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Increasing Rice Seedling Growth under Induced Water-Deficient Conditions Using Indole-3-Acetic Acid Produced from *Enterobacter* sp. Bacteria

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ABSTRACT

Purpose: *Water deficiency stress affects rice growth and productivity around the world while reducing indole-3-acetic acid (IAA) levels within plants. This study aims to determine the effects of IAA, produced from the endophytic bacteria Enterobacter sp. (RD4-1-1), on the germination and seedling growth of two lowland rice varieties, namely, RD31 and RD41, under water deficiency stress.*

Research Method: *Water deficiency was simulated in culture medium [using the percentage of polyethylene glycol 6000 (PEG)] and soil cultivation (using different watering frequencies). The effects of RD4-1-1 IAA at various concentrations on rice under water deficiency stress were assessed by measuring a variety of characteristics related to germination and seedling growth.*

Findings: *The effects of RD4-1-1 IAA supplementation in rice depended on the availability of water and the rice variety. RD4-1-1 IAA promoted the germination and growth of rice seedlings according to most measured characteristics, but the effective concentration levels differed with water deficiency in RD31 and RD41 varieties. In particular, the appropriate concentration of bacterial IAA was lower and was in a narrower range when applied to plants in soil compared with that required when IAA was used in plants cultured on medium.*

Originality/ Value: *This study is one of few studies to report the effects of appropriate concentrations of microorganism-produced plant hormones on rice growth under water-deficient conditions.*

Keywords: *Drought stress, Exogenous hormone, Oryza sativa, Plant regulators, Seedling growth*

INTRODUCTION

In modern agriculture, a variety of exogenous substances are used to increase productivity and meet consumer demand, including substances that protect the crops from the effects of climate change (FAO, 2015). However, the substances applied to improve plant productivity under climate change conditions must be considered carefully in terms of the resources and methods used and their residual effects; otherwise, improper practices can create problems that affect the environment (Yohannes, 2016).

Among the stresses caused by climate change, including increase in temperature and light intensity and shortage in rainfall, drought stress is a particularly important stress, as the available water in the soil is insufficient to meet the plant's needs, thereby affecting the various metabolisms in the plant and plant physiology (Seleiman *et al*., 2021; Oguz *et al*., 2022). Drought stress can alter both biochemical and physiological processes in plants, such as the metabolism of phytohormones, antioxidants, ion uptake, and

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stomatal closure (Oguz *et al*., 2022). Water deficiency affects rice plants at all growth stages and reduces productivity, which is a major problem given the status of rice as a staple food crop in many countries (Mustikarini *et al*., 2017). Thus, planting rice varieties that can tolerate or resist dehydrating conditions is a first-line strategy for farmers. However, although a rice variety may be tolerant or resistant to a specific stress, it can also have genetic limitations. Thus, the use of supplementing factors is necessary when growing some rice varieties, and market acceptance of such supplementation must be considered.

Supplementation with plant hormones is one method used to alleviate stress in plants. Indole-3-acetic acid (IAA) is an auxin naturally produced within plants that affects their growth and development, both in the shoot and root tissues, and controls cell division and elongation related to all stages of plant growth (Majda and Robert, 2018). However, IAA production decreases in plants grown under drought conditions (Chaoxing and Jing, 1990). Thus, IAA supplementation has been suggested as a means of enhancing rice growth under stressful conditions, such as water deficiency (Khan *et al*., 2016). Notably, the use of exogenous hormones will only arise if they are used at proper concentrations (Shahab *et al*., 2009). In previous studies, the use of microbial IAA or application of IAA-producing bacteria by inoculation into rice seeds or spraying during the growth stage was shown to efficiently alleviate drought stress in rice; moreover, it is considered one of the most environmentally friendly supplementation methods available (Ahemad and Kibret, 2014; Devarajan *et al*., 2020).

Although a wide range of microbial species is capable of producing plant hormones (Arshad and Frankenberger, 1991), few studies have investigated the proper concentrations at which the hormones produced by these microorganisms should be used in plants. Thus, in the present study, we investigated the effects of a range of IAA concentrations on the water stress conditions applied to germination and during the growth of seedlings of two rice varieties commonly used in Thailand.

MATERIALS AND METHODS

Plant Hormone and Plant Material

IAA produced from endophytic bacteria (*Enterobacter* sp. RD4-1-1) was isolated from upland rice seeds. During the process of IAA production, 0.1 mg/ml tryptophan was added to nutrient broth containing 1% bacterial inoculum, and the nutrient broth was incubated at 30°C for 72 h with mixing rotation. Subsequently, the inoculated suspension product was centrifuged (3×10^3 rpm for 15 min), and the supernatant was collected. The supernatant was sterilized at 121°C for 15 min using an autoclave, and the concentration of IAA in the sterile supernatant was determined according to the method described by Phetcharat and Duangpaeng (2012) before the study progressed. Two lowland non-photosensitive rice varieties namely, var. *RD31* and *RD41*, both of which are commonly grown in Thailand were used in the study.

Simulating Water Deficiency

Water deficiency was simulated using two methods. In a culture medium method, polyethylene glycol 6000 (PEG; catalogue no. 5720, AJAX Finechem, Thermo Fisher Scientific Australia Pty Ltd., Australia) was prepared in MS medium (Murashighe and Skoog, 1962) to simulate different water availability conditions under aseptic conditions. In a soil cultivation method, autoclaved sandy loam soil was used with different soil watering frequencies, which were chosen based on preliminary testing of field capacity percentages in soil reported by Chauhan and Abugho (2013), i.e.

$$Pecentage\ of\ field\ capacity = 100 \times \left[\frac{weight\ of\ soil - weight\ of\ dry\ soil}{weight\ of\ dry\ soil}\right]$$

Treatments and Experiments

Two experiments were conducted to assess the effects of RD4-1-1 IAA at five concentrations, viz., namely, 0, 2.5, 25, 50 and 100 μM, under two planting conditions, i.e. in culture medium (experiment I) and in soil (experiment II). Each experiment was performed to assess the influence of RD4-1-1 IAA under different water deficit simulations of the planting materials.

In experiment I, the five concentrations of RD4-1-1 IAA and four percentages of 6000 PEG (0%, 10%, 20% and 30% constituting the four levels 0, −0.15, −0.49 and −1.03 MPa, respectively) were used as treatments in cultured medium. For each treatment, sterile un-husked seeds were soaked with RD4-1-1 IAA for 8 h, after which the husked seeds were sown on sterile MS medium in a Petri dish for one week under 16/8-h light/dark conditions at 25°C–26°C.

In experiment II, the five concentrations of RD4-1-1 IAA and three watering frequencies (once every 2, 4 or 7 days) were used as treatments. For each treatment, sterile un-husked seeds were soaked with RD4-1-1 IAA for 24 h, after which they were incubated in moist conditions until the coleorhiza protruded. The germinated seeds were planted in plastic blocks containing 4 kg of sterile soil. One week after epicotyl emergence, 100 ml of RD4-1-1 IAA was sprayed once per treatment onto individual plants. Subsequently, spraying with 250 ml of distilled water was conducted according to treatment frequency.

Determination of Plant Characteristics

In experiment I, various characteristics were recorded for rice germination on MS medium. For experiment II, characteristics were recorded for seed emergence above the soil. Thus, the recorded characteristics differed between experiments I (germinated seeds) and II (emerged seeds), despite the definitions being similar.

Below, the formulae used to quantify characteristics are described for experiment I. For experiment II, the word ‘emergence’ or ‘E’ was used instead of ‘germination’ or ‘G’. In experiment I, the following characteristics were measured: germination percentage (GP), mean time to germination (MTG), speed germination index (SGI), germination rate (GR), co-efficient of germination (CG), vigour index (VI), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW) and SDW/RDW ratio. The formulae used to calculate these characteristics are given below.

$$\mathrm{GP} = \frac{GN}{SN} \times 100,$$

where is the total number of seeds germinated and is the total number of seeds tested (Belwal *et al*., 2015).

$$\mathrm{MTG} = \sum \frac{(ni \times di)}{\mathrm{N}},$$

where *ni* is the number of germinated seeds in day *i*, *di* is the accumulation of days since sowing and *N* is the total number of germinating seeds (Maraghni *et al*., 2010).

The SGI was calculated according to AOSA (1983) and Yousof and El-Saidy (2014) as below:

$$\mathrm{SGI} = \frac{No.of\ germinated\ seeds\ in\ first\ count}{Days\ of\ first\ count} + \cdots + \cdots + \frac{No.of\ germinated\ seeds\ in\ final\ count}{Days\ of\ final\ count},$$

$$\mathrm{GR} = \frac{a + (a+b) + (a+b+c) + \ldots + (a+b+c+m)}{n(a+b+c+m)},$$

where *a*, *b* and *c* are the number of germinated seeds counted at the first, second and third, respectively; m is the number of germinated seeds counted on the last day; and n is the total number of seeds counted (Bartlett, 1937; Yousof and El-Saidy, 2014).

$$\mathrm{CG} = \frac{100\ (A1 + A2 + \ldots + An)}{A1T1 + A2T2 + \ldots + AnTn},$$

where *A* is the number of germinated seeds, *T* is time (days) corresponding to *A* and *N* is the number of days to the final count (Copeland and McDonald, 1976; Yousof and El-Saidy, 2014).

$$\mathrm{VI} = \frac{\%\mathrm{GR} \times \mathrm{MSH}}{100},$$

where %GR is the final GP and MSH is the mean seedling height (Abdul-Baki and Anderson, 1973; Alizadeh *et al*., 2013).

In experiment II, seedling growth was measured in weeks 1–3 after planting. The fresh and dry weights of shoots and roots of all plants in all replicates were measured by cutting and separating each part, weighing for fresh weight, oven-drying at 60°C for 2 days and then weighing for dry weight. The dry weight ratio between the shoot and root tissues was also calculated.

Experimental Design and Data Analysis

ANOVA was used to analyse data in this study with a significant level of $P < 0.05$. A 5 × 4 factorial completely randomised design (CRD) with 10 replications (1 Petri dish per replication; 10 seeds per Petri dish) was used in experiment I. Two factors were the five concentrations of RD4-1-1 IAA used for seed soaking and the four concentrations of 6000 PEG in the MS medium. The 5 × 3 factorial CRD was used in experiment II. Two factors were the five concentrations of RD4-1-1 IAA used for soaking the seeds and seedling spraying and the three different watering frequencies. Experiment II had four replications, with 1 plastic block per replication and 50 plants per plastic block.

RESULTS AND DISCUSSION

Effects of IAA and PEG on Germination and Seedling Characteristics

In experiment I, for *RD31*, 5 of 11 characteristics were significantly affected by the IAA × PEG interaction, including VI, SL, RL, SDW and SDW/RDW (Table 01). Of the six characteristics not significantly affected by this interaction, PEG only had a significant effect on three characteristics: GP, SGI and RDW. Therefore, SGI was the only speed of germination-related trait (the others being MTG, GR and CG) that was significantly affected by the stress of unavailable water. Compared with the GP value at 0% PEG (99.8%), GP values decreased at 10% PEG (84.5%) and reached zero at 30% PEG (Table 01, see note). Thus, the values of other characteristics could not be detected when the PEG treatment was 30%.

In *RD31*, for four of the five characteristics (VI, SL, RL and SDW) that were significantly affected by the interaction effect between PEG concentrations and RD4-1-1 IAA levels, the highest mean values were observed in seeds soaked with RD4-1-1 IAA at 25–50 µM during culturing, either with 0% or 10% PEG in the MS medium (Figure 01). At the 20% PEG treatment rate, higher values of VI, SL, RL and SDW were observed with applications of 50–100 µM of RD4-1-1 IAA (Fig. 01). For these characteristics, excluding RL and SDW/RDW (data not shown), 10% PEG produced the most pronounced effects, followed by 0% and 20% PEG, respectively.

In *RD41*, 8 of 11 characteristics were significantly affected by the IAA × PEG interaction, excluding SDW, RDW and SDW/RDW (Table 02). Of the three characteristics not significantly affected by this interaction, PEG significantly affected SDW and RDW. Similar to *RD31*, however, the negative effect on GP value was observed starting at 20% PEG (75.2%), and GP and other characteristics were not detected at 30% PEG (Table 02, see note).

Table 01: Mean and ANOVA data for 11 characteristics [6 germination-related traits (a) and 5 seedling-related traits (b)] in var. *RD31* when seeds were soaked with indole-3-acetic acid (IAA) produced from bacteria (RD4-1-1) and cultured on sterile solid agar containing polyethylene glycol 6000 (PEG).

(a)

Factors	GP (%)	SGI	MTG	GR	CG	VI
RD4-1-1 IAA	65.5-71.9 NS	2.18-2.39 NS	3.00NS	1.00NS	33.28-33.33 NS	1.49-2.68 **
PEG	0-99.8**	0-3.32**	3.00NS	1.00NS	33.30-33.33 NS	0.38-3.66**
P-value (RD4-1-1 IAA x PEG)	0.998 NS	0.998 NS	0.362 NS	0.362NS	0.362 NS	1.29×10^{-4} **
Overall mean	70.31	2.34	3.00	1.00	33.32	1.97

(b)

Factors	SL (cm)	RL (cm)	SDW (g)	RDW	SDW/RDW
RD4-1-1 IAA	1.49-2.68**	2.47-3.90**	4.57×10^{-3}-8.42×10^{-3}**	0.012-0.018 NS	0.393-0.936*
PEG	0.38-3.66**	0.64-4.12**	3.38×10^{-3} -10.28×10^{-3}**	0.0046-0.0307**	0.381-0.956**
P-value (RD4-1-1 IAA x PEG)	1.29×10^{-4}**	9.16×10^{-3}**	2.97×10^{-3}**	0.613NS	0.0210*
Overall mean	1.97	2.87	6.23×10^{-3}	0.0153	0.650

*Note: (a) GP = germination percentage; SGI = speed germination index; MTG = mean time of germination; GR = germination rate; CG = co-efficient of germination; VI = vigour index.; GP (%): 0% PEG = 99.8 a; 10% PEG = 84.5 b; 20% PEG = 97.0 a; 30% PEG = 0 c.; SGI: 0% PEG = 3.32 a; 10% PEG = 2.81 b; 20% PEG = 3.23 a; 30% PEG = 0 c.; (b) SL = shoot length; RL = root length; SDW = shoot dry weight; RDW = root dry weight.; Not available (NA) with 30% PEG for MTG, GR, CG, VI, SL, RL, SDW, RDW, and SDW/RDW. RDW (g): 0% PEG = 0.0137 b; 10% PEG = 0.0307 a; 20% PEG = 0.0046 c; 30% PEG = NA.;*P<0.05 and **P<0.01; NS, non-significant difference at P≥0.05. a, b, c, different letters indicate significant differences at P<0.05.*

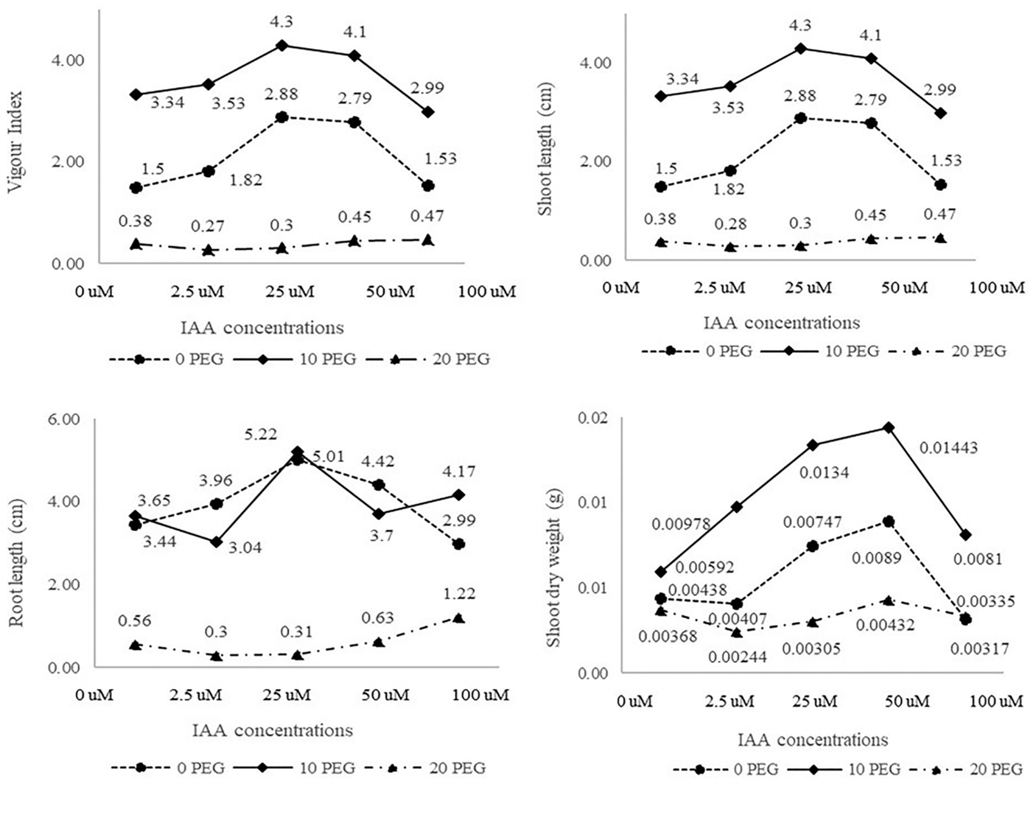

Figure 01: Changes in the mean values of five characteristics measured at the germination and early seedling stages in lowland rice var. *RD31* when seeds were soaked with different RD4-1-1 IAA concentrations and cultured with different polyethylene glycol 6000 (PEG) levels.

Table 02: Mean and ANOVA data for 11 characteristics [6 germination-related traits (a) and 5 seedling-related traits (b)] in var. *RD41* when seeds were soaked with indole-3-acetic acid (IAA) produced from bacteria (RD4-1-1) and cultured on sterile solid agar containing polyethylene glycol 6000 (PEG).

(a)

Factors	GP (%)	SGI	MTG	GR	CG	VI
RD4-1-1 IAA	60.3-72.9**	1.84-2.16**	3.40-3.76*	0.89-0.945*	28.60-29.99*	0.94-1.60*
PEG	0-99.0**	0-3.28**	3.02-4.57**	0.777-0.997**	22.51-33.10**	0.16-2.05**
P-value (RD4-1-1 IAA x PEG)	8.77×10^{-10}**	6.08×10^{-14}**	2.25×10^{-7}**	8.1×10^{-8}**	1.49×10^{-9}**	5.89×10^{-11}**
Overall mean	67.4	2.03	3.56	0.921	29.29	1.17

(b)

Factors	SL (cm)	RL (cm)	SDW (g)	RDW (g)	SDW/RDW
RD4-1-1 IAA	0.97-1.65**	1.40-2.13**	$0.68\text{-}1.52\times10^{-2}$NS	0.0111-0.0270*	0.497-1.336NS
PEG	0.18-2.08**	0.29-3.49**	$0.217\text{-}1.45\times10^{-2}$**	0.0033-0.0301**	0.535-1.375NS
P-value (RD4-1-1 IAA x PEG)	1.04×10^{-9}**	6.23×10^{-4}**	0.3614NS	0.2123NS	0.381NS
Overall mean	1.24	1.79	0.0105	0.0212	0.903

*Note: (a) GP = germination percentage; SGI = speed germination index; MTG = mean time of germination; GR = germination rate; CG = co-efficient of germination; VI = vigour index.; (b) SL = shoot length; RL = root length; SDW = shoot dry weight; RDW = root dry weight.; Not available (NA) with 30% PEG for MTG, GR, CG, VI, SL, RL, SDW, RDW, and SDW/RDW. GP (%): 0% PEG = 99.0 a; 10% PEG = 95.9 a; 20% PEG = 75.2 b; 30% PEG = 0% c. SDW (g): 0% PEG = 0.0141 a; 10% PEG = 0.0145 a; 20% PEG = 0.0022 b; 30% PEG = NA. RDW (g): 0-μM IAA = 0.0270 a; 2.5-μM IAA = 0.0255 a; 25-μM IAA = 0.0111 b; 50-μM IAA = 0.0193 ab; 100-μM IAA = 0.0233 a; 0% PEG = 0.0301 a; 10% PEG = 0.0229 a; 20% PEG = 0.0033 b; 30% PEG = NA.; *P<0.05 and **P<0.01; NS, non-significant difference at P≥0.05.; a, b, different letters indicate significant differences at P<0.05*

For *RD41*, the highest mean values of the characteristics, excluding MTG, were detected at 0% PEG, followed by 10% and 20% PEG, respectively (Fig. 02). For many characteristics, higher values were observed at 25–50 μM RD4-1-1 at 0% or 10% PEG. However, at 20% PEG, GP and the speed germination-related traits (SGI, MTG, GR and CG) had higher values when IAA was applied at 2.5–25.0 μM. All seedling growth-related traits (VI, SL and RL) decreased with PEG at 20% and RD4-1-1 applied at all concentrations.

In experiment I of this study, a small decrease in the GP value was shown (~15%) at osmotic potential from approximately −0.15 to −0.49 MPa (osmotic potential; i.e. ~10%–20% PEG), although GP was reduced by 100% at approximately −1 MPa (30% PEG). The reduction in GP recorded in the present study was lower than the reduction reported by Pirdiashit *et al*. (2003) in 15 rice lines (reductions of approximately 50%, 70%, and 100% at −0.3, −0.5, and −1 MPa, respectively), which may have been affected by soaking the seeds in RD4-1-1 IAA solution at different concentrations before they were sown. Negative impact on the germination of seeds of many plant species at high osmotic potentials of more than −1.0 MPa has been previously reported (Mohammadkhani and Heidari, 2008). Thus, in *RD31*, VI, SL, and SDW had higher values at 10% PEG (approximately −0.15 MPa) than at 0% PEG. However, when high levels of PEG was used, i.e. PEG at 20% (approximately −0.49 MPa), the values of VI, SL, RL, and SL decreased markedly. Pirdashti *et al*. (2003) reported a reduction in the value of plumule dry weight in rice that began at −0.3 MPa for nonprimed seeds sown under various PEG treatment conditions. Although the data are not shown here, the highest SDW/RDW value was observed with treatment at 20% PEG, indicating that water deficiency at the early seedling stage affected the root tissue more than it affected the shoot tissue.

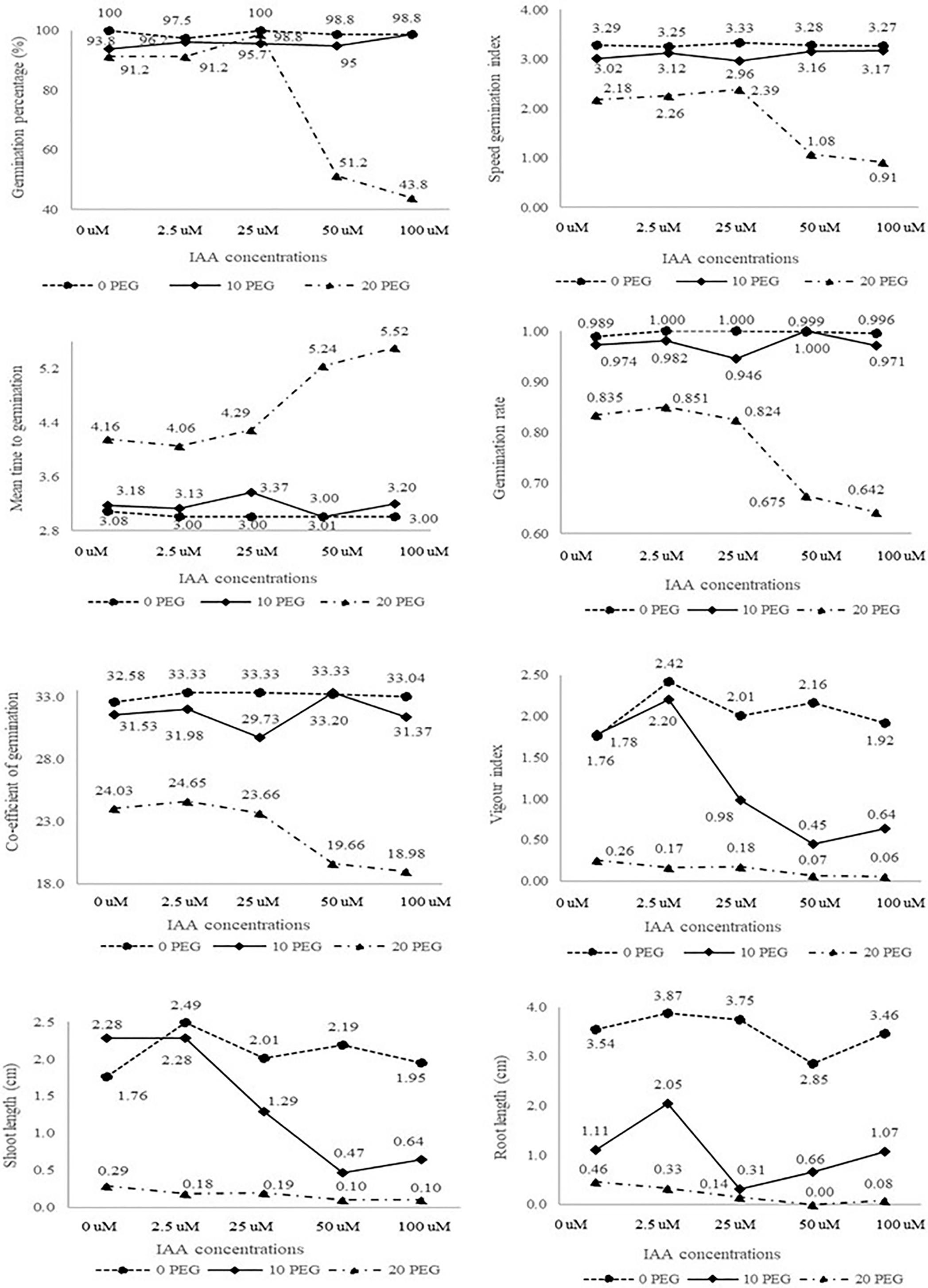

Figure 02: **Changes in the mean values of eight characteristics measured at the germination and early seedling stages in lowland rice var. *RD41* when seeds were soaked with different RD4-1-1 IAA concentrations and cultured with different polyethylene glycol 6000 (PEG) levels.**

PEG is a substance frequently used to study water availability stress in growing plants because of its high molecular weight. With treatment, water cannot transmit through plant cell walls, resulting in controlled water potential and cytorrhysis (Mohammadkhani and Heidari, 2008). When osmotic potential is less than −0.6 to −0.8 MPa, water is still available to start the germination mechanism (phases I and II) and root cell growth (phase III), although these abilities may be reduced under such conditions (Mohammadkhani and Heidari, 2008; Pant and Bose, 2016; Hellal *et al*., 2018). Accordingly, in *RD31* treated with an increased exogenous IAA concentration (50–100 µM) and 20% PEG, higher values were detected for many characteristics compared with the values observed when PEG was 0% and 10% and exogenous IAA was 25–50 µM.

For both *RD31* and *RD41* varieties, the effect of PEG began at 10%. However, *RD41* was seemingly more sensitive to water deficiency and responded to a lower concentration of IAA when compared with *RD31*. Plant hormone expression is dominated by genetics, the environment and the interaction between the two (Zhang *et al*., 2009). Thus, our results differed between the tested rice varieties (Figure 01 and Figure 02). Although IAA levels have been associated with dehydration stress in plants, the results are complex, including either a decrease in the exposure to stress or an increase in the adaptation process (Park, 2007).

Effects of IAA and Watering Frequencies on Emergence and Seedling Characteristics

In experiment II, for *RD31*, 3 of 12 characteristics were significantly affected by the IAA × watering interaction, including, EP, CE, and VI. The concentration of IAA significantly affected three characteristics that were not affected by IAA × watering, which were SEI, ER, and SL, at week 3. Additionally, bacterial IAA supplementation significantly affected EP and other characteristics related to speed of emergence (i.e. SEI, ER and CE), excluding MTE (Table 03).

Table 03: Mean and ANOVA data for 12 characteristics [6 germination-related traits (a) and 6 seedling-related traits (b)] in var. *RD31* when seeds were soaked and seedlings sprayed with indole-3-acetic acid (IAA) produced from bacteria (RD4-1-1) and grown in soil with various watering frequencies.

(a)

Factors	EP (%)	SEI	MTE	ER	CE	VI
RD4-1-1 IAA	72-100**	1.21-1.64**	2.62-3.67NS	0.78-0.91*	29.54-37.15*	4.41-10.84**
Watering	86.25-97.50**	1.22-1.56**	2.89-3.28NS	0.82-0.88NS	32.75-36.14NS	6.20-10.24**
P-value (RD4-1-1 IAA x Watering)	3.79×10^{-4}**	0.3235NS	0.105NS	0.0614NS	0.0336*	0.0387*
Overall mean	90.83	1.37	3.13	0.85	34.53	7.76

(b)

Factors	SL_W3 (cm)	RL_W3 (cm)	RC_W3	SDW_W3(g)	RDW_W3(g)	SDW/RDW
RD4-1-1 IAA	17.30-23.75**	7.08-8.39NS	3.62-4.46NS	0.011-0.002NS	4.87×10^{-3} -6.22×10^{-3}NS	1.864-2.555NS
Watering	18.90-22.62**	6.57-9.80**	3.70-4.41*	0.013-0.014NS	5.07×10^{-3} -5.77×10^{-3}NS	2.086-2.463NS
P-value (RD4-1-1 IAA x Watering)	0.3015NS	0.126NS	0.5976NS	0.372NS	0.395NS	0.242NS
Overall mean	20.61	7.81	3.98	0.014	0.0055	2.297

*Note: (a) EP = emergence percentage; SEI = speed emergence index; MTE = mean time of emergence; ER = emergence rate; CE = co-efficient of emergence; VI = vigour index.; SEI: 0-µM IAA =1.21 b; 2.5-µM IAA = 1.64 a; 25-µM IAA = 1.36 b; 50-µM IAA = 1.24 b; 100-µM IAA = 1.40 b; watering every 2 days = 1.56 a; every 4 days = 1.33 b; every 7 days = 1.22 b.; ; (b) SL = shoot length; RL = root length; RC = root score; SDW = shoot dry weight; RDW = root dry weight; W3 = week 3.; SL (cm): 0-µM IAA =19.95 b; 2.5-µM IAA = 23.75 a; 25-µM IAA = 22.91 a; 50-µM IAA = 17.30 b; 100-µM IAA = 19.11 b; watering every 2 days = 22.62 a; every 4 days = 20.30 b; every 7 days = 18.90 b.; RL (cm): watering every 2 days = 6.57 b; every 4 days = 9.80 a; every 7 days = 7.07 b.; RC: watering every 2 days = 3.70 b; every 4 days = 4.41 a; every 7 days = 3.81 b.; *P<0.05 and **P<0.01; NS, non-significant difference at P≥0.05; a, b, different letters indicate significant differences at P<0.05*

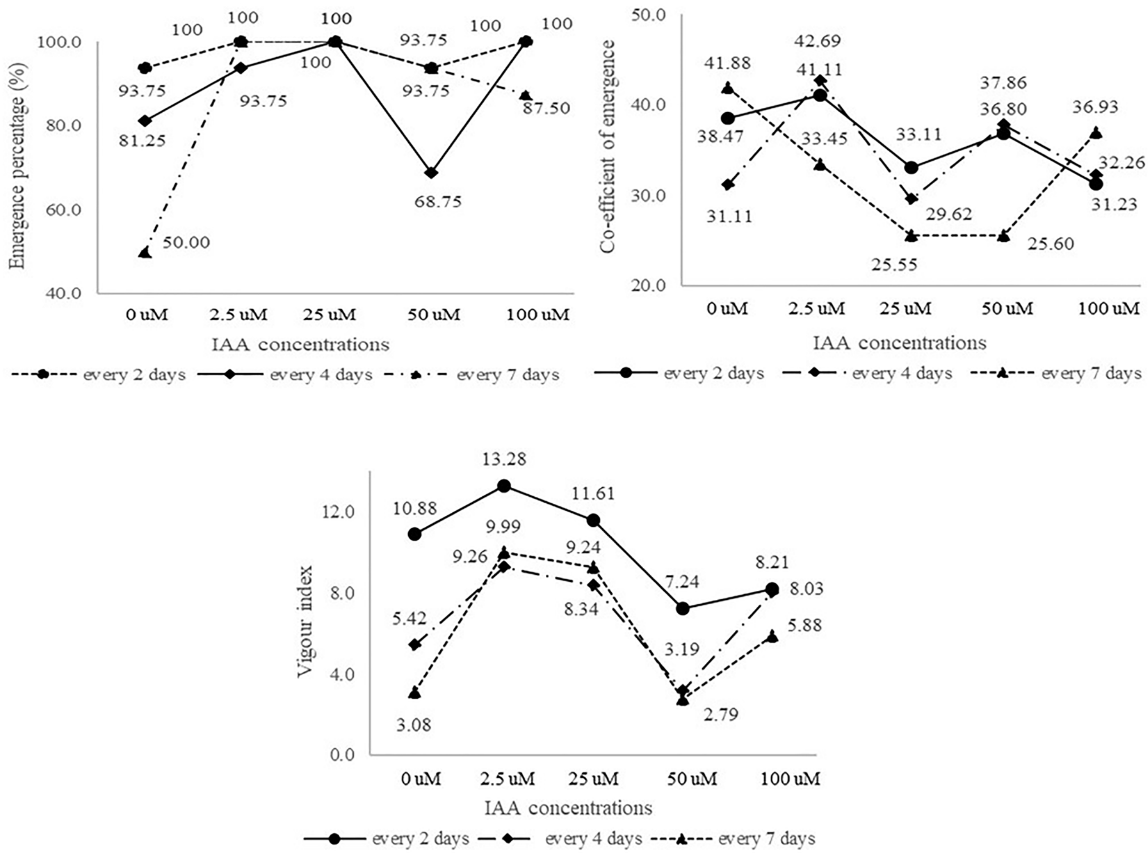

Figure 03: **Changes in the mean values of three characteristics measured at the germination and early seedling stages in lowland rice var. RD31 when seeds were soaked and seedlings sprayed with different RD4-1-1 IAA concentrations and grown in soil with different watering frequencies.**

In *RD31*, the mean values of three characteristics, EP, CE, and VI, were significantly affected by the IAA × watering interaction (Fig. 03). For each watering frequency, treatments with RD4-1-1 IAA were applied to the seeds by soaking, which led to the increase in the EP values. The highest EP and VI values were observed at IAA concentrations of 2.5–25.0 µM for all frequencies of watering. For the CE trait, watering every 2 or 4 days produced the highest values with 2.5 µM IAA. However, when watering occurred every 7 days, IAA at 100 µM produced the highest CE values.

In *RD41*, only 2 of 12 characteristics were significantly affected by the IAA × watering interaction, i.e. SEI and CE (Table 04). Of those characteristics that were not significantly affected, either IAA concentration or watering frequency significantly affected different traits. Characteristics related to germination (EP, SEI, CE and VI) and seedling growth (SL, RL and RC) were significantly affected by the concentration of IAA, whereas all dry weight characteristics (SDW, RDW and SDW/RDW) were significantly affected by frequencies of watering. In *RD41*, IAA RD4-1-1 supplementation at 2.5 µM by seed soaking or seedling spraying produced the highest values for many characteristics, including EP, VI and SL (data not shown).

During soil cultivation, the emergence of seedlings from the soil is dependent on the energy and vigour of the seedling, especially when the soil is firm and hard due to dehydration (Shi *et al*., 2020). Water in the soil not only contributes to activity within the seedling but also enables the plant to absorb nutrients from the soil. In the present study, there was a significant positive effect of IAA on most characteristics when it was used for seed soaking regardless of watering frequencies for *RD31* (2.5–25.0 µM IAA) and *RD41* (2.5 µM IAA). Notably, exogenous supplementation with hormones may change the hormonal balance in plants under both stress and non-stress conditions (El-Mergawi *et al*., 2020). Exogenous auxin application has been reported to affect the biosynthesis of abscisic acid and gibberellin, which are the main hormones in seed germination (Shuai *et al*., 2017). Moreover, the proper concentration of exogenous IAA varies across species, which could be due to several factors, including plant genetics, hormone activity and stage of testing.

Increasing the early vigour of seedlings using IAA supplementation has many advantages, especially in relation to rice fields. Stand establishment of rice seedlings in the field provides the ability to intercept solar energy and outcompete weeds or other species that have contaminated the growing area. Consequently, IAA supplementation is an effective practice for farmers, along with watering, fertilising and weeding. Given these advantages, seedling vigour can be considered one of the important criteria to consider when deciding on the benefits of using external IAA (McKenzie *et al*., 1994).

Table 04: Mean and ANOVA data for 12 characteristics [6 germination-related traits (a) and 6 seedling-related traits (b)] in var. *xx* when seeds were soaked and seedlings sprayed with indole-3-acetic acid (IAA) produced from bacteria (RD4-1-1) and grown in soil with various watering frequencies..

(a)

Factors	EP (%)	SEI	MTE	ER	CE	VI
RD4-1-1 IAA	85.42-100*	1.07-1.52**	2.74-3.65NS	0.76-0.89NS	28.43-37.21**	5.75-10.22**
Watering	91.25-97.50NS	1.25-1.34NS	3.18-3.43NS	0.80-0.83NS	31.52-32.09NS	6.41-10.72**
P-value (RD4-1-1 IAA x Watering)	0.5950NS	0.0206*	0.0616NS	0.0616NS	0.0254*	0.6280NS
Overall mean	95	1.30	3.31	0.81	31.84	8.19

(b)

Factors	SL_W3(cm)	RL_W3(cm)	RC_W3	SDW_W3(g)	RDW_W3(g)	SDW/RDW
RD4-1-1 IAA	17.00-21.30*	5.23-7.72**	3.31-4.43**	0.018-0.028NS	0.0098-0.0143NS	1.706-2.454NS
Watering	16.54-22.58**	6.45-6.80NS	3.60-4.06NS	0.016-0.032**	0.0096-0.0141**	1.656-2.386*
P-value (RD4-1-1 IAA x Watering)	0.8997NS	0.3643NS	0.2711NS	0.0712NS	0.8744NS	0.1014NS
Overall mean	19.21	6.65	3.87	0.022	0.0114	1.995

*Note: (a) EP = emergence percentage; SEI = speed emergence index; MTE = mean time of emergence; ER = emergence rate; CE = co-efficient of emergence; VI = vigour index.; P (%): 0-µM IAA =93.75 ab; 2.5-µM IAA = 97.92 a; 25-µM IAA = 100 a; 50-µM IAA = 85.42 b; 100-µM IAA = 97.92 a.; VI: 0-µM IAA = 8.46 ab; 2.5-µM IAA = 9.95 a; 25-µM IAA = 7.76 b; 50-µM IAA = 4.90 c; 100-µM IAA = 4.90 c; watering every 2 days = 11.07 a; every 4 days = 6.90 b; every 7 days = 7.57 b.; (b) SL = shoot length; RL = root length; RC = root score; SDW = shoot dry weight; RDW = root dry weight; W3 = week 3.; SL (cm): 0-µM IAA = 18.81 abc; 2.5-µM IAA = 21.30 a; 25-µM IAA = 18.72 bc; 50-µM IAA = 17.00 c; 100-µM IAA = 20.17 ab; watering every 2 days = 22.58 a; every 4 days = 18.46 b; every 7 days = 16.54 b.; RL (cm): 0-µM IAA = 6.57 ab; 2.5-µM IAA = 7.14 a; 25-µM IAA = 6.59 a; 50-µM IAA = 5.23 b; 100-µM IAA = 7.72 a.; RC: 0-µM IAA = 3.35 bc; 2.5-µM IAA = 4.38 a; 25-µM IAA = 3.85 ab; 50-µM IAA = 3.31 c; 100-µM IAA = 4.43 a.; DW(g): watering every 2 days = 0.032 a; every 4 days = 0.017 b; every 7 days = 0.016 b.; RDW(g): watering every 2 days = 0.0141 a; every 4 days = 0.0096 b; every 7 days = 0.0105 b. SDW/RDW ratio: watering every 2 days = 2.386 a; every 4 days = 1.940 ab; every 7 days = 1.656 b. *P<0.05 and **P<0.01; NS, non-significant difference at P≥0.05.; , b, c, different letters indicate significant differences at P<0.05*

CONCLUSIONS

For the lowland rice varieties *RD31* and *RD41*, increasing PEG levels in MS medium resulted in a decreased GP, and the rice was unable to germinate at 30% PEG. In *RD31*, characteristics related to seed germination (GP, SGI and VI) and seedling growth (SL, RL, SDW, RDW and SDW/RDW) were affected by the individual factors, i.e. RD4-1-1 IAA and PEG concentrations, as well as the interaction between these factors. In *RD31*, VI, SL, RL and SDW values were highest at 0% and 10% PEG when IAA was at 25–50 µM, but IAA needed to increase to 50–100 µM when PEG increased to 20%. In *RD41*, almost every characteristic (both germination- and seedling growth-related characteristics, excluding SDW/RDW) was significantly affected by either the individual, combined or interacting factors. Higher values for characteristics were detected with PEG at 0% or 10% when seeds were soaked with 25–50 µM IAA. However, IAA concentration showed a positive effect on germination-related characteristics at levels <25 µM when PEG was at 20%. Although a significant reduction in the values of characteristics was observed for both *RD31* and *RD41* rice varieties in medium containing PEG at 10% or above, *RD41* was more sensitive to water deficiency than *RD31*.

In our soil cultivation experiment, the individual factors (i.e. RD4-1-1 IAA and watering frequency) or the interaction between these factors affected characteristics in both rice varieties, but the effects varied. In *RD31*, soaking the seeds with 2.5–25 µM IAA promoted an increase in the mean values of most characteristics regardless of watering frequency. However, only soaking the seeds at 2.5 µM IAA enhanced the characteristics of *RD41*. Thus, using exogenous RD4-1-1 IAA at the lowest concentration efficiently improved many plant characteristics in rice cultivated in soil compared with rice cultured in MS medium.

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