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Case Report

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Disease-Free Seed Ginger Production Technology (G3, G4 And G5) in the Field

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Abstract

Increasing threats of ginger from bacterial wilt: *Ralstonia solanacearum*, is impacted to ginger production and market of farmer in Thailand. The objective of this study was to find the integrated ginger production system that are produced high yield stability and able to replanted in the same area for farmer's well-being sustainable development. This study evaluated the integrated ginger production technology consist of cultivate management, bioproduct from *Bacillus subtilis* BS-DOA 24 strain for controlling bacterial wilt of ginger and disease-free rhizomes seeds from tissue culture of ginger three generations (G3, G4 and G5) at Phetchabun Highland Agricultural Research Center during October 2016- September 2019. The rhizomes seed production after planting 11 months was free from bacterial wilt disease. The sprouting ginger seed germination percentage after 2 months of G3, G4 and G5 were 92, 97 and 99%, respectively. Total yield of DOA's technology in G3, G4 and G5 showed 3,248, 10,133 and 10,568 kg/rai (20.3, 63.33, and 66.05 tons/hectare), respectively that the yield of DOA's G5 was significantly higher than farmer method (6,725 kg/rai or 42.03 tons/hectare).

The number of DOA's G3 branches per rhizome (17 branches) was higher than other generation. In contrast, rhizome weight of G3 in DOA technology (433 g/rhizome, respectively) was lower than another treatment however DOA's G5 (1,409 g/rhizome) was significantly heavier than farmer's technology (910 g/rhizome). The production costs of rhizomes ginger in G3 (28,817 baht/rai or 6,003.54 USD/hectare) were higher than another treatment due to high value of seeds prices in this generation. Net income of DOA's G5 showed the highest of value as 211,360 baht/rai or 44,033 USD/hectare therefore the profit (186,060 baht/rai or 38,762.5 USD/hectare) also was higher than farmer's technology. Moreover, 19 farmers were established field demonstration by using DOA's techniques and these knowledges were transferred to 137 farmers for apply in their farming. Diseases-free rhizome seeds (9,040 kg) were planted in 20.1 rais (3.216 hectare) of farmer fields at Khao Kho, Phetchabun and Kantharawichai, Maha Sarakham. In summary, the disease-free ginger production was shown to increase productivity, decrease unit cost and increase net income and net profit, and BS-DOA 24 strain bioproduct was controlled bacterial wilt in all generations after replanted in the same area.

Keywords: The disease-free seed, production, bacterial wilt, *Bacillus subtilis*, ginger.

Introduction

Ginger (Zingiber officinale) is an important crop of the world economy. It's a functional foods and medicinal plant in the Zingiberaceae family [1]. In 2016, the ginger export in Thailand was 44,322 tons, a value of shift million and the ginger export volume increased more than 50% against 2017 indices. The importing countries of ginger from Thailand were Pakistan, Japan, America, New Zealand and China [2]. The development of ginger quality for exporting is an important issue to produce rhizome ginger of

farmer. However, the problem of ginger production in Thailand are the bacteria wilt in ginger cause by *Ralstonia solanacearum* [3] that it causes yield losses over 50% during harvests [4]. Disease symptoms show that water-soaked patches or linear streaks on the collar region of the pseudostems. Later leaves become flaccid with intense yellowish bronze color and droop. The leaves roll up and the whole plant dries in 5-7 days. Pseudostems come off easily with a gentle pull. Milky bacterial exudate oozes out on pressing

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the rhizome gently [5]. According to the problem, the farmers planted to new lands for ginger production due to avoid bacterial wilt. They used seed rhizome reserved from own production then often infected with this disease [6]. Farmer replanting ginger three generations in the same area showed that, the percent yield in the first year as 80%, reduced 50% in the second year and remained 30% in the third year [7]. Moreover, the disease-free seed rhizome from tissue culture quite small size then the affect to high seed prices and high unit cost [8].

Integrated ginger production system, seed rhizome from tissue culture technique and bioproduct from *Bacillus subtilis* BS-DOA 24 strain that able prevented bacterial wilt (approximately 60%) in the field [9]. In addition, the management of soil preparation with soil fumigation was mixed with urea fertilizer (0-0-46) and lime rate of 80:800 kg/rai or 0.5:5.0 tons/hectare (1:10 ratio) to reduce the population of bacteria *R. solanacearum* before planting ginger and during ginger plant [10]. Furthermore, *B. subtilis* also increased the yield of ginger [11] and potato [12]. The objective of this study was to finding integrated ginger production system that are produced high yield stability and able to replanted in the same area for farmer's well-being sustainable development, environmental conservation and natural resource recovery.

Materials and Methods

Plant material

The integrated ginger production of DOA's technology, disease-free rhizomes seeds from tissue culture of three generations (G3, G4 and G5) combined with soil preparation (urea: lime in a ratio of 1:10) and bioproduct from *Bacillus subtilis* BS-DOA 24 strain for controlling bacterial wilt of ginger from the Department of Agriculture (DOA), Thailand were compared with farmer's technology (Table 1). The experiment was conducted in research farm at the Phetchabun Highland Agricultural Research Center (PBHARC), khao Kho, Phetchabun (16035'35"N, 100057'50"E 742 m) and extended the technology to 17 farmer's ginger production in Khao Kho, Phetchabun and Kantharawichai, Maha Sarakham during 2016-2019. A loam soil texture derived from sedimentary and metamorphic rocks, and an average annual rainfall of approximately 1,200 mm.

Testing Integrated Technology of Ginger Production

DOA Ginger Production Technology

In the first year, the G3 disease-free seed rhizome of ginger production was produced from G2 seed rhizome in Chiang Rai Horticulture Research Center, Mueang, Chiang Rai during 2016-2017. After removed weeds, the management of soil preparation in one rai (0.16 hectare) with soil fumigation was mixed with urea fertilizer (0-0-46) and lime rate of 80:800 kg/rai or 0.5:5.0 tons/hectare (1:10 ratio). The land was plowed two times and solarized of

beds in four weeks for decrease the pathogens and contamination. Beds of about 70 cm width, 25 cm height and convenient length were prepared with an interspace of 70 cm in between beds. Chicken manure were applied in furrow before planting with 250 kg/rai (1.56 tons/hectare). The seed rhizomes 7,600 plants/rais or 47,500 plants/hectare were cut into small pieces of 5-7.5 cm length each having three or four good sprouts.

These seeds were dipped with mancozeb fungicide (50 mL/20 L of water) and cypermethrin pesticide (50 mL/20 L of water) for 30 minutes, shade dried for 30-60 minutes after that treated with B. subtilis 'BS-DOA 24' (40 mL/ 20 L of water) for 30 mins, shade dried. The seed rhizome bits were planted at a spacing of 30 × 70 cm and placed in shallow pits and covered with a thin layer of soil (2-5 cm). Mulching the bed with rice straw or grass straw were covered the row for protect the moisture, sun burning, and weed competition then an enclosed 1 m high of the net fence to protect from human and animal contamination. NPK fertilizers (15-15-15) was applied in the rate of 50 kg/rai (0.31ton/hectare) 2 months after planting and 50 kg/rai (0.31 ton/hectare) of NPK (13-13-21) was applied at 4 months after planting in a furrow after removed weed, and made a horn. Spraying BS-DOA 24 bioproduct (40 mL/ 20 L of water) every month continue to four months. However, the infection of bacterial wilt in ginger plantlet was close from the field and combined lime and urea (46-0-0) with 10:1 ratio at 500 g/ plant.

After that, covered hold with soil and applied BS-DOA suspension (40 mL/20 L of water) was applied at 30-50 mL/plant in the evening until the disease symptom appearing. The cultural and management practices i.e. irrigation and spraying for insect pest and disease control were carried out uniformly for all treatments. The ginger plants were harvested at 10-11 months after planting or when the plant's foliage or stem had died back. After collection, the seed rhizomes were determined. The G4 seed rhizomes were stored in the shade after 2 months for produced G5 seed rhizome production and testing the integrated technology of DOA's ginger production compared with farmer's technology in PBHARC during 2018-2019 (Table 1).

Farmer's ginger production technology

In the third year, the G4 seed rhizome of farmer were compared with the integrated technology of DOA's ginger production in PBHARC during 2018-2019 (Table 1). Gingers were planted in an acquiring the new area that land preparation in one rai (0.16 hectare) not less than one month. The land was plowed two times and solarized of beds in four weeks. Beds of about 70 cm width, 25 cm height and of convenient length were prepared with an interspace of 70 cm in between beds. Chicken manure were applied in furrow before planting with 250 kg/rai (1.56 tons/hectare). The seed rhizomes were cut into small pieces of 5-7.5 cm length

each having three or four good buds. These seeds were dipped with mancozeb fungicide (50~mL/20~L of water) and cypermethrin pesticide (50~mL/20~L of water) for 30 minutes, shade dried for 30-60 minutes.

The seed rhizome bits were planted at a spacing of 30×70 cm and placed in shallow pits and covered with a thin layer of soil (2-5 cm). Mulching the bed with rice straw or grass straw were covered the row for protect the moisture, sun burning, and weed competition then an enclosed 1 m high of the net fence to protect from human and animal contamination. NPK fertilizers (15-15-15) was applied in the rate of 50 kg/rai (0.31 ton/hectare) 2 months after planting and 50 kg/rai (0.31 ton/hectare) of NPK (13-13-21) was applied at 4 months after planting in a furrow after removed weed, and made a horn. The cultural and management practices i.e. irrigation and spraying for insect pest and disease control were carried out uniformly for all treatments. The ginger plants were harvested at 10-11 months after planting or when the plant's foliage or stem had died back. After collection, the seed rhizomes were determined.

The Extension and Transfer of Technology

Knowledge management in the integrated ginger seed rhizome production of DOA's technology was prepared for transfer to farmers. The seed rhizome field demonstration was established in PBHARC during 2019-2020. In addition, extension of transfer DOA's technology in ginger production for farmers via training courses and filed demonstrations at Khao Kho Agricultural Productivity Efficiency Increasing Learning Center (KKAPEILC), Phetchabun and Highland Herb Community Enterprise, Khao Kho, Petchabun.

Finally, research presentation, publication and research service were conducted.

Statistical analysis

The data included the percentage of sprout germination, number of branches per rhizome, weight per rhizome, production per rai and cost were recoded. The experiment was laid out using a randomized completely block design (RCBD) and analyzed by using the independent t-test ($p \le 0.05$) with two treatments and four replications. Statistical analyses were carried out using the IRRISTAT program.

Results and Discussion

Testing Integrated Technology of Ginger Production

The Yield Components and Total Yield of Ginger

Sprout germination rate of ginger rhizome seed in raised beds after planting 2 months increased in each generation (Table 2). DOA's rhizome seed in G3 generation showed 92% sprout germination, 97% in G4 and 99% in G5. In addition, G5 rhizome seed of DOA's technology was lower sprout germination than seed of farmer's technology (98%). Sprouts start to germinate because both respiration and moisture loss increase rapidly [13]. Ginger is a quantitative short-day plant and that long days tend to enhance vegetative growth while rhizome swelling is promoted by short days [14]. The optimum soil temperature for germination is between 25-26°C, and for growth it needs 27.5°C. A temperature in excess of 32°C can cause sunburn; on the other hand, low temperatures induce dormancy. Rhizome swelling promoted as the daylength was decreased from 16 to 10 hours [15].

Table 1: Ginger production technology of DOA and Thai farmer.

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		DOA's Ginger Production Technology	Farmer's Ginger Production Technology						
	1	Using the disease-free rhizomes seeds from tissue culture of G3, G4 and G5.	Using the ginger rhizomes seed that selected from the year before planting.						
	2	Soil preparation: replanting in the same area with disinfects of pathogens (urea fertilizer and lime rate of 1:10 ratio).	Planting in the new area due to avoid pathogens.						
	3	Apply bioproduct from BS-DOA 24 strain (40 mL/ 20 L of water) to protect of pathogens.	Non-apply bioproduct from BS-DOA 24 strain.						

Table 2: The total yield and yield components of DOA's technology (G3, G4 and G5 rhizome seed) were compared with farmer's technology in ginger production during 2016-2019.

Yield and Yield Components	DOA's Technology		Farmer's Technology	P-value	
	G3	G4	G5	G5	
Sprout germination (%)	92	97	99	98	-
Sprout number (sprouts/rhizome)	17	12	7	9	0.055 ^{ns}
Rhizome weight (g/rhizome)	433	1,351	1,409	910	0.002*
Yield (tons/hectare)	20.3	63.33	66.05	42.03	-

Remarks: - Means followed by the same letter within a column are not significantly different at 5% level of significance by the independent t-test method

- DOA's technology = cultivate management, bioproduct from *B. subtilis* BS-DOA 24 strain and disease-free seeds rhizomes of ginger (G3, G4 and G5 generations).
- Farmer's technology = cultivate management and farmer's rhizome seed.

The number of branches per rhizome decreased in each generation due to initial generation (G3) of seed size represented small size and became large in G5 (Table 2). Then, branches number in G5 of DOA's technology (7 branches/rhizome) was less than G5 of farmer's technology (9 branches/rhizome) but not significantly different from the farmer treatments. However, the rhizome size of DOA's technology was larger than rhizome of farmer's technology. These results are similar with the study of [16] revealed that ginger plantlets from meristem micropropagation produced more tillering and rhizome branching when compared with conventional method (old rhizomes). The weight of rhizome in G3 (433 g/rhizome), G4 (1,351 g/rhizome) and G5 (1,409 g/rhizome) increased in each generation (Table 2). Rhizome weight in G5 that treated with DOA's technology was significantly heavier than that from the farmer's technology (910 g/rhizome). Our results demonstrate that, besides to those of three biocontrol agents (BCAs) namely B.subtilis, Trichoderma album and Trichoderma hamatum enhanced the average tuber weight of potato [17].

Total yield per rai of rhizome in G5 of DOA's technology (10,568 kg/rai or 66.05 tons/hectare) was higher than G4 (10,133 kg/rai or 63.33 tons/hectare) and G3 (3,248 kg/rai or 20.30 tons/hectare), respectively (Table 2). There was higher than ginger that treated with farmer's technology (6,725 kg/rai or 42.031 tons/hectare). These results are similar to those of [11] reported that BS-DOA

24 improved the ginger production at 4,288.03 kg/rai which more than control [12] indicated that the application of a microbial product of *B. subtilis* strain increased tuber yield by fostering the recruitment of beneficial bacteria in the potato rhizosphere [12]. In addition, *B. subtilis* had properties to be plant growth promoting microorganism and mechanism to suppress disease caused by pathogen which increase crop productivity [18].

The Production Costs of Ginger

The important production cost of ginger production was the rhizome seed in each generation (Table 3). The rhizome seed cost in G5 of farmer's technology (10,108 baht/rai or 2,105.83 USD/hectare) was higher than DOA's technology in G3 (6,156 baht/rai or 1,282.50 USD/hectare), G5 (2,128 baht/rai or 443.33 USD/hectare) and G4 (1,748 baht/rai or 364.17 USD/hectare), respectively. The bioproduct from BS-DOA 24 strain for controlling bacterial wilt of ginger that treated in the DOA's technology was increased production cost in each generation. However, the integrated ginger production of DOA's technology were increased yield of ginger then also increased income and profit of ginger production. DOA's technology income in G5 (211,360 baht/rai or 44,033.33 USD/hectare) and G4 (202,660 baht/rai or 42,220.83 USD/hectare) was higher than G5 of farmer's technology (134,500 baht/rai or 28,020.83 USD/hectare).

Table 3: The production cost of DOA's technology (G3, G4 and G5 rhizome seed) were compared with farmer's technology in ginger productions during 2016-2019.

Item	DOA's Technology			Farmer's Technology
	G3	G4	G5	G5
Ginger rhizome seed (Baht/rai)	6156	1748	2128	9975
Ginger rhizome seed (USD/hectare)	1282.5	364.17	443.33	2078.13
Material cost (USD/hectare)	2804.38	3016.04	2910.83	2076.46
B. subtilis (USD/hectare)	916.67	916.67	916.67	-
Labor cost (USD/hectare)	1000	1000	1000	1000
Total costs (USD/hectare)	6003.54	5296.88	5270.83	5182.29
Income (USD/hectare)	13533.33	42220.83	44033.33	28020.83
Profit (USD/hectare)	7529.79	36923.96	38762.5	22838.54
BCR	1,25	6.97	7.35	4.41

Remark: planting area of 1 rai = 6.25 hectares.

Similarly, ginger production in G5 of DOA's technology (186,060 baht/rai or 38,762.50 USD/hectare) was showed the highest net profit production. NdaNmadu revealed that labor, seed, fertilizer and capital inputs were significant in explaining the output [19]. The mean technical efficiency of 0.799 indicate that an average ginger farmer in the study area will enjoy an output increase of 18.55% if management techniques are improved and attains the level of the most efficient ginger farmer. Among the various inputs, cost of labour (50.57%) and planting materials (30.38%)

contributed higher to the total variable cost of production [20]. However, bioproduct BS-DOA 24 strain increased productivity then increased the profitability of ginger production [12,18].

The Extension and Transfer of Technology

Knowledge management in the integrated ginger seed rhizome production of DOA's technology was consisted knowledge of soil preparation, the seed rhizomes preparation, cultivation and farm maintenance, pest and disease management, fertilizer and

nutrient management, harvest and postharvest, packaging and transportation. These techniques were emphasized cultivation by using the disease-free rhizomes seeds from tissue culture of G3, G4 and G5, soil preparation by using urea fertilizer (0-0-46) and lime rate of 80:800 kg/rai or 0.5:5.0 tons/hectare (1:10 ratio) and applying bioproduct from BS-DOA 24 bacteria of 40 mL/ 20 L of water for 30 mins to protect of pathogens.

The seed rhizome field demonstration of DOA's technology was established in PBHARC during 2019-2020. 17 farmers from Khao Kho, Phetchabun and Kantharawichai, Maha Sarakham received disease-free seed rhizome of ginger approximately 9,040 kg in 3.056-hectares (19.1 rais) area planting and BS-DOA 24. Furthermore, they were confident DOA's technology and applied this technique in their farm. In addition, extension of transfer DOA's technology in ginger production for farmers via filed demonstrations at Khao Kho Agricultural Productivity Efficiency Increasing Learning Center (KKAPEILC). Supporting the G5 and G6 disease-free seed rhizomes was as 800 kg and two kg of BS-DOA 24 to learning center. In 2019, the learning center produced 8,000 kg seed rhizomes and distribute to other farmers then increased net profit about 25,000 USD/hectare (120,000 baht/rai). Moreover, the integrated seed rhizome production of DOA's extended to Phetchabun and Highland Herb Community Enterprise, Khao Kho, Petchabun in 2019.

The seed rhizome (1,000 kg) planted in field demonstration and treated with 2 kg of BS-DOA 24 bioproduct. At least 100 farmers in organization improved their skills and knowledge via DOA's training courses. They were satisfied and applied this technology to produce in Zingiberaceae family such as cassumunar (Zingiber cassumunar Roxb.), turmeric (Curcuma longa) and Black Galingale (Kaempferia parviflora Wallich. ex Baker.). Moreover, 50 farmers improved knowledge and 9,040 kg seed rhizomes were distributed to 4.56 hectares (28.5 rais) of farmers in ginger farming. Furthermore, to represent PR performance e.g. four papers of research presentation and publication, research service and welcome visiting delegations from Khao Kho, Phetchabun more than 250 persons.

Conclusion

The integrated ginger production DOA's technology was shown to be an appropriate method for soil preparation (urea: lime in a ratio of 1:10) combine with disease-free rhizomes seeds from tissue culture of three generations (G3, G4 and G5) and bioproduct from *B. subtilis* BS-DOA 24 strain for controlling bacterial wilt of ginger. This technology increased yield and rhizome weight and decreased the total costs of ginger production. The farm income increased then increased quality of a livelihood in the farming households. Moreover, 19 farmers were established field demonstration by using DOA's techniques and these knowledges were transferred to

137 farmers for apply in their farming.

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