

Yield and quality of raw material derived from plants of common St. John's wort (*Hypericum perforatum* L.) propagated *in vitro* and generatively

INTRODUCTION

The herb of St. John's wort has a varied chemical composition and a wide range of therapeutic effects. The content of bioactive compounds present in this herb, however, depends to a large degree on the genetic and developmental factors. The raw material for herbal industry derived from the plants harvested from natural sites and from cultivation, is very variable, which makes the preparation of standardized extracts difficult. *In vitro* production of St. John's wort cuttings seems to be a good method for the preparation of a genetically, and thus chemically homogeneous, industrial raw material. The aim of this study was to determine the suitability of cuttings obtained from *in vitro* cultures as an initial plant material to establish plantations of St. John's wort.

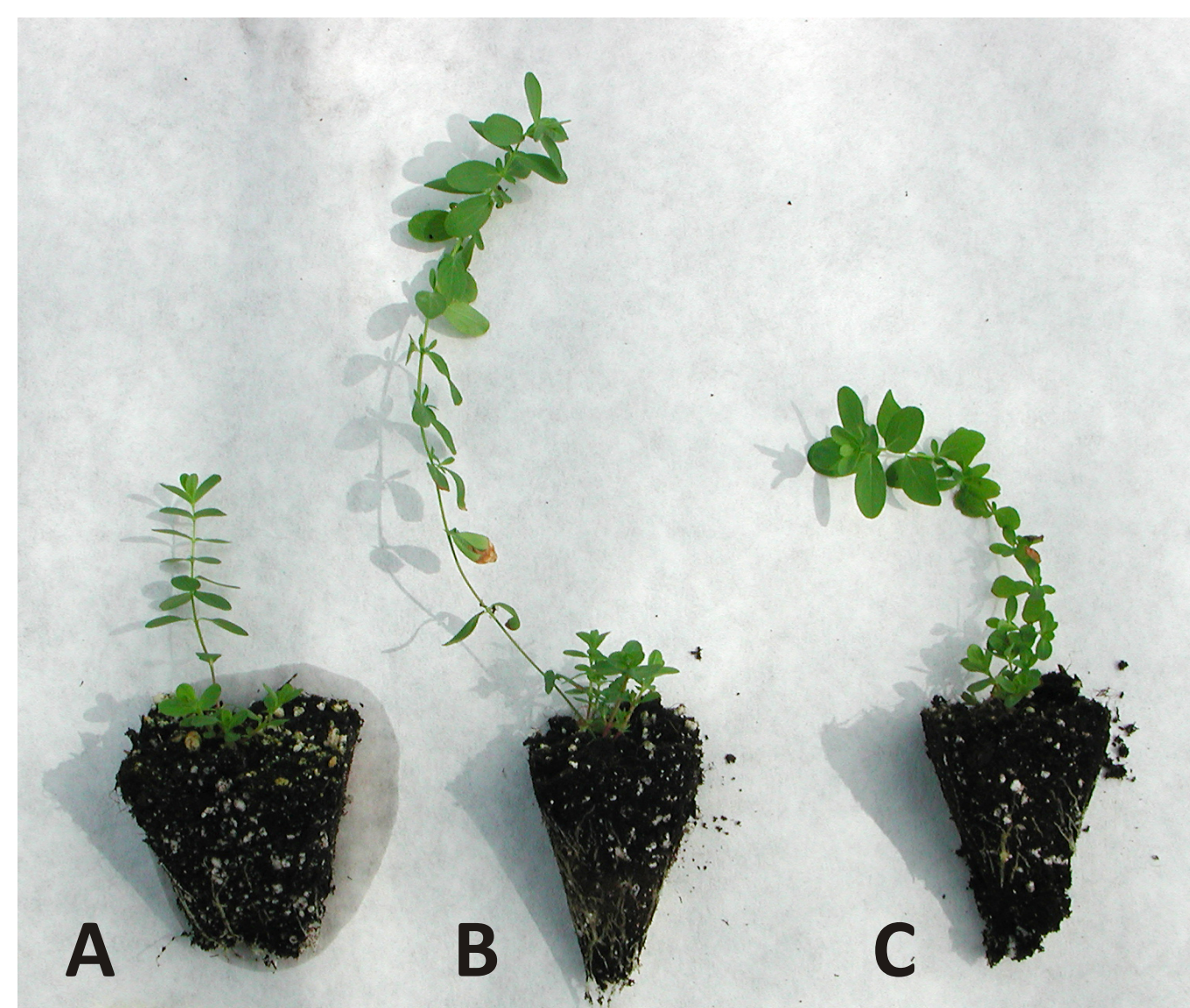
MATERIAL AND METHODS

Two populations of St. John's wort (No. 25 and 31) selected in the Department of Vegetable and Medicinal Plants and the cultivar 'Topas' were used as the source of plant material for propagation.

The field experiment was carried out in the randomized complete block design with 4 replications at the Experimental Field in Wilanów. Plantations of St. John's wort were established by direct sowing, planting of seedlings, and planting of cuttings obtained by micropropagation. The seeds were sown in the first decade of April at 40 cm row spacing. The seedlings prepared in the greenhouse and cuttings from *in vitro* culture were planted in the field in the second decade of April at the spacing of 40 × 30 cm. The herb was collected in the second year of cultivation in the vegetative stage of plant growth, at the beginning of the generative stage, and in full flowering. The herb was dried at 30–40°C. The fresh and dry weight of raw material were determined. The composition of biologically active compounds in the air-dry herb was investigated. The content of hypericins (hypericin and pseudohypericin) was determined by HPLC method. The total content of flavonoids (expressed as hyperoside) was determined spectrophotometrically according to Polish Pharmacopoeia. HPLC was used for quantitative determination of flavonoid compounds. The total content of polyphenolic acids was determined according to Polish standard PN-91/R-87019. Moreover, the qualitative analysis of polyphenolic acids was carried out by GC method.

RESULTS

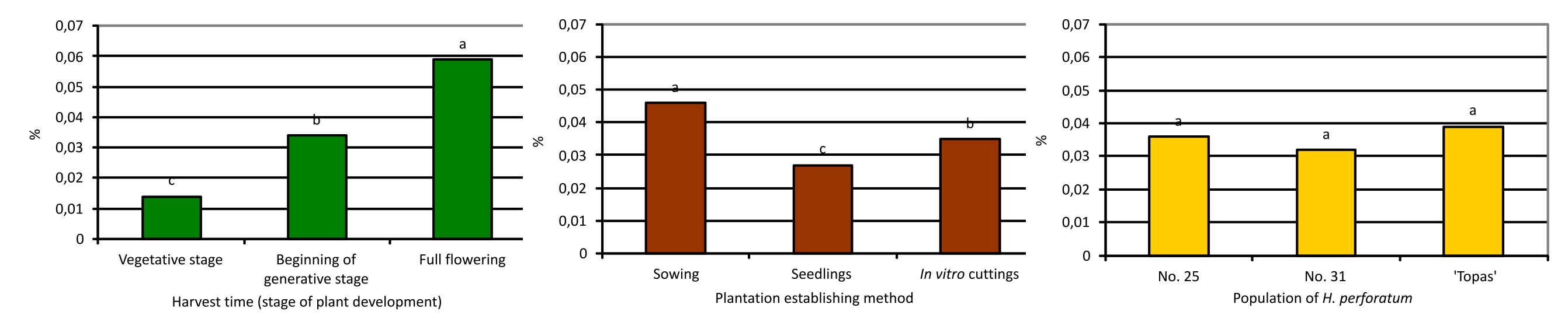
Field investigation showed that yield of St. John's wort herb collected from the plantation established from vegetatively propagated plants and from seedlings prepared in the greenhouse was comparable and exceeded the yield of plants originating from the seeds sown directly in the field. The way of plant propagation did not affect the content of biologically active compounds in herb. The highest content of hypericins was found in plants originating from the seeds sown directly in the field, while the highest concentration of polyphenolic acids was determined in plants obtained from seedlings. Investigated plants did not differ in respect of the content of flavonoids. Chromatographic analysis resulted in identification of 6 flavonoid compounds (rutin, hyperoside, isoquercetin, luteolin 7-glucoside, quercitrin, quercetin) and 17 free polyphenolic acids. There was no clear relationship between the method of plant propagation and the content of particular compounds in the herb. It was also found that the content of biologically active compounds in herb depended on the developmental stage of harvested plants. The highest content of hypericin and polyphenolic acids was found in the herb collected in the stage of full flowering, and flavonoid compounds – at the beginning of the generative stage.



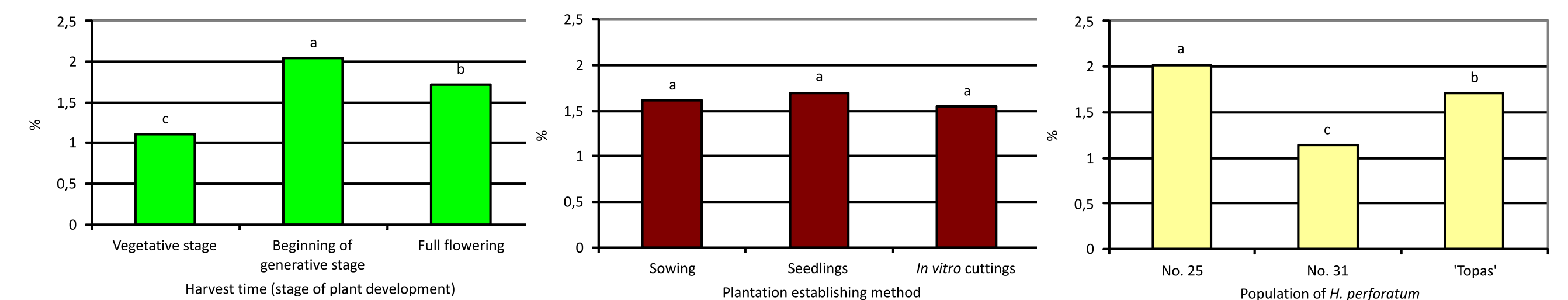
A – seedlings; B, C – *in vitro* cuttings ('Topas')



The effect of experimental factors on the yield of the air-dry herb



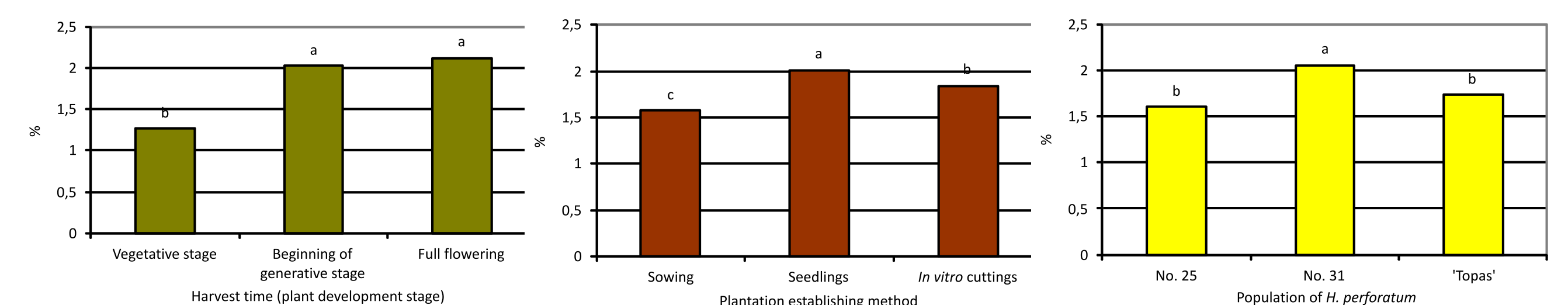
The effect of experimental factors on the hypericins content in the air-dry herb



The effect of experimental factors on the total flavonoids content (expressed as hyperoside) in the air-dry herb

Content of selected flavonoid compounds in the air-dry herb harvested in full flowering stage (%)

Flavonoid compound	Plantation establishing method	Population of <i>H. perforatum</i>			Mean
		No. 25	No. 31	'Topas'	
Rutin	Sowing	1.11	1.20	0.75	1.02
	Seedlings	0.77	0.93	0.76	0.81
	<i>In vitro</i> cuttings	0.80	1.33	0.76	0.96
	Mean	0.90	1.15	0.76	0.93
Hyperoside	Sowing	0.38	0.40	0.51	0.43
	Seedlings	0.37	0.37	0.59	0.44
	<i>In vitro</i> cuttings	0.31	0.41	0.60	0.44
	Mean	0.35	0.40	0.57	0.44
Isoquercetin	Sowing	0.42	0.35	0.48	0.42
	Seedlings	0.38	0.29	0.58	0.42
	<i>In vitro</i> cuttings	0.58	0.45	0.53	0.52
	Mean	0.46	0.36	0.53	0.45



The effect of experimental factors on the total polyphenolic acids content in the air-dry herb

Content of identified free polyphenolic acids in the air-dry herb harvested in full flowering stage, (means from 3 populations, %)

Polyphenolic acids	Plantation establishing method		
	Sowing	Seedlings	<i>In vitro</i> cuttings
benzoic	1.99	2.62	2.50
chlorogenic	0.95	-	1.00
salicylic	0.86	1.11	0.96
m-hydroxybenzoic	-	2.86	2.90
cinnamic	-	0.067	0.05
o-hydroxyphenylacetic	0.51	0.14	0.49
p-hydroxybenzoic	13.62	16.19	15.20
pyrocatechuic	0.28	0.29	0.29
γ-resorcylic	7.32	7.32	7.34
gentisic	0.84	1.45	1.22
o-coumaric	0.34	-	-
α-resorcylic	1.48	-	1.30
β-resorcylic	8.44	6.89	9.22
syringic	0.67	0.92	-
p-coumaric	4.00	2.50	-
caffeic	-	0.53	0.52
sinapic	0.15	0.37	0.35
Catechin	14.29	8.60	12.56

CONCLUSIONS

1. The plants of St. John's wort obtained by micropropagation can provide an alternative source of genetically homogeneous material for setting up the field plantations of this species.
2. The yield of herb collected from the plantation established from cuttings obtained *in vitro* and from seedlings was comparable and exceeded the yield of plants originating from the seeds sown directly in the field.
3. The content of biologically active compounds in herb was not affected by the type of propagation material used for establishing the plantation but depended on the developmental stage of harvested plants.