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Seed priming with melatonin improves seed germination and root system architecture in wheat (*Triticum aestivum* L.)

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Abstract
Melatonin, a multifunctional hormone found in various organisms, has gained significant attention in plant biology due to its potential role in regulating various physiological processes. A crucial phase of a plant's life cycle is seed germination. Recent studies have demonstrated that melatonin plays a critical role in promoting germination and influence root architecture, Here, to analyze the effect of melatonin in modulating the germination indices and root development, seeds of wheat cvHD2967 were primed with a series of melatonin at MT1 (15 mgl^{-1}), MT2 (30 mgl^{-1}), MT3 (50 mgl^{-1}), MT4 (60 mgl^{-1}), and MT5 (75 mgl^{-1}) and water alone as control. MT3 (50 mgl^{-1}), and MT4 (60 mgl^{-1}) substantially increased radical length, shoot length, fresh weight, dry weight, root attributes and germination potential. In conclusion, melatonin emerges as a key regulator of seed germination and root development in plants. Its ability to enhance the germination rates, promote root growth and alleviates stress effects underscores it as a potential and valuable tool in plant science and crop improvement strategies.

Keywords: Melatonin, seed priming, wheat, root architecture, germination, fresh weight

Introduction

Depending on the plant species and the experimental setup, melatonin has been documented to have both stimulatory and inhibitory effects on seed germination. Several studies have demonstrated that exogenous application of melatonin enhances seed germination. For instance, Liang *et al*. (2015) [7] found that melatonin treatment increased the germination rate, germination potential and seedling vigour of tomato seeds. On the other hand, melatonin can also exhibit inhibitory effects on seed germination. Exogenous melatonin application in lettuce seeds delayed germination and reduced germination percentage. These inhibitory effects may be attributed to the inhibitory action of melatonin on gibberellin synthesis or its interaction with other hormones involved in germination regulation. Melatonin controls primary growth, lateral root creation, and root hair elongation, all of which are essential for the development of root architecture. Exogenous melatonin treatment has been reported to promote root growth in various plant species. For example, Zhang *et al*. (2014) [14] demonstrated that melatonin treatment enhanced root length, surface area and volume in cucumber seedlings. Melatonin stimulates root growth by increasing cell division and elongation, enhancing auxin signalling and modulating the balance of reactive oxygen species (ROS) in roots. Melatonin also regulates lateral root formation. Zhang *et al*. (2016) [15] shown that melatonin promote lateral root development in Arabidopsis thaliana by modulating auxin transport and distribution. Additionally, melatonin can influence root hair elongation, a crucial factor for nutrient and water uptake. In maize, exogenous melatonin treatment increased root hair length and density (Wang *et al*., 2019) [13]. This effect is mediated by melatonin's ability to enhance auxin signalling and modulate the expression of genes involved in root hair elongation. So, in order to analyse the effect of different concentration of melatonin, we designed this study to first optimise the effective concentration of MT in boosting germination and wheat seed germination indices, seedling growth and overall root architecture.

Materials and Methods

Plant material, seed priming and growth conditions

Seeds of wheat (*Triticum aestivum* L.) cv HD 2967were used in this experiment and were obtained from Division of Genetics, ICAR-Indian Agriculture Research Institute (IARI), New Delhi, India. Healthy seeds were separated and surface sterilised for 3 min in a solution of 1%

sodium hypochlorite. Seeds were rinsed with sterilised water, allowed to air dry on filter paper and further treated with the fungicide Bavistin 1% for another 2hours followed by air-drying. Seeds were primed by soaking them in MT solution of varying strength. Melatonin (molecular weight: 232.28) was purchased from Sigma Aldrich with >99% purity. A suitable volume of anhydrous ethanol was used to dissolve 75 mg MT to create a stock solution. Then, deionized water was added, bringing the solution's total volume to 1ml. Further dilutions were performed to create the melatonin concentrations MT1 (15 mgl-1), MT2 (30 mgl-1), MT3 (50 mgl-1), MT4 (75 mgl-1), and NP (No Priming, just water). Seeds were then immersed in the produced solutions for 24 hours at 22 °C ± 2 °C in the dark. After that seeds were air-dried thoroughly at room temperature and germinated in petri plates containing moistened blotting paper. After 1-2 days when radicle emergence started, seeds were transferred to pots containing soilrite for further growth in a growth chamber at 22 °C. Another set of seeds were germinated in germination papers and continued there for measurement of plant architecture and growth parameters.

Measurement of shoot-root, Fresh weight, Dry weight, Germination percentage

Daily measurements of the length of the roots and shoots were done according to the Association of Official Seed Analysts' (1983) manual. At the conclusion of the experiment, the final germination percentage (GP) was computed by multiplying the ratio of the total number of seeds to the number of seeds that germinated by 100. At the conclusion of each experiment, the final root and shoot lengths of the emerging seedlings were measured.

Germination percentage (GP) = (Total number of normal seeds germinated/Total number of seeds sown initially) × 100.

Fresh weight of samples was taken directly. For dry weight determination, plants were oven-dried at 80 °C for 1-2 days and weight measured.

Root Architecture System

Primed wheat seedlings that were ten days old were carefully removed from germination sheets, washed with tap water until the roots were clean, and then dried on blotting sheets. The seedlings were then scanned by a lighted flatbed scanner at 300 dpi, and the scans were analysed by Win RHIZO software (Win RHIZO LA2400 Scanner, Regent Instruments Canada Inc.). Total root length, surface area, root volume, number of tips, number of forks, and average diameter were all measured.

Statistical Analysis

The findings are presented as means and standard error (S.E.). Duncan's multiple range tests were used to identify the significant difference (at $p<0.05$) between treatments for all morphological and physiological parameters, and analysis of variance (ANOVA) was used to assess the results. SPSS 10.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel were used to calculate the ANOVA and critical difference value.

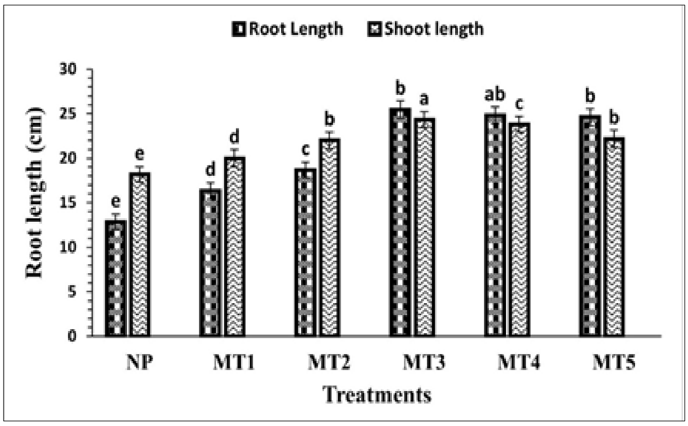
Result and Discussion

Melatonin Priming improves the growth parameters in wheat seedlings

For measurements of the root and shoot lengths, seedlings that were 10 days old were employed. The treatments differed significantly ($p< 0.05$) when primed with various melatonin concentrations. Under MT3 and MT4 priming, the shoot length rose dramatically by 33.94% and 31.00%, respectively, while the root length increased by 98.44% and 93.25% compared to the comparable control. (Fig. 1a).

As compared to controls, various melatonin doses enhanced the morphology and germination indices differently, which improved the overall physiological state of the plants. Furthermore, the germination potential increased by 7% in MT1, 10.4% in MT2, 18.7% in MT3, 17.9% in MT4 and 17.5% in MT5 respectively over the control (Fig. 1b).

However, the fresh and dry biomass of germinating wheat seeds were dramatically enhanced by the optimised melatonin concentrations.



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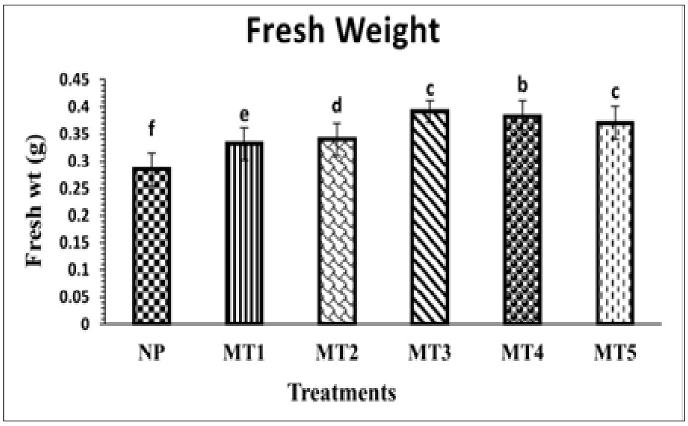

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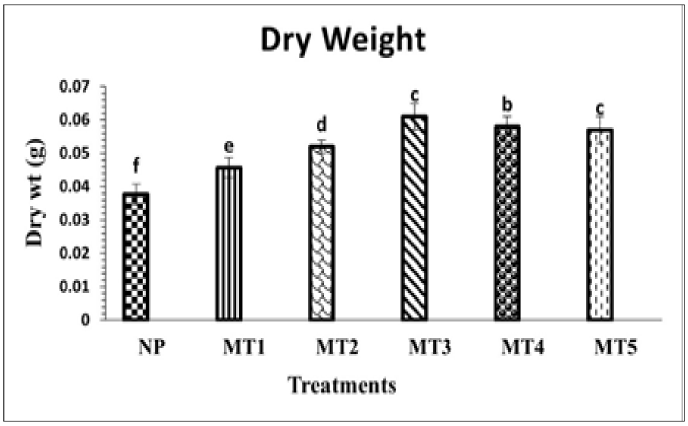

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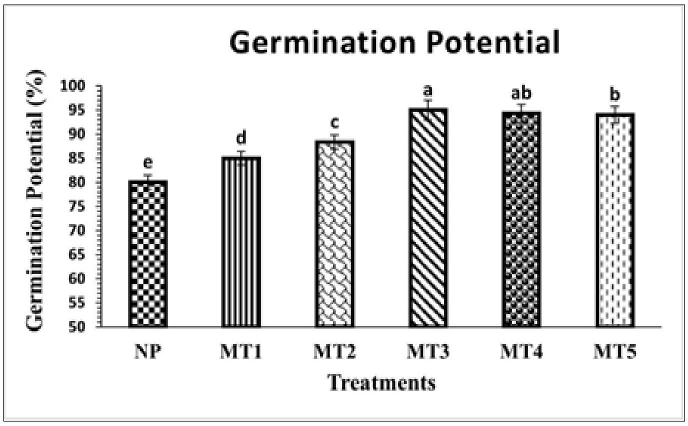


(d)

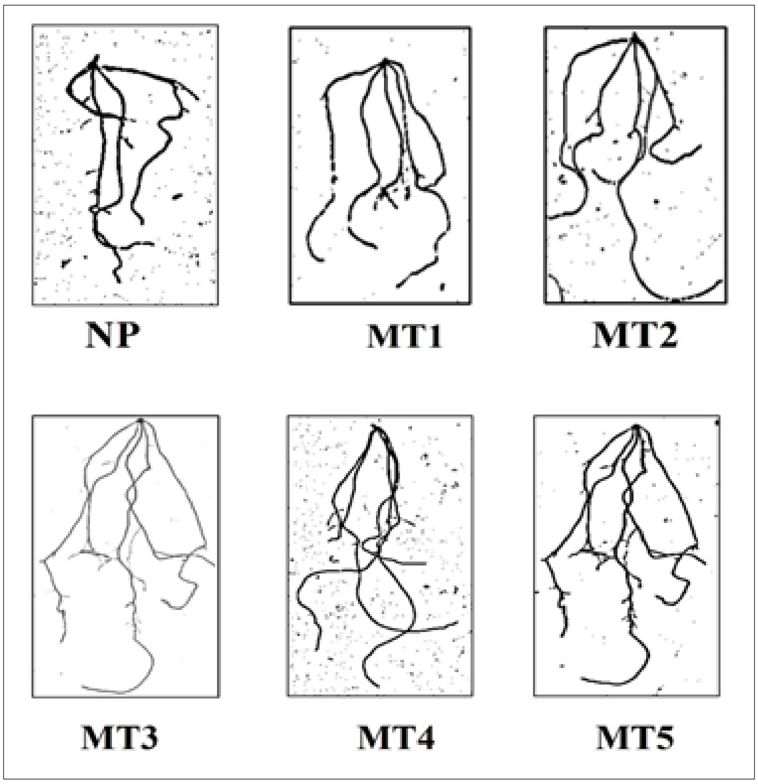
Fig 1: Initial seed priming effect at seedling stage: Effect of different concentrations of melatonin on different germination parameters. Values are the means ± SD (n=3). Root and shoot length data were recorded at 1stday of emergence up to 10 days. a. Root shoot length b. Fresh weight c. Dry weight d. Germination percentage.NP (No Priming), MT1 (15mgl-1), MT2 (30mgl-1), MT2 (50mgl-1), MT2 (60mgl-1), MT2 (75mgl-1). Vertical bars represent ±standard error of the mean and values followed by different letters of significant differences ($p < 0.05$) probability level determined using Duncan's multiple range test

Melatonin priming positively impacts root architecture system

The root architecture of primed plants with different melatonin concentration is depicted here in Fig 2a. When total root length was measured from seeds subjected to MT1, MT2, MT3, MT4 and MT5, it showed 31.2%, 36.1%, 45.8%, 36.1%, and 34.8% increase over control condition (Fig 2b).

In case of surface area a significant increase was recorded for MT1, MT2 and MT3, MT4 and MT5 at 23.79%, 34.8%, 38.4%, 25.9 and 41.2% over control (Fig 2c).

In case of root volume plants studied, MT3 and MT4 led to significant increase of 99.5% and 56.14%, while MT1, MT2 and MT5 showed 26.3%, 47.8% and 51.5% over corresponding control (Fig 2d).MT3 recorded highest increase in case of no of tips followed by MT5, MT4, MT2 and MT1 with 23.47%, 23%, 18.7%, 13.1% and 10.7% respectively (Fig 2e). Similar trend was observed in no of forks and average diameter with highest increase in MT3 with 26.3% and 43.0% over control condition (Fig 2f, g).



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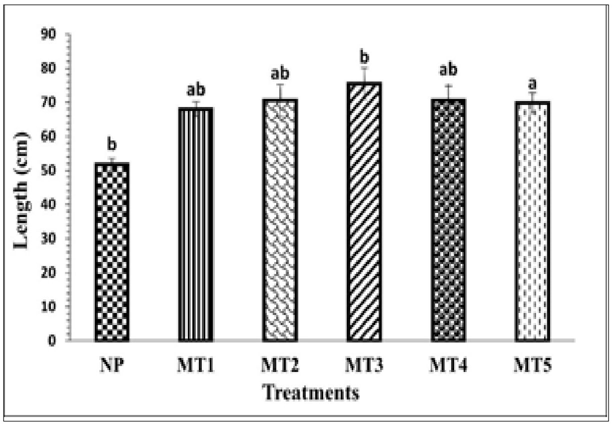

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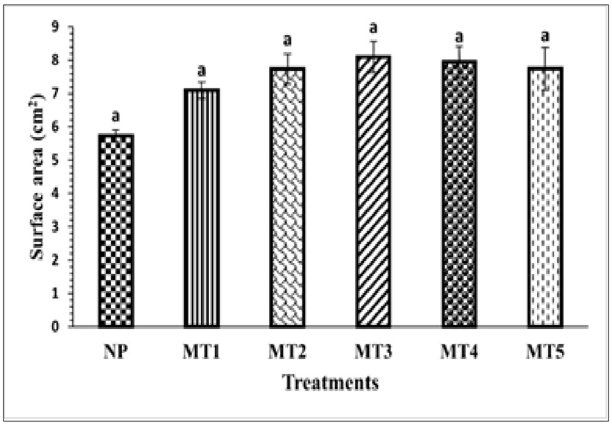

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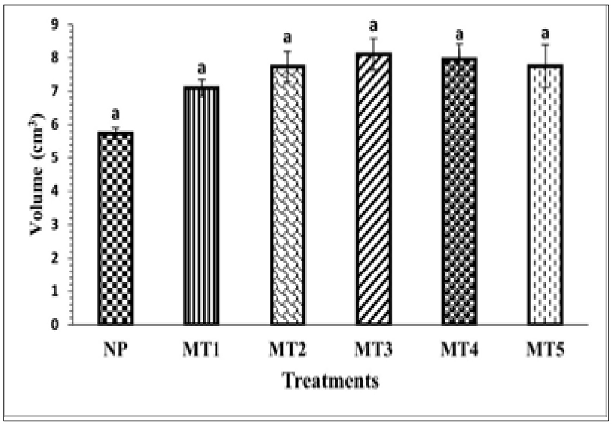

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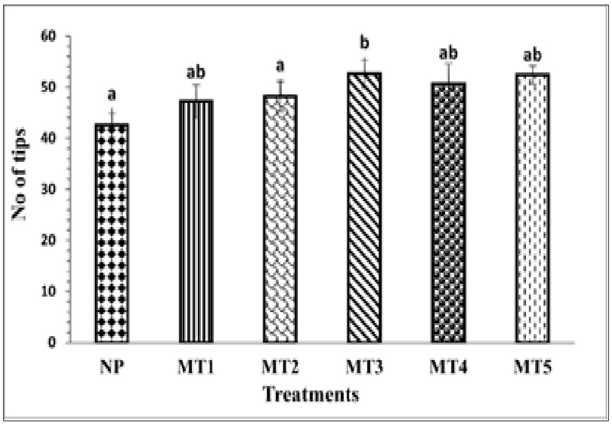

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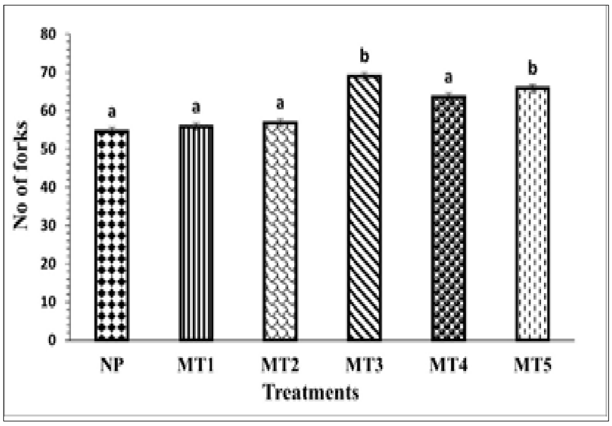

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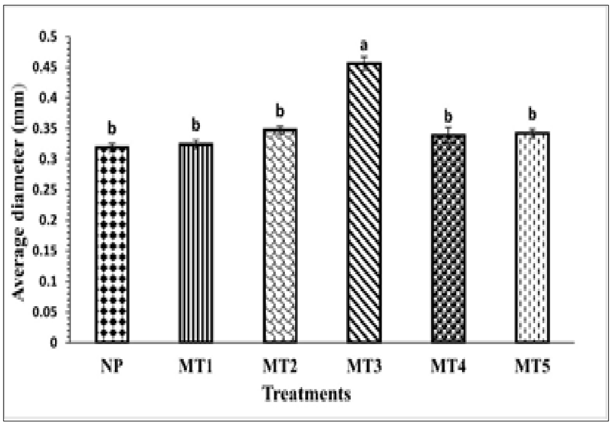

(g)

Fig 2: Effect of priming with different concentration of melatonin on root architecture system (RSA) of 10 days old seedling. NP (No Priming), MT1 (15mgl^{-1}), MT2 (30mgl^{-1}), MT3 (50mgl^{-1}), MT4 (60mgl^{-1}), MT5 (75mgl^{-1}), (a) Primed wheat root under different melatonin concentration and control, (b) Root length (c)Surface area (d) Volume (e) No of tips (f) No of forks (g) Average diameter Vertical bars represent means ± SE and the lowercase letters refer to significant differences between the treatments at the ($p < 0.05$) levels

The above study investigated the role of melatonin in seed germination and root architecture in plants regulating the basic plant growth processes under different melatonin concentration having MT3 and MT4 shows better result over other concentrations. These results are in line with Arnao *et al*. (2019) [1] and Fan *et al*. (2018) [10] who explained melatonin as a multi regulatory molecule with many diverse role in plants including plant growth, seed germination, root architecture, photosynthesis. Xiao *et al*. (2019) [2] documented the promotional effects of melatonin at different concentration on cotton seed germination, germination potential, germination rate, fresh weight. These findings support our findings.

Conclusion

According to the results of our investigation, melatonin may have a function in the differential regulation and enhancement of germination indices and other physiological processes. The germination potential, branch length, radical length, fresh weight, and dry weight of wheat seed were all significantly improved by pre-treatment with melatonin at doses of 50 mgl-1 and 60 mgl-1. The results of this study imply that melatonin at these precise doses (50 mgl-1 and 60 mgl-1) can improve root architecture by increasing root length, root volume, and root surface area.

Further investigations are needed to fully uncover the molecular mechanisms underlying theses effects and exploit the practical applications of melatonin in agriculture.

Acknowledgements

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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