Topolin Metabolism and Its Implications for In Vitro Plant Micropropagation

6

Karel Doležal and Magdalena Bryksová

Contents

Abstract

Topolins are a relatively recent discovery, following search for viable alternatives to 6-benzylaminopurine (BAP) which while effective and affordable has important disadvantages for certain crops. This chapter reviews some biochemical and technical aspects of topolin metabolism in relation to in vitro plant micropropagation.

Keywords

Meta-topolin · Topolin · Cytokinin · Metabolism · Plant tissue culture

6-Benzylaminopurine (BAP, also known as N^6-benzyladenine: BA) was long the most widely used cytokinin (CK) in micropropagation systems, due to its efficacy and affordability (Holub et al. 1998). However, its adverse effects on the growth, rooting, and acclimatization of some recalcitrant species and induction of other physiological disorders (Aremu et al. 2012a) prompted the search for and subsequent

K. Doležal (✉) · M. Bryksová
Department of Chemical Biology, Faculty of Science, Palacký University, Olomouc, Czech Republic

Laboratory of Growth Regulators, Institute of Experimental Botany, AS CR, Palacký University, Olomouc, Czech Republic
e-mail: karel.dolezal@upol.cz

N. Ahmad, M. Strnad (eds.), *Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture*, https://doi.org/10.1007/978-981-15-9046-7_6

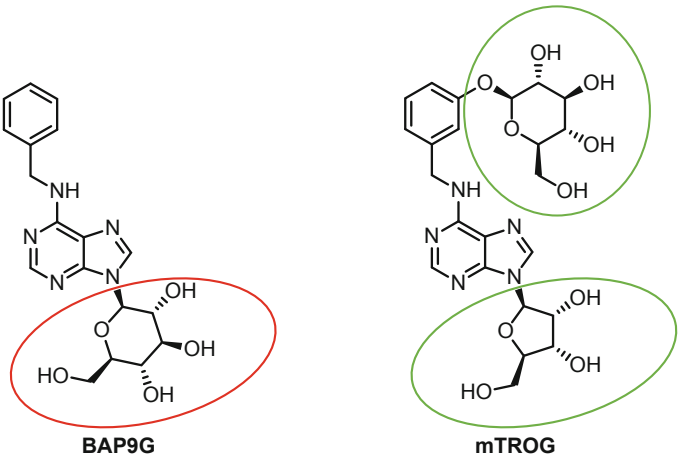

Fig. 6.1 Chemical structure of 6-benzylamino-9-β-D-glucopyranosylpurine (BAP9G) and 6-(3-*O*-β-D-glucopyranosyl)benzylaminopurine-9-β-D-riboside (*m*TROG)

discovery of viable alternatives: the choice of CK remains critical to the success or failure of any newly developed micropropagation protocol (Bairu et al. 2007; Werbrouck 2010; Aremu et al. 2012a; Valero-Aracama et al. 2010). The increasing importance of hydroxylated derivatives commonly referred to as topolins (Strnad et al. 1997) in micropropagation has been critically reviewed more recently (Aremu et al. 2012a, 2017).

Following description of the basic aspects of CK activity of topolin ribosides (Kaminek et al. 1987) and unambiguous identification of 6-(3-hydroxybenzylamino) purine (*meta*-topolin, *m*T) and its derivatives as naturally occurring CKs *in planta* (Strnad 1996, 1997; Strnad et al. 1997), pioneering work was published on the metabolism and in vitro effects of *m*T in micropropagated *Spathiphyllum floribundum* (Werbrouck et al. 1996). *m*T was compared to BAP, 6-benzylamino-9-(2-tetrahydropyranyl)purine (BAP9THP), and 6-benzylaminopurine-9-β-D-riboside (BAPR) using an HPLC separation system, coupled to a tandem quadrupole mass spectrometer (MS/MS) equipped with an electrospray interface (ESI). In vitro, BAP and its 9-substituted derivatives BAP9THP and BAPR were mainly converted into the stable and inactive metabolite, 6-benzylamino-9-β-D-glucopyranosylpurine (BAP9G) (Fig. 6.1), located mostly in the basal part of the micropropagated plant (Werbrouck et al. 1995). In contrast, 6-(3-*O*-β-D-glucopyranosyl) benzylaminopurine-9-β-D-riboside (*m*TROG) (Fig. 6.1) was identified as the main metabolite of *m*T. This new cytokinin-*O*-glucoside, which was present in all plant parts, was formed much faster than BAP9G during acclimatization process. The effect of BAP and *m*T on in vitro shoot and root production and ex vitro rooting was then compared (Werbrouck et al. 1996). Only *m*T combined sufficient shoot production with acceptable in vitro root formation. The plants developed on medium with 10 μM or more *m*T rooted better also during acclimatization in comparison with those grown on medium with comparable BAP concentrations.

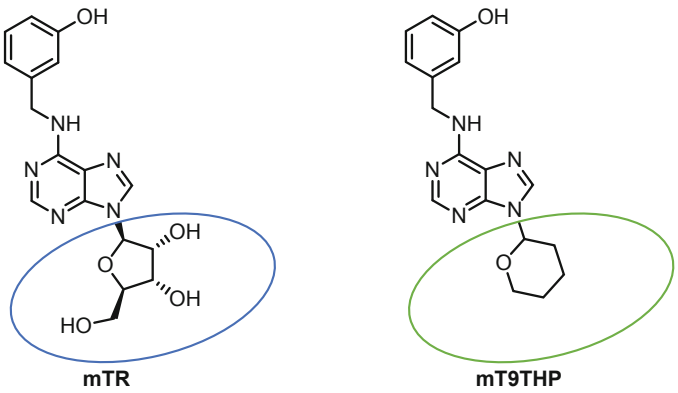

Fig. 6.2 Chemical structure of 6-(3-hydroxybenzylamino)purine-9-β-D-ribofuranoside (*m*TR) and 6-(3-hydroxybenzylamino)-9-tetrahydropyran-2-ylpurine (*m*T9THP)

Very similar results were later obtained by Bairu et al. (2011) in micropropagated *Harpagophytum procumbens* tissues, where changes in endogenous CK profiles and the physiological implications of this in relation to shoot-tip necrosis (STN) and CK treatments were also studied. Generally, necrotic shoots contained more total CKs compared to normal shoots, and CK accumulation was higher in the basal section. More importantly, further analysis of structural and functional CK forms revealed the inability of BAP to form *O*-glucosides as well as excessive accumulation of 9-glucosides (irreversible deactivation product) in necrotic and basal callus like tissues of BAP-treated shoots (Bairu et al. 2011). The addition of IAA enhanced the formation of 9-glucosides in BAP-treated cultures. The symptom of STN could therefore be attributed to conversion of active CK to other forms such as 9-glucoside (Bairu et al. 2011). On the other hand, the presence of a hydroxyl group in their molecule gives topolins a structural advantage over BAP. This was reflected in the presence of a generous amount of *O*-glucosides in topolin-treated samples and hence little or no CK shortage (Bairu et al. 2011). Moreover, the level of irreversible inactivation (9-glucoside formation) of *m*T was found to be even lower when exogenously applied in the form of 9-riboside, compared with free base application.

As the most efficient plant growth regulator (PGR), 10 μM 6-(3-hydroxybenzylamino)purine-9-β-D-ribofuranoside (*m*TR, Fig. 6.2) treatment also produced the highest number of shoots (approximately five shoots per explant) during clonal propagation of *Lachenalia montana*, a species endemic to Southern Africa and extensively traded as ornamental plants in the international floriculture industry (Aremu et al. 2017b). Based on the concentrations of endogenous CKs subsequently determined, 10 μM *m*TR regenerants also had the highest CK levels which were mainly of the aromatic type (98%). In terms of the functional role of the CKs, *O*-glucosides were again the dominant CK metabolites in the regenerants of the 10 μM *m*TR treatment. On the other hand, the insufficient rooting, predominantly in regenerants of the BAP treatments, was closely related to the high accumulation of

N9-glucosides compared to regenerants from other treatments. These findings provided further evidence of the interrelationship between exogenous topolin application, positive phenotypic responses, and endogenous CK levels in the in vitro regenerants (Aremu et al. 2017b).

Based on these studies, a series of attempts was made to prepare other topolin derivatives substituted at the 9-position using various protective groups to improve the specific biological functions of the CKs already routinely used in the plant micropropagation industry. For example, inspired by discovery of the protective role of ribose in the 9-position (Bairu et al. 2009). Szüčová et al. (2009) prepared several substituted 6-benzylamino-9-tetrahydropyran-2-ylpurine (THPP) and 9-tetrahydrofuran-2-ylpurine (THFP) derivatives, with hydroxy and methoxy functional groups at various positions on the benzyl ring. The new compounds were synthesized by condensation of 6-chloropurine with 3,4-dihydro-2H-pyran or 2,3-dihydrofuran and then by the reaction of these intermediates with the corresponding benzylamines (in *n*-propanol or *n*-butanol, in the presence of triethylamine). Identity and purity of the prepared compounds were confirmed by CHN analysis, TLC, HPLC, melting point determinations, CI+ MS, and 1H NMR spectroscopy. The CK activity of the prepared derivatives was determined in three classical cytokinin bioassays (tobacco callus, wheat leaf senescence, and *Amaranthus* bioassay).

In another study (Podlešáková et al. 2012), in contrast to canonical CKs, the 9-tetrahydropyranyl derivative of *m*T (Fig. 6.2) and its methoxy counterpart showed negative effects on root development at only three orders of magnitude higher concentration. The methoxy derivative also demonstrated a positive effect on lateral root branching and leaf emergence in nanomolar concentration range in comparison with untreated plants. Tetrahydropyranyl substitution at the *N9*-position of CK purine ring was also found to significantly enhance acropetal transport of a given CK. Together with the methoxy substitution, inhibition of the formation of non-active CK glucosides in roots allows gradual release of the active base and has a significant effect on the distribution and amount of endogenous isoprenoid CKs in different plant parts (Podlešáková et al. 2012). These results provided a basis for anticipating that the use of novel aromatic CK derivatives could improve the expected hormonal effects in plant propagation methodology in the future.

This led to 9-THP topolin derivative (Fig. 6.2) being successfully used in various micropropagation systems, for example, two widely used medicinal plants, *Aloe arborescens* and *Harpagophytum procumbens* (Amoo et al. 2014). In terms of *A. arborescens* shoot multiplication, *m*TTHP and *m*T (at equimolar level) showed similar effects, and both were comparably better than the control and BAPR. In *H. procumbens*, *m*T-treated cultures were the most responsive to treatment at 2.5 μM compared to the control. At 5.0 μM concentration, *m*T9THP and *m*TR demonstrated a similar activity on shoot proliferation. Particularly at low concentrations, *m*T9THP had a better rooting stimulatory activity than the other CKs in both plant species. It is conceivable that *m*T9THP is another viable alternative topolin with the added advantage of inducing rooting at low concentrations (Amoo et al. 2014). Later (Amoo et al. 2015), this compound was also successfully used to improve

micropropagated *Merwilla plumbea* shoot production without rooting inhibition as well as its positive carry-over effect on ex vitro growth. Unlike *m*TTHP treatments, an increase in concentration of *m*TR or TDZ, other tested CKs, beyond 0.5 μM resulted in a significant decrease in the concentrations of all the photosynthetic pigments quantified. Even after 6 months of ex vitro growth, regenerated plants of the 0.5 μM *m*TTHP treatment had the significantly higher total leaf area, total leaf fresh weight, and bulb size compared to all *m*TR- and TDZ-treated plants (Amoo et al. 2015).

In a similar study on *Merwilla plumbea* (Lindl.) Speta, a popular and highly sought-after South African medicinal plant with diverse therapeutic uses (Aremu et al. 2014), the effect of another *meta*-topolin derivative, 6-(3-methoxybenzylamino)-9-tetrahydropyran-2-ylpurine (Me*m*TTHP), was evaluated on the growth and level of endogenous CKs during micropropagation and acclimatization stages. A total of 37 (22 isoprenoid and 15 aromatic) CKs were determined in both in vitro and ex vitro acclimatized plants. Based on their metabolic function, these were separated into five different groups, including free bases, ribosides, ribotides, and *O*- and 9-glucosides. In addition to enhancing our understanding of the hormone physiology in *M. plumbea*, the current findings were discussed in line with the effect of exogenously applied CK on the observed differences in growth before and after the important stage of acclimatization. The observed dynamics in endogenous CK can provide an avenue to optimize the in vitro growth and development of investigated species.

Another successful attempt to enhance the anti-senescence properties of topolins was described by Doležal et al. (2017) and Matušková et al. (2020), by preparing their 9-β-D-arabinofuranosyl or 9-β-D-2′-deoxyribofuranosyl derivatives (Fig. 6.3) via a one-step reaction. The starting material, optically pure unprotected 9-(2′-deoxy-β-D-ribofuranosyl)hypoxanthine (2′-deoxy-9-β-D-inosine) or 9-(β-D-arabinofuranosyl)hypoxantine, and BOP [(benzotriazol-1yloxy)tris

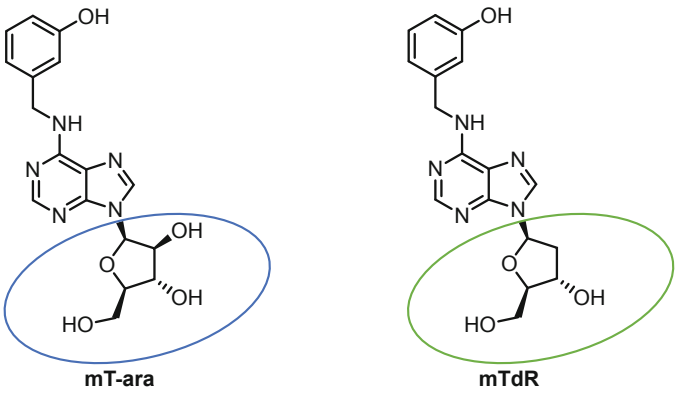

Fig. 6.3 Chemical structure of 6-(3-hydroxybenzylamino)purine-9-β-D-arabinofuranoside (*m*T-ara) and 6-(3-hydroxybenzylamino)purine-9-(2′-deoxy-β-D-ribofuranoside) (*m*TdR)

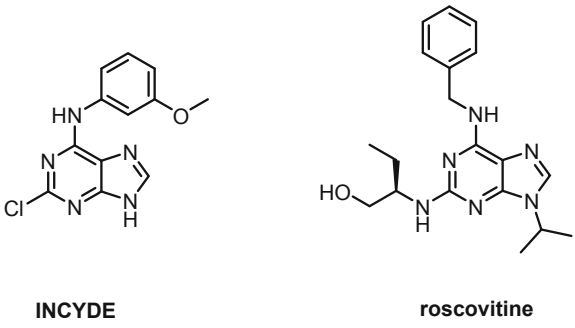

Fig. 6.4 Chemical structure of 2-chloro-6-(3-methoxyphenyl) aminopurine (INCYDE) and 2-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine (roscovitine)

(dimethylamino)phosphonium hexafluorophosphate] were dissolved in dry DMF (3 mL) under nitrogen or argon atmosphere at 55–60 °C, and DIPEA was added, followed by appropriate substituted benzylamine (1.2 eq.) as the last component. The resulting white solid was isolated by filtration and re-crystallized from EtOH. The synthesized compounds were characterized by CHN and melting point analysis, analytical thin layer chromatography, high-performance liquid chromatography, ES^+ MS spectrometry, and ^1H NMR. The positive effect of selected derivatives on shoot proliferation in *Harpagophytum procumbens* and *Amelanchier alnifolia* as well as control of shoot-tip necrosis in in vitro cultures of the medicinal plant *Gymnosporia buxifolia* was then demonstrated (Doležal et al. 2017).

Modulating the CK status with inhibitors of CK perception and/or degradation may affect the general physiology of the plant (Dwivedi et al. 2010; Motte et al. 2013; Zatloukal et al. 2008). For this reason, regulation of phytohormone metabolism may be another potential way to improve plant growth and development during micropropagation.

The effect of supplementing either *m*T or BAP requiring cultures with INCYDE (2-chloro-6-(3-methoxyphenyl)aminopurine) (Fig. 6.4), an inhibitor of CK degradation (Spichal et al. 2012), on the endogenous CK profiles and physiology of banana in vitro was hence investigated (Aremu et al. 2012b).

Another interesting alternative approach for decreasing levels of irreversible topolin deactivation in banana tissue cultures was developed by Aremu et al. (2012b). An inhibitor of 9-glucosylation, roscovitine 2-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine (Fig. 6.4), which was previously discovered among a number of 2,6,9-trisubstituted purines, tested as potential *N*-glucosylation inhibitors (Blagoeva et al. 2004; Letham et al. 1977; Dwivedi et al. 2010). Aremu et al. (2012b) demonstrated that its application simultaneously with exogenous CK (s) in the cultivation media has the potential to change endogenous CK pools, thereby influencing the rooting and ex vitro acclimatization of in vitro-derived *Musa* spp. It was observed that plantlets regenerated from *m*T + roscovitine media produced the most shoots. They also had the highest total CK content (661 pmol/g FW) with the roots having approximately 68-fold more than the shoots (Aremu et al. 2012b).

A general trend observed was that the addition of roscovitine and/or INCYDE with *m*T improved the total CK pool in both roots and shoots of the tissue-cultured 'Williams' banana regenerants (Aremu et al. 2012b). A similar pattern was determined in the shoots when BAP was supplemented with roscovitine and INCYDE; however, both compounds reduced the total CK pool in the roots as well as the sum total in the plantlets. It is noteworthy to highlight that the reduction in total CK pool was mainly due to the decrease in the level of 9-glucosides, which are generally detrimental to plant growth (Aremu et al. 2012b).

6.1 Future Perspectives: Application of Fluorinated Compounds

Fluorination has a long tradition in nucleoside chemistry. It was demonstrated that replacement of the 2′ or 3′ hydroxyl groups of a nucleoside with a fluorine atom causes only a minor change in the total structure, but significantly affects the stereoelectronic properties of the sugar moiety (Thibaudeau et al. 1998). Fluorine substitution has been extensively investigated in drug research and biochemistry as a means of increasing biological activity and enhancing chemical or metabolic stability. However, to date only a few fluorinated CK derivatives have been prepared and their biological activity tested (Clemenceau et al. 1996; Doležal et al. 2006, 2007). Later, Murvanidze et al. (2019) evaluated the impact of 6-(3-fluorobenzylamino) purine (*m*F-BAP) and its 9-β-D-riboside (mF-BAPR) (Fig. 6.5) on in vitro cloning of *Phalaenopsis* hybrids, which are usually characterized by slow growth and low multiplication rates. The plantlets formed significantly more but smaller new shoots when treated with *m*F-BAPR (25.3) compared to *m*F-BAP (14.6) and BAP (7.0). The results suggest the following strategy: massive micropropagation of small shoots for a number of cycles on *m*F-BAPR in closed containers, followed by a

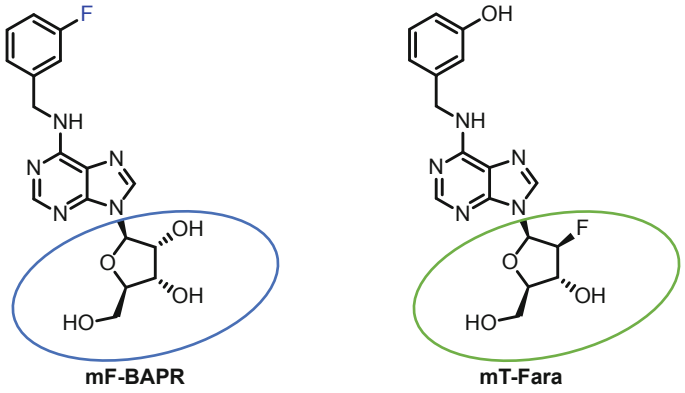

Fig. 6.5 Chemical structure of 6-(3-fluorobenzylamino)purine-9-β-D-ribofuranoside (*m*F-BAPR) and 6-(3-hydroxybenzylamino)purine-9-(2′-fluoro-2′-deoxy-β-D-arabinofuranoside) (*m*T-Fara)

final step with BAP in filter vessels to produce large shoots with roots. For this reason, the use of fluorinated topolins might present a breakthrough in the in vitro micropropagation of *Phalaenopsis* (Murvanidze et al. 2019). Another class of N^6-substituted-2′-deoxy-2′-fluoro-9-β-D-arabinofuranosylpurine derivatives (Fig. 6.5) was very recently prepared, and their biological activity is currently being evaluated (Bryksová et al. 2020).

Acknowledgements We would like to thank Alex Oulton, PhD, for the careful and critical reading of the chapter. This work was funded by the project "Plants as a tool for sustainable global development" (No. CZ.02.1.01/0.0/0.0/16_019/0000827) within the program Research, Development and Education (OP RDE) and the Internal Grant Agency of Palacký University (IGA_PrF_2021_011).

References

Amoo SO, Aremu AO, Moyo M, Szüčová L, Doležal K, Van Staden J (2014) Physiological effects of a novel aromatic cytokinin analogue in micropropagated *Aloe arborescens* and *Harpagophytum procumbens*. Plant Cell Tiss Org Cult 116:17–26

Amoo SO, Aremu AO, Moyo M, Sunmonu TO, Plíhalová L, Doležal K, Van Staden J (2015) Physiological and biochemical effects of a tetrahydropyranyl-substituted meta-topolin in micropropagated *Merwilla plumbea*. Plant Cell Tiss Org Cult 121:579–590

Aremu AO, Bairu MW, Doležal K, Finnie JF, Van Staden J (2012a) Topolins: a panacea to plant tissue culture challenges? Plant Cell Tiss Org Cult 108:1–16

Aremu AO, Bairu MW, Novák O, Plačková L, Zatloukal M, Doležal K, Finnie JF, Strnad M, Van Staden J (2012b) Physiological responses and endogenous cytokinin profiles of tissue-cultured 'Williams' bananas in relation to roscovitine and an inhibitor of cytokinin oxidase/dehydrogenase (INCYDE) treatments. Planta 236:1775–1790

Aremu AO, Plačková L, Bairu MW, Novák O, Plíhalová L, Doležal K, Finnie JF, Van Staden J (2014) How does exogenously applied cytokinin type affect growth and endogenous cytokinins in micropropagated *Merwilla plumbea*? Plant Cell Tiss Org Cult 118:245–256

Aremu AO, Doležal K, Van Staden J (2017a) New cytokinin-like compounds as a tool to improve rooting and establishment of micropropagated plantlets. Acta Hortic 1155:497–503

Aremu AO, Plačková L, Masondo NA, Amoo SO, Moyo M, Novák O, Doležal K, Van Staden J (2017b) Regulating the regulators: responses of four plant growth regulators during clonal propagation of *Lachenalia montana*. Plant Growth Regul 82:305–315

Bairu MW, Stirk WA, Doležal K, Van Staden J (2007) Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can *meta*-topolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell Tiss Org Cult 90(1):15–23

Bairu MW, Novák O, Doležal K, Van Staden J (2011) Changes in endogenous cytokinin profiles in micropropagated *Harpagophytum procumbens* in relation to shoot-tip necrosis and cytokinin treatments. Plant Growth Regul 63:105–114

Blagoeva E, Dobrev PI, Malbeck J, Motyka V, Strnad M, Hanuš J, Vaňková R (2004) Cytokinin N-glucosylation inhibitors suppress deactivation of exogenous cytokinins in radish, but their effect on active endogenous cytokinins is counteracted by other regulatory mechanisms. Physiol Plant 121:215–222

Bryksová M, Hybenová A, Hernándiz AE, Novák O, Pěnčík A, Spíchal L, De Diego N, Doležal K (2020) Hormopriming for mitigating abiotic stress effects: a case study of N9-substituted cytokinin derivatives with fluorinated carbohydrate moiety. Frontiers in Plant Sciences, submitted.

Clemenceau D, Cousseau J, Martin V, Molines H, Wakselman C, Mornet R, Nogue F, Laloue M (1996) Synthesis and cytokinin activity of two fluoro derivatives of N^6-isopentenyladenine. J Agric Food Chem 44:320–323

Doležal K, Popa I, Kryštof V et al (2006) Preparation and biological activity of 6 benzylaminopurine derivatives in plants and human cancer cells. Bioorg Med Chem 14:875–884

Doležal K, Popa I, Hauserová E et al (2007) Preparation, biological activity and endogenous occurrence of N^6-benzyladenosines. Bioorg Med Chem 15:3737–3747

Doležal K, Plíhalová L, Vylíčilová H, Zatloukal M, Plíhal O, Voller J, Strnad M, Van Staden J, Baskaran P, Kumari A, Aremu AO (2017) 6-aryl-9-glycosylpurines and use thereof. US 0326168

Dwivedi S, Vanková R, Motyka V, Herrera C, Zizkova E, Auer C (2010) Characterization of *Arabidopsis thaliana* mutant ror-1 (roscovitine-resistant) and its utilization in understanding of the role of cytokinin N-glucosylation pathway in plants. Plant Growth Regul 61:231–242

Holub J, Hanuš J, Hanke DE, Strnad M (1998) Biological activity of cytokinins derived from *ortho*- and *meta*-hydroxybenzyladenine. Plant Growth Regul 26(2):109–115

Kaminek M, Vaněk T, Motyka V (1987) Cytokinin activities of N6-Benzyladenosine derivatives hydroxylated on the side-chain phenyl ring. J Plant Growth Regul 6:113–120

Letham DS, Parker CW, Duke CC, Summons RE, MaCleod JK (1977) O-glucosylzeatin and related compounds - a new group of cytokinin metabolites. Ann Bot 41:261–263

Matušková V, Zatloukal M, Voller J, Grúz J, Pěkná Z, Briestenská K, Mistríková J, Spíchal L, Doležal K, Strnad M (2020) New aromatic 6-substituted 2′-deoxy-9-(β)-D-ribofuranosylpurine derivatives as potential plant growth regulators. Bioorg Med Chem 28(2):115230

Motte H, Galuszka P, Spíchal L, Tarkowski P, Plíhal O, Šmehilová M, Jaworek P, Vereecke D, Werbrouck S, Geelen D (2013) Phenyl-adenine, identified in a LIGHT-DEPENDENT SHORT HYPOCOTYLS4-assisted chemical screen, is a potent compound for shoot regeneration through the inhibition of CYTOKININ OXIDASE/DEHYDROGENASE activity. Plant Physiol 161:1229–1241

Murvanidze N, Dolezal K, Werbrouck SPO (2019) Fluorine containing topolin cytokinins for *Phalaenopsis amabilis* (L.) Blume micropropagation. Propag Ornam Plants 19(2):48–51

Podlešáková K, Zalabák D, Čudejková M, Plíhal O, Szűčová L, Doležal K, Spíchal L, Strnad M, Galuszka P (2012) Novel cytokinin derivatives do not show negative effects on root growth and proliferation in submicromolar range. PLOS One 7, e39293

Spichal L, Gemrotova M, Zatloukal M, Frebortova J, Galuszka P, Werner T, Schmulling T, Dolezal K, Strnad M (2012) Substituted 6-anilinopurine derivatives as inhibitors of cytokinin oxidase/dehydrogenase and preparations containing these derivatives. US 08222260

Strnad M (1996) Enzyme immunoassays of N^6-benzyladenine and N^6-(meta-hydroxybenzyl)adenine cytokinins. J Plant Growth Regul 15:179–188

Strnad M (1997) The aromatic cytokinins. Physiol Plant 101:674–688

Strnad M, Hanuš J, Vaněk T et al (1997) Meta-Topolin, a highly active aromatic cytokinin from poplar leaves (*Populus x canadensis* Moencho cv. Robusta). Phytochemistry 42:213–218

Szüčová L, Spíchal L, Doležal K, Zatloukal M, Greplová J, Galuszka P, Kryštof V, Voller J, Popa I, Massino FJ, Jørgensen JE, Strnad M (2009) Synthesis, characterization and biological activity of ring-substituted 6-benzylamino-9-tetrahydropyran-2-yl and 9-tetrahydrofuran-2-ylpurine derivatives. Bioorg Med Chem 17:1938–1947

Thibaudeau C, Plavec J, Chattopadhyaya J (1998) A new generalized karplus-type equation relating vicinal proton-fluorine coupling constants to H-C-C-F torsion angles. J Org Chem 63 (15):4967–4984

Valero-Aracama C, Kane M, Wilson S, Philman N (2010) Substitution of benzyladenine with *meta*-topolin during shoot multiplication increases acclimatization of difficult- and easy-to acclimatize sea oats (*Uniola paniculata* L.) genotypes. Plant Growth Regul 60:43–49

Werbrouck SPO (2010) Merits and drawbacks of new aromatic cytokinins in plant tissue culture. Acta Hortic 865:103–108

Werbrouck SP, van der Jeugt B, Dewitte W, Prinsen E, Van Onckelen HA, Debergh PC (1995) The metabolism of benzyladenine in *Spathiphyllum floribundum* 'Schott Petite' in relation to acclimatisation problems. Plant Cell Rep 14:662–665

Werbrouck SP, Strnad M, Van Onckelen HA, Debergh PC (1996) Meta-topolin, an alternative to benzyladenine in tissue culture? Physiol Plant 98(2):291–297

Zatloukal M, Gemrotová M, Doležal K, Havlíček L, Spíchal L, Strnad M (2008) Novel potent inhibitors of *A. thaliana* cytokinin oxidase/dehydrogenase. Bioorg Med Chem 16:9268–9275