

Article

Effect of Melatonin on Germination and Seedling Growth in Aging Seeds or under Drought Conditions

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Abstract: Seed germination (GS) and seedling growth are vital plant stages that can be affected by stresses such as drought and aging, which cause deterioration and reduce seed viability. With the aim of homogenizing and improving GS, priming treatments with biostimulants such as the antioxidant melatonin are commonly used in seeds. In this study, the effects of melatonin on germination and seedling growth in two different situations, i.e., aging seeds of rice, barley, and sorghum and under polyethylene glycol (PEG)-induced drought stress in sorghum, were studied. Aged seeds were primed for 7 days in different concentrations of melatonin, and drought stress seeds were primed for 24 h before PEG treatment for 7 days, and germination and initial growth parameters were monitored. Aging-seeds of rice and barley showed the maximum response in terms of germination percentage at 20 μ M melatonin and 0.05 μ M respectively; while aging-seeds of sorghum showed improvement in germination for practically all concentrations studied, even the highest tested at 50 μ M. Regarding the effect of melatonin treatments on drought stress in sorghum seeds, all the studied parameters showed a significant attenuation of the adverse effects of drought stress, alleviating them, for all concentrations tested but especially at 200 μ M melatonin. The results obtained confirm that priming seeds with melatonin under low germinability conditions relieves stress and improves both germination and seedling growth.

Keywords: melatonin; seeds; germination; abiotic stress; aging; drought; seedling growth



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1. Introduction

Seeds are the units of sexual reproduction of plants, and their function is to propagate, perpetuate, and disperse the species to which they belong. Seed germination (SG) is a vital stage in plant development and can be considered as a determinant for plant productivity [1]. The major food seeds are those of cereals and legumes; their annual world production is approaching 3 billion tons [2]. SG refers to the physiological process culminating in the emergence of the embryo from its enclosing coverings [3]. It begins with water imbibition, the mobilization of food reserve, protein synthesis, and radicle protrusion. Physiological and biochemical changes followed by morphological changes during germination are strongly related to seedling survival rate and vegetative growth, which affect yield and quality [4].

SG and early seedling growth are two critical stages particularly vulnerable to environmental stress conditions, particularly water stress condition [1]. Drought is a severe limitation for plant growth, development, and productivity, and the response characteristics of plants exposed to drought stress have become a crucial environmental research topic [5]. Drought stress reduces imbibition and increases osmotic potential of growth medium, thus reducing germination percent and seedling vigor [6]. Polyethylene glycol (PEG) with a molecular weight of 6000 Da, is a natural water-soluble and non-ionic polymer [7], which

it is generally used to induce drought stress in studies with higher plants [8]. The use of PEG6000 will increase in the osmotic potential of the growth medium, reducing the imbibition capacity by seeds.

Many seeds are capable of surviving dehydration at maturity, and in this state, they can survive for long periods (up to hundreds of years in some cases) and resume growth when rehydrated. However, deteriorative chemical processes continue in dry seeds, resulting in their gradual loss of vigor and eventual death [3]. Viability is a measure of the percentage of seeds with the capacity to germinate and generate seedlings under favorable environmental conditions. The aging of a seed occurs when the viability of a seed in a state of dormancy for long periods of time is affected by deterioration, which will cause a delay in its germination or even its total cessation and/or the reduction in growth seedling rates [9]. Aging involves the impairment of metabolism during germination, with the reduction in the mitochondrial respiratory activity, the production of toxic substances such as oxidized free fatty acids, the reduction in some enzymatic activities, and the loss of soluble compounds due to the abnormal permeability of cell membranes [10].

A good imbibition phase followed by a progressive germination rate, including a rapid and uniform radicle emergence, will improve crop production. It has been observed that the differences in these stages are generally not recovered, directly affecting crop yield [11]. To increase the yield of commercial seed lots, the SG rate can be induced by priming the seeds in different media or immersing them for short times in solutions containing some biostimulant compounds such as phytohormones or others, with the aim of homogenizing and improving SG. Priming technique can promotes quick and uniform germination, improves seedling responses, ensures successful seedling establishment, leading to better plant growth [12]. Seed priming can also enhance the germination of weak, damaged, or aged seeds, even under unfavorable environmental conditions [13].

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine derived from tryptophan that was first detected in the bovine pineal gland in 1958 [14]. A few years later, in 1995, it was identified in several plant species using radioimmunoassay and HPLC-MS [15–17]. Melatonin contents in plants are highly variable, ranging from picograms to micrograms per gram of plant material analyzed. Phytomelatonin (plant melatonin) has been detected in a wide variety of plants, from edible to wildtypes, and in all tissues and organs such as seeds, stems, leaves, roots, flowers and fruits [18,19].

This molecule with a molecular weight of 232.2 Da and a chemical formula of C₁₃H₁₆N₂O₂ is being considered as a new plant hormone, due to its involvement in multiple physiological functions of plant development [20–22]. The identification of the first melatonin receptor (PMTR1/CAND2, a receptor coupled to a G protein) in *Arabidopsis thaliana* was a major breakthrough [23].

At the cellular level, melatonin biosynthesis is located mainly in chloroplasts since they contain the main enzyme responsible for its synthesis, serotonin N-acetyltransferase, which was identified in rice chloroplasts [24] and later in red algae [25]. Other enzymes that participate in the biosynthesis pathway are located in the endoplasmic reticulum, in the cytoplasm and occasionally in the mitochondria [26,27]. In total, five enzymes are involved in its biosynthesis: tryptophan decarboxylase (TDC), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), acetylserotonin methyltransferase (ASMT), and caffeic acid O-methyltransferase (COMT) [28]. These routes are widely studied in *Arabidopsis thaliana* and rice, although some aspects of serotonin biosynthesis still need to be elucidated [29–31]. The biosynthesis route is very similar in both plants and animals, except in certain steps, such as the hydroxylation of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase (TPH), since this enzyme has not yet been identified in plants, but its substrate and product are present in some plant species [30,32].

Due to the diversity of actions of melatonin described by several authors [20,33,34] either affecting plants in different processes such as germination, rooting, growth, photosynthesis efficiency, parthenocarpy, fruiting, ripening, and senescence, as well as related actions or factors such as stomatal conductance, intercellular CO₂, Rubisco activity, photosystem

efficiency, etc., phytomelatonin has been proposed as a master regulator in plants [21]. This denomination is also due to the wide characterization of melatonin as a regulator of gene expression and its relationship with other phytohormones such as auxin, cytokinins, gibberellins, abscisic acid, jasmonic acid, salicylic acid, and ethylene [35,36].

Phytomelatonin contents can be regulated intrinsically (e.g., circadian clock, phytohormones) and extrinsically by biotic and abiotic stressors through several second messengers such as reactive oxygen and nitrogen species (ROS and RNS), gasotransmitter signals (nitric oxide and hydrogen sulfide), and crosstalking with other plant hormones [35,36].

Another characteristic role of melatonin is its function as an antioxidant. This antioxidant capacity has been confirmed in both plant and animal studies. Thus, it has been described that, in plants treated with melatonin and subjected to cadmium stress [37], it indirectly enhances the expression of several antioxidant enzymes, including peroxidases, catalases, and superoxide dismutases [21], and the stress caused by ROS and nitric oxide is regulated [38]. In addition, it has also been seen that melatonin is capable of protecting plant cells against biotic stress caused by pathogens such as fungi, nematodes, bacteria, and viruses [28,39].

Seed priming can improve SG under stress conditions compared to unprimed seeds, as it achieves a rapid and uniform germination, leading to successful plant establishment. The germination-promoting activity of melatonin was demonstrated in early studies on red cabbage and cucumber [40,41]. In this study, we investigated the optimal concentration of melatonin to mitigate the adverse effect on germination and seedling growth in seeds, under two conditions: aging-seeds (in rice, (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) and sorghum (*Sorghum* spp.)) or drought stress induced by polyethylene glycol (PEG) (in sorghum), determining the adaptative response in the presence of melatonin.

2. Materials and Methods

2.1. Plant and Chemical Materials

Sorghum, rice, and barley seeds used for bioassay I were obtained from a local specialized store in edible seeds (Murcia, Spain) in 2018 and stored at 4 °C for 5 years. For bioassay II, sorghum seeds were purchased from the same local store in 2023.

All seeds used in the different bioassays were classified according to their size and healthy appearance, discarding damaged or small seeds [42].

In all studies, the seeds were sterilized with 10% sodium hypochlorite for 10 min and then washed 3 times with plenty of distilled water to remove traces of the disinfectant.

Melatonin (*N*-acetyl-5-methoxytryptamine) and polyethylene glycol (PEG) were obtained from Sigma-Aldrich (Madrid, Spain). The different melatonin solutions were prepared in distilled water from an initial 10^{-2} M ethanol solution. The drought study was carried out with a PEG6000 solution prepared in distilled water at a concentration of 18% (*w/v*), which generated an osmotic potential of $\psi = -0.4$ MPa.

2.2. Study of the Effect of Melatonin Treatment on Aging Seeds

Bioassay I: The study was carried out on the following seeds: rice, barley, and sorghum. A total of 20 seeds previously sterilized were placed per Petri dishes containing two filter paper disks. For priming, 20 mL of distilled water (control) or different concentrations of melatonin (0.05, 0.1, 0.2, 1, 20, 50 μ M) was added to each plate (this range of melatonin concentrations was selected by us in previous assays). The plates were placed in a culture chamber with controlled temperature at 25 ± 2 °C and a photoperiod of 16-h light/8-h dark. Each treatment (consisting of 3 petri dishes of 20 seeds each) was repeated 3 times.

2.3. Study of the Effect of Melatonin Treatment on Sorghum Seeds Subjected to Drought Stress

Bioassay II: The drought stress was imposed artificially using polyethylene glycol (PEG6000). Sorghum seeds that were previously sterilized were dipped in different melatonin concentrations, 50, 100, 200, and 300 μ M, with distilled water as the control, for 24 h,

at 25 ± 2 °C in the dark. After the incubation time, 20 seeds of each treatment were placed in Petri dishes with 2 filter paper discs containing 20 mL of a solution of PEG 18% p/v (-0.4 MPa) [43]. Two types of controls were utilized: seeds not treated with melatonin were sown in water without PEG as control (CK) or sown in PEG 18% (stress control).

The plates were placed in a culture chamber with a controlled temperature of 25 ± 2 °C and a photoperiod of 16 h light/8 h dark. At least three replicates were performed per treatment, and the bioassay was repeated 3 times.

2.4. Determination of Parameters and Indices of Germination

The number of germinated seeds was recorded daily from day 4 to 7 after sowing, for each treatment and repetition. Seed germination was considered to have occurred when the seed coat was broken and a radicle was visible ≥ 0.2 cm. Different indices, germination (G) and germination potential (GP), were calculated according to the formula of [44], relative seed germination (RSG), relative root length (RRL), and germination index (GI) according to the formula of [45], and vigor index (VI) according to [46,47], and by applying following equations:

$$G(\%) = \frac{\text{Number of germinated seeds}}{\text{Number of seeds kept for germination}} \times 100 \quad (1)$$

$$GP(\%) = \frac{\text{Number of germinated seeds on the 4rd day}}{\text{Number of seeds kept for germination}} \times 100 \quad (2)$$

$$RSG(\%) = \frac{\text{number of seeds germinated per treatment}}{\text{number of seeds germinated in control}} \times 100 \quad (3)$$

$$RRL(\%) = \frac{\text{Mean root length per treatment}}{\text{Mean root length in control}} \times 100 \quad (4)$$

$$GI(\%) = \frac{GRS \times RRG}{100} \quad (5)$$

$$VI = \frac{\text{seedling (radicles + plumules) length} \times G(\%)}{100} \quad (6)$$

2.5. Determination of Seedling Growth

The shoot length of the seedlings was measured, from day 4 to 7 after sowing, for each of the treatments and repetitions. The length was noted from the neck region to the apical end of the leaf. The root length of the seedlings was measured, from day 4 to 7 after sowing, for each of the treatments and repetitions. The length was noted from the base of the stem to the apical end of the main root. On day 7, the dry weight of the seeds that had germinated was determined for each treatment and repetition. For this purpose, seedlings were placed in the oven at 60 °C for 48 h to obtain a constant dry weight, and this weight was recorded on an analytical balance and expressed in gram per plant.

2.6. Stress Tolerance Index (STI)

The stress tolerance index is a useful tool to determine the high yield and stress tolerance potential of seeds assessed in bioassay II (drought stress). Stress tolerance indices for root and shoot growth were estimated on the 7th day. The stress tolerance index in shoot (sSTI) and stress root tolerance index (rSTI) in root were calculated using the following equations [48].

$$sSTI(\%) = \frac{\text{Shoot length of stress plant}}{\text{Shoot length of control plant}} \times 100 \quad (7)$$

$$rSTI(\%) = \frac{\text{Root length of stress plant}}{\text{Root length of control plant}} \times 100 \quad (8)$$

2.7. Statistical Analyses

The obtained experimental data were statistically analyzed using the IBM SPSS Statistics 22.0 program (International Business Machines Corporation (IBM), New York United States, <https://www.ibm.com/support/pages/spss-statistics-220-available-download> (accessed on 30 June 2024)). An ANOVA test and a post hoc test with a Tukey “honestly significant difference” (HSD) test were applied for treatment comparisons, with $p < 0.05$, to detect differences between means.

3. Results

3.1. Bioassay I

3.1.1. Germination Study of the Effect of Melatonin Treatment on Aging Seeds

We performed germination bioassays with different concentrations of melatonin (0–50 μM) to determine how it affects the germination of aging seeds. The seeds began to germinate on the third day of incubation at 25 °C, and we recorded the result of the daily germination percentage for each treatment from day 4 to 7. Figure 1 illustrates the germination speed of each treatment for the species tested in bioassay I (rice, barley, and sorghum). The different calculated parameters are shown in Table 1, Table 2, and Table 3, respectively.

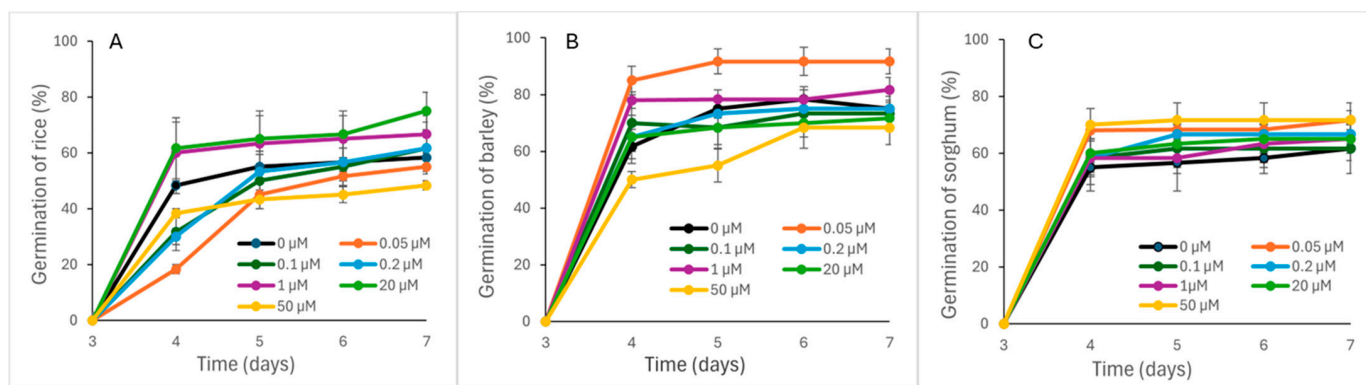


Figure 1. Bioassay I: germination percentage of aging seeds of rice (A), barley (B), and sorghum (C) primed with different concentrations of melatonin from day 4 to day 7. Vertical bars represent data means $\pm$ SE ($n = 3$).

Table 1. Effect of different melatonin treatments on the germination and growth of aging rice seeds.

Rice Seeds						
Treatments (μM)	Germination (%)	Germination Potential (%)	Relative Seed Germination (%)	Relative Root Length (%)	Germination Index	Vigor Index
0	58.3 $\pm$ 5.0 ^{b,*}	48.3 $\pm$ 9.3 ^c	100.0 $\pm$ 4.1 ^b	100.0 $\pm$ 21.9 ^b	104.7 $\pm$ 25.8 ^b	1.9 $\pm$ 0.5 ^a
0.05	55.0 $\pm$ 0.1 ^b	18.3 $\pm$ 1.7 ^a	101.6 $\pm$ 0.1 ^{bc}	69.3 $\pm$ 11.8 ^a	70.3 $\pm$ 12.0 ^a	1.2 $\pm$ 0.2 ^a
0.1	61.7 $\pm$ 9.3 ^c	31.7 $\pm$ 6.7 ^b	113.9 $\pm$ 17.1 ^c	69.7 $\pm$ 12.0 ^a	83.4 $\pm$ 27.0 ^{bc}	1.4 $\pm$ 0.4 ^a
0.2	61.7 $\pm$ 6.0 ^c	30.0 $\pm$ 2.9 ^b	114.6 $\pm$ 11.3 ^c	70.6 $\pm$ 12.5 ^a	81.7 $\pm$ 18.5 ^{bc}	1.4 $\pm$ 0.2 ^a
1	66.7 $\pm$ 4.4 ^{cd}	60.0 $\pm$ 2.5 ^d	111.4 $\pm$ 8.6 ^{bc}	154.7 $\pm$ 10.2 ^c	172.5 $\pm$ 18.1 ^c	3.1 $\pm$ 0.4 ^b
20	68.3 $\pm$ 6.7 ^d	61.7 $\pm$ 2.5 ^d	114 $\pm$ 14.3 ^c	155.8 $\pm$ 9.1 ^c	180.4 $\pm$ 30.9 ^c	3.8 $\pm$ 0.3 ^b
50	48.3 $\pm$ 1.7 ^a	38.3 $\pm$ 1.7 ^b	77.1 $\pm$ 4.9 ^a	88.0 $\pm$ 4.5 ^b	68.3 $\pm$ 7.4 ^a	1.5 $\pm$ 0.1 ^a

* Values are mean $\pm$ standard error (SE, $n = 3$). Different letters indicate significant differences using the Tukey HSD test ($p < 0.05$).

Table 2. Effect of different melatonin treatments on the germination and growth of aging barley seeds.

Barley						
Treatments (μM)	Germination (%)	Germination Potential (%)	Relative Seed Germination (%)	Relative Root Length (%)	Germination Index	Vigor Index
0	75.0 ± 0.1 ^{b,*}	61.7 ± 6.0 ^b	100.0 ± 0.1 ^b	100.0 ± 4.3 ^d	100.0 ± 4.3 ^d	5.2 ± 0.1 ^c
0.05	91.7 ± 4.4 ^d	85.0 ± 5.0 ^d	122.2 ± 5.9 ^d	82.7 ± 2.4 ^c	101.0 ± 4.8 ^d	6.1 ± 0.3 ^d
0.1	73.3 ± 6.0 ^{ab}	70.0 ± 8.7 ^c	97.8 ± 8.0 ^{ab}	87.2 ± 0.5 ^d	85.2 ± 6.4 ^c	4.9 ± 0.3 ^c
0.2	75.0 ± 2.9 ^b	65.0 ± 5.0 ^{bc}	100.0 ± 3.8 ^b	90.0 ± 0.9 ^d	90.1 ± 4.4 ^c	5.0 ± 0.2 ^c
1	81.7 ± 4.4 ^c	80.0 ± 2.5 ^d	108.9 ± 5.9 ^c	79.4 ± 1.0 ^c	86.4 ± 4.3 ^c	4.8 ± 0.4 ^c
20	71.7 ± 4.4 ^{ab}	65.0 ± 12.5 ^{bc}	96.7 ± 10.0 ^{ab}	73.3 ± 4.8 ^b	70.0 ± 5.7 ^b	4.0 ± 0.5 ^b
50	68.3 ± 6.0 ^a	51.7 ± 3.3 ^a	91.1 ± 8.0 ^a	54.0 ± 3.4 ^a	49.3 ± 5.7 ^a	2.5 ± 0.3 ^a

* Values are mean ± standard error (SE, *n* = 3). Different letters indicate significant differences using the Tukey HSD test (*p* < 0.05).

Table 3. Effect of different melatonin treatments on the germination and growth of aging sorghum seeds.

Sorghum						
Treatments (μM)	Germination (%)	Germination Potential (%)	Relative Seed Germination (%)	Relative Root Length (%)	Germination Index	Vigor Index
0	61.7 ± 1.7 ^{a,*}	62.5 ± 2.5 ^a	100.0 ± 3.6 ^a	100.0 ± 11.1 ^a	100.5 ± 14.2 ^a	2.0 ± 0.1 ^a
0.05	71.7 ± 3.3 ^b	70.0 ± 2.9 ^b	116.2 ± 5.4 ^b	118.7 ± 15.2 ^a	139.3 ± 22.7 ^b	2.7 ± 0.5 ^b
0.1	61.7 ± 8.8 ^a	58.3 ± 9.3 ^a	100.0 ± 14.3 ^a	181.5 ± 8.0 ^b	179.4 ± 19.3 ^c	3.1 ± 0.3 ^{bc}
0.2	66.7 ± 3.3 ^{ab}	58.3 ± 6.7 ^a	108.1 ± 5.4 ^{ab}	186.4 ± 4.3 ^b	201.1 ± 6.2 ^c	3.4 ± 0.2 ^c
1	65.0 ± 7.6 ^{ab}	58.3 ± 11.7 ^a	105.4 ± 12.4 ^{ab}	178.9 ± 38.9 ^b	197.2 ± 62.7 ^c	4.1 ± 0.9 ^d
20	65.0 ± 5.0 ^{ab}	60.0 ± 12.5 ^a	101.4 ± 12.2 ^{ab}	103.9 ± 0.3 ^a	109.6 ± 8.6 ^{ab}	2.5 ± 0.4 ^{ab}
50	71.7 ± 6.0 ^b	70.0 ± 5.8 ^b	116.2 ± 9.7 ^b	105.1 ± 8.0 ^a	123.5 ± 18.1 ^{ab}	2.8 ± 0.4 ^b

* Values are mean ± standard error (SE, *n* = 3). Different letters indicate significant differences using the Tukey HSD test (*p* < 0.05).

The evolution of the germination percentage from the beginning of data collection, sampling from day 4 to day 7 from sowing is shown in Figure 1. The final germination data of day 7 for each tested seed type (rice, barley, and sorghum) are shown in Table 1, Table 2, and Table 3, respectively. In general, it is observed that the maximum germination rate occurs on day 4 for all treatments and seeds, and from this day, the germination rate slows down and remains the same. This behavior is especially appreciated in sorghum, while in rice and barley, some variation is observed between days 4 and 5.

In aging rice seeds, treatments containing 0.1, 0.2, 1, and 20 μM of melatonin induced an increase in germination percentage (G) compared to the control, with significant differences according to Tukey's post hoc test (*p* < 0.05) (Table 1). The 20 μM melatonin treatment was the most effective, with an increase in the germination percentage of 10.0% compared to the control (Table 1). It should be noted that the maximum tested concentration of 50 μM melatonin had a deleterious effect, with a G reduction of 10.0% compared to the control, and this behavior is also observed in the values obtained for GI and RSG, i.e., the parameters that inform about the behavior of the treated seeds in relation to the control (Table 1). According to Table 1, 20 μM melatonin treatment was, of all the tested concentrations, the one that shows an improved value in all the calculated parameters compared to the control; thus, it can be concluded that, in aging rice seeds, treatment with 20 μM melatonin represents a significant improvement in germination.

In aging barley seeds, 0.05 and 1 μM melatonin treatments caused a significant increase in the germination percentage (G) compared to control (Figure 1), but of these two, 0.05 μM melatonin was clearly more effective, with an increase compared to the control for G of 16.74% compared to the increase of 6.7% caused by 1 μM melatonin (Table 2). Furthermore, 0.05 μM melatonin shows a significant improvement, not showed by melatonin 1 μM , for most of the calculated parameters, with 0.05 μM melatonin being the only one that improve the vigor index (VI) with respect to the control. In barley seeds, as in rice seeds, the maximum tested concentration (50 μM) most affects germination (G) compared to the control, reducing it to 68.3 (−6.7%). This effect is even more deleterious than in rice, since all the calculated parameters are affected and exhibit decreased values for all the tested concentrations. It can be concluded that the concentration of melatonin capable of improving germination in barley is the lowest tested concentration (0.05 μM).

In sorghum, as shown in Table 3, and unlike what happens with the other two species of studied aging seeds (rice and barley, Tables 1 and 2, respectively), at all tested melatonin concentrations (except 0.1 μM) and for calculated parameters, an improvement is observed with respect to the control, resulting in higher (0.05 and 50 μM melatonin) or at least equal values (without significant difference with the control, 0.2, 1, and 20 μM) for G, GP, SRG RRL, IG, and VI. It should be noted that the concentration of melatonin with the highest VI (parameter that combines the values of the germination percentage with the growth of the seedling) is 1 μM followed by 0.2 μM , which induce a VI that is 2.1 and 1.7 times higher the control, respectively. It can be concluded that, in sorghum seeds, the melatonin concentration capable of further improving germination was the lowest tested concentration (0.05 μM). Notably, the highest tested melatonin concentration (50 μM) reduces G for both aging rice and barley, unlike the response of aging sorghum where G clearly improves with 50 μM melatonin.

3.1.2. Effect of Melatonin Treatment on Seedling Growth

In rice, in relation to shoot and root growth (Figure 2A,D), the results revealed statistically significant improvements in 1 and 20 μM melatonin treatments compared to the control. Rice seeds treated with 1 μM melatonin showed the greatest effect in relation to stem elongation, reaching a value of 1.86 cm, 45.31% higher than that in the control with a value of 1.28 cm (Figure 2A). For root elongation, 20 μM melatonin treatment showed the greatest effect, reaching a value of 3.13 cm, 51.94% higher than that for the control (Figure 2D).

In barley, 0.05 and 0.1 μM melatonin treatments induced shoot length with respect to the control. Among these treatments, 0.05 μM melatonin showed the greatest increase in shoot length, reaching a value of 3.86 cm, which represents an increase of 10% compared to the control. A 0.2–50 μM melatonin interval provoked some decrease in shoot growth, which increases as the tested concentration of melatonin increases (Figure 2B). In relation to the root growth of barley, a decrease in root length is observed at all tested concentrations of melatonin, with this effect being greater in the highest concentrations, i.e., 20 and 50 μM melatonin (Figure 2E).

In sorghum, a significant increase in shoot growth compared to the control was observed for all tested melatonin treatments except for the 0.05 μM melatonin (Figure 2C). The maximum shoot length-promoting effect was observed at 1 μM melatonin, with a height of 2.59 cm, 112% higher than that of the control; higher melatonin treatments provoked lower length responses in sorghum shoots (Figure 2C). In relation to the roots, a significant increase in root length was observed compared to the control for the 0.1, 0.2, and 1 μM melatonin treatments, showing a typical Gaussian bell response, with a maximum response at 0.1 μM melatonin, with a value of 4.36 cm, which represents an increase of 122% compared to the control (Figure 2F).

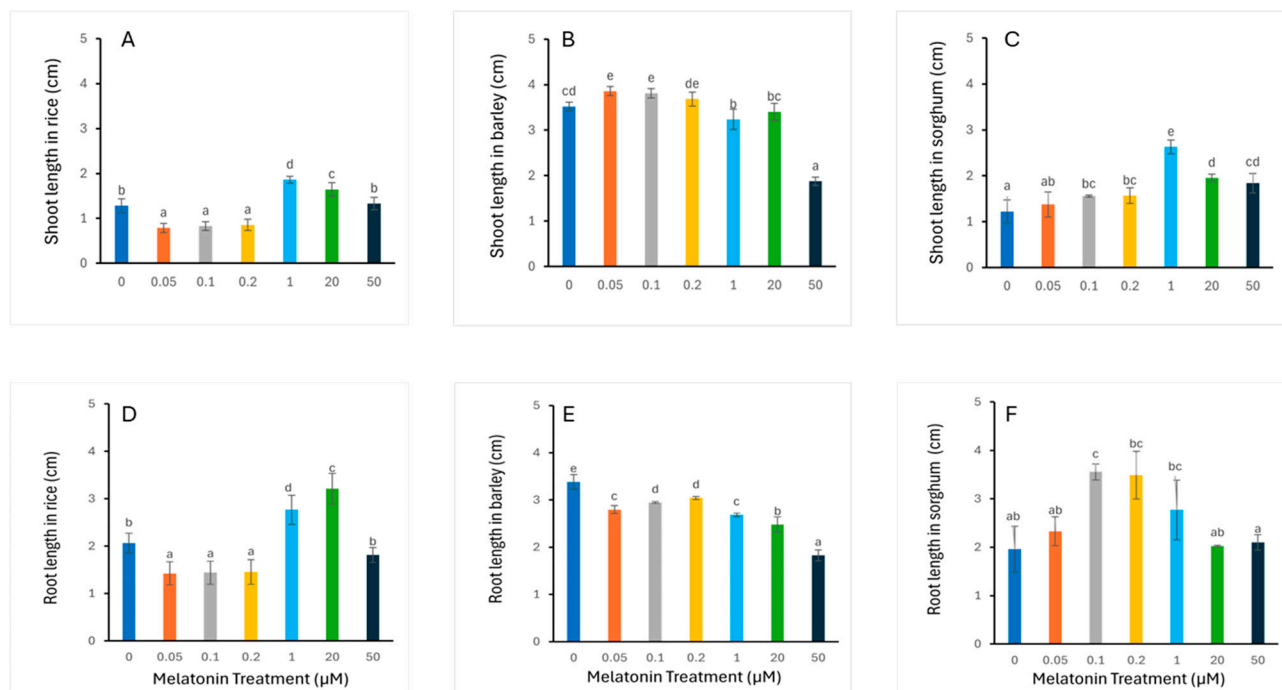


Figure 2. Bioassay I: effect of melatonin treatment on shoot and root growth in aging seeds of rice (A,D), barley (B,E), and sorghum (C,F) at 7 days. Different letters indicate significant differences based on a Tukey HSD test with a significance level of $p < 0.05$. Vertical bars represent data means $\pm$ SE ($n = 3$).

In relation to the effect of melatonin treatments on dry weight seedlings (Figure 3), a similar behavior is observed in rice and sorghum, with a very notable increased value at 0.2 μ M melatonin compared to the control. Thus, the dry weight of sorghum seedlings increased by approximately 4-fold while that of rice seedlings increased by 1.5-fold compared to the control. On the other hand, barley does not show improvement with any of the tested melatonin treatment.

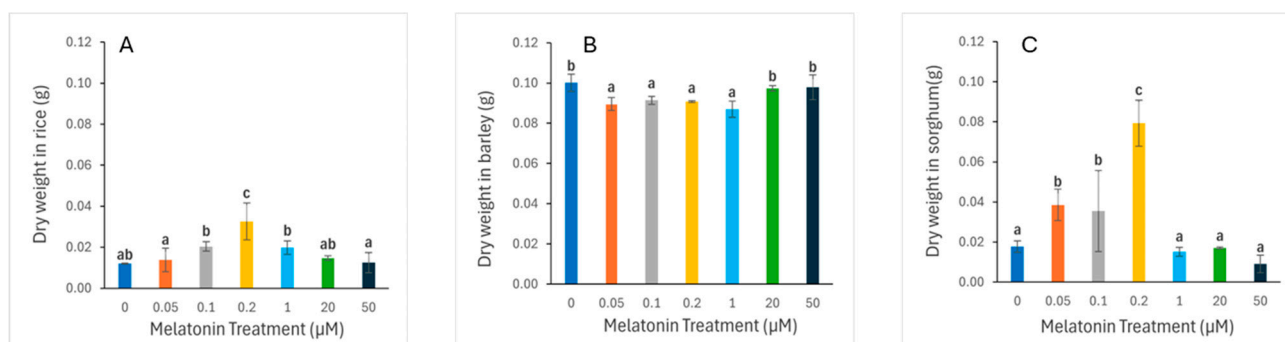


Figure 3. Bioassay I: dry weight of seedlings from melatonin-treated aging seeds of rice (A), barley (B), and sorghum (C) at day 7 of sowing. Different letters indicate significant differences based on a Tukey HSD test with a significance level of $p < 0.05$. Vertical bars represent data means $\pm$ SE ($n = 3$).

3.2. Bioassay II

3.2.1. Germination Study of the Effect of Melatonin in Drought Stress Conditions

Sorghum seeds (non-aging) subjected to drought stress induced by 18% PEG show a decrease in the germination percentage of 15.3% compared to the control (sown in water), which denotes the effectiveness of PEG to provoke drought stress in seeds (Table 4). Regarding the studied germination parameters in melatonin-primed seeds in the presence of PEG, a general improvement in their germination can be observed for all tested melatonin

concentrations (50, 100, 200, and 300 μM). It should be noted that 200 μM melatonin is even capable of increasing germination (G) by 8.4% more than that in the control. The behavior observed with the different concentrations of melatonin is similar to the Gaussian bell that is observed in the classic growth test of coleoptiles incubated with the auxin indole 3-acetic acid, where both low and high concentrations cause a weak effect either due to either lack or excess of phytohormone (probably due to saturation that causes toxicity). This same behavior was observed for all calculated parameters GP, RSG, GI, and VI. It should be noted that the VI has a similar value for 100 and 200 μM melatonin treatments, being approximately 2.5-times higher in the PEG control, not exceeding in any case the value of the control (CK), although as it has been mentioned above, the germination induced by 200 μM melatonin is greater than that of CK. This is because VI is a parameter that encompasses germination and plant growth, and the growth under drought stress conditions is affected by the low availability of water, which plays an important role in cell elongation. Thus, PEG-induced drought stress effectively inhibits sorghum seed germination, with 200 μM melatonin being an improvement treatment alleviating the drought stress.

Table 4. Germination parameters in sorghum drought stress seeds primed with different melatonin concentrations.

Sorghum + PEG 18%						
Treatments (μM)	Germination (%)	Germination Potential (%)	Relative Seed Germination (%)	Relative Root Length (%)	Germination Index	Vigor Index
CK	68.3 $\pm$ 10.1 ^{bc,*}	68.3 $\pm$ 10.1 ^{bc}	100.0 $\pm$ 14.8 ^{bc}	100.2 $\pm$ 2.8 ^e	100.2 $\pm$ 15.5 ^e	1.7 $\pm$ 0.4 ^d
P	53.3 $\pm$ 10.9 ^a	53.3 $\pm$ 10.9 ^a	77.9 $\pm$ 16.0 ^a	37.6 $\pm$ 3.9 ^a	28.1 $\pm$ 2.9 ^a	0.4 $\pm$ 0.1 ^a
PM50	66.7 $\pm$ 4.4 ^b	66.7 $\pm$ 4.4 ^b	97.3 $\pm$ 6.4 ^{bc}	61.0 $\pm$ 5.7 ^c	58.7 $\pm$ 2.0 ^c	0.8 $\pm$ 0.1 ^{bc}
PM100	73.3 $\pm$ 6.0 ^{bc}	73.3 $\pm$ 6.0 ^{bc}	104.1 $\pm$ 10.0 ^{bc}	61.3 $\pm$ 6.2 ^c	65.0 $\pm$ 13.0 ^c	0.9 $\pm$ 0.2 ^c
PM200	76.7 $\pm$ 6.0 ^c	76.7 $\pm$ 7.5 ^c	111.9 $\pm$ 8.8 ^c	70.7 $\pm$ 4.2 ^d	79.1 $\pm$ 8.2 ^d	1.0 $\pm$ 0.1 ^c
PM300	65.0 $\pm$ 2.9 ^b	65.0 $\pm$ 2.9 ^b	94.9 $\pm$ 7.3 ^b	48.4 $\pm$ 6.1 ^b	45.6 $\pm$ 5.3 ^b	0.5 $\pm$ 0.1 ^{ab}

* Values are mean $\pm$ standard error (SE, $n = 3$). Different letters indicate significant differences using the Tukey HSD test ($p < 0.05$). CK, control absolute; P, PEG stress without melatonin; PM50, PEG stress with 50 μM melatonin; PM100, PEG stress with 100 μM melatonin; PM200, PEG stress with 200 μM melatonin; and PM300, PEG stress with 300 μM melatonin.

3.2.2. Melatonin Effect on Sorghum Seedling Growth in Drought Stress Conditions

Sorghum shoot length decreased significantly in the presence of PEG compared to the control seedlings (Figure 4A). A slight improvement in the shoot growth of the stressed seeds is observed for all the melatonin treatments, except for the one with the highest concentration (300 μM melatonin). In the case of the effect on root growth, PEG-stressed seeds showed a drastic decrease (more than double) than control seeds (without PEG), with values of 0.53 cm (P0 in Figure 4B) and 1.42 cm (CK in Figure 4B), respectively. As in sorghum shoots, a general improvement was observed when seeds were primed with melatonin; the melatonin-induced effect on root growth was stronger, showing all PEG-stressed seeds primed with melatonin (50, 100, 200, and 300 μM), an increased growth response compared to the PEG control.

The results of shoot and root growth were reflected in the dry weight values of seedling as shown in Figure 4C, where it is observed that the lowest dry weight value is obtained for the control with PEG, with better values for the seedlings treated with melatonin. In any case, shoot, root, and dry weight reached the respective values presented by the non-stressed seeds (Figure 4).

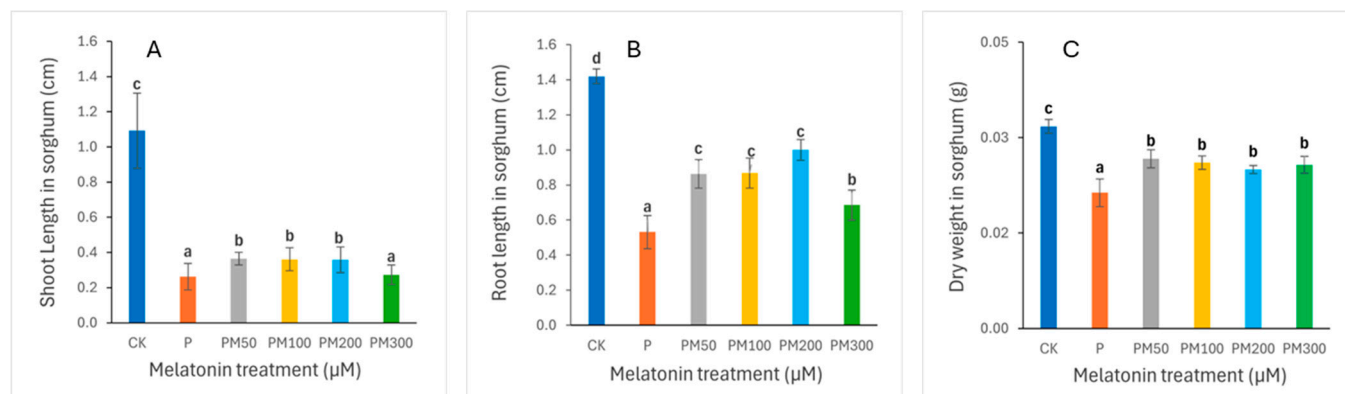


Figure 4. Bioassay II: effects of melatonin treatment on the seedling growth of sorghum under drought stress induced by PEG 18%: (A) shoot length, (B) root length, and (C) dry weight. CK, control; P, PEG stress without melatonin; PM50, PEG stress with 50 μM melatonin; PM100, PEG 18% stress with 100 μM melatonin; PM200, PEG stress with 200 μM melatonin; and PM300, PEG stress with 300 μM melatonin. Different letters indicate significant differences based on a Tukey HSD test with a significance level of $p < 0.05$. Vertical bars represent data means $\pm$ SE ($n = 3$).

3.2.3. Stress Tolerance Index (STI) in Melatonin-Primed Sorghum Seeds

The tolerance to drought stress of seeds treated with melatonin was improved compared to the untreated seeds in both roots and shoots of seedlings (Table 5). It was observed that stress tolerance increased by 13% for 50, 100, and 200 μM of melatonin for the shoots. The effect of increasing tolerance is stronger in the roots than that in the shoots, and the highest improvement was observed at 200 μM melatonin, presenting an increase in tolerance of 33.1% compared to the control. The rest of the applied melatonin concentrations also improve tolerance, with the highest used concentration of melatonin (300 μM) having a minor effect.

Table 5. Results of PEG-induced drought stress tolerance indices for primed sorghum seeds with different melatonin concentrations.

Sorghum +PEG 18%		
Treatments (μM)	Stress Tolerance Index in Shoot (%)	Stress Tolerance Index in Root (%)
CK	100.0 $\pm$ 19.0 ^{b,*}	100.0 $\pm$ 2.9 ^e
P	20.6 $\pm$ 3.2 ^a	37.6 $\pm$ 3.9 ^a
PM50	33.6 $\pm$ 3.4 ^a	61.0 $\pm$ 6.2 ^c
PM100	33.0 $\pm$ 5.5 ^a	61.3 $\pm$ 5.7 ^c
PM200	32.7 $\pm$ 6.7 ^a	70.7 $\pm$ 4.2 ^d
PM300	24.2 $\pm$ 4.0 ^a	48.4 $\pm$ 6.1 ^b

* Values are mean $\pm$ standard error (SE, $n = 3$). Different letters indicate significant differences using the Tukey HSD test ($p < 0.05$). CK, control; P, PEG stress without melatonin; PM50, PEG stress with 50 μM melatonin; PM100, PEG stress with 100 μM melatonin; PM200, PEG stress with 200 μM melatonin; and PM300, PEG stress with 300 μM melatonin.

In summary, treatments with melatonin through the priming of seeds help to attenuate the effect of drought stress on seedlings, with roots being the organ that responds best to the presence of melatonin.

4. Discussion

The results obtained in this study show that melatonin is a promising agent for improving SG and growth of seedlings, under both studied conditions, i.e., aging seeds and drought-stressed seeds. In general, the effect of melatonin seem to be conditioned by the concentration used [49,50], as it has been demonstrated in previous studies that high concentrations of melatonin inhibited or did not exert a stimulating effect on germination,

while low concentrations exerted a stimulating effect [51–55]. Here, a similar behavior is observed with aging seeds of barley and rice, where the maximum effect on the germination percentage is obtained with a melatonin concentration of 0.05 μM in barley and 20 μM in rice (16.7 and 10% more germination than the control, respectively), and it was inhibited in both species at 50 μM melatonin, the highest tested concentration (6.7 and 10% less germination than control, respectively). While aging seeds of sorghum show an improvement in germination for practically all studied concentrations, even the highest tested concentration of 50 μM .

In relation to the effect of melatonin on the elongation of shoots and roots, it was observed that the response of both organs was different and dependent on the concentration of melatonin used, influencing the sensitivity of the organ. This behavior is already well known in the case of auxin, where roots are much more sensitive to auxin than stems or leaves. The influence of melatonin on plant growth was first described by Arnao and colleagues [50,56]. Our study found that melatonin, similar to IAA, promoted vegetative growth in the etiolated hypocotyls of *Lupinus albus* L. and the etiolated coleoptiles of some monocotyledons (canary seed, wheat, barley, and oat) and significantly inhibited root growth. In the current study, melatonin also inhibited the root growth of barley compared to that of the control. However, in sorghum, melatonin promoted the growth of both roots and shoots, with roots showing greater sensitivity compared to shoots, which requires a ten-fold higher concentration. In rice seedlings, melatonin promoted both root and shoot growth at similar concentrations.

In other studies of wild mustard seedlings [57], at low melatonin concentrations, the biosynthesis of IAA was stimulated, and, on the contrary, when melatonin concentration was increased, then, it exerted an inhibitory effect on IAA. This result coincides with that of other recent studies, in which the cause of this behavior is revealed: the higher concentrations of auxin promote cell expansion in shoots but inhibit cell expansion in roots. Auxin is involved in the roots in the activation of two signaling pathways that act antagonistically, i.e., (1) a transmembrane kinase1 (TMK1) pathway and (2) the transport inhibitor response1 and auxin-signaling f-box (TIR1/AFB) pathway, where the first is based on the acidification of the apoplast, which facilitates cell expansion, and the second on its alkalization [58]. Furthermore, the growth mechanisms in melatonin-treated seedlings remain uncertain. In previous studies, the ability of melatonin to positively affect the development of the shoots and radicles was studied by the reduction in intercellular pH and the relaxation of the cell wall induced by melatonin, as could be inferred from the elongation and expansion of the cell wall in lupin [59].

Therefore, there is a relationship between IAA and melatonin, which is associated with the fact that both hormones use the same precursor, L-tryptophan. Furthermore, both compounds mutually modulate their contents: the low levels of exogenous IAA enhance melatonin production, while the high levels of melatonin decrease IAA production and also reduce the levels of auxin-transporting PIN proteins [57,60]. In general, it is observed that there is an interaction between melatonin and IAA in their transcriptional regulation, both upstream and downstream. All these interactions seen together would modulate the development of plants and their adaptation to stress [61].

Aging affects seeds, causing a loss of germinability. The ability to retain germinability, vigor, and viability varies widely among plant species [62]. Aging effect has been studied in different seeds such as *Arabidopsis* and common beech [63,64], and in general, it was observed that seed aging reduces germination by increasing ROS production (superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals), lipid peroxidation membrane injury, and DNA alterations [65]. Therefore, the antioxidant capacity of melatonin would justify the results obtained pertaining to the increase in germination in aging rice, barley, and sorghum seeds assessed in this study. A few studies have been published that have assessed the participation of melatonin in the viability or aging of seeds during long-term storage. It is worth highlighting the studies carried out on pepper [66] and lettuce [67] consisted of pretreating the seeds in melatonin solutions for 24 h and then drying them

and storing them for 1 year for pepper or 2 years for lettuce. Pepper seeds pretreated with 25 μM melatonin resulted in a higher germination percentage and lower germination rate and electrical conductivity values during storage. In lettuce seeds, it was observed that the quality of non-pretreated seeds deteriorated rapidly when stored at 25 °C, but pretreatment with melatonin significantly reduced this deterioration by increasing the activities of antioxidant enzymes and restoring membrane properties. The authors conclude that melatonin could be used to slow the natural aging process of seeds and may also have important practical applications, especially in storing seeds of endangered species or valuable breeding material.

About the effect of drought stress induced by PEG in sorghum seeds (bioassay II), this stress could inhibit their germination and plant growth. A significant decrease in the germination percentage, vigor index, germination index, and shoot and root growth of sorghum seeds sown at -0.4 MPa was observed. However, after melatonin treatment, all the above studied indicators (G, GP, RSG, RRL, GI, and VI) showed a significant attenuation of adverse effects of drought stress, alleviating them, which was confirmed when the tolerance stress indices of the shoot and root were calculated (Table 5), where tolerance increased with the majority of tested melatonin concentrations (except for the highest concentration used, 300 μM). These results are consistent with the previous studies carried out in cucumber [68], rice [44], and carrot. In carrot, the effect of osmopriming melatonin on SG was closely related to melatonin treatment, being the optimal melatonin concentration capable of promoting SG between 50 and 200 μM [69]. This behavior can be explained by the antioxidant capacity of melatonin that can reduce the amount of ROS that is produced in the presence of PEG. Studies carried out on seed soybean stressed with PEG showed an excess of intracellular ROS, capable of damaging the plant membranes, thereby causing lipid peroxidation. Melatonin treatment reduced this effect, by decreasing H_2O_2 content and O_2^- production [70]. Similar results have been observed in a study on triticale seeds (*Triticale hexaploide* L.), where priming seeds with 20 μM melatonin alleviated the adverse effects of drought stress on germination and seedling growth induced by PEG6000. The results suggested that the priming of seeds with melatonin promotes ROS-scavenging capacity and improves energy supply and antioxidant enzymatic activities, to alleviate the adverse effects of drought stress on triticale [71].

Melatonin improve germination and seedling growth through the modulation of plant hormone contents in tissues, mainly on auxin, gibberellins, cytokinins, abscisic acid, ethylene, jasmonic acid, salicylic acid, and brassinosteroids [20]. In aging or stressful conditions, SG is clearly affected. To achieve a sustainable level of crop yield, it is important to improve SG under abiotic stress conditions. A recent review has been published on the effects of stressors on SG and the regulatory role that melatonin can play due to its ability to interact with different physiological mechanisms [65]. This review highlights that melatonin induces specific responses to stress, such as the regulation of ionic homeostasis and hydrolysis of storage proteins under salinity stress, the activation of aquaporins, and the accumulation of osmolytes under drought stress. Melatonin also modulates common responses such as its role in the regulation of gibberellin biosynthesis, abscisic acid catabolism, redox homeostasis, and Ca^{2+} signaling, all of which are important players in germination.

5. Conclusions

- The exogenous application of melatonin in aging seeds has a biostimulator effect; this effect depends on the seed under study and the concentration of melatonin applied.
- As occurs in other cases applying phytohormones, the effectiveness of melatonin depends on the tissue sensitivity, observing induction or inhibition of growing in different tissues (stem and roots) at similar melatonin concentrations.
- In relation to the aged seeds of rice, barley and sorghum tested, the most effective melatonin concentration, especially in the germination parameters and vigor index, has been 20, 0.05 and 1 μM respectively.

- PEG-induced drought stress in sorghum is alleviated by almost all melatonin concentrations (50, 100 and 200 μ M) tested, but the 200 μ M concentration stands out for being the most effective in improving parameters of germination, early growth and stress.

To conclude, the data obtained in this study focus on the biostimulatory role of melatonin in the germination process, reinforcing seedlings against abiotic stressors, through the modulation of redox network and plant hormonal responses.

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References

1. Ali, A.S.; Elozeiri, A.A.; Ali, A.S.; Elozeiri, A.A. Metabolic Processes During Seed Germination. In *Advances in Seed Biology*; IntechOpen: London, UK, 2017; ISBN 978-953-51-3622-4.
2. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. Structure and Composition. In *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.; Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., Eds.; Springer: New York, NY, USA, 2013; pp. 1–25, ISBN 978-1-4614-4693-4.
3. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. Longevity, Storage, and Deterioration. In *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.; Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., Eds.; Springer: New York, NY, USA, 2013; pp. 341–376, ISBN 978-1-4614-4693-4.
4. Bewley, J.D. Seed Germination and Dormancy. *Plant Cell* **1997**, *9*, 1055–1066. [[CrossRef](#)] [[PubMed](#)]
5. Ahmad, S.; Ahmad, R.; Ashraf, M.; Ashraf, M.; Waraich, E. Sunflower (*Helianthus annuus* L.) Response to Drought Stress at Germination and Seedling Growth Stages. *Pak. J. Bot.* **2009**, *41*, 647–654.
6. Anjum, S.A.; Ashraf, U.; Zohaib, A.; Tanveer, M.; Naeem, M.; Ali, I.; Tabassum, T.; Nazir, U. Growth and Developmental Responses of Crop Plants under Drought Stress: A Review. *Zemdirb.-Agric.* **2017**, *104*, 267–276. [[CrossRef](#)]
7. Chazen, O.; Hartung, W.; Neumann, P.M. The Different Effects of PEG 6000 and NaCl on Leaf Development Are Associated with Differential Inhibition of Root Water Transport. *Plant Cell Ent.* **1995**, *18*, 727–735. [[CrossRef](#)]
8. Hajihashemi, S.; Sofo, A. The Effect of Polyethylene Glycol-Induced Drought Stress on Photosynthesis, Carbohydrates and Cell Membrane in *Stevia Rebaudiana* Grown in Greenhouse. *Acta Physiol. Plant.* **2018**, *40*, 142. [[CrossRef](#)]
9. Kozłowski, T.T. *Germination Control. Metabolism, and Pathology*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 1972; ISBN 978-0-12-424303-3.
10. Anderson, J.D.; Baker, J.E. Deterioration of Seeds during Aging. *Phytopathology* **1983**, *73*, 321–325. [[CrossRef](#)]
11. Rajjou, L.; Debeaujon, I. Seed Longevity: Survival and Maintenance of High Germination Ability of Dry Seeds. *Comptes Rendus Biol.* **2008**, *331*, 796–805. [[CrossRef](#)]
12. Abid, M.; Hakeem, A.; Shao, Y.; Liu, Y.; Zahoor, R.; Fan, Y.; Suyu, J.; Ata-Ul-Karim, S.T.; Tian, Z.; Jiang, D.; et al. Seed Osmopriming Invokes Stress Memory against Post-Germinative Drought Stress in Wheat (*Triticum aestivum* L.). *Environ. Exp. Bot.* **2018**, *145*, 12–20. [[CrossRef](#)]

13. Domokos-Szabolcsy, E.; Marton, L.; Sztrik, A.; Babka, B.; Prokisch, J.; Fari, M. Accumulation of Red Elemental Selenium Nanoparticles and Their Biological Effects in *Nicotiana glauca*. *Plant Growth Regul.* **2012**, *68*, 525–531. [\[CrossRef\]](#)
14. Lerner, A.B.; Case, J.D.; Takahashi, Y.; Lee, T.H.; Mori, W. Isolation of Melatonin, the Pineal Gland Factor That Lightens Melanocytes. *J. Am. Chem. Soc.* **1958**, *80*, 2587. [\[CrossRef\]](#)
15. Dubbels, R.; Reiter, R.J.; Klenke, E.; Goebel, A.; Schnakenberg, E.; Ehlers, C.; Schiwara, H.W.; Schloot, W. Melatonin in Edible Plants Identified by Radioimmunoassay and by HPLC-MS. *J. Pineal Res.* **1995**, *18*, 28–31. [\[CrossRef\]](#)
16. Hattori, A.; Migita, H.; Ito, M.; Yamamoto, K.; Ohtani-Kaneko, R.; Hara, M.; Suzuki, T.; Reiter, R.J. Identification of Melatonin in Plants and Its Effects on Plasma Melatonin Levels and Binding to Melatonin Receptors in Vertebrates. *Biochem. Mol. Biol. Int.* **1995**, *35*, 627–634. [\[PubMed\]](#)
17. Kolar, J.; Machackova, I.; Illnerova, H.; Prinsen, E.; van Dongen, W.; van Onckelen, H. Melatonin in Higher Plant Determined by Radioimmunoassay and Liquid Chromatography-Mass Spectrometry. *Biol. Rhythm. Res.* **1995**, *26*, 406–409.
18. Arnao, M.B. Phytomelatonin: Discovery, Content, and Role in Plants. *Adv. Bot.* **2014**, *2014*, e815769. [\[CrossRef\]](#)
19. Arnao, M.B.; Hernández-Ruiz, J. Phytomelatonin, Natural Melatonin from Plants as a Novel Dietary Supplement: Sources, Activities and World Market. *J. Funct. Foods* **2018**, *48*, 37–42. [\[CrossRef\]](#)
20. Arnao, M.B.; Cano, A.; Hernández-Ruiz, J. Phytomelatonin: An Unexpected Molecule with Amazing Performances in Plants. *J. Exp. Bot.* **2022**, *73*, 5779–5800. [\[CrossRef\]](#)
21. Arnao, M.B.; Hernández-Ruiz, J. Melatonin: A New Plant Hormone and/or a Plant Master Regulator? *Trends Plant Sci.* **2019**, *24*, 38–48. [\[CrossRef\]](#)
22. Arnao, M.B.; Hernández-Ruiz, J. Melatonin: Plant Growth Regulator and/or Biostimulator during Stress? *Trends Plant Sci.* **2014**, *19*, 789–797. [\[CrossRef\]](#)
23. Wei, J.; Li, D.-X.; Zhang, J.-R.; Shan, C.; Rengel, Z.; Song, Z.-B.; Chen, Q. Phytomelatonin Receptor PMTR1-Mediated Signaling Regulates Stomatal Closure in *Arabidopsis thaliana*. *J. Pineal Res.* **2018**, *65*, e12500. [\[CrossRef\]](#)
24. Byeon, Y.; Lee, H.Y.; Lee, K.; Park, S.; Back, K. Cellular Localization and Kinetics of the Rice Melatonin Biosynthetic Enzymes SNAT and ASMT. *J. Pineal Res.* **2014**, *56*, 107–114. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Byeon, Y.; Yool Lee, H.; Choi, D.-W.; Back, K. Chloroplast-Encoded Serotonin N-Acetyltransferase in the Red Alga *Pyropia yezoensis*: Gene Transition to the Nucleus from Chloroplasts. *J. Exp. Bot.* **2015**, *66*, 709–717. [\[CrossRef\]](#)
26. Tan, D.-X.; Reiter, R.J. An Evolutionary View of Melatonin Synthesis and Metabolism Related to Its Biological Functions in Plants. *J. Exp. Bot.* **2020**, *71*, 4677–4689. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Zhao, D.; Yu, Y.; Shen, Y.; Liu, Q.; Zhao, Z.; Sharma, R.; Reiter, R.J. Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. *Front. Endocrinol.* **2019**, *10*, 249. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Hernández-Ruiz, J.; Giraldo-Acosta, M.; El Mihyaoui, A.; Cano, A.; Arnao, M.B. Melatonin as a Possible Natural Anti-Viral Compound in Plant Biocontrol. *Plants* **2023**, *12*, 781. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Back, K. Melatonin Metabolism, Signaling and Possible Roles in Plants. *Plant J.* **2021**, *105*, 376–391. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Back, K.; Tan, D.-X.; Reiter, R.J. Melatonin Biosynthesis in Plants: Multiple Pathways Catalyze Tryptophan to Melatonin in the Cytoplasm or Chloroplasts. *J. Pineal Res.* **2016**, *61*, 426–437. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Tan, D.-X.; Hardeland, R.; Back, K.; Manchester, L.C.; Alatorre-Jimenez, M.A.; Reiter, R.J. On the Significance of an Alternate Pathway of Melatonin Synthesis via 5-Methoxytryptamine: Comparisons across Species. *J. Pineal Res.* **2016**, *61*, 27–40. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Tan, D.-X.; Manchester, L.C.; Esteban-Zubero, E.; Zhou, Z.; Reiter, R.J. Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism. *Molecules* **2015**, *20*, 18886–18906. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Wang, S.-Y.; Shi, X.-C.; Wang, R.; Wang, H.-L.; Liu, F.; Laborda, P. Melatonin in Fruit Production and Postharvest Preservation: A Review. *Food Chem.* **2020**, *320*, 126642. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Xu, T.; Chen, Y.; Kang, H. Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Front. Plant Sci.* **2019**, *10*, 1388. [\[CrossRef\]](#)
35. Arnao, M.B.; Hernández-Ruiz, J. Melatonin as a Regulatory Hub of Plant Hormone Levels and Action in Stress Situations. *Plant Biol.* **2021**, *23*, 7–19. [\[CrossRef\]](#)
36. Arnao, M.B.; Hernández-Ruiz, J. Melatonin and Its Relationship to Plant Hormones. *Ann. Bot.* **2018**, *121*, 195–207. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Gu, Q.; Chen, Z.; Yu, X.; Cui, W.; Pan, J.; Zhao, G.; Xu, S.; Wang, R.; Shen, W. Melatonin Confers Plant Tolerance against Cadmium Stress via the Decrease of Cadmium Accumulation and Reestablishment of microRNA-Mediated Redox Homeostasis. *Plant Sci.* **2017**, *261*, 28–37. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Reiter, R.J.; Rosales-Corral, S.; Tan, D.X.; Jou, M.J.; Galano, A.; Xu, B. Melatonin as a Mitochondria-Targeted Antioxidant: One of Evolution's Best Ideas. *Cell. Mol. Life Sci.* **2017**, *74*, 3863–3881. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Moustafa-Farag, M.; Almoneafy, A.; Mahmoud, A.; Elkelish, A.; Arnao, M.B.; Li, L.; Ai, S. Melatonin and Its Protective Role against Biotic Stress Impacts on Plants. *Biomolecules* **2020**, *10*, 54. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Posmyk, M.M.; Balabusta, M.; Wieczorek, M.; Sliwinska, E.; Janas, K.M. Melatonin Applied to Cucumber (*Cucumis sativus* L.) Seeds Improves Germination during Chilling Stress. *J. Pineal Res.* **2009**, *46*, 214–223. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Posmyk, M.M.; Kuran, H.; Marciniak, K.; Janas, K.M. Presowing Seed Treatment with Melatonin Protects Red Cabbage Seedlings against Toxic Copper Ion Concentrations. *J. Pineal Res.* **2008**, *45*, 24–31. [\[CrossRef\]](#) [\[PubMed\]](#)

42. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.; Springer: New York, NY, USA, 2013; ISBN 978-1-4614-4692-7.
43. Jincy, M.; Prasad, V.B.R.; Jeyakumara, P.; Senthila, A.; Manivannan, N. Evaluation of Green Gram Genotypes for Drought Tolerance by PEG (Polyethylene Glycol) Induced Drought Stress at Seedling Stage. *Legume Res.* **2019**, *44*, 684–691. [\[CrossRef\]](#)
44. Megala, R.; Kalarani, M.K.; Jeyakumar, P.; Senthil, N.; Pushpam, R.; Umapathi, M. Standardization of Optimum Melatonin Concentration for Drought Tolerance at Germination and Early Development Stage in Rice (CO-54). *J. Appl. Nat. Sci.* **2022**, *14*, 1022–1030. [\[CrossRef\]](#)
45. Wang, G.; Yang, Y.; Kong, Y.; Ma, R.; Yuan, J.; Li, G. Key Factors Affecting Seed Germination in Phytotoxicity Tests during Sheep Manure Composting with Carbon Additives. *J. Hazard. Mater.* **2022**, *421*, 126809. [\[CrossRef\]](#)
46. Hernández-Herrera, R.M.; Santacruz-Ruvalcaba, F.; Ruiz-López, M.A.; Norrie, J.; Hernández-Carmona, G. Effect of Liquid Seaweed Extracts on Growth of Tomato Seedlings (*Solanum lycopersicum* L.). *J. Appl. Phycol.* **2014**, *26*, 619–628. [\[CrossRef\]](#)
47. Kamran, M.; Wang, D.; Xie, K.; Lu, Y.; Shi, C.; EL Sabagh, A.; Gu, W.; Xu, P. Pre-Sowing Seed Treatment with Kinetin and Calcium Mitigates Salt Induced Inhibition of Seed Germination and Seedling Growth of Choysum (*Brassica rapa* Var. *Parachinensis*). *Ecotoxicol. Environ. Saf.* **2021**, *227*, 112921. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Amin, H.; Arain, B.A.; Amin, F.; Surhio, M.A. Phytotoxicity of Chromium on Germination, Growth and Biochemical Attributes of *Hibiscus esculentus* L. *Am. J. Plant Sci.* **2013**, *4*, 2431–2439. [\[CrossRef\]](#)
49. García-Sánchez, S.; Cano, A.; Hernández-Ruiz, J.; Arnao, M.B. Effects of Temperature and Light on the Germination-Promoting Activity by Melatonin in Almond Seeds without Stratification. *Agronomy* **2022**, *12*, 2070. [\[CrossRef\]](#)
50. Hernández-Ruiz, J.; Cano, A.; Arnao, M.B. Melatonin: A Growth-Stimulating Compound Present in Lupin Tissues. *Planta* **2004**, *220*, 140–144. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Korkmaz, A. The Effects of Seed Treatment with Melatonin on Germination and Emergence Performance of Pepper Seeds under Chilling Stress. *Tarim Bilim. Derg.-J. Agric. Sci.* **2017**, *23*, 167–176.
52. Lv, Y.; Pan, J.; Wang, H.; Reiter, R.J.; Li, X.; Mou, Z.; Zhang, J.; Yao, Z.; Zhao, D.; Yu, D. Melatonin Inhibits Seed Germination by Crosstalk with Abscissic Acid, Gibberellin, and Auxin in Arabidopsis. *J. Pineal Res.* **2021**, *70*, e12736. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Simlat, M.; Ptak, A.; Skrzypek, E.; Warchol, M.; Morańska, E.; Piórkowska, E. Melatonin Significantly Influences Seed Germination and Seedling Growth of Stevia Rebaudiana Bertoni. *PeerJ* **2018**, *6*, e5009. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Xiao, S.; Liu, L.; Wang, H.; Li, D.; Bai, Z.; Zhang, Y.; Sun, H.; Zhang, K.; Li, C. Exogenous Melatonin Accelerates Seed Germination in Cotton (*Gossypium hirsutum* L.). *PLoS ONE* **2019**, *14*, e0216575. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Zhang, M.; Liu, S.; Tong, S.; Zhang, D.; Qi, Q.; Wang, Y.; Wang, X.; An, Y.; Lu, X. Effects of Melatonin Priming on Suaeda Corniculata Seed Germination, Antioxidant Defense, and Reserve Mobilization: Implications for Salinized Wetland Restoration. *Front. Ecol. Evol.* **2022**, *10*, 941032. [\[CrossRef\]](#)
56. Hernández-Ruiz, J.; Cano, A.; Arnao, M.B. Melatonin Acts as a Growth-Stimulating Compound in Some Monocot Species: Melatonin as Growth Promoter in Plants. *J. Pineal Res.* **2005**, *39*, 137–142. [\[CrossRef\]](#)
57. Chen, Q.; Qi, W.; Reiter, R.J.; Wei, W.; Wang, B. Exogenously Applied Melatonin Stimulates Root Growth and Raises Endogenous Indoleacetic Acid in Roots of Etiolated Seedlings of *Brassica juncea*. *J. Plant Physiol.* **2009**, *166*, 324–328. [\[CrossRef\]](#)
58. Li, L.; Verstraeten, I.; Roosjen, M.; Takahashi, K.; Rodriguez, L.; Merrin, J.; Chen, J.; Shabala, L.; Smet, W.; Ren, H.; et al. Cell Surface and Intracellular Auxin Signalling for H⁺ Fluxes in Root Growth. *Nature* **2021**, *599*, 273–277. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Arnao, M.B.; Hernández-Ruiz, J. Melatonin Promotes Adventitious- and Lateral Root Regeneration in Etiolated Hypocotyls of *Lupinus albus* L. *J. Pineal Res.* **2007**, *42*, 147–152. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Ren, S.; Rutto, L.; Katuuramu, D. Melatonin Acts Synergistically with Auxin to Promote Lateral Root Development through Fine Tuning Auxin Transport in *Arabidopsis thaliana*. *PLoS ONE* **2019**, *14*, e0221687. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Zhang, M.; Gao, C.; Xu, L.; Niu, H.; Liu, Q.; Huang, Y.; Lv, G.; Yang, H.; Li, M. Melatonin and Indole-3-Acetic Acid Synergistically Regulate Plant Growth and Stress Resistance. *Cells* **2022**, *11*, 3250. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Merritt, D.J.; Senaratna, T.; Touchell, D.H.; Dixon, K.W.; Sivasithamparan, K. Seed Ageing of Four Western Australian Species in Relation to Storage Environment and Seed Antioxidant Activity. *Seed Sci. Res.* **2003**, *13*, 155–165. [\[CrossRef\]](#)
63. Rajjou, L.; Lovigny, Y.; Groot, S.P.C.; Belghazi, M.; Job, C.; Job, D. Proteome-Wide Characterization of Seed Aging in Arabidopsis: A Comparison between Artificial and Natural Aging Protocols. *Plant Physiol.* **2008**, *148*, 620–641. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Ratajczak, E.; Małecka, A.; Bagniewska-Zadworna, A.; Kalembe, E.M. The Production, Localization and Spreading of Reactive Oxygen Species Contributes to the Low Vitality of Long-Term Stored Common Beech (*Fagus sylvatica* L.) Seeds. *J. Plant Physiol.* **2015**, *174*, 147–156. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Wang, L.; Tanveer, M.; Wang, H.; Arnao, M.B. Melatonin as a Key Regulator in Seed Germination under Abiotic Stress. *J. Pineal Res.* **2024**, *76*, e12937. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Korkmaz, A.; Köklü, S.; Yakupoglu, G. Investigating the Effects of Melatonin Application on the Ageing Process of Pepper Seeds. In Proceedings of the Acta Horticulturae; International Society for Horticultural Science (ISHS), Leuven, Belgium, 30 June 2018; pp. 9–16.
67. Yakupoglu, G.; Köklü, S.; Karaca, A.; Düver, E.; Reiter, J.R.; Korkmaz, A. Fluctuations in Melatonin Content and Its Effects on the Ageing Process of Lettuce Seeds during Storage. *Acta Sci. Pol. Hortorum Cultus* **2021**, *20*, 77–88. [\[CrossRef\]](#)

68. Zhang, H.; Zhang, N.; Yang, R.; Wang, L.; Sun, Q.; Li, D.; Cao, Y.; Weeda, S.; Zhao, B.; Ren, S.; et al. Melatonin Promotes Seed Germination under High Salinity by Regulating Antioxidant Systems, ABA and GA₄ Interaction in Cucumber (*Cucumis sativus* L.). *J. Pineal Res.* **2014**, *57*, 269–279. [[CrossRef](#)]
69. Rosińska, A.; Andrzejak, R.; Kakkerla, V. Effect of Osmopriming with Melatonin on Germination, Vigor and Health of *Daucus Carota* L. Seeds. *Agriculture* **2023**, *13*, 749. [[CrossRef](#)]
70. Zhang, M.; He, S.; Qin, B.; Jin, X.; Wang, M.; Ren, C.; Cao, L.; Zhang, Y. Exogenous Melatonin Reduces the Inhibitory Effect of Osmotic Stress on Antioxidant Properties and Cell Ultrastructure at Germination Stage of Soybean. *PLoS ONE* **2020**, *15*, e0243537. [[CrossRef](#)] [[PubMed](#)]
71. Guo, Y.; Li, D.; Liu, L.; Sun, H.; Zhu, L.; Zhang, K.; Zhao, H.; Zhang, Y.; Li, A.; Bai, Z.; et al. Seed Priming with Melatonin Promotes Seed Germination and Seedling Growth of *Triticale hexaploide* L. Under PEG-6000 Induced Drought Stress. *Front. Plant Sci.* **2022**, *13*, 932912. [[CrossRef](#)] [[PubMed](#)]

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