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MORPHOGENETIC RESPONSE TO PLANT GROWTH REGULATORS IN TRANSFORMED AND UNTRANSFORMED *HYPERICUM PERFORATUM* L. CLONES

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This study examined the effects of different exogenous auxins and cytokinins at 0.1-5.0 mg·l^{-1} concentration on shoot cuttings of two *H. perforatum* clones transformed with a wild agropine strain of *A. rhizogenes* and one untransformed clone. Their sensitivity to the auxins varied and showed concentration-dependent behavior, and the response to auxins differed between the transgenic clones. The number of cuttings of transgenic clones capable of root formation, and the onset of rooting on most of the media with auxins lagged behind the control. The number of differentiated shoots of the transgenic clones on hormone-free medium was two to three times higher than that of the untransformed control. Regenerated shoots of the transgenic clones on basal medium branched much less than the nontransgenic clone. The transgenic and control clones differed in their ability to form shoots on media supplemented with cytokinins. Increased cytokinins led to differentiation of shorter shoots with fewer leaf pairs. Because gene expression studies have shown integration of *rolABC* genes, their possible impact on the type of morphogenetic response is discussed.

Key words: Hairy root-regenerants, plant growth regulators, *rol* genes, shoot cuttings, St. John's wort.

INTRODUCTION

Like some other species of the genus *Hypericum*, *Hypericum perforatum* is a widely studied medicinal plant. Interest is concentrated on the pharmacological activity of hypericins and acylphloroglucinols (reviewed by Kubin et al., 2005; Medina et al., 2006) and other secondary products, but also on biotechnological ways of enhancing the biosynthetic ability of valuable metabolites. Modification of the plant genome through genetic transformation provides a tool for study and random or directed manipulation of plant characteristics. Recently, efficient *Agrobacterium rhizogenes*-mediated transformation protocols have been developed for *H. perforatum* (Di Guardo et al., 2003; Vinterhalter et al., 2006). *Agrobacterium rol* genes integrated into the plant genome may cause changes in the hormone balance or in hormone signal perception (Christey, 2001) and result in morphological and physiological alterations. Previously published data revealed induction of the hairy root phenotype (DiGuardo et al., 2003) and biochemical and morphological alterations (Koperdáková et al., 2009) in *H. perforatum* plants transformed by a wild agropine strain of *A. rhizogenes*. On the other hand, plants modified by *A. rhizogenes* strain A4M70GUS showed a phenotype similar to the untransformed controls (Vinterhalter et al., 2006). Phenotypic features of modified plants depend on the site and copy number of the integrated genes. As it is likely that the genes from pRi are integrated at random sites of the plant genome, different traits can be affected, including biosynthetic ability or hormone perception.

The aim of this study was to examine the morphoregulatory effect of exogenously applied auxins and cytokinins on shoot cuttings of two transgenic *H. perforatum* clones cultured in vitro, as compared with untransformed controls.

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Abbreviations: PGRs – plant growth regulators; 2iP – 6-γ,γ-dimethylallylaminopurine; BAP – 6-benzylaminopurine; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; Kin – kinetin; NAA – 1-naphthalene acetic acid.

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MATERIALS AND METHODS

The experiments used shoots grown in vitro from two transgenic clones, B and L expressing the hairy root phenotype, and untransformed clone K of *Hypericum perforatum* L. Transgenic clones were developed from hairy roots obtained by transformation of leaf (clone L) and root (clone B) cuttings of clone K with a wild agropine type *Agrobacterium rhizogenes*, strain ATCC 15834. Clone K, the original plant used for the transformation, was derived by in vitro propagation of wild *H. perforatum* of Italian provenance (Di Guardo et al., 2003).

The basal medium contained mineral salts according to Linsmaier and Skoog (1965), B5 vitamins according to Gamborg et al. (1968), 100 $mg \cdot l^{-1}$ myo-inositol, 2 $mg \cdot l^{-1}$ glycine, 30 $g \cdot l^{-1}$ sucrose and 0.6% (w/v) agar, with pH adjusted to 5.6 before autoclaving. To test the effects of different plant growth regulators, the auxins indole-3-acetic acid (IAA) (Sigma), indole-3-butyric acid (IBA) (Sigma) and 1-naphthalene acetic acid (NAA) (Jansen), and the cytokinins 6-benzylaminopurine (BAP) (Sigma), 6-γ,γ-dimethylallylaminopurine (2iP) (Sigma) and kinetin (Kin) (Sigma) were applied at concentrations of 0.1, 0.5, 1.0 or 5.0 $mg \cdot l^{-1}$. The morphogenetic effects of plant growth regulators on shoot cuttings comprising two leaf-pairs were evaluated. Thirteen cuttings per plant were placed on 13 different types of culture media containing auxins and cytokinins. Five cuttings per flask in three replicates of each clone (clones L and B and the untransformed clone K) were placed on the surface of the basal medium with different plant growth regulators. Control samples were cultured on basal medium without growth regulators.

The cultures for cytokinin bioassays were kept in the culture room at 23°C under a 16 h photoperiod with fluorescent lighting (33 $\mu mol \cdot m^{-2}s^{-1}$) and 70% relative humidity, while those used for auxin bioassays were stored in the dark. All cultures were subcultured at 6-week intervals. The morphogenetic responses were registered after 12 weeks of cultivation.

The effect of the different auxins within the 0.1–5.0 $mg \cdot l^{-1}$ concentration range on shoot sections of transformed clones B and L and untransformed clone K was compared with control samples of each clone cultivated on basal hormone-free medium under the same culture conditions.

The presence and copy number of the *rolABCD* genes and the genes encoding for enzymes involved in the alternative biosynthetic pathway of auxins (*aux1, 2*) of *A. rhizogenes* T-DNA in the analyzed clones were detected by quantitative real time PCR (qPCR) with SYBR Green I binding dye according to Koperdáková et al. (2009). The genomic fragment of the *hyp-1* gene (GenBank acc. n.: AY148090) was used as the internal reference. Total genomic DNA for the analysis was isolated from 100 mg of plant material using the DNeasy Plant Mini Kit (Qiagen). The reaction conditions in 30 µl reaction volume were as follows: 1x iQ SYBR Green Supermix (BioRad), 0.5 µM forward and reverse primer and 50 ng DNA. The PCR amplifications (5 min at 95°C; 40 cycles of 30 s at 94°C, 30 s at 60°C – *rolABCD*, 61°C – *aux1,2* or 52°C – *hyp-1*, 30 s at 72°C, and 5 min at 72°C) were performed in the iCycler iQ Real Time PCR Detection System (BioRad).

Expression of *rolABC* genes was verified by RT-PCR. Total RNA was isolated from fresh plant material according to Jaakola et al. (2001). Residual DNA co-precipitated during isolation was digested by DNase I (Invitrogen). Reverse transcription (RT) was performed using 200U M-MLV reverse transcriptase (Promega) and 10 mM anchored oligoT primer at 42°C according to the manufacturer's instructions. RT and RT-PCR were performed in an MJ Mini Thermal Cycler (BioRad). For RT-PCR, 50 ng cDNA was used according to the reaction and amplification conditions used by qPCR.

RESULTS

EFFECTS OF AUXINS ON ROOT FORMATION

The differences in the morphogenetic response of the transformed clones to different auxins and concentration levels were evaluated.

The rooting ability of the studied clones on basal hormone-free medium varied widely (Tab. 1). Cuttings of untransformed clone K differentiated on average 3 branching roots on basal medium. The number of roots differentiated from shoot sections of transgenic clone B ranged from none to many abundantly branching roots. Shoot sections of clone L showed almost no rooting ability.

The sensitivity of the shoot cuttings of the studied clones to different auxins varied and showed concentration-dependent behavior (Tab. 1). Clone K shoot sections had more roots at lower concentrations of IAA but not IBA than on hormone-free medium. Concentrations of IAA and IBA higher than 0.5 $mg \cdot l^{-1}$ as well as the lowest concentration of NAA resulted in the formation of multiple roots with reduced geotropism. Higher concentrations of NAA led to callus formation (Fig. 1).

The reaction of clone B to the different auxins varied (Fig. 2). Lower concentrations had no effect (IAA) or caused differentiation of plentiful roots with reduced geotropism (IBA), or callus formation followed by differentiation of short roots (NAA). Higher concentrations of all auxins resulted in the formation of callus, from which multiple root regeneration occurred under the influence of IAA and IBA.

Clone L had lower rooting ability than the other clones after auxin administration. The number of differentiated roots per cutting and number of

TABLE 1. Morphogenic effect of auxins

Medium supplemented with	Clone	% of root-differentiating cuttings	Intensity of rooting	% of callus-producing cuttings	% of shoot-differentiating cuttings
no PGR	B	33.3	+	66.6	73.3
	L	6.7	+	73.3	20.0
	K	66.7	+	100.0	93.3
0.1 mg·l^{-1} NAA	B	46.7	○	93.3	20.0
	L	26.7	○	93.3	6.7
	K	53.3	+++	80.0	26.7
0.5 mg·l^{-1} NAA	B	20.0	○	100.0	0.0
	L	0.0		100.0	0.0
	K	86.7	○	93.3	0.0
1 mg·l^{-1} NAA	B	46.7	○	100.0	0.0
	L	0.0		93.3	0.0
	K	86.7	○	100.0	6.7
5 mg·l^{-1} NAA	B	0.0		80.0	0.0
	L	0.0		80.0	0.0
	K	33.3	○	100.0	0.0
0.1 mg·l^{-1} IAA	B	26.7	+	80.0	40.0
	L	26.7	+	80.0	66.7
	K	73.3	++	100.0	86.7
0.5 mg·l^{-1} IAA	B	80.0	+	100.0	6.7
	L	60.0	+	93.3	66.7
	K	80.0	++	100.0	66.7
1 mg·l^{-1} IAA	B	73.3	+++	93.3	26.7
	L	66.7	+	86.7	40.0
	K	80.0	+++	100.0	53.3
5 mg·l^{-1} IAA	B	86.7	+++	93.3	6.7
	L	57.1	++	92.9	7.1
	K	80.0	+++	60.0	13.3
0.1 mg·l^{-1} IBA	B	66.7	+++	93.3	13.3
	L	80.0	+	93.3	6.7
	K	86.7	+	93.3	33.3
0.5 mg·l^{-1} IBA	B	60.0	+++	86.7	26.7
	L	73.3	++	100.0	26.7
	K	100.0	+++	100.0	46.7
1 mg·l^{-1} IBA	B	86.7	+++	93.3	26.7
	L	80.0	++	86.7	0.0
	K	80.0	+++	86.7	66.7
5 mg·l^{-1} IBA	B	66.7	+++	93.3	0.0
	L	70.0	++	100.0	0.0
	K	100.0	+++	100.0	46.7

○ morphogenic callus, + 1–3, ++ >3, +++ multiple roots per cutting

rooting cuttings increased on media with lower IAA and IBA concentrations. The shoot cuttings of this clone tended to form compact callus under higher IAA and IBA and all NAA concentrations. On media supplemented with 1.0 or 5.0 mg·l^{-1} IAA, or with 0.5 or 1.0 mg·l^{-1} IBA, several roots differentiated from calli. The number of cuttings of transgenic clones capable of root formation and

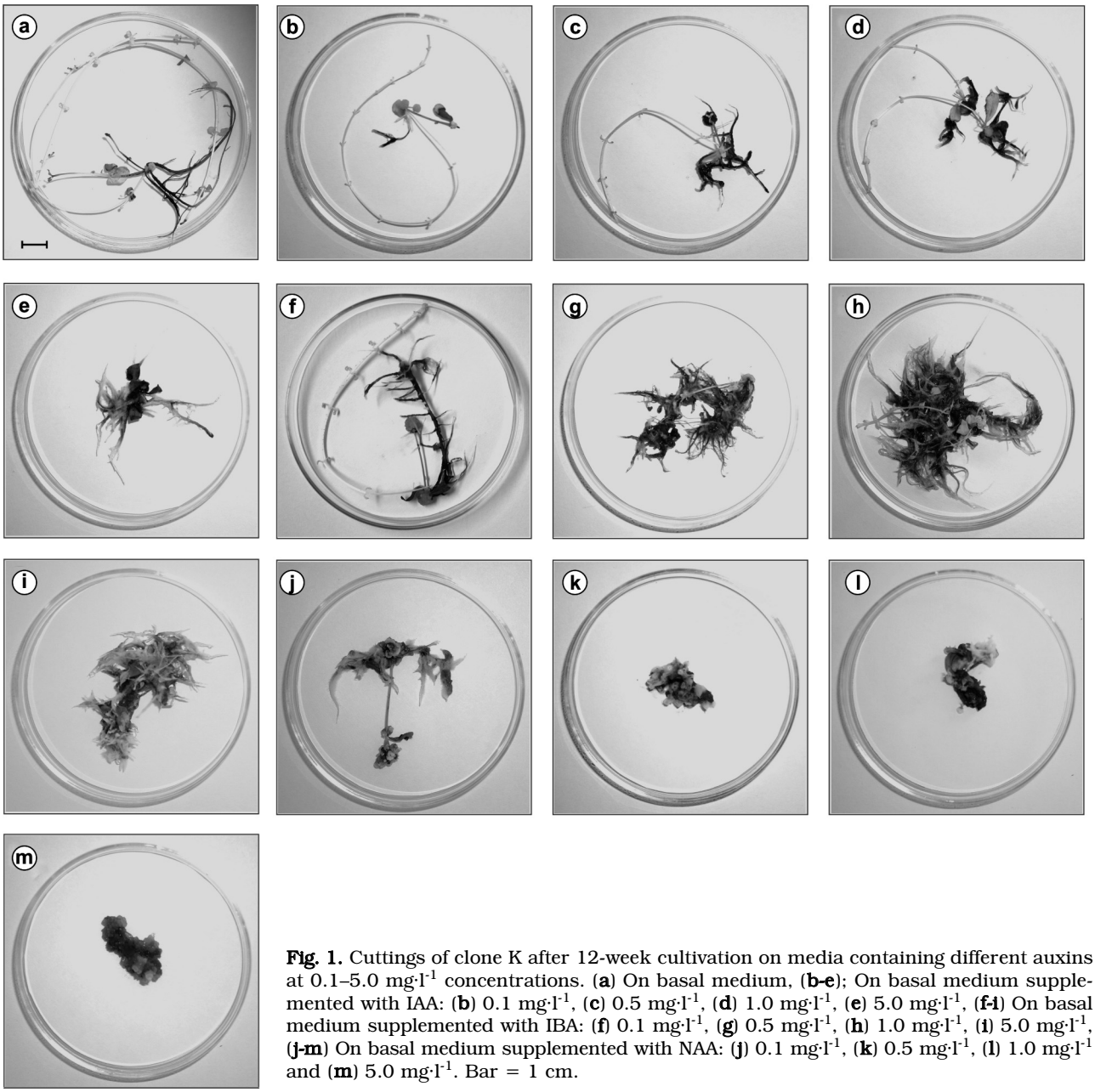

Fig. 1. Cuttings of clone K after 12-week cultivation on media containing different auxins at 0.1–5.0 mg·l^{-1} concentrations. (**a**) On basal medium, (**b-e**); On basal medium supplemented with IAA: (**b**) 0.1 mg·l^{-1}, (**c**) 0.5 mg·l^{-1}, (**d**) 1.0 mg·l^{-1}, (**e**) 5.0 mg·l^{-1}, (**f-i**) On basal medium supplemented with IBA: (**f**) 0.1 mg·l^{-1}, (**g**) 0.5 mg·l^{-1}, (**h**) 1.0 mg·l^{-1}, (**i**) 5.0 mg·l^{-1}, (**j-m**) On basal medium supplemented with NAA: (**j**) 0.1 mg·l^{-1}, (**k**) 0.5 mg·l^{-1}, (**l**) 1.0 mg·l^{-1} and (**m**) 5.0 mg·l^{-1}. Bar = 1 cm.

the onset of rooting on most media lagged behind the control.

EFFECTS OF CYTOKININS ON SHOOT DIFFERENTIATION

Different cytokinins within the 0.1–5.0 mg·l^{-1} concentration range were used to compare their morphoregulatory effect on shoot formation. On hormone-free medium the transgenic clones had slightly fewer differentiated shoots than the untransformed control. The cuttings of control clone K and some of the transgenic clones differentiated compact green light callus on the cut. In these conditions the regenerated shoots of the transgenic clones branched much less than the nontransgenic clone.

The morphogenetic response of shoot sections of untransformed clone K to the different cytokinins showed differences and concentration-dependency. Lower concentrations of 2iP had no effect on the number of differentiated shoots, but did increase branching and caused dark callus formation. The number of shoots per cutting increased on media with 0.1 mg·l^{-1} Kin. Callus formation followed by multiple shoot differentiation was observed on all media with BAP and on media supplemented with higher concentrations of

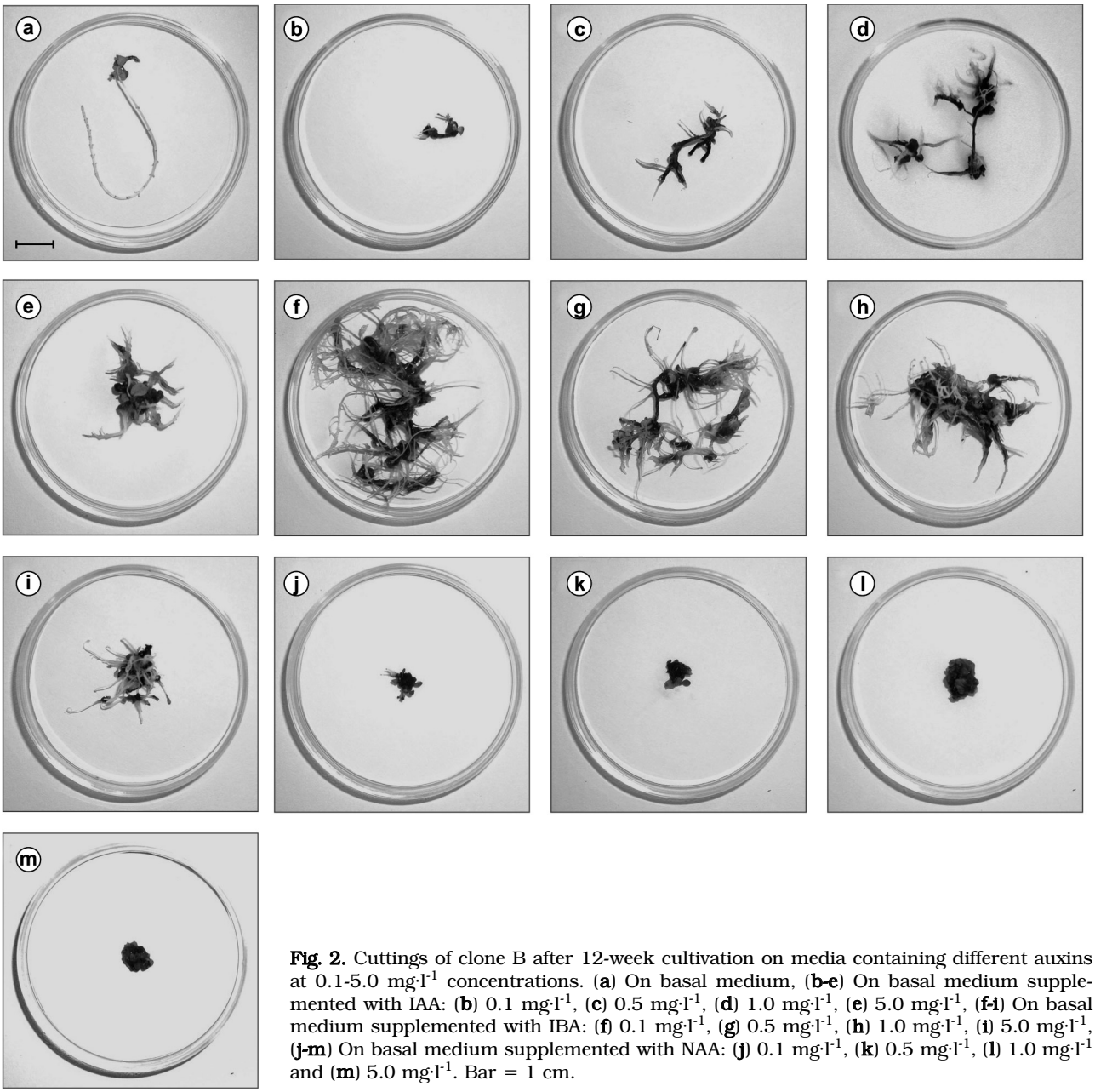

Fig. 2. Cuttings of clone B after 12-week cultivation on media containing different auxins at 0.1-5.0 mg·l^{-1} concentrations. (**a**) On basal medium, (**b-e**) On basal medium supplemented with IAA: (**b**) 0.1 mg·l^{-1}, (**c**) 0.5 mg·l^{-1}, (**d**) 1.0 mg·l^{-1}, (**e**) 5.0 mg·l^{-1}, (**f-i**) On basal medium supplemented with IBA: (**f**) 0.1 mg·l^{-1}, (**g**) 0.5 mg·l^{-1}, (**h**) 1.0 mg·l^{-1}, (**i**) 5.0 mg·l^{-1}, (**j-m**) On basal medium supplemented with NAA: (**j**) 0.1 mg·l^{-1}, (**k**) 0.5 mg·l^{-1}, (**l**) 1.0 mg·l^{-1} and (**m**) 5.0 mg·l^{-1}. Bar = 1 cm.

2iP and Kin. Addition of BAP led to plentiful branching of shoots and to altered morphology: internode shortening, differentiation of smaller leaves and suppression of rooting (Fig. 3).

The clone B cuttings formed numerous shoots on media with Kin and BAP (Tab. 2). Shoot length and the number of leaf pairs decreased with increased concentrations of these cytokinins. Addition of 2iP only slightly improved shoot differentiation. Higher concentrations of all cytokinins caused callus formation and suppressed rooting of differentiated shoots. The morphogenetic response of transgenic clone L was similar to that of clone B, with more frequent callus formation followed by shoot differentiation (Fig. 4). Addition of low concentrations of BAP and Kin increased not only shoot formation but also the percentage of root-differentiating cuttings of transgenic clone L, from 26.7% to 53.3–60.0% (Tab. 2).

T-DNA GENE INTEGRATION AND EXPRESSION IN TRANSGENIC CLONES

Integration of *rolA, B, C* genes into the genomes of clone B and L was confirmed, but integration of *rolD* and *aux* genes was not. Quantitative real time PCR (qPCR) revealed similar copy numbers of

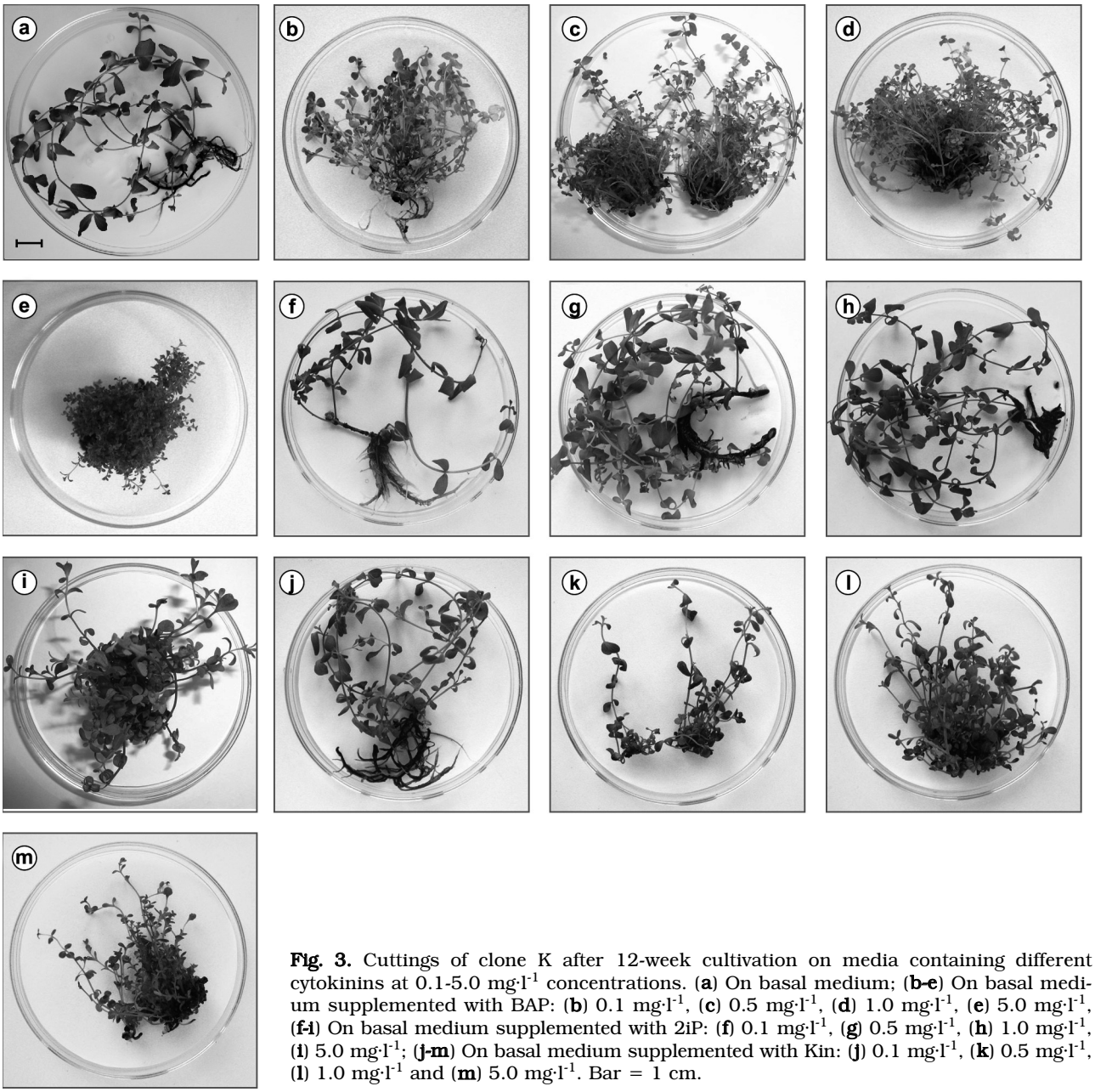

Fig. 3. Cuttings of clone K after 12-week cultivation on media containing different cytokinins at 0.1-5.0 mg·l^{-1} concentrations. (**a**) On basal medium; (**b-e**) On basal medium supplemented with BAP: (**b**) 0.1 mg·l^{-1}, (**c**) 0.5 mg·l^{-1}, (**d**) 1.0 mg·l^{-1}, (**e**) 5.0 mg·l^{-1}, (**f-i**) On basal medium supplemented with 2iP: (**f**) 0.1 mg·l^{-1}, (**g**) 0.5 mg·l^{-1}, (**h**) 1.0 mg·l^{-1}, (**i**) 5.0 mg·l^{-1}; (**j-m**) On basal medium supplemented with Kin: (**j**) 0.1 mg·l^{-1}, (**k**) 0.5 mg·l^{-1}, (**l**) 1.0 mg·l^{-1} and (**m**) 5.0 mg·l^{-1}. Bar = 1 cm.

integrated *rolABC* genes in both clones (Tab. 3). Expression of the introduced genes was shown by RT-PCR at the level of the presence of *rolABC* gene transcripts.

DISCUSSION

The transgenic clones used in this study were derived from unique transformation events and from different types of tissues: clone L from leaf and clone B from root cuttings of the control clone. This, together with the semi-random nature of *A. rhizogenes* T-DNA integration into different genomic regions, might lead to phenotypic differences between transgenic clones B and L. Transgene expression resulted in the 'hairy root' phenotype, accompanied by altered morphology and changes in biosynthetic ability (Koperdáková et al., 2009). Since *rolABC* gene expression was confirmed in both clones, different integration sites of the transgene may have been involved.

Most known functions of *Agrobacterium* T-DNA gene products are related to altered auxin and

TABLE 2. Morphogenic effect of cytokinins

Medium supplemented with	Clone	% of shoot-differentiating cuttings	Intensity of shoot differentiation	% of callus producing cuttings	% of root-differentiating cuttings
no PGR	B	86.7	+	33.3	60.0
	L	100.0	+	30.0	26.7
	K	100.0	+	80.0	60.0
0.1 mg·l^{-1} 2iP	B	86.7	+	100.0	46.7
	L	100.0	+	100.0	40.0
	K	100.0	+	100.0	73.3
0.5 mg·l^{-1} 2iP	B	80.0	+	100.0	60.0
	L	93.3	+	93.3	20.0
	K	100.0	+	100.0	73.3
1 mg·l^{-1} 2iP	B	100.0	+	100.0	26.7
	L	93.3	+	100.0	13.3
	K	100.0	+	100.0	66.7
5 mg·l^{-1} 2iP	B	86.7	+	80.0	26.7
	L	93.3	+	93.3	0.0
	K	86.7	+++	93.3	60.0
0.1 mg·l^{-1} BAP	B	80.0	+++	80.0	46.7
	L	93.3	+++	93.3	53.3
	K	100.0	+++	100.0	46.7
0.5 mg·l^{-1} BAP	B	93.3	+++	86.7	6.7
	L	100.0	+++	100.0	0.0
	K	100.0	+++	100.0	6.7
1 mg·l^{-1} BAP	B	100.0	+++	93.3	0.0
	L	100.0	+++	100.0	0.0
	K	100.0	+++	100.0	0.0
5 mg·l^{-1} BAP	B	93.3	+++	100.0	0.0
	L	100.0	+++	100.0	0.0
	K	100.0	+++	100.0	0.0
0.1 mg·l^{-1} Kin	B	86.7	+	86.7	40.0
	L	93.3	+	93.3	60.0
	K	100.0	++	100.0	60.0
0.5 mg·l^{-1} Kin	B	78.6	++	78.6	26.7
	L	100.0	++	100.0	60.0
	K	100.0	+++	93.3	40.0
1 mg·l^{-1} Kin	B	66.7	++	80.0	26.7
	L	93.3	+++	93.3	46.7
	K	93.3	+++	93.3	46.7
5 mg·l^{-1} Kin	B	93.3	+++	73.3	20.0
	L	100.0	+++	86.7	0.0
	K	100.0	+++	100.0	33.3

+ 1–4, ++ >4, +++ multiple shoots per cutting

cytokinin metabolism. The altered hormonal response includes increased tolerance to high levels of auxins and abscisic acid, and a higher sensitivity to various cytokinins (Schmülling et al., 1993). Despite much research over the years and some evidence connecting the action of *rol* genes with plant

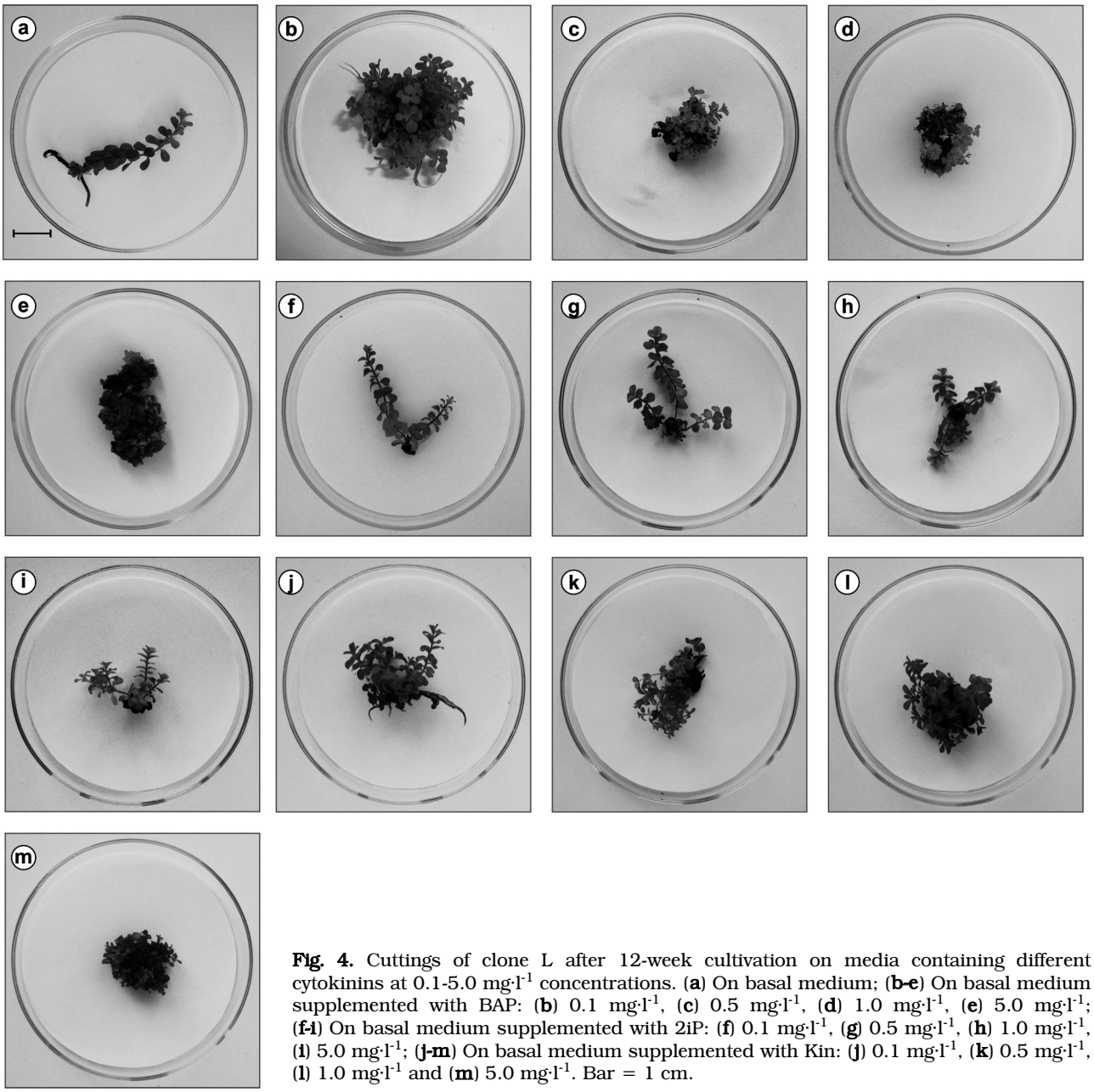

Fig. 4. Cuttings of clone L after 12-week cultivation on media containing different cytokinins at 0.1-5.0 mg·l^{-1} concentrations. (**a**) On basal medium; (**b-e**) On basal medium supplemented with BAP: (**b**) 0.1 mg·l^{-1}, (**c**) 0.5 mg·l^{-1}, (**d**) 1.0 mg·l^{-1}, (**e**) 5.0 mg·l^{-1}; (**f-i**) On basal medium supplemented with 2iP: (**f**) 0.1 mg·l^{-1}, (**g**) 0.5 mg·l^{-1}, (**h**) 1.0 mg·l^{-1}, (**i**) 5.0 mg·l^{-1}; (**j-m**) On basal medium supplemented with Kin: (**j**) 0.1 mg·l^{-1}, (**k**) 0.5 mg·l^{-1}, (**l**) 1.0 mg·l^{-1} and (**m**) 5.0 mg·l^{-1}. Bar = 1 cm.

hormone effects, the biochemical functions of these genes remain poorly understood, reflecting the complexity of the system (Casanova et al., 2005).

We compared the morphogenetic reaction of the clones to different PGRs and different concentrations with their response to hormone-free medium. Cuttings on media containing auxins were cultivated in the dark, while those on media supplemented with cytokinins were cultivated under light.

An interesting effect of light on root differentiation of cuttings cultivated on PGR-free media in different light conditions served as a control for the auxin and cytokinin assays. While the percentage of root-differentiating cuttings of control clones on basal media remained almost the same in different light conditions, cuttings of both transgenic clones were much more prone to differentiate roots under light than in the dark (Tabs. 1, 2). It turned out that the transgenic cuttings' endogenous auxin level was rather low, and root differentiation proceeded much more effectively after regeneration of shoots under light. It has been shown that nontransgenic adventitious shoots of *H. perforatum* can be easily rooted on PGR-free medium (e.g., Karppinen et al., 2006).

TABLE 3. Copy number of *rolABC* genes integrated into B and L clones of *H. perforatum* detected by quantitative real time PCR

Clone	Mean copy number of integrated T-DNA genes ± SD		
	rolA	*rolB*	*rolC*
B	3.37± 0.75	3.30 ± 0.65	2.62 ± 0.62
L	4.80 ± 0.96	2.59 ± 0.46	1.81 ± 0.37
K	–	–	–

The level of endogenous auxin in shoots was already sufficient for rooting, and exogenously applied auxin inhibited the growth of already formed root primordia (Wojcik and Podstolski, 2007).

Auxins and cytokinins have coordinated functions as both long-distance and local signals. These hormones are produced not only in root tips and shoot apices, as was thought previously, but at various sites of the plant (Ljung et al., 2001; Nordstrom et al., 2004). The importance of plant growth regulators in plant tissue culture is well documented. Earlier studies on the morphoregulatory role of auxins and cytokinins on seedlings and shoots of *H. perforatum* cultured in vitro also showed concentration-dependent root-inducing effects of IAA and IBA (Čellárová and Kimáková, 1999; Gadzovska et al., 2005), and callus formation resulting from higher concentrations of them or from the presence of NAA and 2,4-D. Shoot differentiation is promoted especially by BAP (Karppinen et al., 2006; Wojcik and Podstolski, 2007) and Kin, but not in the presence of 2iP (Čellárová and Kimáková, 1999). Low levels of BAP accelerate shoot regeneration from root, leaf and hypocotyl explants (Franklin and Dias, 2006). Higher BAP concentrations often resulted in red coloration of tissues (Franklin and Dias, 2006; Wojcik and Podstolski, 2007) as we also observed in our experiments. Recently, it was shown that low levels of BAP not only promote shoot formation but also increase the content of the active compounds hypericin and hyperforin (Charchoglyan et al., 2007; Liu et al., 2007).

To our knowledge this is the first report of morphogenetic effects of PGRs on transgenic *H. perforatum* clones. The reaction of these transgenic clones reveals some similarities but also features unique to their transgenic behavior. There were also differences between clones in their sensitivity to auxins and cytokinins, reflected in altered morphogenetic reactions. Cuttings of the clones differed in their ability to form shoots, and the shoot sections of transgenic clone L were less sensitive to exogenous auxins than those of untransformed clone K. The expression of *rolABC* was confirmed and the copy numbers of the integrated *rol* genes in the clones were similar; the differences in morphogenetic response may have resulted from differences in the integration sites, which could affect the expression of the integrated genes and/or interfere with some processes of normal plant development.

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REFERENCES

Casanova E, Trillas MI, Moysset L, and Vainstein A. 2005. Influence of rol genes in floriculture. *Biotechnology Advances* 23: 3–39.

Čellárová E, and Kimáková K. 1999. Morphoregulatory effect of plant growth regulators on *Hypericum perforatum* L. seedlings. *Acta Biotechnologica* 19: 163–169.

Charchoglyan A, Abrahamyan A, Fujii I, Boubakir Z, Gulder TAM, Kutchan TM, Vardapetyan H, Bringmann G, Ebizuka Y, and Beerhues L. 2007. Differential accumulation of hyperforin and secohyperforin in *Hypericum perforatum* tissue cultures. *Phytochemistry* 68: 2670–2677.

Christey MC. 2001. Use of Ri-mediated transformation for production of transgenic plants. *In Vitro Cellular and Developmental Biology-Plant* 37: 687–700.

Di Guardo A, Čellárová E, Koperdáková J, Pistelli L, Rufonni B, Allavena A, and Giovannini A. 2003. Hairy root induction and plant regeneration in *Hypericum perforatum* L. *Journal of Genetics and Breeding* 57: 269–278.

Franklin G, and Dias ACP. 2006. Organogenesis and embryogenesis in several *Hypericum perforatum* genotypes. *In Vitro Cellular and Developmental Biology-Plant* 42: 324–330.

Gadzovska S, Maury S, Ounnar S, Righezza M, Kascakova S, Refregiers M, Spasenoski M, Joseph C, and Hagčge D. 2005. Identification of hypericin and pseudohypericin in different *Hypericum perforatum* L. *in vitro* cultures. *Plant Physiology and Biochemistry* 43: 591–601.

Gamborg OL, Miller RA, and Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cultures. *Experimental Cell Research* 50: 148–151.

Jaakola L, Pirtilla AM, Halonen M, and Hohtola A. 2001. Isolation of high quality RNA from Bilberrry (*Vaccinium myrtillus* L.) fruit. *Molecular Biotechnology* 19: 201–204.

Karppinen K, Gyorgy Z, Kauppinen M, Tolonen A, Jalonen J, Neubauer P, Hohtola A, and Haggman H. 2006. *In vitro* propagation of *Hypericum perforatum* L. and accumulation of hypericins, pseudohypericins and phloroglucinols. *Propagation of Ornamental Plants* 6: 170–179.

Koperdáková J, Komarovská H, Košuth J, Giovannini A, and Čellárová E. 2009. Characterization of hairy root-phenotype in transgenic *Hypericum perforatum* L. clones. *Acta Physiologiae Plantarum* 31: 351–358.

Kubin A, Wierrani F, Burner U, Alth G, and Grünberger W. 2005. Hypericin – the facts about a controversial agent. *Current Pharmaceutical Design* 11: 233–253.

Linsmaier EM, and Skoog F. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum* 18: 100–127.

Liu XN, Zhang XQ, and Sun JS. 2007. Effects of cytokinins and elicitors on the production of hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum perforatum*. *Plant Growth Regulation* 53: 207–214.

Ljung K, Bhalerao RP, and Sandberg G. 2001. Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *Plant Journal* 28: 465–474.

Medina MA, Martinez-Poveda B, Amores-Sanchez MI, and Quesada AR. 2006. Hyperforin: More than an antidepressant bioactive compound? *Life Sciences* 79: 105–111.

Nordstrom A, Tarkowski P, Tarkowska D, Norbaek R, Astot C, Dolezal K, and Sandberg G. 2004. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin-regulated development. *Proceedings of the National Academy of Sciences of the United States of America* 101: 8039–8044.

Schmülling T, Fladung M, Grossmann K, and Schell J. 1993. Hormonal content and sensitivity of transgenic tobacco and potato plants expressing single *rol* genes of *Agrobacterium rhizogenes* T-DNA. *Plant Journal* 3: 371–382.

Vinterhalter B, Ninkovic S, Cingel A, and Vinterhalter D. 2006. Shoot and root culture of *Hypericum perforatum* L. transformed with *Agrobacterium rhizogenes* A4M70GUS. *Biologia Plantarum* 50: 767–770.

Wojcik A, and Podstolski A. 2007. Leaf explant response in in vitro culture of St. John's wort (*Hypericum perforatum* L.). *Acta Physiologiae Plantarum* 29: 151–156.