Commentary

Strategies to generate melatonin-enriched transgenic rice to respond to the adverse effects on rice production potentially caused by global warming

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ABSTRACT

Global warming is predicted to reduce the yield of rice, which feeds more than half of the world's population. A rise in temperature will inevitably hamper rice production by causing drought and flooding. Melatonin has the capacity to ameliorate such adverse effects. Here, we propose multiple genetic means of producing melatonin-enriched, high-yield rice variants to adapt upcoming global warming.

Key words: melatonin, global warming, arsenic, high temperature, drought

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Global warming has a negative effect on agricultural production (1). In 2014, the International Panel on Climate Change (IPCC) initially predicted a 1.5–2.0°C increase in global temperature by the end of this century, but in 2017 this was reevaluated to a 5°C increase (2). Rice yields are more vulnerable to the nighttime temperature changes than that in daytime (3). Ceccarelli *et al*. (4) have predicted that a temperature increase will reduce rice production up to 41% by the end of the 21st century and reduce maize production up to 50% by 2080. This prediction is linked to a report that yields of rice grain may decline by 10% for each 1°C rise above growing-season's normal temperature (5). Temperatures higher than the optimum decrease photosynthesis (6) and cause drought; thereby, reducing rice yield as well (7). A high temperature during the flowering and grain-ripening stages significantly decreases seed fertility and grain-filling rate, as well as rice yield (8). Worldwide, rice production is hampered by high temperature, drought, floods, pathogens, salt stress, and arsenic stress (9). Clearly, a means of overcoming the adverse effects of global warming on rice production is needed.

Globally, annual rice production is around 782 million tons and this grain contributes to the food supply of more than half of the world's population (10). To maintain current rice production levels, efforts have focused on improving cultivation practices and breeding new rice variants that are more resistant to stresses caused by global warming.

Melatonin is an indoleamine with diverse physiological roles in animals and plants (11). Since its discovery in plants in 1995 (12, 13), approximately 800 papers on melatonin in plants have been published (14). Melatonin as a potent antioxidant can quench up to 10 molecules of reactive oxygen species (ROS) and lacks prooxidant activity (15, 16). In fact, melatonin protects plants from chemical stressors that generate ROS (17). These stressors include cadmium, lead, aluminum, copper, vanadium, methyl viologen, and herbicides. Exogenous application of melatonin or an increase in endogenous melatonin in transgenic plants enhances tolerance of plants to these stressors (18). With regard to arsenic accumulation in rice grain, melatonin confers arsenic stress tolerance by enhancing antioxidant activity in soybean (19) and tea plants and decreases the arsenic content of tea (20), suggesting that it blocks arsenic translocation from roots to aboveground tissues, as it also does for cadmium (21, 22). Melatonin also enhances tolerance to abiotic stresses that cause oxidative damage by directly scavenging toxic radicals and by elevating the activities of antioxidant enzymes via induction of the expression of signaling and defense genes (23, 24). These abiotic stresses include drought, salinity, heat, cold, intense light, alkalinity, and acidity (25). Biotic stresses including bacteria, fungi, and viruses have also been attenuated by exogenous melatonin application and in melatonin-enriched transgenic plants (26, 27).

To generate melatonin-enriched transgenic rice plants, it needs to understand melatonin biosynthetic and catabolic pathways in plants. Melatonin synthesis begins with the conversion of tryptophan into tryptamine by tryptophan decarboxylase (TDC). In the rice genome, there are at least three *TDC* genes, of which TDC1 (AK069031) and TDC3 (Os08g0140500) have 87% amino acid homology, compared to 47% and 48% homology to TDC2 (AK103253), respectively (28). The optimum *TDC* gene for melatonin production is *TDC3*. Tryptamine 5-hydroxylase (T5H) in rice catalyzes the conversion of tryptamine to serotonin. Therefore, ectopic overexpression of *T5H* in rice could increase melatonin levels (29). Serotonin *N*-acetyltransferase (SNAT) is responsible for *N*-acetylserotonin synthesis from serotonin. Two *SNAT* isogenes (*SNAT1* and *SNAT2*) have been functionally characterized (24), but there are others in the rice genome (30). Ectopic overexpression of *SNAT1* and *SNAT2* increases melatonin in rice but the role of the rice ortholog of apple *SNAT3* (AK109295) is unknown. Finally, *N*-acetylserotonin *O*-methyltransferase (ASMT) converts *N*-acetylserotonin into melatonin. A diverse array of *O*-methyltransferase enzymes shows ASMT activity, including caffeic acid *O*-methyltransferase (COMT) (24). The melatonin synthetic pathway is illustrated in Figure 1.

In an earlier study on rice, overexpression of three *ASMT* genes (*ASMT1*, *ASMT2*, *ASMT3*) increased melatonin content, albeit only slightly (31). By contrast, *COMT*-overexpressing transgenic rice plants produce more melatonin than *ASMT1–3*-overexpressing plants (32). The putative rice ASMT4 (XM_015782901), which is orthologous to apple MzASMT9, localizes to chloroplasts (33) and has potential to enhance melatonin production (24). Overexpression of a novel *ASMT5* gene (AK068864) in rice increases melatonin production twofold (34).

Several melatonin catabolic genes have been cloned, including melatonin 2-hydroxylase (*M2H*), melatonin 3-hydroxylase (*M3H*), and *N*-acetylserotonin deacetylase (*ASDAC*) (24). M2H catalyzes conversion of melatonin into 2-hydroxymmelatonin whereas M3H converts melatonin into cyclic 3-hydroxymelatonin. Interestingly, ASDAC catalyzes the conversion of *N*-acetylserotonin into serotonin (35). These genes can be manipulated by genome-editing technologies, such as the CRISPR-Cas system. *ASDAC* (AK072557) exists as a single copy in the rice genome, enabling the generation of *ASDAC*-knockout mutant rice by CRISPR-Cas. The rice *ASDAC* mutant has blocked the reverse way of melatonin synthesis from *N*-acetylserotonin to serotonin, leading to melatonin accumulation. Rice possesses at least four copies of *M2H* (36) and at least three copies of *M3H* (37) with different V_{max} values. There require stepwise mutant generations from quadruple to triple in *M3H* (*m3h1/2/3/4*) and *M2H* (*m2h1/2/3*) mutation to generate mutant rice with elevated melatonin synthesis. Melatonin production also can be increased using melatonin biosynthetic genes from other plants or animal species as well as by modifying melatonin biosynthesis, such as by overexpressing

COMT in chloroplasts (38) (Figure 1).

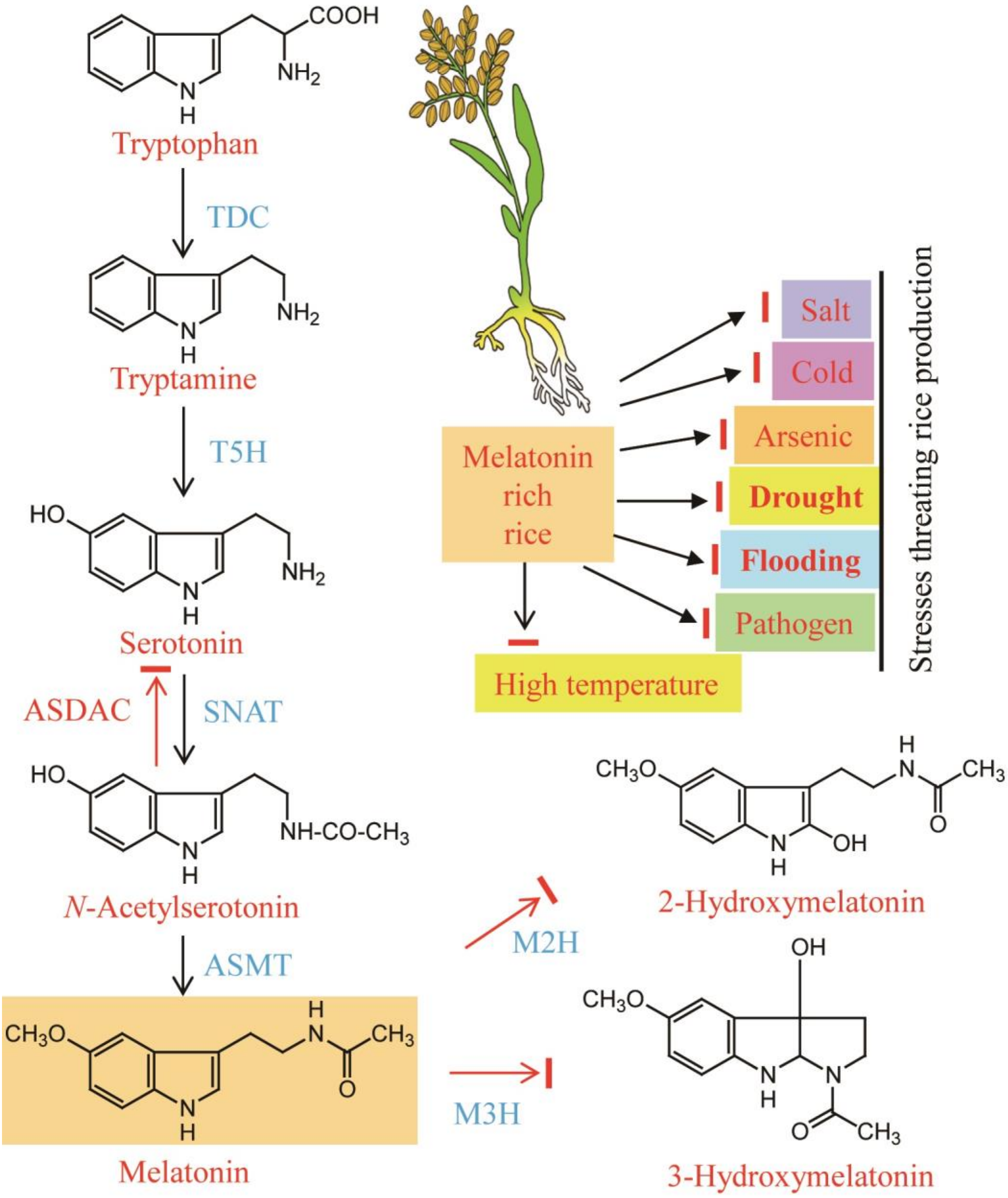

Fig. 1. Melatonin synthetic pathway and the enzymes which can be genetically manipulated to increase endogenous melatonin levels in rice.

The common abiotic stresses in rice plants including drought and flooding associated with global warming are highlighted in bold letters. Genes involved in melatonin biosynthesis and catabolism which can be used to increase the endogenous melatonin content of rice are marked as blue color. The bars indicate the block effect. The genetic modifications can be achieved by constitutively overexpressing melatonin biosynthetic genes under the control of a strong constitutive promoter, such as that of maize ubiquitin. Genes involved in melatonin catabolism (M2H, M3H) and the reverse pathway (ASDAC) can be knocked out by genome editing using the CRISPR-Cas system to increase the melatonin levels. TDC, tryptophan decarboxylase; T5H, tryptamine 5-hydroxylase; SNAT, serotonin N-acetyltransferase; ASMT, N-acetylserotonin O-methyltransferase; COMT, caffeic acid O-methyltransferase; ASDAC, N-acetylserotonin deacetylase; M2H, melatonin 2-hydroxylase; M3H, melatonin 3-hydroxylase.

In sumary, melatonin-enriched rice plants can be produced by overexpressing melatonin synthetic genes or knocking out melatonin catabolic genes. The most effective genes must be determined empirically to select transgenic rice plants with high melatonin contents but no growth inhibition or yield reduction. Melatonin-enriched rice not only has high yield but most importantly, be tolerant to climate change, such as global warming.

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AUTHORSHIP

All authors contributed to the conceptualization, writing and editing of this article.

CONFLICT INTEREST

The authors report no conflict of interest.

REFERENCES

1. Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. *Environ. Exp. Bot.* **61**: 199-223.
2. Le Quéré C. et al. (2017) Global carbon budget 2017. In *Earth System Science Data Discussion* 1-79.
3. Mohammed AR, Tarpley L (2011) High nigh temperature and plant growth regulator effects on spikelet sterility, grain characteristics and yield of rice (*Oryza sativa* L.) plants. *Can. J. Plant Sci.* **91**: 283-291.
4. Ceccarelli S, Grando S, Maatougui M, Michael M, Slash M, Haghparast R, Rahmanian M, Taheri A, Al-Yassin A, Benbelkacem A, Labdi M, Mimoun H, Nachit M (2010) Plant breeding and climate changes. *J. Agric. Sci.* **148**: 627-637.
5. Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci. USA* **101**: 9971-9975.
6. Wise RR, Olson AJ, Schrader SM, Sharkey TD (2004) Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant Cell Environ.* **27**: 717-724.
7. Muehe EM, Wang T, Kerl CF, Planer-Friedrich B, Fendorf S (2019) Rice production threatened by coupled stresses of climate and soil arsenic. *Nature Commun*. **10**: 4985.
8. Shah F, Huang J, Cui K, Nie L, Shah T, Chen C, Wang K (2011) Impact of high-temperature stress on rice plant and its traits related to tolerance. *J. Agric. Sci.* **149**: 545-556.
9. Ogasa N (2021) Rice in trouble. Drought and floods threaten this worldwide food staple. *Science News.* September 25, 16-19.
10. Carrijo DR, Lundy ME, Linquist BA (2017) Rice yields and water use under alternative wetting and drying irrigation: a meta-analysis. *Field Crops Res.* **203**: 173-180.
11. Zhao L, Chen L, Gu P, Zhan X, Zhang Y, Hou C, Wu Z, Wu YF, Wang QC. (2019) Exogenous application of melatonin improves plant resistance to virus infection. *Plant*

Pathol. **68**: 1287-1295.

12. Hattori A, Migitaka H, Iigo M, Itoh M, Yamamoto K, Ohtani-Kaneko R, Hara M, Suzuki T, Reiter RJ (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem. Mol. Biol. Int.* **35**: 627–634.
13. Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwara HW, Schloot W (1995) Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* **18**: 28–31.
14. Murch SJ, Erland LAE (2021) A systemic review of melatonin in plants: an example of evolution of literature. *Front. Plant Sci.* **12**: 683047.
15. Zhang HM, Zhang Y (2014) Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. *J. Pineal Res.* **57**: 131-146.
16. Arnao MB, Hernández-Ruiz J (2019) Melatonin and reactive oxygen and nitrogen species: a model for the plant redox network. *Melatonin Res.* **2**: 152-168.
17. Khan A, Numan M, Khan AL, Lee IJ, Imran M, Asaf S, Al-Harrasi A (2020) Melatonin: awakening the defense mechanisms during plant oxidative stress. *Plants* **9**: 407.
18. Moustafa-Farag M, Elkelish A, Dafea M, Khan M, Arnao MB, Abdelhamid MT, El-Ezz AA, Almoneafy A, Mahmoud A, Awad M, Li L, Wang Y, Hasanuzzaman M, Ai S (2020) Role of melatonin in plant tolerance to soil stressors: salinity, pH and heavy metals. *Molecules* **25**: 5359.
19. Bhat JA, Faizan M, Bhat MA, Huang F, Yu DY, Ahmad A, Bajguz A, Ahmad P (2021) Defense interplay of the zinc-oxide nanoparticles and melatonin and in alleviating the arsenic stress in soybean (*Glycine max* L.). *Chemosphere.* Doi:https://doi.org/10.1016/j.chemosphere.2021.132471.
20. Li X, Ahammed GJ, Zhang XN, Zhang L, Yan P, Zhang LP, Fu JY, Han WY (2021) Melatonin-mediated regulation of anthocyanin biosynthesis and antioxidant defense confer tolerance to arsenic stress in *Camellia sinensis* L. *J. Hazard. Mater.* **403**: 123922.
21. Hasan MK, Ahammed GJ, Yin L, Shi K, Xia X, Zhou Y, Yu J, Zhou J (2015) Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. *Front. Plant Sci.* **6**: 601.
22. Jiang M, Dai S, Wang B, Xie Z, Li J, Wang L, Li S, Tan Y, Tian B, Shu Q, Huang J (2021) Gold nanoparticles synthesized using melatonin suppress cadmium uptake and alleviate its toxicity in rice. *Environ. Sci. Nano* **8**: 1042.
23. Wang Y, Reiter RJ, Chan Z (2018) Phytomelatonin: a universal abiotic stress regulator. *J. Exp. Bot.* **69**: 963-974.
24. Back K (2021) Melatonin metabolism, signaling and possible roles in plants. *Plant J.* **105**: 376-391.
25. Sun C, Liu L, Wang L, Li B, Jin C, Lin X (2021) Melatonin: a master regulator of plant development and stress responses. *J. Integr. Plant Biol.* **63**: 126-145.
26. Zhao D, Yu Y, Shen Y, Liu Q, Zhao Z, Sharma R, Reiter RJ (2019) Melatonin synthesis and function: evolutionary history in animals and plants. *Front. Endocrinol.* **10**: 249.
27. Zhao D, Wang H, Chen S, Yu D, Reiter RJ (2021) Phytomelatonin: an emerging regulator of plant biotic stress resistance. *Trends Plant Sci.* **26**: 70-82.
28. Byeon Y, Park S, Lee HY, Kim YS, Back K (2014) Elevated production of melatonin in transgenic rice seeds expressing rice tryptophan decarboxylase. *J. Pineal Res.* **56**: 275-282.
29. Park S, Byeon Y, Back K (2013) Transcriptional suppression of tryptamine 5-hydroxylase, a terminal serotonin biosynthetic gene, induces melatonin biosynthesis in rice (*Oryza sativa* L.) *J. Pineal Res.* **55**: 131-137.

30. Wang L, Feng C, Zheng X, Guo Y, Zhou F, Shan D, Liu X, Kong J (2017) Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. *J. Pineal Res.* **63**: e12429.
31. Park S, Byeon Y, Back K (2013) Functional analysis of three *ASMT* gene family members in rice plants. *J. Pineal Res.* **55**: 409-415.
32. Byeon Y, Choi GH, Lee HY, Back K (2015) Melatonin biosynthesis requires *N*-acetylserotonin methyltransferase activity of caffeic acid *O*-methyltransferase in rice. *J. Exp. Bot.* **66**: 6917-6925.
33. Zheng X, Tan DX, Allan AC, Zuo B, Zhao Y, Reiter RJ, Wang L, Wang Z, Guo Y, Zhou J, Shan D, Li Q, Han Z, Kong J (2017) Chloroplastic biosynthesis of melatonin and its involvement in protection of plants from salt stress. *Sci. Rep.* 7: 41236.
34. Hong Y, Zhang Y, Sinumporn S, Yu N, Zhan X, Shen X, Chen D, Yu P, Wu W, Liu Q, Cao Z, Zhao C, Cheng S, Cao L (2018) Premature leaf senescence 3, encoding a methyltransferase, is required for melatonin biosynthesis in rice. *Plant J.* **95**: 877-891.
35. Lee K, Hwang OJ, Back K (2020) Rice *N*-acetylserotonin deacetylase regulates melatonin levels in transgenic rice. *Melatonin Res*. **3**: 32-42.
36. Byeon Y, Back K (2015) Molecular cloning of melatonin 2-hydroxylase responsible for 2-hydroxymelatonin production in rice (*Oryza sativa*). *J. Pineal Res.* **58**: 343-351.
37. Lee K, Zawadzka A, Czarnocki Z, Reiter RJ, Back K (2016) Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (*Oryza sativa*). *J. Pineal Res.* **61**: 470-478.
38. Choi G-H, Lee HY, Back K (2017) Chloroplast overexpression of rice caffeic acid *O*-methyltransferase increase melatonin production in chloroplasts via the 5-methoxytryptamine pathway in transgenic rice plants. *J. Pineal Res.* **63**: e12412.

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