International Journal of Agricultural Technology 2022Vol .18(5):2089-2108
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Alleviation of salinity stress at the early seedling stage in rice by using exogenous indole-3-acetic acid (IAA) produced from *Enterobacter* sp.

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Na Chiangmai, P., Khianngam, S., Pinwised, W., Anuphanchai, J., Rienghlam, P. and Meetum, P. (2022). Alleviation of salinity stress at the early seedling stage in rice by using exogenous indole-3-acetic acid (IAA) produced from *Enterobacter* sp. International Journal of Agricultural Technology 18(5):2089-2108.

Abstract The effectiveness of indole-3-acetic acid (IAA) produced by isolated-endophytic bacterium, *Enterobacter* sp. RD4-1-1 from upland rice seed compared with synthetic IAA on seed/seedling emergence and growth and the effectiveness of external IAA derived from in rice *Enterobacter* sp. RD4-1-1 growing under salty stress by varied sodium chloride (NaCl) concentrations were reported. The results showed statistical significance in many emergence-related characteristics and most of them showed higher value at 2.5 µM RD4-1-1 IAA. However, low concentration of IAA from RD4-1-1 (2.5 µM) had similar effectiveness to high concentration of synthetics IAA at 2.5 µM in stimulating seed emergence and seedling growth. The positive effect of using exogenous IAA on soaked seeds before seeding showed that it can alleviate salinity stress, increased rice germination, and promoted seedling growth. The speed germination index (SGIs) was evaluated as appropriate to indicate salinity tolerance in rice varieties and exhibited the change of values under salinity stress was consistent with vigor index (VI) and shoot length (SL). Although there were many characteristics in terms of germination and seedling growth, it showed significantly affected by the interaction between factors (IAA x salinity) in these rice varieties. The concentration of exogenous IAA that was appropriated for using in priming the seeds in sterile medium at 2.5 µM. However, under testing in culture media, the proper IAA concentration was increased at 25 µM. Moreover, increasing the IAA concentration to 50 or 75 µM IAA is recommended when salinity level (≥ 8 dS/m) increased which depended on the trait in each variety.

Keywords: Exogenous hormone, Endophytic bacteria, Salt stress, Plant growth regulators, *Oryza sativa*

Introduction

Salt stress is one of the most severe abiotic stresses to plant production. The expected increase of this stress in many areas is a consequence from both

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global warming and improper agricultural practices by anthropogenic activities (Yadav *et al*., 2011). About 800 million hectares of the land (~ 6%) in the world and about 450 million hectares of land (~14%) in Asia, the Pacific, and Australia have reported these areas were affected by either salinity or sodicity (Rengasamy, 2010; Yadav *et al*., 2011). About one in three of irrigated areas in the world affected by the problem of salt stress (Rahman *et al*., 2017). Moreover, salt stress problem will increase annually as estimated from the increasing of the salinized area at around 10 percent (Shrivastava and Kumar, 2015). Finally, for arable land around 50 percent of areas is estimated to be affected by salinity stress in 2050 (Jamil *et al*., 2011).

Rice (*Oryza sativa* L.) is the staple food of people all over the world—the food production is about 40% in global—and has a growing area spread across many continents as well (Herman *et al*., 2015). However, rice has been categorized as a moderately salt-sensitive crop (Aref and Rad, 2012) in a glycophyte type (Reddy *et al*., 2017), in which salinity interferes in many stages of rice production (i.e., germination, growth, and development) both in vegetative and reproductive phases as reported by Hakim *et al*. (2010), and Gupta and Huang (2014). For this reason, using the tolerant-salinity rice varieties as the first choice to alleviate the salinity stress problem in planting areas was suggested. Unfortunately, rice-growing farmers in Thailand do not have any rice varieties that are resistant to planting in extremely saline soil condition. For this reason, farmers will apply additional fertilizers in the fields, in extreme amounts, to increase rice growth and the yield of rice affected by salinity. The use of fertilizers, especially chemical fertilizers, that exceeds the usability of crops, especially in saline soil conditions, increases the farming cost. In addition to this, fertilizers that cannot be absorbed by those plants can leach and contaminate the groundwater. That is one issue causing more environmental problems (Sunitha *et al*., 2013).

The effect of salinity which firstly affects the ability of germination of rice. For practice in the field, transplanting of seedling has been suggested to alleviate the stress of salinity in the germination stage of rice (Reddy *et al*., 2017). However, there is a lack of labor and the cost of rice transplanting practice is high in Thailand, either manually or by machines. This has allowed the Thai farmers to choose from sowing germinated seeds instead of transplanting the seedlings for rice cultivation in many areas affected by salinity problems. Other methods for alleviating rice planting problems in saline soils include the use of external substances such as plant growth regulators. As widely reported by Pieterse *et al*. (2012), the supplying of exogenous plant growth regulators to plants in the stress condition can adjust hormones to balance the inside of the plant. This has also been confirmed by

Javid *et al*. (2011a, 2011b) who stated that for substances such as plant-growth regulators, the supplying of exogenous indole-3-acetic acid (IAA) has been popularly reported to relieve salt stress on seed germination and plant growth. In addition, Egamberdieva (2009) also attested the positive roles of synthetic IAA on germination and growth characteristics of seedlings in cereals. However, the use of bacterial hormones, including IAA has been reported to be effective to plant production and environmentally friendly (Shi *et al*., 2017).

There is an IAA currently being produced from a variety of genus of bacteria, such as *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Acetobacter*, *Klebsiella*, *Enterobacter*, *Arthrobacter* and *Xanthomonas*, but their use must also be considered to the concentration levels that are necessary to plant growth (Tsavkelova *et al*., 2006; Ahmad *et al*., 2008; Li *et al*., 2018). In symbiosis relationship between bacteria and plant, the bacterial auxin can affect various auxin-related processes in the host plant. Thus, the use of exogenous auxin produced from bacteria may interfere with the growth process in the target plant as well (Spaepen and Vanderleyden, 2011). In addition to using external IAA on plant growth-promoting at the normal condition, it was also used to solve crop problems under certain unsuitable environments such as the elongation of coleoptile in lowland rice grown under waterlogging conditions (Kefford, 1961). More than waterlogging for lowland rice practice, IAA producing bacteria was reported to promote seedling root growth in wheat under salt stress as well (Egamberdieva, 2009). However, many previous studies on bacterial IAA production employed bacterial suspension method, which mostly did not specify the concentration of the hormone (Tabatabaei *et al.*, 2016; Saengsange, 2018). For studies on synthetic IAA, various concentrations have been reported to successfully promote seed germination, depending on plant species, varieties, and cultural patterns. Therefore, the comparison between synthetic IAA and bacterial IAA need to be investigated in this research.

Hence, the objective of the study was to assess the influence of IAA produced by endophytic bacteria on various characteristics in germination and early growth of seedlings.

Materials and methods

The following experiments were carried out at the Plant Tissue Culture Laboratory of Animal Sciences and Agricultural Technology Faculty, Silpakorn University, IT Campus, Cha-Am, Phetchaburi, Thailand, from July to September in 2019.

Enterobacter sp. RD4-1-1 and IAA preparation

Enterobacter sp. RD4-1-1, an endophytic bacterium, was isolated from upland rice seed. This bacterium was cultured in Nutrient Agar (NA) slant and kept at Microbiology Laboratory of Animal Sciences and Agricultural Technology Faculty, Silpakorn University.

IAA product of *Enterobacter* sp. RD4-1-1 was prepared with using Nutrient Broth (NB) medium mixing with 100 ug/ml L-Tryptophan and culture of the 1% inoculum under incubated at 30◦C for 72 h on a rotary shaker. The cultural bacteria was centrifuged at 3,000 rpm for 15 minutes and collected the supernatant. The IAA quantity was detected as described by Phetcharat & Duangpaeng (2012). The control was NB + 100 μg/ml L-Tryptophan but was not added in culture.

Before experiments, the IAA was sterilized at 121 ℃ for 15 minutes using an autoclave for sterilization. The sterilized IAA supernatant was used in the study according to the IAA concentration treatment, and was used as one factor for testings.

Plant materials and experimental treatments

The study selected both the material and treatments based on the salinity levels and rice variety name which were set up from the real practice and information of rice planting area of farmers who are facing salinity problem in Ban Laem Subdistrict, Cha-am District, Phetchaburi Province, Thailand. This area is near the coast, thus, the location is experiencing saline soil problems. Two lowland rice varieties— var. RD31 and var. RD41, both well-known rice varieties in Thailand—were used in this study. The rice varieties were selected as non-photosensitive, which could tolerate low levels of salinity. However, these varieties are popular with farmers in Thailand because thery are of good quality. These seed varieties were collected at the maturity stage from the farmers' field in 2018.

Four experiments (A-D) were conducted in this study. In experiment A, evaluating the usefulness of IAA produced from bacteria compared with commercial synthetic IAA; for seed emergence and seedling growth in early stage after germination. Two main factors included two rice varieties and seven sources and concentrations of IAA [including as 0 μM IAA (nil IAA, control treatment): 0.25, 2.5, 25 μM RD4-1-1 IAA and 0.25, 2.5, 25 μM synthetic IAA). Rice seeds of RD31 and RD41 varieties were sterilized and soaked according to each IAA treatment for eight hours. After that, seeds were sowed in autoclaved sand (4 kg in weight) in a plastic basket; 100 seeds per basket,

with four replications. The sterile nil water was sprayed two times each day at 250 ml per basket each time for three weeks after sowing. About one week after sowing, the epicotyl was observed above the soil. Seedling at week 2-3 was then sprayed with Hoagland solution (pH 5.6) at 35 ml once per week (Hoagland and Arnon, 1950). Then, 10 plants in each basket were kept for plant height measurement and plant growth calculation.

For experiments B-D, only IAA produced by bacteria (RD4-1-1) was used for evaluation. In experiment B, steriled unhusked rice seeds of both RD31 and RD41 were soaked with IAA-produced by bacteria at five concentrations (0, 0.25, 2.5, 25, and 50 μM IAA) for eight hours. Then, soaked seeds were sowed on sterile medium (8 g/L agar, 30 g/L sucrose in MS medium and pH was adjusted at 5.8), as followed from Murashige and Skoog (1962) containing five different salinity levels (0, 4, 6, 8, and 10 dS/m) by NaCl concentrations. The results from experiment B were used to adjust the concentration of the IAA-derived bacteria in experiment C.

In experiment C, IAA concentration was adjusted (0, 2.5, 25, 50, and 100 μM RD4-1-1 IAA) but the salty (NaCl) level (0, 4, 6, 8, and 10 dS/m) was the same as in experiment B for evaluation on seedling emergence and seedling growth. For this experiment, it was assessed only with RD31, the lowland rice variety. Steriled unhusked seeds were soaked with each IAA treatment for 24 hours. After root germination was observed, 20 germinated seeds were sowed in autoclaved soil in each plastic pot; in which 2 kg soil was carried. Watering with 100 ml NaCl solution according to each treatment of salinity level was done for 4 days per week (once per day), and then switched with 100 ml sterile nil water for three days per week (once per day). For the 100 ml IAA solution, it was sprayed at week 2-3 once per week. This experiment was evaluated until three weeks after the epicotyl emergence. The results from experiment C were used to adjust the level of salinity in experiment D.

In experiment D, unhusked rice seeds were soaked with five IAA concentrations (0, 2.5, 25, 50, and 75 μM) for eight hours. Soaked seeds of two rice varieties were placed to culture on sterile MS medium; pH at 5.8, containing six different salinity levels (0, 4, 6, 8, 10, and 16 dS/m) by NaCl concentrations. All experiments (A-D) were conducted in 16/8 hours photoperiod (25,000 lux illuminated with fluorescent tube) and dark period in constant temperature at 26 ℃.

Characteristic determination

Two parameters were recorded and calculated in seed planted in sterile sand (experiment A) and sterile soil (experiment C), which included the final

percentage of seedling emergence (EPf) of the coleoptile and speed seedling emergence index (SEIs), were measured after sowing. The calculated formula of these parameters was modified from Abari *et al*. (2011). Ten seedlings per basket and twenty seedlings per pot were measured for shoot length (SL) in each week (three weeks) after seed/seedling emergence in experiments A and C, respectively. Only in experiment A at week three after seed emergence, seedlings were withdrawn to determine characteristics including root length (RL), root score (RS), and adventitious root number (ARN). For RS, it was evaluated according to the degrees of density and length of the fibrous root. The score of the root was classified into five scores: (1) very low, (2) low, (3) moderate, (4) quite high, (5) high. After that, the seedling was cut and shoot and root parts were separated and dried in a hot air oven at 60^{o}C for 48 hours; then weighed. Shoot dry weight (SDW), root dry weight (RDW), and the ratio between these traits (SDW/RSW ratio) was also recorded.

For seed testing under sowing and culture on sterile medium: final percentage of germination (GPf) and speed (shoot) germination index (SGIs) from experiments B and D were recorded (Abari *et al*., 2011). The other ten characteristics were only recorded in experiment D. The characteristics related to germination— time of average germination (TAG) (Mavi *et al*., 2010), germination of seed rate (GSR) and germination coefficient (GC) (Yousof and El-Saidy, 2014)—were recorded within a week after the germination had begun. However, for the characteristics associated with seedling growth: vigor index (VI) (Alizadeh *et al*., 2013), RS, SL, RL, SDW, RDW, and SDW/RDW ratio, it was recorded after germination of about two weeks.

Experimental design and data analysis

The two factors factorial in completely randomized design (CRD) for were used in all experiments (A-D). Concentrations of IAA in experiment A, both produced from bacteria and commercial synthesis, and the two lowland rice varieties were arranged using 7x2 factorial in CRD for four replications. Factors including bacteria-derived IAA concentrations and salinity levels were arranged in each rice variety was conducted in experiment B and C (5x5 factors) and D (5x6 factors), respectively. These experiments were conducted with ten replications. Ten petri dishes per treatment for evaluation by culture seed in sterile medium and ten seeds were put on a medium per petri dish. All data were analyzed by analysis of variance (ANOVA) and the mean differences were compared by Duncan's multiple range test (DMRT) on probability at 0.05 by R program version 3.4.1 (R Core Team 2017).

Results

In experiment A, the interaction between lowland rice varieties and IAA treatment had a significant difference in 4 out of the 12 characteristics: SEIs, SL at week 1, RDW, and SDW/RDW ratio (data not shown). In most characteristics, the highest mean of both varieties (var. RD31 and RD41) was found in soaked seeds with 0.25-25 µM RD4-1-1 IAA and 25 µM synthetic IAA. For the variety factor, RD31 showed higher values more than RD 41with statistical significance on 7 characteristics, such as SEIs, SL at week 1-3, ARN, SDW, and RDW (data not shown).

Mean and statistic results of each factor and interaction between factors, varieties x IAA were presented in four characteristics which include SEIs, EPfs, ARN, and RS. The effect of different IAA treatments had a significant difference in all characterisitics. The influence of IAA treatments showed that 2.5 µM RD4-1-1 IAA had the highest value in SEIs and EPf. While, control treatment (0 µM IAA) had the lowest value in all four characterisitcs. For ARN characteristic, higher values were found in treatments with 0.25 µM and 2.5 µM RD4-1-1 IAA; while higher values in RS were identified at 2.5 µM RD4-1-1 IAA and 0.25 µM synthetic IAA (Table 1).

Table 1. Means of the germination- and growth-related characterisitcs in lowland rice varieties (var. RD31 and RD41) received by soaked seed and sprayed with different exogenous indole-3-acetic acid (IAA) produced by bacteria (RD4-1-1) and commercial product (synthetic IAA) in experiment A

IAA (µM)	SEIs[1]	EPf(%)[2]	ARN[3]	RS[4]
P-value (IAA)	0.001**[6]	8.7x10^{-6}**	0.004**	0.034*
0 uM IAA	4.0 C[7]	29.9 D	6.3 C	2.99 B
0.25µM bac.	7.6 AB	56.1 AB	7.6 A	3.20 AB
2.5µM bac.	9.1 A	64.0 A	7.2 AB	4.10 A
25µM bac.	6.5 BC	45.5 BC	6.6 BC	3.36 AB
0.25µM syn.	7.0 AB	52.9 A-C	6.8 BC	3.81 A
2.5µM syn.	5.2 BC	42.5 CD	6.4 C	3.02 B
25µM syn.	7.4 AB	62.1 A	7.3 AB	3.70 AB
P-value (varieties)	2.7x10^{-10}**	0.842NS[5]	5.1x10^{-12}**	0.586NS
	RD31,9.1X	RD31,50.8	RD31,7.7X	RD31,3.38
	RD41,4.3Y	RD41,50.1	RD41,6.0Y	RD41,3.54
P-value (varieties.xIAA)	0.015*	0.064NS	0.208NS	0.613NS
CV(%)	33.14	23.86	9.99	19.72
Overall mean	6.70	50.43	6.86	3.46

[1/] SEIs = the speed (seed) emergence index. [2/] EPf = the final percentage of seed emergence. [3/] ARN = adventitious root number. [4/] RS = root score. [5/] NS means non significant difference at 0.05 level of proability. [6/] *,** means significant different at 0.05 and 0.01 levels of proability, respectively. [7/] Different upper case letters (A, B, C or X, Y) in the same column means significant difference at 0.05 level of probability.

From experiment A, higher values on emergence- and seedling growth-related characteristics were observed in bacteria-derived IAA at lower concentration. Thus, only the IAA -derived from *Enterobacter* sp. RD4-1-1 was used for experiment B, by adjusting the concentration and testing under the range of salt concentrations (0-10 dS/m) in medium.

In experiment B, only the factor of concentration of IAA derived from *Enterobacter* sp. RD4-1-1 affected on GPf values, both in RD31 and RD41. The GPf in both varieties found no significant differences as affected from either the salinity levels or the interaction between salinity levels and IAA concentrations (Table 2).

Table 2. Means of the final percentage of germination (GPf) (in parentheses) and the speed (shoot) germination index (SGIs) of two lowland rice varieties (var. RD31 and RD41) received by soaked seed with exogenous indole-3-acetic acid (IAA); produced by bacteria, and cultured on different salt concentrations medium in experiment B

IAA (µM) RD4-1-1	**RD31; SGIs and GPf (% in parentheses) Salinity levels (dS/m)**					
	0	**4**	**6**	**8**	**10**	**Means (IAA)**
0	2.72a-d[1] (97)	2.62c-i (92)	2.62c-h (95)	2.22kl (92)	2.24kl (96)	2.48B[2] (94.4A)
0.25	2.62b-f (95)	2.62b-g (94)	2.68b-c (97)	2.35gik (93)	2.18kl (93)	2.49B (94.4A)
2.5	2.99a (95)	3.00a (98)	2.97a (98)	2.61d-i (92)	2.37f (92)	2.79A (95.0A)
25	2.02l (90)	2.33hjk (91)	2.44e-k (86)	2.35gik (91)	2.04l (86)	2.24C (88.8B)
50	2.80a-d (98)	2.88ab (95)	2.88abc (99)	2.37g-k (92)	2.68b-e (96)	2.72A (96.0A)
Means (salinity)	2.63X[3] (95.0)	2.69X (94.0)	2.72X (95.0)	2.38Y (92.0)	2.30Y (92.6)	
SGIs: P-value (Salt) $<2x10^{-16}$ **[4], P-value (IAA) $<2x10^{-16}$ **, P-value (Salt x IAA) $<9.06x10^{-5}$ **, CV(%) 10.29						
GPf; %: P-value (Salt) 0.191 NS[5], P-value (IAA) $<5.56x10^{-5}$ **, P-value (Salt x IAA) 0.580 NS, CV(%) 8.35						
IAA (µM)	**RD41; SGIs and GPf (% in parentheses) Salinity levels (dS/m)**					
	0	**4**	**6**	**8**	**10**	**Means (IAA)**
0	2.65efg[1] (95)	2.65def (95)	2.35hij (94)	2.02kl (94)	2.03kl (96)	2.34C[2] (94.8AB)
0.25	2.68c-f (97)	2.64efg (95)	2.35hij (90)	2.07kl (93)	1.86l (92)	2.32C (93.4B)
2.5	3.17a (100)	3.08ab (99)	2.78cde (93)	2.33hij (98)	2.22ijk (97)	2.72A (97.4A)
25	2.83cde (95)	2.90bc (99)	2.59efg (96)	2.10jk (93)	2.10jk (95)	2.50B (95.6AB)
50	2.63efg (99)	2.89bcd (95)	2.55fgh (95)	2.49fgh (95)	2.41ghi (99)	2.59B (96.6A)
Means (salinity)	2.79X[3] (97.2)	2.83X (96.6)	2.52Y (93.6)	2.20Z (94.6)	2.12Z (95.8)	
SGIs: P-value (Salt) $<2x10^{-16}$ **[4], P-value (IAA) $<2x10^{-16}$ **, P-value (Salt x IAA) 0.0016 **, CV(%) 9.74						
GPf; %: P-value (Salt) 0.0514 NS[5], P-value (IAA) 0.0311*, P-value (Salt x IAA) 0.6970 NS, CV(%) 7.01						

[1/] Different lowercase letters) a, b, c, …) means significant difference at 0.05 level of probability .
[2/] Different uppercase letters) A, B, C (means significant difference at 0.05 level of probability. [3/] Different uppercase letters) X, Y(means significant difference at 0.05 level of probability. [4/] *, ** means significant difference at 0.05 and 0.01 level of proability, respectively. [5/] NS means non significant difference at 0.05 level of probability.

Differently for SGIs, this trait had highly significant difference affected by either each factor: salinity levels and IAA concentrations, or the interaction between them. In which these findings were consistent in both varieties studied (Table 2). Significant reductions in SGIs were observed in seed culturing on salty mediums at 8 and 6 dS/m in var. RD31 and RD41, respectively. In assessing the effect of IAA-produced by bacteria *Enterobacter* sp. RD4-1-1 (supplementation by soaking the seeds before culturing), the highest SGIs values were found at 2.5 µM IAA in both rice varieties (Table 2). The effect of this interaction resulted in a slight difference between var. RD31 and RD41. Higher values on SGIs in RD31 and RD41 were observed at 2.5 µM IAA when cultured on medium at 0-8 dS/m and 0-6 dS/m, respectively. While at 10 dS/m and 8-10 dS/m in RD31 and RD41, respectively, the highest values on SGIs in seeds treated by soaking were assessed with 50 µM IAA.

Form Experiment B, the concentration application of IAA derived from *Enterobacter* sp. RD4-1-1 (0-100 µM) was adjusted for experiment C. The riceseeds were soaked with IAA, plnated in autoclaved soil in the same range of salinity levels (0-10 dS/m), and sprayed with IAA.

In experiment C, the interaction between two factors (salinity level and RD4-1-1 IAA level) showed a statistically significant difference on four characteristics (SEIs, EPf, and SL at first, and second weeks) (Table 3). In addition, those characteristics also showed significant differences affected by either salinity levels or IAA concentrations (Table 3). In most of those characteristics, the salinity level at 0 dS/m showed the highest values.

In RD31, higher SEIs and EPf were observed in seeds soaked by 25-100 µM and 25-50 µM IAA RD4-1-1, respectively. The reduction of SEIs and EPf, compared with control treatment (0 dS/m) occurred since 4 dS/m and 6 dS/m, respectively. Range of salinity levels was between 0-6 dS/m; the highest values on SEIs and EPf were found at 25 µM IAA. At higher salinity level (8-10 dS/m), however, the highest value of SEIs and EPf was recorded at 50 µM IAA (Table 3, above).

SL at one week and two weeks old seedling showed a significant difference affected by all factors: salinity level, IAA concentration, and interaction between them (Table 3). Considering the IAA concentration factor, there were higher values on SL at 25 and 25-100 µM IAA for seedlings at one week old and two weeks old, respectively; the lowest was found at 0 µM IAA. However, one- and two-week old seedlings shoot length had similar results, showingthe decreasing values since 4 dS/m onward. The effect of interaction between salinity and IAA concentrations on SL, for both at one week and two weeks old seedling, aslo showed similar trend. At ranges between 0-6 dS/m salinity, the highest values on SL in these seedling ages were observed using 25

µM IAA for priming the seeds. Nevertheless at 8-10 dS/m salinity in these seedling ages, the highest value on SL was observed at 50 µM IAA.

Table 3. Means of the speed (seedling) emergence index (SEIs), the final percentage of seedling emergence (EPf) and shoot length (SL) at weeks one and two in lowland rice variety (var. RD31) soaked seed and sprayed with different concentration of indole-3-acetic acid (IAA) produced by bacteria (RD4-1-1) grown under different salinity levels in experiment C

IAA (µM) RD4-1-1	**SEIs and EPf (%) (in parentheses) Salinity levels (dS/m)**					
	0 dS/m	**4 dS/m**	**6 dS/m**	**8 dS/m**	**10 dS/m**	**Means (IAA)**
0 µM	1.70c-g[1] (58b-f)	0.76i (29 g)	0.82hi (34 fg)	1.86b-g (68a-d)	1.80b-g (61a-e)	1.39B[2] (49.8B)
2.5 µM	2.62ab (76abc)	1.52e-i (50c-g)	1.28 f-i (50c-g)	1.35 f-i (45d-g)	1.18g-i (48d-g)	1.59B (53.8B)
25 µM	2.88a (76abc)	2.18a-f (68a-d)	2.54abc (79ab)	1.77b-g (61a-e)	2.09a-f (75abc)	2.29A (71.8A)
50 µM	2.03a-g (63a-e)	2.60abc (83ab)	2.03a-g (62a-e)	1.86b-g (64a-e)	2.64ab (86a)	2.23A (71.4A)
100 µM	2.43a-d (64a-e)	2.42a-e (64a-e)	1.28f-i (40efg)	1.63d-h (48d-g)	1.96b-g (63a-e)	1.94A (55.7B)
Means (salinity)	2.33X[3] (67X)	1.89Y (59XY)	1.59Y (53Y)	1.69Y (57XY)	1.93Y (67X)	
SEIs: P-value (Salt) $4.2x10^{-4}$** [4], P-value (IAA) $4.0x10^{-7}$** , P-value (Salt x IAA) $1.17x10^{-3}$ **, CV(%) 28.14				EPf (%):P-value (Salt) 0.018*, P-value (IAA) $6.9x10^{-6}$**, P- value (Salt x IAA) 6.09×10^{-3} **, CV(%) 25.82		

IAA (µM) RD4-1-1	**Shoot length (cm) at weeks one and two (in parentheses) after emergence Salinity levels (dS/m)**					
	0 dS/m	**4 dS/m**	**6 dS/m**	**8 dS/m**	**10 dS/m**	**Means (IAA)**
0 µM	5.4d-i[1] (18.7c-f)	2.7ghi (15.3e-h)	2.0hi (13.2f-i)	6.1d-i (16.0e-h)	2.4hi (10.0hi)	3.73C[2] (14.62 B)
2.5 µM	13.1ab (25.6ab)	7.3c-g (17.8d-g)	3.7f-i (13.8fgh)	4.9e-i(13.8fgh)	1.3i (7.1i)	6.08B (15.64 AB)
25 µM	15.7a (28.3a)	9.5b-e (20.7b-e)	8.6b-e (20.5b-e)	5.3e-i (11.7ghi)	3.6f-i (10.2hi)	8.55A (18.29 A)
50 µM	7.9c-f (21.8b-e)	8.7b-e (21.3b-e)	6.5c-h (17.0efg)	5.4d-e (13.2f-i)	5.1e-i (14.1fgh)	6.73B (17.46 A)
100 µM	10.1bcd(23.6a-d)	10.9bc (24.3abc)	3.3f-i (14.2fgh)	4.8e-i (12.7f-i)	3.4f-i (11.9ghi)	6.51B (17.34 A)
Means (salinity)	10.47W[3](23.60W)	7.83X (19.90X)	4.83YZ (15.73Y)	5.31Y (13.48Y)	3.15Z (10.63Z)	
Shoot height at weeks one: P-value (Salt) $3.6x10^{-11}$** [4], P-value (IAA) $5.7x10^{-5}$**, P-value (Salt x IAA) 0.003**, CV(%) 45.51				Shoot height at week two (in parentheses): P-value (Salt) $2.0x10^{-16}$ **, P-value (IAA) 0.026*, P-value (Salt x IAA) 0.014*, CV(%) 23.39		

[1/]Different lower case letter (a, b, c, …) means significant difference at the 0.05 level of probability. [2/]Different upper case letter (A, B, C) means significant difference at 0.05 level of probability. [3/]Different upper case letter (W, X, Y, Z) means significant difference at 0.05 level of probability. [4/]*,** means significant difference at 0.05 and 0.01 levels of probability, respectively.

The results from experiment C led to adjust the concentration application of IAA derived from *Enterobacter* sp. RD4-1-1 (0-75 µM) and increase the range of salt concentrations application (0-16 dS/m) of the cultured medium in experiment D.

From the 12 characteristics in experiment D, there were significant differences affected by the interaction of IAA and salinity concentrations between 4 to 11 characteristics in RD31 and RD41; excluded SDW, respectively (Figures 1 and 2).

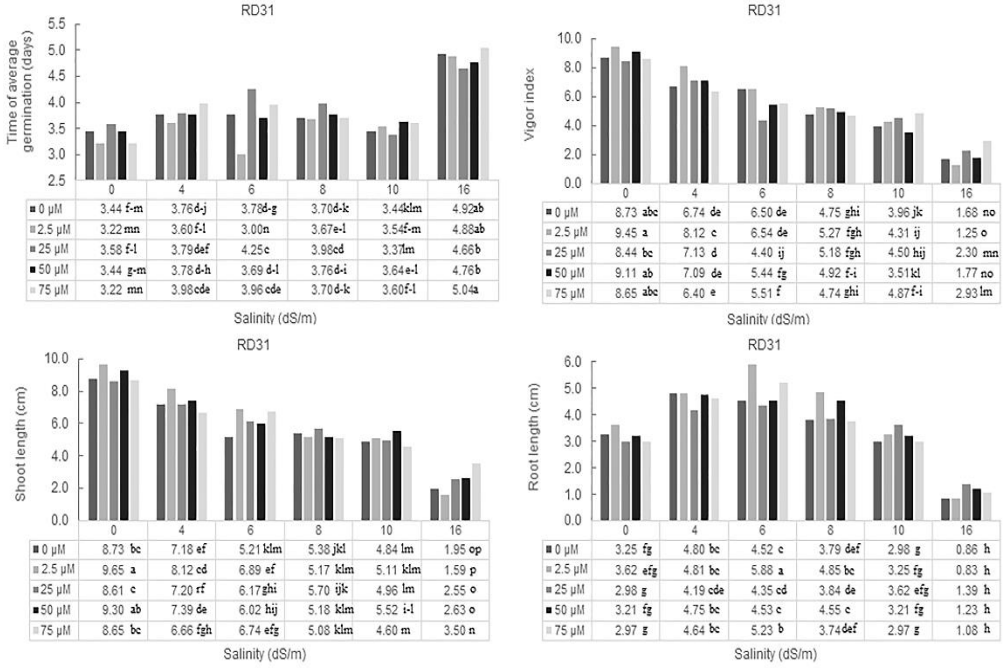

Figure 1. Changeable means of some traits related to germination stage in lowland rice var. RD31 under various salinity levels time of average germination in days (TAG, upper left); vigor index (VI, upper right); shoot length in cm (SL, lower left); root length in cm (RL, lower right) (experiment D)

In var. RD31, there was a significant difference affected by the interaction between IAA and salinity concentrations on four characteristics (TAG, VI, SL, and RL) as shown in Figure 1. When increasing the salinity level, increasing values were observed in TAG trait while other two characteristics (VI and SL) showed decreasing values. Opposite with the RL trait, there were higher values at the salinity levels range between 4-8 dS/m.

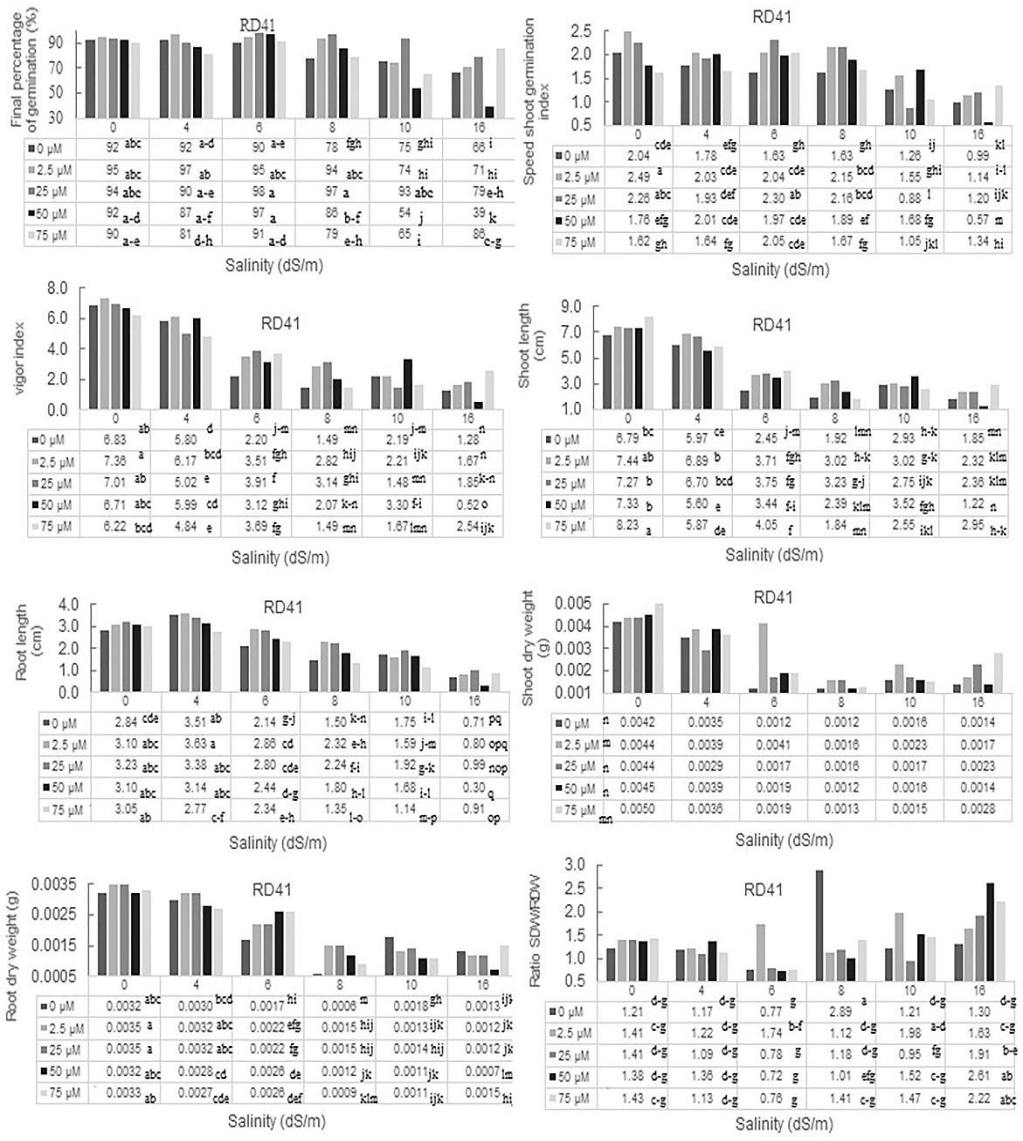

Figure 2. Changeable means of some traits related to early seedling stage in lowland rice var. RD41 under various salinity level; final percentage of germination (GPf, line 1 left); speed shoot germination index (SGIs, line 1 right); vigor index (VI, line 2, left); shoot length in cm (SL, line 2 right); root length in cm (RL, line 3 left); shoot dry weight in g (SDW, line 3 right); root dry weight in g (RDW, line 4 left); ratio of shoot/root dry weight (ratio SDW/RDW, line 4 right) (experiment D)

In var. RD41, only seven from eleven characteristics showed an explicit impact from the factor of interaction between IAA x salinity levels with a significant difference presented in Figure 2. Although SDW was insignificant due to the interaction between these factors (IAA x salinity levels), it is an important characteristic. For GPf trait, the effect of soaking seed with IAA concentration showed a significant positive effect in the range at 8-16 dS/m (Figure 2). Oppositely, other seven traits showed positive effect of soaking seed with IAA since at 0 dS/m.

Using 2.5 and 50 μM IAA to soak the seeds could promote VI trait on salinity level at 0-8 dS/m. However, the appropriate concentration of IAA to support VI traits was changed at 10 and 16 dS/m were 50 and 75 μM IAA, respectively (Figure 2). VI traits were calculated by multiplying between two traits included GPf and SL.

Among the time using for germination traits (TAG, SGIs, GSR, and GC), only SGIs showed consistently with VI and GPf for the highest values in seeds supplemented with various IAA levels (2.5 μM at 0-8 dS/m, 50 μM at 10 dS/m, and 75 μM at 16 dS/m). Therefore, only SGIs are exhibited in Figure 2.

Four characteristics—VI, SL, SDW, and RDW—showed the highest values at 0-4 dS/m, these values reduced followed by the increase of salinity levels (Figure 2). The concentration of IAA at 2.5-50 μM seemed to support all traits in salinity levels range between 6-10 dS/m.; excluding SDW/RDW ratio. However, at 16 dS/m salinity, higher values on these traits were observed at 75 μM IAA; excluding SDW/RDW ratio. For SDW/RDW ratio, there was a variation of the mean values affected by different IAA concentrations in each salinity level. Nevertheless, at 6-10 dS/m and 16 dS/m, a high ratio of values between SDW/RDW were observed at IAA concentrations between 2.5-25 μM and 50-75 μM, respectively (Figure 2).

Discussion

In experiment A, although soaked seeds with IAA derived either from bacteria *Enterobacter* sp. RD4-1-1 or the commercial product, showed higher values in characteristics compared with nil IAA treatment in both rice varieties (data not shown). Seeds soaked with IAA produced from bacteria in sterile supernatant showed higher values at fewer concentrations (0.25 μM and 2.5 μM IAA) compared with synthetic IAA (at 2.5-25 μM IAA) in SEIs, EPf, ARN, and RS characteristics. However, considering all the characteristics, the seemingly best concentration in both RD31 and RD41 was 2.5 μM RD4-1-1 IAA. The use of IAA produced by bacteria that are effective at less concentration compared to synthetic IAA could due to the compatabiliy

between the bacteria and the host plants (Afzal *et al*., 2019). Generally, the host plants and bacteria depend on each other in symbiosis relationship. Hence, it is assumed that plant hormones produced by symbiosis bacteria are effective in promoting growth in host plants. In this study, endophytic bacteria (*Enterobacter* sp. RD4-1-1) that produce RD4-1-1 IAA was isolated from the seeds of upland rice, and RD4-1-1-produced IAA was then tested on lowland rice seeds. Both upland and lowland rice are the same species, possibly making the use of bacteria-producing IAA effective for the germination and growth of rice seedlings in this study. Although the chemical structure of synthetic IAA and natural IAA produced by bacteria are the same, synthetic IAA generally does not possess impurities (Hao and Yang, 2010; Spaepen and Vanderleyden, 2011). In this study, IAA produced by bacteria was employed as solution form that may contain a mixture of the precursors of RD4-1-1 IAA. Moreover, IAA, a major natural auxin that is synthesized by microorganisms, does not only promotes plants growth, but also reported to be a signal molecule affecting gene expression in bacteria (Spaepen and Vanderleyden, 2011). Therefore, the bacteria-produced IAA may in-turn be able to influence other processes involving the production of other bacterial substances, resulting in extra substances within the IAA solution. However, these are still unproven hypotheses. These may be the reasons that the efficacy of RD4-1-1 IAA (2.5 µM) was higher than synthetic IAA (25 µM) when assessed at optimal concentrations of these IAA sources for the promotion of characteristics in this study.

The optimal concentration of 2.5 µM RD4-1-1 IAA can promote all the characteristics (in RD31 and RD41). This value was lower than that reported by Abiri *et al.* (2016) whose study found that a higher value of synthetic IAA (at 285 µM and 570 µM) was used to stimulate the germination in indica rice varieties. In other study, the effectiveness for using IAA also depended on plant species and varieties (Suzuki *et al*., 2003). Moreover, the improper concentration of IAA also depends on the source of IAA deriving effectiveness of IAA dependent on the kind of bacterial species. Likewise, there was a report about the use of IAA derived from *Pseudomonas* (5-10 mg/l IAA) that have a negative effect on the percentage of germination compared to not using at all for priming seeds (Tabatabaei *et al*., 2016). However, the IAA derived from *Enterobacter* sp. NRRU-N13, a rhizobacterium isolated from soil, demonstrated effectiveness in promoting seedling growth in lowland rice (var. KDML105) (Saengsanga, 2018). As mentioned earlier, this study used IAA produced from endophytic bacteria; *Enterobacter* sp. isolated from the upland rice seeds. Both the bacterial species (*Enterobacter*) and the compatibility with the same species of the host plant (rice) may contribute to its efficiency and

better use in lower concentrations. Tabatabaei *et al*. (2016) suggested that the suitable concentration of external IAA in applying to plants should be lower than that found as a suitable level in plant root. For root formation, it is determined during embryo formation (embryogenesis). However, in both during- and post-embryogenesis, the role of hormones in the plant and collaboration with other factors also affect both shape and amount of lateral root (Overvoorde *et al*., 2010). For this reason, the external use of IAA can also affect both the number and shape of adventitious roots rice as shown in this study.

Under medium culture (experiment B), only the differences in concentration of IAA derived from *Enterobacter* sp. RD4-1-1 that affected to GPf trait in both RD31 and RD41. It may indicate that soaking the seeds promote taking up water into the seeds for the following processes: seed imbibition, preparing both enzymes and substrates, for seed germinating (Zhang *et al*., 2015). Increasing salinity levels affected to increase germination time, consequence to delay germination and delayed growth in aboveground and underground, resulting in photosynthesis and survival ability (Keshavarzi, 2011). In this study, RD31 (8 dS/m) showed the beginning of SGIs values reducing at salinity levels was higher than in RD41 (6 dS/m). For this result, if evaluating only the germination speed (SGIs), var. RD31 should be able to withstand salinity (tolerance) for rapid germination more than var. RD41. Although auxin was reported for seed dormancy, it has the role in seed germination as well. Kaya *et al*. (2009) reported using IAA externally could alleviate the salinity stress in plants through many processes such as making the balance between concentrations of ions (Na^+, Ca^{2+}, and K^+), promote chlorophyll synthesis, etc. The IAA concentration at 2.5 uM seems more appropriate to support all these traits grown between <8 dS/m salinity in both rice varieties; although the concentration was an increase in higher salinity levels (depended on traits in each rice variety).

Testing in soil (experiment C) with continuous saline watering showed that the use of external IAA required an increase in concentration compared to experiment B. In RD31, in zero–to-moderate salinity levels (at 0-6 dS/m), using external IAA produced from bacteria at 25 µM IAA) is positively affected by the promotion of seedling emergence through SEIs characteristic. However, at high salinity (8-10 dS/m), using higher concentration of IAA was necessary at 50 µM IAA. In soil testing, it was not only the strong effect of salinity stress, but also the hardness of soil that affected on EPf resulting to the values lower than 75 percent. For this reason, soaking seeds with auxin is good for seed germination under salinity stress preparing seed ready through metabolic process, increase vigor of seed during emergence, and repair the membrances

etc. (Ibrahim, 2016). The speed of seed emergence testing (SEIs) in this study was recorded two times per day; in the morning and afternoon or about six hours apart. The 6-hour interval is sufficient to study the growth of epicotyl in the assessment of SEIs traits. Breviario *et al*. (1992) found that the use of IAA affects the growth of epicotyl of rice after using for only four hours. Which had the result of the cells expanded within two hours by using IAA at a concentration of 10 µM.

A seedling at one week old showed that seeds when soaked with external IAA produced from *Enterobacter* sp. RD4-1-1 did not only promote seedling emergence characteristics (speed and percent of seedling emergence) but shoot height as well. An increased shoot height could mean a relation to increase leaf area for photosynthesis (Ribeiro *et al*., 2019). For seedling growth, spraying the exogenous IAA at start-week is recommended. The proper concentration of IAA showed higher promotion at the start of shoot height at one week old seedling was at 2.5 µM. However, at higher salinity level of 10 dS/m, higher IAA concentration at 25 µM should be chosen. The proper concentration of IAA for spraying for two weeks old seedling at salinity levels at 0-6 dS/m and at 8-10 dS/m were 25 µM and 50 µM RD4-1-1 IAA, respectively. Therefore, the use of increased concentration of external IAA to promote rice growth in salinity conditions may be related to plant growth stage or length of exposure to salinity, or both.

Both increasing values in TAG trait resulted to delay of seed germination, or decreasing the values in VI and SL when cultured the seeds on high salinity medium, indicated that these characteristics are impacted by salinity. Increasing root length either hairy root or lateral root is a characteristic that found to be spontaneous response of the plant to survive under salinity or dehydration conditions. This plasticity changes of root to increase the surface area to uptake water and nutrients and exhibited under mild stress of saltly (Franco *et al*., 2008; Zolla *et al*., 2010; Ma *et al*., 2018). Therefore, the high salt concentration affected the length of the roots or reduced ability to adapt and withstand the intense stress. For this explaination, RL is high at mild-to-medium salt concentrations of 4-8 dS/m salinity. As observed by Ma *et al*. (2018), the behavior of increasing either on length or number of adventitious/lateral root in rice was one criterion to promote that plant could survive when growing under abiotic stress conditions, and effected by IAA factor (Peret *et al*., 2009).

For GPf trait in RD41, the graph was quite flat between different salt concentrations in GPf. This result of soaking the seeds before culturing reduced germination problems under salty conditions. The difference of treatments between or within each salinity level in VI was reflected in the difference from

SL traits; VI trait was assessed using the two characteristics, GPf and SL. The VI trait is an important characteristic to evaluate seedling strength and obviously indicated the light interception ability for photosynthesis of plants which are related to leaf area, leaf width, and the angular coefficient value (Weraduwage *et al*., 2015). For this reason, the use of external IAA derived from bacteria can promote both germination and shoot length, increasing seedling vigor, which is critical for seedling to survive under saline conditions. Moreover, productive factors such as fertilizer, plant hormone, etc., are also effective for management. IAA produced from bacteria has also been reported to increase the length of shoots (increasing 28.57 percent) in wheat at salinity level of 10 dS/m (Egamberdieva, 2009).

All experiments concluded that using different concentrations of IAA derived from *Enterobacter* sp. RD4-1-1 bacteria to soak the seeds could promote many traits on salinity level at 0-6 dS/m between culture in sterile condition and under growing in culture media at 2.5 and 25 μM, respectively. However, the appropriate concentration of IAA to support these germination-related traits was changed at 8-10 and 16 dS/m to 50 and 75 μM IAA, respectively. For spray in seedling, the average across all salinity levels, using 25 and 50 μM IAA derived from bacteria were proper for one week and two weeks old seedling, respectively. However, the study in rice fields facing salinity stress require further studies on the efficacy of IAA produced by *Enterobacter* sp.

Acknowledgements

The authors would like to thank the THAIWEST program under the Office of the Higher Education, Ministry of Education, and Silpakorn University Research and Development Institute (SURDI) in Thailand (grant numbers 02/2560 and 7/2561) for the financial and administrative support. The authors would also like to offer particular thanks to the Royal Project of Agronomy and Horticultural Demonstration Center under the Chaipattana Foundation for providing information about the farmers and research area.

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(Received: 11 July 2021, accepted: 30 July 2022)