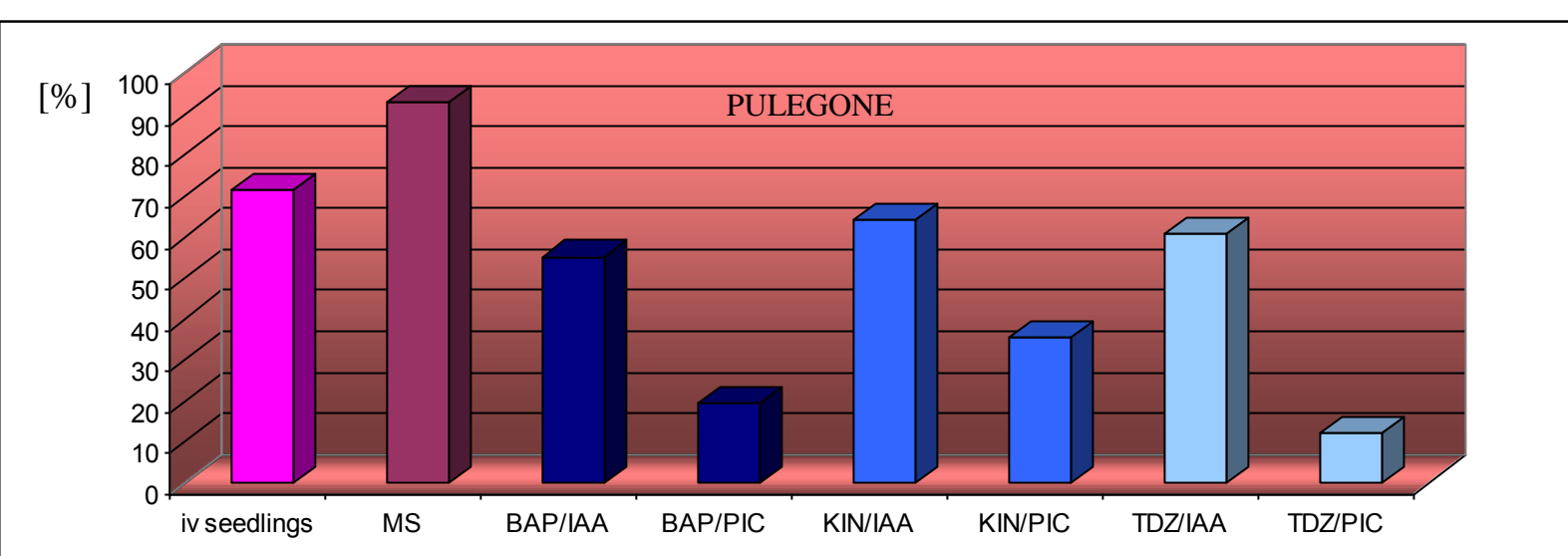
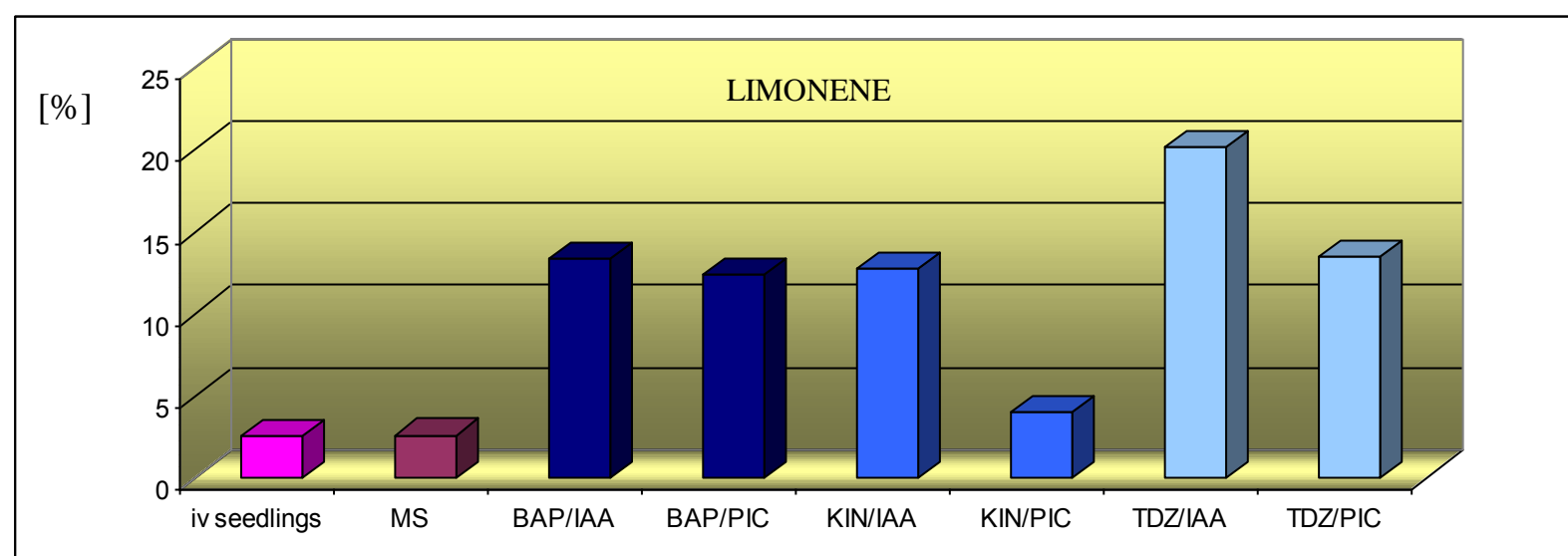
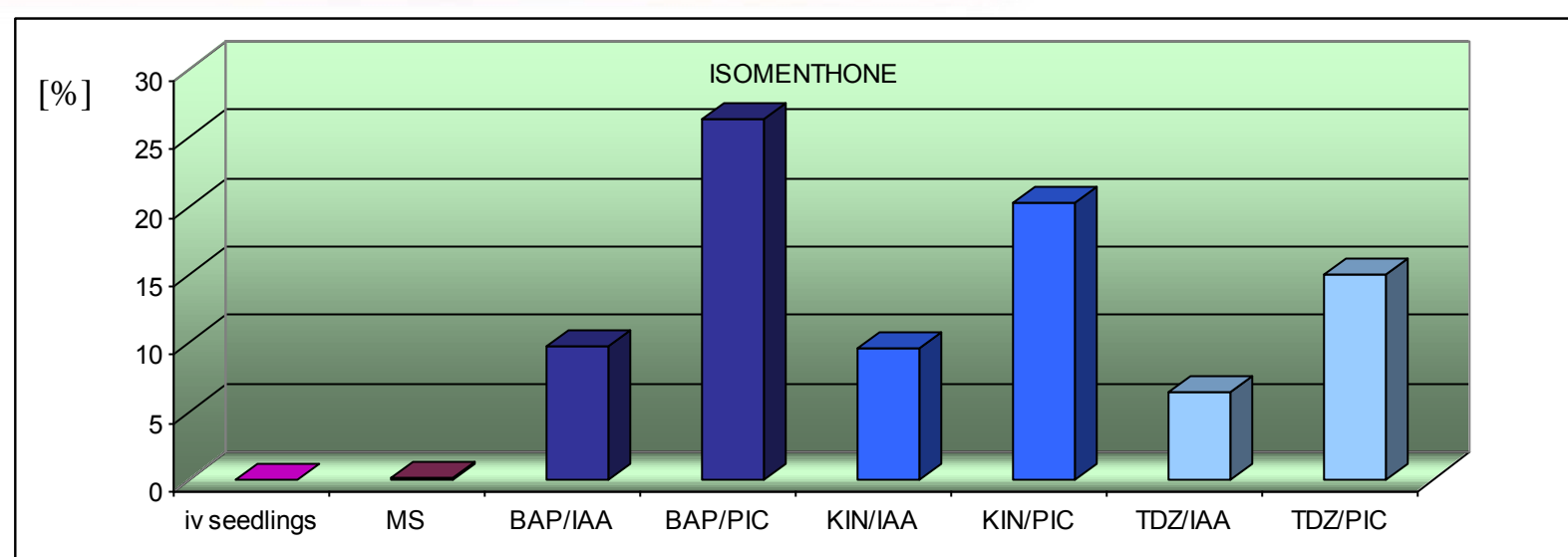
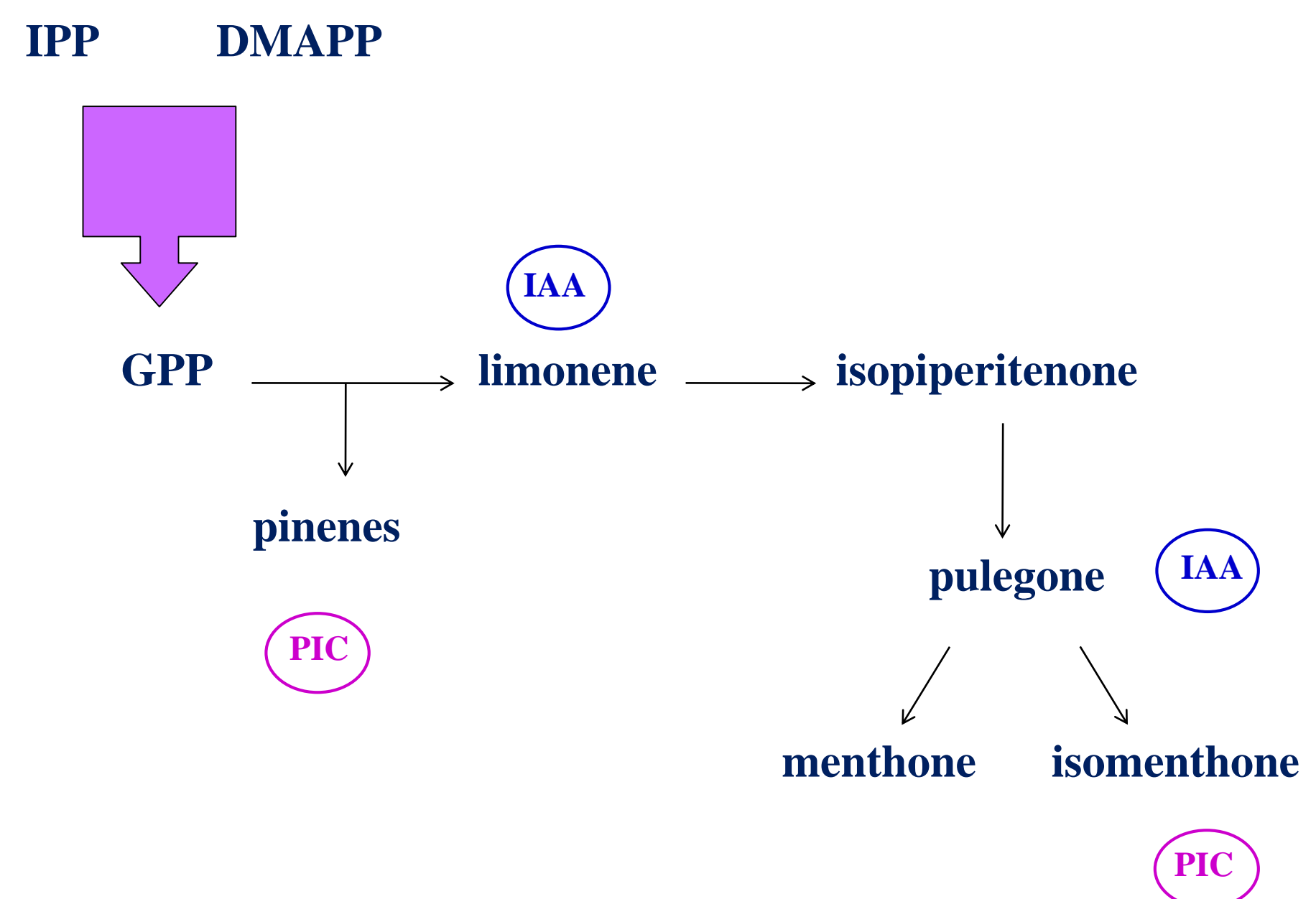
$\alpha$ -pinene, *l*-isomenthone, *l*-pulegone), and small amounts of sesquiterpenes (caryophyllene, calamene). The roots of *A. rugosa* contain several oleanane type triterpenes, oxidized abietane diterpenes, and rosmarinic acid as a main polyphenol.

The essential oil has antibacterial, antifungal, and anti-complement activity, attributed to estragole content, that makes up at least half of the oil fraction. Floral essential oil, containing estragol, *d*-limonene, and anisaldehyde is antimutagenic, cytotoxic and immunostimulating.

The present study was focused on the influence of *in vitro* conditions and plant growth regulators on the biosynthetic routes and metabolic profile of volatile phenylpropanoids and monoterpenes.



*in vitro* encapsulated microshoot during conversion scale: 1 cm



*in vitro* shoot cultures, scale: 1 cm

### Establishment of *in vitro* cultures

Four-week-old *in vitro*-germinated seedlings were used for volatile extraction and for the establishment of shoot cultures. Shoot tips were excised and individually transferred to a shoot proliferation medium – Murashige and Skoog (MS) basal medium without plant growth regulators or supplemented with specific concentrations of one of three cytokinins: BAP (benzyladenine), KIN (kinetine), TDZ (thidiazuron), and one of two auxins: IAA (indoleacetic acid) or PIC (picloram).

### Headspace solid-phase microextraction - gas chromatography - mass spectrometry (HS-SPME-GC-MS)

The volatiles were extracted with solid-phase microextraction (SPME) technique using a syringe-like cartridge with the divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) coated fiber. Identification was based on the comparison of the retention indices (RI) and mass spectra with available libraries (Wiley 8<sup>th</sup> edn, NIST 05, MassFinder 4) and with literature data.

### Essential oil distillation

Essential oil was obtained by hydro-distillation in Deryng's apparatus following the method of Polish Pharmacopea VI.



*Agastache rugosa* *in vitro* blooming plant scale: 1 cm