

Auxin Priming Promotes Seed Germination and Seedling Growth of Spinach (*Amaranthus tricolor*)

Fadzil Suhaimi Fadzillah Adibah^{1*}, Raj Ragunathan Darshan¹, Nor Hasima Mahmod², Mohd Nozulaidi Nordin³, Muhamad Hanis Abd Razak¹, Fathul Nabila Abd Karim¹, and Wan Nur Aimi Najwa Wan Mohd Nor⁴

¹Department of Science and Technology, Faculty of Technology, Design and Management, UCYP University, Lot PT 88929, Jalan Tanjung Lumpur, Kg Peramu, 26060 Kuantan, Pahang, Malaysia

²School of Agriculture Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, 22200 Besut, Terengganu, Malaysia

³Department of Agronomy and Fertiliser Technology, FGV R&D Sdn. Bhd., Pusat Penyelidikan Pertanian Tun Razak, 26400 Jengka, Pahang, Malaysia

⁴Business Development and Commercialisation Department, KYP Education Sdn Bhd, 26060 Kuantan, Pahang, Malaysia

ABSTRACT

Seed priming is a successful method to promote germination in agriculture production. The result of auxin priming in promoting the germination and seedling growth of *Amaranthus tricolor* remains to be examined. Therefore, the aim of this research was to examine the effects of auxin priming on seed germination and seedling growth of *A. tricolor*, a leafy vegetable with high nutritional value and potentially contribute to food security, which in line with SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production). In this study, 50 seeds of *A. tricolor* were soaked in each Petri dish contained three different concentrations of auxin [A0- 0 μ M (control), A1- 50 μ M, and A2- 100 μ M] for eight hours before germinate on the germination paper. The Petri dishes were laid out based on a completely randomised design (CRD) with five replicates. Data for seed germination and seedling growth of *A. tricolor* were recorded. The data were analysed using the one-way analysis of variance (ANOVA) followed by the Duncan's post-hoc test. Results showed that A1 and A2

significantly enhanced germination percentage and germination index meanwhile only A2 significantly increased seedling length and hypocotyl length compared to the control. These finding suggests that auxin priming promotes seed germination and seedling growth of *A. tricolor*, potentially improving agricultural productivity.

Keywords: Auxin priming, *A. tricolor*, indole-3-butyric acid, germination, seedling growth

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E-mail addresses:

fadzillahadibah@ucyp.edu.my (Fadzil Suhaimi Fadzillah Adibah)

darshan5121.dr@gmail.com (Raj Ragunathan Darshan)

norhasima@unisza.edu.my (Nor Hasima Mahmod)

nozulaidi.n@fgvholdings.com (Mohd Nozulaidi Nordin)

muhamadhanis@ucyp.edu.my (Muhamad Hanis Abd Razak)

fathulnabila@ucyp.edu.my (Fathul Nabila Abd Karim)

aiminajwamohdnor@gmail.com

(Wan Nur Aimi Najwa Wan Mohd Nor)

* Corresponding author

INTRODUCTION

Seed germination happens when the radicle protrudes from seed with the presence of optimum phytohormones, water, light and temperature (Farooq et al., 2022). The process of germination happens in three phases; first is imbibition, second is activation phase and third is root protrusion (Zulfiqar, 2021). The process of seed germination involves two kinds of hormones, first is abscisic acid (ABA) and second is gibberellic acid (GA) which act antagonistically (Farooq et al., 2022).

Auxin in Greek word meaning to grow (Gomes & Scortecci, 2021) or to expand (Jing et al., 2023). Auxin is a phytohormone acts as a key regulator of plant physiological processes (Cohen & Strader, 2024) and promotes the formation of plant organs (Gao et al., 2024). A few studies reported that auxin able to alleviate abiotic stress (Yu et al., 2022). On the other hand, auxin crosstalk with other hormones like abscisic acid (ABA) and jasmonic acid (JA) antagonistically (Ali et al., 2025).

In this study, seed priming method was implemented to improve seed germination rates (Pappalettere et al., 2024). Priming is classified into different categories depend on the priming agents (Liu et al., 2022). Current study used auxin as hormonal priming method. This method has been successfully implemented to stimulate and coordinate germination (Pereira et al., 2021).

Amaranthus tricolor L., a purple-red-green leafy vegetable belongs to the genus *Amaranthus* in the family Amaranthaceae (Jahan et al., 2022) and order Caryophyllales (Wang et al., 2023) is widely called as Bayam Separa Merah in Malaysia. It was reported that *A. tricolor* contains high carbohydrate (39.80%), protein (26.60%), potassium (1080.02 mg/100g), calcium (39.76 mg/100g) (Jahan et al., 2022), phenolic content (TPC), and total flavonoid content (TFC) (Sarker et al., 2024). Hence, these specialities attract food industry player interest to invent supplement benefits to human health (Sarker et al., 2022).

There are several reports on the effects of auxin priming on seed germination and seedling growth of *Gossypium hirsutum* L. (Zhao et al., 2020) and *Abelmoschus esculentus* L. (Sarath et al., 2022). However, the study on auxin priming in promoting the germination and seedling growth of *A. tricolor* remains to be examined. The objectives of current research are to (1) determine the effects of auxin priming on seed germination and (2) investigate the effects of auxin priming on seedling growth of *A. tricolor*.

MATERIALS AND METHODS

Experimental Design

This research was conducted in the Biology Laboratory, UCYP University. *A. tricolor* seeds (Crop Power, Malaysia) and auxin (SERBAJADI, Malaysia) were used in this experiment. Fifty of *A. tricolor* seeds were soaked in different Petri dishes containing three concentrations of auxin [A0- no auxin (control), A1- 50 μ M (8.76 mg/L of auxin)

and A2- 100 μ M (17.52 mg/L of auxin)] for eight hours before sowing (Lyalina et al., 2023). Each of the Petri dishes was arranged based on a completely randomised design (CRD) with five replications (Table 1). After priming, *A. tricolor* was rinsed with water three times and was germinated on wet germination paper (Zhao et al., 2020). The seeds were maintained under temperature (24 ± 2 °C) and relative humidity (60 ± 10 %) (Abdullahi et al., 2025). Data for seed germination and seedling growth were recorded from the first to the seventh day after sowing (DAS).

Table 1
Completely Randomised Design (CRD) with five replicates

A0R1	A1R2	A2R2
A1R3	A2R3	A0R2
A2R1	A0R3	A1R4
A0R5	A1R5	A2R4
A1R1	A2R5	A0R4

Determination of Seed Germination Parameters of *A. Tricolor*

Determination of Germination Percentage (G %)

Germination percentage (G %) of *A. tricolor* seeds were recorded on the first DAS based on the formula (Ellouzi et al., 2024):

$$G\% = \left(\frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds Sowed}} \right) \times 100$$

Determination of germination index (GI)

The germination index (GI) was determined based on the formula below to measure the speed and uniformity of seed germination (Ismaeil et al., 2022):

$$GI = \sum \left(\frac{Gt}{Tt} \right)$$

Where, Gt = Number of seeds germinated on first to third DAS; Tt = Number of days from first to third DAS

Measurement of Seedling Growth Parameters of *A. Tricolor*

Measurement of Seedling Length and Hypocotyl Length

Seedling length was measured starting from the tip of the plumule to the end of the radicle (Ali et al., 2021) meanwhile hypocotyl length was measured from the cotyledon to the radicle (Yaakob et al., 2020) on seventh DAS by using ruler.

Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) followed by Duncan’s post-hoc test by using Statistical Package for the Social Sciences (SPSS) version

29. The mean values were considered significant when $p < 0.05$. All data were presented as mean \pm standard deviation (STD).

RESULTS AND DISCUSSION

Effects of Auxin Priming on Seed Germination Parameters of *A. Tricolor*

Germination Percentage (G %)

The effects of auxin on germination percentage of *A. tricolor* were presented (Figure 1A). Different letters show there are significantly different ($p < 0.05$). The germination percentage of *A. tricolor* treated with A1 (98.4%) and A2 (99.2%) increased significantly compared to the treatment A0 (32%). However, treatment A1 and A2 showed no significant differences. The results of current study align with previous report that highlight the critical role of auxins in seed germination and early plant development (Mekonnen et al., 2024). Moreover, germination percentage of hormonal primed *D. carota* seed was the second highest (84%) compared to hydro primed (85%) and nutri primed (77%) (Dessalew et al., 2022). In contrast, other study reported that high concentration (50, 100 and 150 ppm) of auxin inhibits the germination of onion seeds (Song et al., 2020).

Germination Index

According to Figure 1B, *A. tricolor* treated with A1 (90.87) and A2 (91.27) were significantly higher compared to treatment A0 (56.87). However, the germination index of treatment A1 and A2 have no significant differences. Earlier study has presented that auxin is also involved in the transformation of seed from dormant stage to germination (Wu et al., 2020). Instead of that, biopriming with seaweed and microbes increased germination index of *Abelmoschus esculentus* (L.) Moench (Makhaye et al., 2021). Conversely, germination index treated with sheep manure was high compared to pig and chicken manure due to high ammonium nitrogen content (Wang et al., 2022).

Effects of Auxin Priming on Seedling Growth Parameters of *A. Tricolor*

Seedling Length

Auxin priming effects on seedling length are displayed in Figure 1C. Seedling length treated with A1 (2.25 cm) was insignificantly higher than A0 (1.91 cm) meanwhile A2 (2.35 cm) was significantly higher than A0. Earlier study reported that hormo priming with gibberellic acid enhanced seedling length of *Tanacetum parthenium* (L.) Sch. Bip. Auxin might increase enzyme activity and enhance plasma membrane integrity (Alizadeh et al., 2022). Furthermore, bio priming with *Trichoderma virens* increased seedling length of *Glycine max* L. The increment of seedling length may be due to the increment of zinc and iron uptake (Dhal et al., 2022).

Hypocotyl Length

Figure 1D presented that the hypocotyl length treated with A2 (1.08 cm) is significantly higher compared to A1 (0.81 cm) and A0 (0.62 cm). Auxin promotes hypocotyl elongation (Yu et al., 2023) by enhancing the BZR1, a central component of the brassinosteroid (BR) signalling pathway which is vital for plant growth (Yu et al., 2023). The production of antioxidant in sprouts treated with plant hormone increased compare to the untreated sprouts (Yan et al., 2024). In contrast, hypocotyl length of hormo and nutri primed *L. sativa* seed was decreased than control (Adhikari et al., 2022).

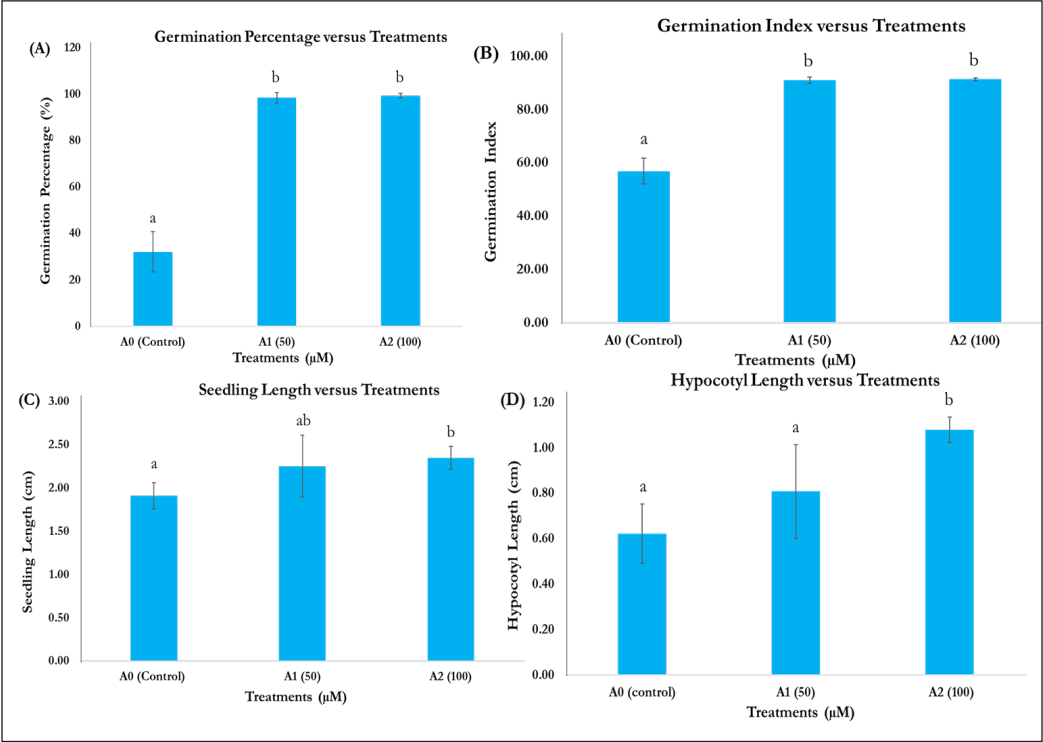


Figure 1. Effects of auxin priming on germination percentage (A), germination index (B), seedling length (C) and hypocotyl length (D) of *A. tricolor*. The results are indicated as mean value \pm standard deviation. Different letters show there are significantly different ($p < 0.05$)

CONCLUSION

The results of this study emphasize that auxin priming could enhance seed germination and seedling growth parameters of *A. tricolor*. This study suggests that 100 μM IBA is the optimum concentration for enhancing germination and seedling growth. It is recommended that further research conduct in field condition to validate the effects of auxin priming under abiotic stress on other important parameters.

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