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REVIEW PAPER

MELATONIN – A NEW PLANT BIOSTIMULATOR?

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Abstract

Melatonin (MEL) is a highly conserved molecule occurring in evolutionarily distant organisms such as bacteria, mono- and multicellular algae, higher plants, invertebrates and vertebrates. Although until recently, this molecule was mainly known as an animal hormone and neurotransmitter, its role in plants is currently being extensively investigated. MEL, *N*-acetyl-5--methoxytryptamine was found in various agronomically important vegetables, fruits, grains and herbs. Its concentration varies from ng to pg per gram fresh weight. MEL is present in all plant organs with its highest level found in seeds. Since the germ tissues are highly vulnerable to oxidative damage, and MEL is well known as an effective amphiphilic free radical scavenger, MEL may play an important role in plant antioxidant defence system. Especially in desiccated seed tissues, where the activity of detoxifying enzymes is limited, MEL may be essential for protecting plant germ and reproductive tissues from oxidative injuries. Recent studies provide solid evidence for MEL acting as plant growth and development regulator as well as a biostimulator, especially under unfavourable environmental conditions. Various plant species rich in MEL exhibit higher capacity for stress tolerance. MEL is also involved in stress-affected developmental transitions including flowering, fruiting and senescence. Plants are equipped with an enzymatic system for MEL biosynthesis; they are also able to synthesize a MEL precursor, tryptophan. In addition to *in vivo* synthesis, plants can also absorb exogenously provided MEL from the environment. These, and particularly the evidence that in plants MEL induces resistance against stresses, suggest that our concept of seed enrichment with exogenous MEL is justified. Our experiments proved that exogenous MEL applied into seeds by pre-sowing treatment (priming) improved their vigour and germination efficiency as well as seedling growth.

Keywords: MEL, phytobiostimulator, seed priming.

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INTRODUCTION

Melatonin is a natural compound commonly occurring in a large number of evolutionarily unrelated eukaryotic and prokaryotic taxa. It was isolated for the first time from a bovine pineal gland in 1958 and considered to be an animal neurohormone performing the critical function in circadian rhythm, seasonal reproductive cycles, and in modulating immunology in mammals (Van Tassel et al. 2001, Agozzino et al. 2003, Hernandez-Ruiz et al. 2004, Pape, Luning 2006). Subsequently, because of its numerous roles in animal organisms, considerable interest arose in MEL utilization as a therapeutic agent in many diseases such as circadian rhythm disorders, insomnia, Alzheimer's disease or even different kinds of cancer (Agozzino et al. 2003).

Since then, MEL has been searched and described in organisms belonging to all kingdoms: *Monera, Protista, Animalia, Plantae* and *Fungi (*Manchester et al. 2000). Much attention was focused on the study of *Gonyaulax polyedra (Lingulodinium polyedrum* 1987*)* belonging to dinoflagellate (Balzer, Hardeland 1991, Van Tassel et al. 2001) and then of feverfew (*Tanacetum parthenium*), St John's Wort (*Hypericum perforatum*) and other medicinal herbs (Murch, Saxena 1997). The presence of endogenous MEL in these organisms directed the attention of scientists to its role in autotrophs, especially in higher plants (Dubbels et al. 1995, Hattori et al. 1995). Since then, the presence of this indoleamine in edible plants and medicinal herbs has been extensively reported (Posmyk, Janas 2009, Paredez et al. 2009, Tan et al. 2012, Arnao 2014). Melatonin has been found *inter alia* in apple, barley, bean, cucumber, grapes, lupin, maize, potato, rice, tomato *etc.* and its concentration in plants varies from ng to pg per gram (Chen et al. 2003, Arnao, Hernández-Ruiz 2006, 2007**,** Paredes et al. 2009, Posmyk, Janas 2009, Manchester et al. 2000, Chen et al. 2009, Tan et al. 2012). It was detected and quantified in roots, shoots, leaves, flowers, fruits and seeds, but its highest level is found in reproductive organs, particularly in seeds. Since MEL was identified in a huge number of edible plants and herbs, its presence in food products and beverages originating from plants is unsurprising. Various beverages e.g. beer, coffee, red vine, and several types of tea (Tan et al. 2012) and also olive and mustard oil (Iriti et al. 2010) were studied as potential natural sources of MEL.

It was observed that MEL concentrations differed not only from species to species but also among varieties of the same species (Tan et al. 2012). It was suggested that variations in MEL contents might result not only from different extraction and detection techniques applied (i.a. radioimmunoassays, high performance liquid chromatography with electrochemical or fluorescent detection, mass spectrometry) but also from the fact that biosynthesis and metabolism of this indoleamine are affected and modified by environmental conditions (i.a. stresses), and MEL levels change during plant ontogenesis (Okazaki, Ezura 2009). It partially explains why different organs of the same plant contain various amounts of MEL during consecutive stages of morphological and physiological development.

Generally, it was noticed that various plant species rich in MEL had greater capacity for stress tolerance (Park, Lee et al. 2013, Bajwa et al. 2014, Zhang et al. 2015) and this fact turned our attention to melatonin as a potential effective factor improving plant stress defence.

THE ROLE OF MELATONIN IN PLANTS

It should be pointed out that evolutionary MEL is a high conserved molecule and cell protection has been its primary role. Plants possess the necessary enzymatic system for MEL biosynthesis. In contrast to animals, they are also able to synthetize the MEL precursor - tryptophan. However, in addition to *in vivo* synthesis, plants can also absorb exogenously provided MEL from the environment and accumulate its high concentrations (Tan et al. 2007*b*, Kołodziejczyk et al. 2015).

Melatonin is a structural analogue of indole-3-acetic acid (IAA) – a common auxin, hence the debate over its potential auxin-like properties is in progress. Biosynthetic pathway of both indoleamines are similar – both are derived from tryptophan. In the case of MEL, it is based on four enzymatic reactions (Figure 1). Firstly, tryptophan decarboxylase (TDC) catalyzes tryptophan transformation into tryptamine. The second step –tryptamine 5-hydroxylase (T5H) derived from cytochrome P450, hydroxylates tryptamine and converts it into serotonin. The third phase – arylalkylamine N-acetyltransferase (AANAT) catalyzes N-acetylserotonin generation in chloroplasts. Finally, N-acetylserotonin is converted into MEL using N-acetylserotonin methyltransferase (ASMT) in the cytoplasm (Kang et al. 2013, Byeon et al. 2014, Zhang et al. 2015).

Currently, the role of MEL in plant physiology is being intensively explored. Much evidence implicates MEL as a growth promoter and plant development factor (Hernández-Ruiz et al. 2004, 2005, Arnao, Hernández-Ruiz 2007, Hernández-Ruiz, Arnao 2008*a*, Chen et al. 2009, Sarrou et al. 2014, Zhang et al. 2014).

Under certain conditions MEL can simulate the auxin effects (Arnao 2014). Evidence for the involvement of MEL in root formation in *Hypericum perforatum* L. (Murch et al. 2001), and etiolated hypocotyls of *Lupinus albus* L. (Arnao, Hernandez-Ruiz 2007) was provided. A similar effect accompanied by an increase in IAA level was observed in the roots of mustard (*Brassica juncea* L. Czern.) after exogenous MEL application (Chen et al. 2009). However, it is unclear whether MEL induces auxin biosynthesis, or whether it may be metabolized and converted to IAA, or whether by structural analogy to IAA MEL exerts auxin-like effects – but these concepts need more studies.

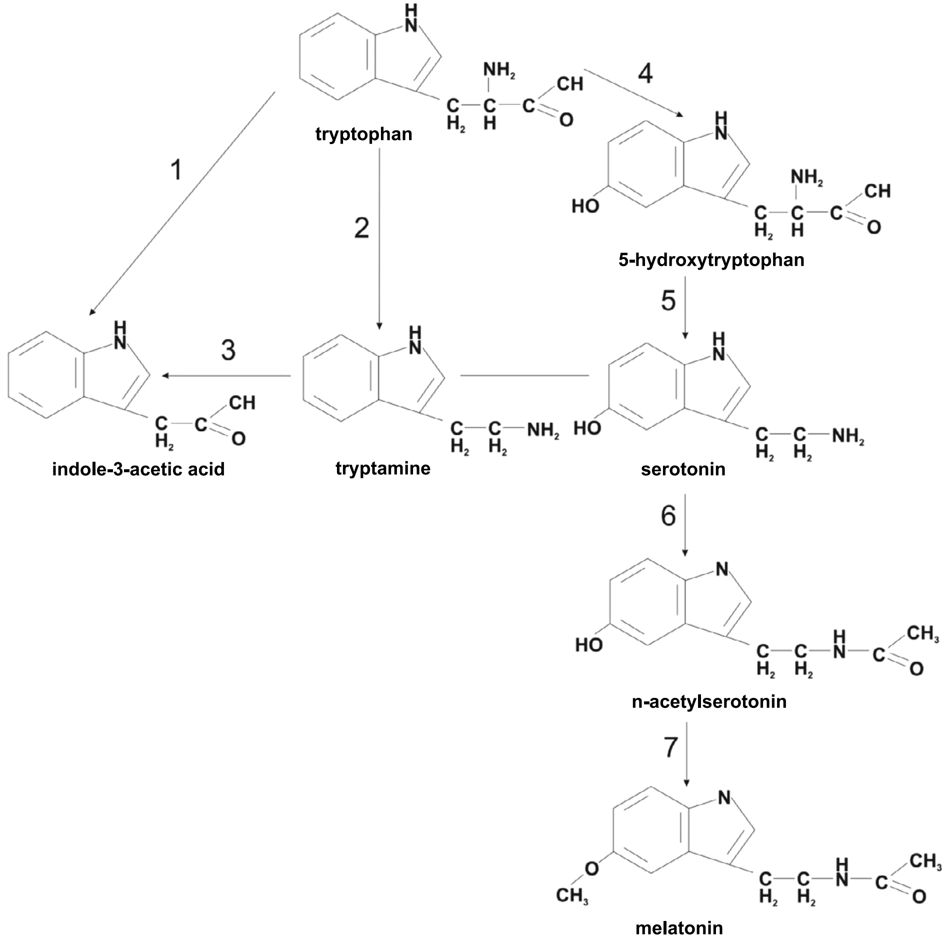

Fig. 1. Melatonin biosynthesis pathway. Enzymes involved: 1 – L-tryptophan transaminase and decarboxylase, 2 – L-tryptophan decarboxylase, 3 – tryptamine deaminase, 4 – tryptophan--5-hydroxylase, 5 – 5-hydroxytryptophan decarboxylase, 6 – serotonin N-acetyltransferase, 7 – acetyloserotonin N-methyltransferase

It was also shown that MEL in a dose-dependent manner stimulated elongation of hypocotyls (Hernández-Ruiz et al. 2004) and cotyledons of lupine – *Lupinus albus* L. (Hernández-Ruiz, Arnao 2008*c*).

Poeggeler et al. (1991) suggested participation of MEL in circadian cycle regulation in unicellular photosynthesizing dinoflagellate *Lingulodinium polyedrum*. A similar rhythm of MEL occurrence was reported in short-day plants, *e.g. Chenopodium rubrum* (Wolf et al. 2001), while daily changes in MEL content were not observed in tomato (Van Tassel et al. 2001). Research of Tan et al. (2007*a*) on the water hyacinth revealed the highest content of MEL and its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) just before sunset, which might confirm the fact that MEL synthesis depends on the intensity of light. This differentiates plants from animals, since in the latter light suppresses MEL production in pineal glands (MURCH et al. 2000). It seems that the MEL content in plants is regulated not only by its biosynthesis rate but also by the intensity of its consumption. Thus, although the correlation of rhythmic MEL content changes with the circadian rhythm depending on the photoperiod was confirmed in some plants, this phenomenon seems to be more complex and susceptible to the influence of additional factors. In general, rhythmic changes in the MEL content in plants, like in other organisms, may be evolutionarily related to the photoperiod. Melatonin synthesized and stored in the dark is consumed in the light in order to reduce photo-oxidative damage so its level during the day falls. It seems that repeated rhythmic fluctuations associated with the synthesis and utilization of MEL could be used by organisms to biochemically measure duration of the day, and consequently to determine changes of the seasons (KOŁODZIEJCZYK et al. 2015). However, it should be taken into account that during evolution plants developed a number of other photoreceptors (phytochrome, cryptochrome, light-dependent photosynthetic apparatus), which effectively regulate the processes of photomorphogenesis.

Certainly, melatonin consumption in the light is associated with protection against photooxidizing processes, to which photosynthetic apparatus of plants is particularly exposed. In plants treated with this indoleamine photosynthesis was more efficient, concentrations of starch, sorbitol and sucrose were higher and chlorophyll degradation during the aging process was slower, while the expression of pheophorbide oxidase (*PAO*) gene (a key gene for the chlorophyll degradation process) and senescence-associated gene 12 (*SAG12)* was inhibited. Moreover, exogenous MEL inhibited the expression of sugar-sensing and senescence-associated hexokinase-1 gene *(HXK1)* (WANG et al. 2013*b*) and effectively prevented the accumulation of H_2O_2, which is a characteristic indicator of the cell aging process (WANG et al. 2012, 2013*a*). In *A. thaliana* MEL treatment inhibited chlorophyllase (*CLH1*) gene expression, an enzyme regulated by light and also involved in chlorophyll degradation (WEEDA et al. 2014). Decreased chlorophyll degradation in barley leaves treated with MEL was also observed and its effects were more pronounced than those invoked by cytokines, widely accepted as anti-aging hormones (ARNAO, HERNANDEZ-RUIZ 2009*a*). A similar impact of exogenous MEL on chlorophyll protection against the effects of oxidative stress was observed in the leaves of *A. thaliana* treated with herbicide paraquat (WEEDA et al. 2014). Studies on rice revealed that treatment with MEL significantly reduced chlorophyll degradation, suppressed transcripts of senescence-associated genes, delayed leaf senescence and enhanced salt stress tolerance (LIANG et al. 2015).

Various stresses inhibit plant growth *via* different mechanisms but all stimulate reactive oxygen species (ROS) production and disturb red-ox homeostasis. It is well known that oxidative stress is a secondary effect of all biotic and some abiotic stresses. Thus, the antioxidant behaviour based on the capability of electron donation resulting in such antioxidant structure reconformation which does not allow for secondary free radical appearance is highly desirable in plant cells. Since MEL is soluble in both water and lipids, it may be a hydrophilic and hydrophobic antioxidant. This fact together with the MEL's small size makes it particularly able to migrate easily between cell compartments in order to protect them against excessive ROS. Moreover, recent evidence indicates that the primary MEL metabolites, especially AFMK, also have high antioxidant abilities. AFMK could be formed by numerous free-radical reactions thus a single MEL molecule is reported to scavenge up to 10 ROS. It is documented that the free radical scavenging capacity of MEL extends to its secondary, tertiary and quaternary metabolites (TAN et al. 2000, 2002, 2007*c*). This process is referred to as the free radical scavenging cascade, which makes MEL even at low concentrations highly effective at protecting organisms against oxidative stress. This cascade reaction is characteristic of MEL making it more efficient than other conventional antioxidants.

As it was mentioned above, various plant species rich in MEL have shown higher capacity for stress tolerance (PARK et al. 2013, BAJWA et al. 2014, ZHANG et al. 2015). Exogenously applied MEL improved resistance to *Marssonina* apple blotch (YIN et al. 2013), this also implies its important role in plant innate immunity. Melatonin is also involved in stress-affected developmental transitions including flowering, fruiting and senescence (KOLAR et al. 2003, ARNAO, HERNÁNDEZ-RUIZ 2009*a*, ZHAO et al. 2013, BYEON, BACK 2014). An increase in the MEL content was detected in sunflower seeds during sprouting (CHO et al. 2008). Since the germ tissue is highly vulnerable to oxidative damage, MEL might be an important component of its antioxidant defense system as a free radical scavenger. Thus, MEL in seeds may be essential for protecting plant germ and reproductive tissues from oxidative injuries (MANCHESTER et al. 2000). It have been described that plant MEL actually scavenges ROS in *Vigna radiata* leaves and protects *Gentiana macrophylla* protoplasts under UV-B exposure (TAN et al. 2012). Generally, oxidative stress elevates amount of endogenous melatonin (BOCCALANDRO et al. 2011). Transgenic plants opulent of endogenic MEL survive negative effects induced by ROS and are able to prevent the damage (WANG et al. 2012). Moreover, it is proved that elevated concentration of melatonin ensures higher levels of GSH, vitamin C and E or carotenoids (TAN et al. 2012, WANG et al. 2012). These suggest that MEL constitutes the first line of antioxidant safeguard.

The aforementioned facts, and particularly the evidence that MEL induced resistance to stresses in plants, indicate that our concept of seed or whole plant enrichment with exogenous MEL is justified.

MELATONIN – A NOVEL PLANT BIOSTIMULATOR

Plant biostimulators – phytostimulators are various kinds of non-toxic substances of natural origin that improve and stimulate plant life processes differently than fertilizers or phytohormones. Their influence on plants is not the consequence of direct metabolic regulation of particular processes but their action could be multidirectional and they influence metabolism more generally. The crucial point is that biostimulators, in contrast to bioregulators, improve plant metabolic processes without changing their natural pathway.

Recent plant neurobiology trends in science based on auxin signaling investigations (Brenner et al. 2006) focus our attention on MEL, which demonstrates parallelism with the plant auxin – indole-3-acetic acid (IAA). Resumption of research on classic plant models used in plant physiology is still necessary to clarify the role and mechanism of action of MEL: (i) as an independent plant growth regulator, (ii) as a factor mediating the activity of other substances influencing plant growth or (iii) as a substance involved in growth regulation but whose activity generally is ascribed to other compounds. However, recent knowledge may qualify MEL as a biostimulator. Its advantages are as follows: (i) it is of natural origin but it can be easily synthesized in laboratories, (ii) it is non-toxic, (iii) it is not expensive, (iv) it dissolves in different solvents: water, alcohols but also lipids – which facilitates the use of various application methods (v) it could be actively uptaken by plants from environment, (vi) it is a small molecule easily penetrating cell compartments, (vii) it has strong antioxidant properties (viii) it improves plant tolerance to stresses.

The quality of seed material is a primary and basic condition determining good harvest. Thus, finding effective methods to improve sowing material by applying biostymulators into seeds is a crucial problem. Generally, it was observed that physiological concentrations of MEL in seeds were very high, for example in white and black mustard seeds they were 129 and 189 ng g^{-1}, respectively (Hattori et al. 1995), much higher than the known physiological blood concentrations of many vertebrates

The known techniques of seed priming: hydro- and osmo-conditioning were tested by Posmyk and colleagues (Posmyk, Janas 2007, Posmyk et al. 2008, 2009, 2009*a*, Janas et al. 2009). Different pre-sowing seed treatments effectively counteract diseases and pests as well as improve seed viability and seedling vigour *per se* (Taylor et al. 1998). All of them are based on controlled seed hydration. These techniques can be combined with other supporting factors such as aeration, light-irradiation, temperature-stratification. Seed priming can also be combined with an application of growth regulators and other bioactive substances (McDonald 1999). Our previous experiments (Posmyk, Janas 2007, Posmyk et al. 2008, 2009, 2009*a*, Janas et al. 2009) proved that exogenous MEL applied into seeds by pre-sowing treatment improved their vigour and germination as well as seedling growth.

Indeed, red cabbage seed hydropriming with MEL proved to be a good tool for vigour improvement (Posmyk et al. 2008). Positive effect of this treatment was visible especially under copper stress conditions. Similarly, experiments with cucumber seeds osmoprimed with MEL (Posmyk et al. 2009*a*, 2009) and with corn seeds hydroprimed with MEL (Janas et al. 2009) proved its positive effects on seeds.

Beneficial effects of priming were not visible in cucumber and corn seed germination tests performed under optimal temperature conditions. Except that primed seeds germinated more uniformly (smaller ±SEM), both parameters: the germination rate and the final germination percentage, were comparable with control seeds (Posmyk et al. 2009*a*, Janas et al. 2009). However, subsequent experiments showed that seedlings grown from seeds conditioned with melatonin tolerated better stresses of suboptimal temperature (10°C) and heavy metal contamination (2.5 mM Cu^{2+}), and also regenerated much better after the relief of stress. It was manifested by better growth (greater weight of seedlings) and higher chlorophyll content and phenolic synthesis in the seedlings grown from seeds hydroprimed with melatonin (Janas et al. 2009).

When in seeds laboratory tests were subjected to stress, differences between the untreated and conditioned variants were apparent. Cucumber seeds osmoprimed with MEL started to germinate at 10°C, which was impossible for the control, untreated ones (Posmyk et al. 2009, 2009*a*). We also obtained interesting results when corn seeds were germinated at 10°C (Janas et al. 2009). The control non-primed seeds germinated after 5 days of imbibition and achieved ~97% germinability after 12 days of experiments, whereas those treated with MEL reduced the lag phase up to 2 days and all seeds were germinated on day 10 of experiment. Hydropriming without MEL also reduced the lag phase to 3 days of imbibition but germinability of the seeds decreased to ~90% (Janas et al. 2009). The seed pre-sowing treatments accelerated germination rates of corn seeds at suboptimal temperature.

Field tests equivocally proved that MEL applied in a specific way to seeds was a perfect biostimulator enhancing plant growth and development as well as increasing yield. Field experiments were performed with the following seeds: *Cucumis sativus* L., *Zea mays* L, *Vigna radiata* L. (Janas, Posmyk 2013). All seeds were primed and re-dried under laboratory conditions, up to one month before being sown in fields. During vegetation, the plants were not supplemented by fertilizers or pesticides – experiments were performed as organic farming. The field experiments showed that plants from the MEL -treated seeds of corn, mung bean and cucumber were greater, better developed, their vegetation was prolonged (they were longer green) and they had higher crop yield than the control ones (Posmyk, Janas 2009, Janas, Posmyk 2013). At harvesting, 50 µM MEL osmoprimed cucumber plants had more fruits than those osmoprimed with MEL 500 µM, osmoprimed without MEL or non-treated plants. We observed that some fruits of MEL-treated plants were larger than those of osmoprimed without MEL and non-osmoprimed ones (Janas, Posmyk 2013). 50 and 500 μM MEL-treated corn plants had more and larger cobs than those hydroprimed without MEL and the non-primed plants. Similar results were observed on mung bean, whose seeds were hydroprimed with MEL at 20, 50 and 500 μM concentrations. Numbers of pods were greater in the plants grown from the seed hydroprimed with 50 μM MEL than in hydroprimed without MEL and non-primed ones (Posmyk, Janas 2009, Janas, Posmyk 2013). It is surprising that a single MEL application into the seeds increased yield of plants growing naturally in a field. It was assessed that the production of corn, cucumber and mung bean primed with MEL was about 10-25% greater in comparison to those primed without MEL and it depended on plant species (Janas, Posmyk 2013).

Since melatonin is safe for animals and humans as well as inexpensive, a conditioning technique using this indoleamine as a plant biostimulator may be a reliable, feasible and cost-effective tool for positive seed quality modification and may be economically beneficial for organic farming (Posmyk, Janas 2009, Janas, Posmyk 2013).

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