The Study and Development of Endophytic Bacteria for Enhancing Organic Rice Growth

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Abstract

Farmers in Han Thao Village, Kud Jab District, Udon Thani Province, cultivate rice (\textit{Oryza sativa} L.), organically. Bio-products from microbial were required to be replacement of agrochemical to reach higher growth and yield. Isolates of endophytic bacteria were extracted from organic rice tissue. PGR producing activity such as indole-3-acetic acid (IAA) like substance was study. The isolates with potential to plant growth promotion were screened. Isolates with high productivity of IAA like substance were inoculated to seeds germinated between paper (BP) at 30 °C for 7 days. Root and shoot length were measured. Ten isolates with high productivity of IAA like substances were poured to plants growing in plastic pots. Growth of plants was measured. There are 35 isolations of endophytic bacteria with high potential of IAA-like substances producing activity. Isolations of O-3-S-3(2) and O-1-S-2 increased shoot and root length of seedlings in paper germination. Inoculation of O-3-S-3(2) and O-1-S-2 into seedlings at 14-days after sowing in pots increased the highest number of shoots per plant and shoot height.

Keywords: endophytic bacteria; organic rice

1. Introduction

Organic rice cultivation is highly dependent on natural systems and avoids the use of synthetic substances that may pollute the environment. The principles of organic agriculture are to emphasize how to restore balance and fertility of the soil by improving the soil with organic matter. The nutrients flow in the soil is balance and complete cycle. Biodiversity in farms is increase (Panyakul, 2004) [1]. Bacteria that live inside plant tissues without any disease symptoms are classified as endophytic bacteria. (Hung...
Endophytic bacteria can live in a certain period or throughout the life span. They infected into plant tissue by several methods such as drift with wind, attached to soil particles, floating on the water, attached to farm equipment and other (Sturz et al, 2000) [3]. Endophytic bacteria in plant cells is capable of fixing nitrogen or produce plant growth regulators (PGRs). Some kind of endophytic bacteria such as Azorarcus spp., Gluconacetobacter diazotrophicus, Herbaspirillum spp. was isolated from sugarcane and rice. Endophytic bacteria inhabit at the rhizosphere of wheat bacteria were Streptomyces, Microbiosa and Micromonospora (Sharma et al, 2005) [4]. Endophytic bacteria have specific to host plants, while some species live together with a host of specific and non specific (Gerhardson and Wright, 2002) [5]. Some species including Azotobacter, Azospirillum, Azoarcus, Herbaspiririllum, Acetobacter, Burkholderia, Klebsiella, Pseudomonas, Enterobacter and Rhizobium, etc. are extracted from some kind of plant tissue, such as sugarcane, rice, corn, sorghum, oil palm and coffee (Bazzicalupo and Okon, 2002) [6]. In rice chemically farming, nitrogen fertilizers are used in large quantities to accelerate growth and improve productivity. To transfer to organic farming systems, which chemical fertilizers are prohibited in the production, the growth of the plant relies on biological processes microorganisms. Elbaltagy, et al. (2001) [7] reported capable of fixing nitrogen by the bacteria isolated from stems of wild rice and trade rice. Herbaspirillum, Ideonella, Enterobacter and Azospirillum were isolated from wild rice. Their living within the tissue was studied using a GFP (Green Fluorescent Protein). Herbaspirillum was found living at intercellular space of leaves tissue of wild rice. Teaumroong, et al. (2001) [8] studied efficiency of nitrogen fixation of endophytic bacteria by acetylene reduction assay (ARA). They produced indole acetic acid (IAA), which promotes the growth of plants. In this research isolations of 71 kinds of endophytic bacteria were isolated from rice tissue and IAA productivity was investigated. The endophytic bacteria with highly IAA productivity were selected and purposed to study their affects on rice seedling growth in paper germination and rice growth in pot cultivation.

2. Material and Methods

Organic rice roots and shoot were sampled from 1st- and 3rd-years organic farm and chemical farm at Han Thoa Village. Endophytic bacteria were isolated by TSA selective medium. Their potential to produce of IAA-like substances was studied using the methods which were reported by Hung and Annapurna, (2004) [2]. 35 isolates of endophytic bacteria with effective in excreting indole acetic acid (IAA) in TSB medium with concentration at $10^6$ CFU/ml. Seeds of rice (Oryza sativa L. cv. RD6) were disinfectant surface with 70% ethanol for 30 seconds and were soak into the endophytic bacteria solution for 30 minutes. Thereafter, they were germinated by using method of between paper (BP). The experimental design was Completely Randomized Design: CRD with 35 treatments and 4 replications per treatment. Shoot height and root length of seedlings were measured at 7 days after germination. To study effect of endophytic bacteria on growth of rice in pot cultivation, 10 isolations with high efficiency to promote root and shoot growth of seedlings were inoculated to rice plants cultivated in pots by soaking seeds before sowing, inoculating at 14 or 28 days after sowing. Growth of rice plants was measured at 60 days after sowing.

3. Results and Discussion

In the results, rice tissue sampling from 3-years organic farm had the larger numbers of isolates than chemical farm and 1-year organic farm, respectively. Isolates from all parts of rice plants had distinct characteristics. There are 10 isolation with highly IAA production between 5.13 - 14.58 μg/ml (Table 1). Jha and Kumar (2009) [9] reported that endophytic bacteria can produce indole acetic acid (IAA) in the tissues of the root and stem of wheat.
Table 1. Activity of endophytic bacteria to produce indole acetic acid (IAA)

| Isolation   | Indole acetic acid (IAA) (μg/ml) |
|-------------|----------------------------------|
| C-R-3       | 5.13                             |
| C-R-5(2)    | 12.68                            |
| C-R-8(2)    | 13.17                            |
| C-S-3       | 7.34                             |
| O-1-R-1     | 8.45                             |
| O-1-R-4 (2) | 14.58                            |
| O-1-N-2 (2) | 13.54                            |
| O-1-S-2     | 5.44                             |
| O-3-R-6(2)  | 8.94                             |
| O-3-S-3(2)  | 5.38                             |

Inoculation of O-3-R-10(2), O-1-S-2 and C-R-3 into rice seeds germinating between papers increased highly root length of rice seedlings with root length of 8.52, 8.93 and 7.42 cm, respectively. Shoot height of seedlings were non-significant in all inoculation (Table 2). Ishii (1973) [10] reported that the effect of auxin to the elongation of rice seedlings stems and roots. Cell elongation occurs when auxin is transported to the leaves.

Table 2. Seedling growth in paper germination after inoculation of endophytic bacteria

| Isolation   | Shoot height (cm.) | Root length (cm.) |
|-------------|--------------------|-------------------|
| C-R-3       | 4.51               | 7.42<sup>ab</sup> |
| C-R-5(2)    | 4.56               | 6.11<sup>ab</sup> |
| C-R-8(2)    | 3.72               | 6.81<sup>ab</sup> |
| C-S-3       | 3.72               | 5.66<sup>a</sup>  |
| O-1-R-1     | 4.33               | 7.34<sup>ab</sup> |
| O-1-R-4 (2) | 4.17               | 7.71<sup>ab</sup> |
| O-1-N-2 (2) | 4.59               | 6.14<sup>ab</sup> |
| O-1-S-2     | 4.76               | 8.93<sup>ab</sup> |
| O-3-R-6(2)  | 4.32               | 6.79<sup>ab</sup> |
| O-3-S-3(2)  | 3.24               | 6.48<sup>ab</sup> |
| F-test      | ns                 | 19.00             |

<sup>1</sup>Mean within the same column followed by the same letter are not significantly different by DMRT at <i>P</i>=0.05  
ns = Non-significant difference at <i>P</i>=0.05, * = Significant difference at <i>P</i> ≤ 0.05

Inoculation of O-3-S-3(2) at 14-days seedlings cultivating in pots increased the highest growth of rice plants such as number of shoots per plant, shoot height, shoot diameter, number of leave per plant, leaf length and width. Inoculation of O-3-S-3(2) at 14 days after sowing encourages the highest number of shoots (31.67 shoots per plant) at 60 days after sowing. Shoot height was 72.65 cm. Inoculation of O-1-S-2 into 14-days seedlings enhanced shoot height, shoot diameter, leaf length and width similar to those of O-3-S-3(2), but had less number of shoots and number of leave per plant than those of O-3-S-3(2). The plant inoculated by O-1-S-2 at 14 days after sowing had 23.33 shoots per plant, while shoot height was 65.02 cm at 60 days after sowing (Table 3).
Table 3. Effect of endophytic bacteria inoculation on growth of rice in pot cultivation at 60 days after sowing

| Treatment               | Num of shoot/plant | Plant height (cm.) |
|-------------------------|--------------------|--------------------|
|                         | O-3-S-3(2)        | 18.50^c-h          | 53.53^c-i          |
|                         | O-3-R6(2)         | 12.38^h            | 57.35^b-h          |
|                         | O-1-S-2           | 16.00^e-c          | 61.53^g-f          |
|                         | O-1-R-4(2)        | 18.50^c-h          | 54.35^b-i          |
|                         | O-1-R-1           | 15.25^e-i          | 51.45^c-i          |
|                         | O-1-N-2(2)        | 19.50^b-f          | 49.92^d-i          |
|                         | C-S-3             | 16.83^d-f          | 58.43^b-g          |
|                         | C-R-8(2)          | 8.67^k             | 43.97^h-i          |
|                         | C-R-5(2)          | 21.50^b-f          | 50.35^c-i          |
|                         | C-R-3             | 25.75^b            | 59.75^g-s          |
| 14 days after sowing    | O-3-S-3(2)        | 31.67^a            | 72.65              |
|                         | O-3-R6(2)         | 18.17^c-h          | 68.38^b            |
|                         | O-1-S-2           | 23.33^d-b          | 65.02^e            |
|                         | O-1-R-4(2)        | 15.00^e-c          | 51.95^e-i          |
|                         | O-1-R-1           | 13.00^e-f          | 56.35^b-h          |
|                         | O-1-N-2(2)        | 8.00^k             | 46.40^b-e          |
|                         | C-S-3             | 24.00^b-c          | 56.30^b-h          |
|                         | C-R-8(2)          | 23.78^b-c          | 60.40^g-s          |
|                         | C-R-5(2)          | 18.67^c-h          | 66.22^d            |
|                         | C-R-3             | 17.33^c-h          | 60.45^g-s          |
| 28 days after sowing    | O-3-S-3(2)        | 13.00^e-f          | 54.30^c-e          |
|                         | O-3-R6(2)         | 10.56^ik           | 55.30^c-e          |
|                         | O-1-S-2           | 17.08^d-k          | 58.20^g-c          |
|                         | O-1-R-4(2)        | 22.50^b-c          | 66.38^b-c          |
|                         | O-1-R-1           | 19.25^b-f          | 53.00^c-i          |
|                         | O-1-N-2(2)        | 18.75^c-h          | 50.55^c-i          |
|                         | C-S-3             | 21.17^b-f          | 63.88^f            |
|                         | C-R-8(2)          | 10.50^ik           | 58.25^g-c          |
|                         | C-R-5(2)          | 23.50^bd           | 57.31^b-i          |
|                         | C-R-3             | 5.67^k             | 41.40              |
| Control                 |                    | 21.50^e-t          | 65.90^d            |
| F-test                  | *                  | *                  |
| C.V. (%)                | 34.92              | 16.15              |

1 Mean within the same column followed by the same letter are not significantly different by DMRT at P=0.05

In this experiment inoculation of O-3-S-3 (2) and O-1-S-2 into seeds before paper germination increase shoot height and root length of seedlings. Govindarajan et al (2007) [11] reported the results of the inoculation of *Burkholderia vietnensis* isolated from tissues of in Vietnam, *Gluconacetobacter diazotrophicus* isolated from root tissue of sugar cane in Brazil, *Azospirillum lipoferum* isolated from rice in France and *Herbaspirillum seropedicae* isolated from root tissues of rice in Vietnam. They can increase rice tillering compared with the control. Meunchang, et al (2004) [12] reported that *Azospirillum* was able to produce IAA. *Azosprillum* a gram-negative rods to move very large cell size of 1.0 um x 3.5 mm with a single flagelum when cultured on MPSS broth and lateral flagella when cultured on MPSS agar at 30 ºC and colonies shaped dark pink on. cultured on MPSS Azospirillum used to produce fertilizer.

4. Conclusion

Isolations of O-3-S-3(2) and O-1-S-2 increased shoot and root length of seedlings in paper germination. Inoculation of O-3-S-3(2) and O-1-S-2 into seedlings at 14-days after sowing in pots increased the highest number of shoots per plant and shoot height.
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