Effects of organic compounds on micropropagation of strawberry 'Pharachatan 80'

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Abstract

This approach was conducted in order to optimize growth and multiplication of micropropagated strawberry 'Pharachatan 80'. A response surface methodology (RSM) was applied to test the Linsmaier and Skoog (LS) organic compounds (comprising of nicotinic acid, pyridoxine, thiamine, glycine, myo-inositol) and adenine sulfate. It was found that individual components of organic compounds in Linsmaier and Skoog (LS) had no significant effects on plant growth and multiplication. However, optimization of individual components as new formulations and adjustment of LS as relative concentrations showed that these adjusted optimized formulations can improve shoot growth and multiplication in strawberry 'Pharachatan 80'.

Keywords: Micropropagation Strawberry, 'Pharachatan 80', Organic compounds

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารประกอบอินทรีย์ต่อการเจริญเติบโตของสตรอว์เบอร์รีพันธุ์พระราชทาน 80 ภายใต้สภาพปลอดเชื้อ โดยประยุกต์ใช้เทคนิคการตอบสนองแบบโครงร่างพื้นผิว จากการศึกษาผลของสารประกอบอินทรีย์แต่ละชนิดที่เป็นองค์ประกอบของสารประกอบอินทรีย์สูตร Linsmaier and Skoog (LS) ได้แก่ nicotinic acid, pyridoxine, thiamine, glycine, myo-inositol และ adenine sulfate พบว่าสารประกอบแต่ละชนิดไม่มีผลต่อการเจริญเติบโตและการเพิ่มจำนวนของสตรอว์เบอร์รี อย่างไรก็ตามหากปรับความเข้มข้นของสารประกอบอินทรีย์สูตร LS ทั้งแบบที่ปรับความ

เข้มข้นของสารแต่ละชนิดและปรับความเข้มข้นของสารประกอบอินทรีย์ LS แบบเท่าตัว พบว่าสามารถช่วยในการเจริญเติบโตและการเพิ่มจำนวนของยอดสตรอว์เบอร์รีพันธุ์พระราชทาน 80 ได้
คำสำคัญ: การเพาะเลี้ยงเนื้อเยื่อพืช สตรอว์เบอร์รีพันธุ์พระราชทาน 80 สารประกอบอินทรีย์

Introduction

Strawberry 'Pharachtan 80' is a famous temperate cultivar grown in many regions of Thailand because it has widely adaptability and produces large fruits with good quality compared to other cultivars. This cultivar is June bearing and cultivated for fresh consumption, food processing and tourist attraction in the North areas such as Chiang Mai, Chiang Rai, or Phayao, etc. Strawberry is one of commercial crops normally propagated through plant tissue culture for mass-production with uniform and healthy mother plants (Rancillac and Nourrisseau, 1988; Rosati, 1992; Jemmali *et al*., 2002). Optimization of culture media for micropropagation is very challenging due to the diverse growth factor requirements of various plant species. Although most *in vitro* plants could synthesize organic compounds themselves, these compounds are still significantly important. Linsmaier and Skoog (1965) reported that vitamins and other organic compounds called LS organic compounds, were required for rapid growth of callus and excised pith tissues of tobacco. These components are often added to culture medium for growth improvement. However, the amount of these factors might not be suitable for shoot culture of strawberry 'Pharachtan 80'. Therefore, this study was aimed to determine and optimize the concentrations of LS organic compounds and adenine sulfate using response surface methodology (RSM) which employed for modeling or optimizing the most significant component factors for *in vitro* plant growth (Niedz and Evens, 2007; Reed *et al*., 2013; Niedz *et al*., 2014; Poothong and Reed, 2014).

Materials and Methods

Plant materials and establishment of shoot cultures.

Shoots of strawberry 'Pharachatan 80' were grown on semi-solid medium with MS inorganic compounds (Murashige and Skoog, 1962) plus LS organic compounds (glycine, myo-inositol, nicotinic acid, pyridoxine and thiamine) (Linsmaier and Skoog 1965) plus 1.0 mg/L N6-benzylaminopurine (BAP), 0.1

mg/L indole-3-butyric acid (IBA), 30 g/L sucrose, and 8 g/L agar (pH 5.7),. All plants were grown at 24±1°C and a 16-h photoperiod with 2,500-3,000 Lux.

Experimental method: optimizing organic compounds.

The experimental treatments were designed using a six-factor RSM design, where the design points (combinations of the six factors) were selected using a modified D-optimal design using the software application Design Expert®8 (Design-Expert, 2010). The medium combinations (41 treatments) which consisted of various concentrations of nicotinic acid, pyridoxine, thiamine, glycine, myo-inositol and adenine sulfate. A basal MS medium and LS organic compounds were used as a control in this experiment. Each treatment included four shoots in each of four bottles (n=16). Shoots were transferred to the same treatment medium at four week intervals and data were collected after 12 weeks. Thereafter, the validation test was conducted as 10 treatments (Trt.) with 4 replications (Table 1). Finally, media with adjusted organic compounds as comparative to LS concentration and adding adenine sulfate were tested (Linsmaier and Skoog 1965).

Data collection and statistical analysis.

Each plant was evaluated for plant quality rating (defined for healthy and appearance) on a scale of 1 (poor quality), 2 (moderate quality) and 3 (good quality), and shoot length of the longest shoot was measured in mm. Leaf color ratings as scores also evaluated on a scale of 1 (discoloration), 2 (pale green) and 3 (healthy green). The number of shoots was counted. Graphic models for each response were produced by modeling a map of the response as a combination of organic compounds using response surface design (Design Expert®8, Stat-Ease Inc., Minneapolis, MN) (Design-Expert, 2010). The best fitting polynomial regression model was obtained for each measured response. The *F* value and *p* value of overall models, analyzed by ANOVA significant at $p=0.05$ were constructed (Niedz and Evens 2007). Plant growth data were calculated and subjected to one-way analysis of variance (ANOVA) for mean comparison. For the validation test and adjusted organic compounds as comparative to LS concentration, the least significant difference (LSD) was used at $p < 0.05$ using IBM SPSS statistic (24.0).

Table 1 The validation test was conducted and designed as 10 treatments (Trt.)

Trt.	The concentrations of each organic compounds (mg/L)					
	Adenine Sulfate	Glycine	Myo-inositol	Nicotinic acid	Pyridoxine	Thiamine
1	0.1	4.0	10.0	1.0	0.5	1.0
2	0.1	4.0	10.0	0.1	1.0	1.0
3	1.0	0.1	10.0	0.1	0.1	1.0
4	0.2	2.1	122.0	0.7	0.3	0.7
5	80.0	4.0	10.0	1.0	0.5	1.0
6	80.0	4.0	10.0	0.1	1.0	1.0
7	80.0	0.1	10.0	0.1	0.1	1.0
8	80.0	2.1	122.0	0.7	0.3	0.7
9	80.0	2.0	100.0	0.5	0.5	0.4
10	0.0	2.0	100.0	0.5	0.5	0.4

Results and Discussion

The effect of organic compounds on the overall quality and plant growth using RSM.

Color contour plots of the regions in the 6-factor design space showing the effect of these factors on overall quality, leaf color, shoot number and shoot length were presented in Fig. 1A-D. The response models were not statistically significant in all parameters. However, the effects of individual factors of organic compounds on overall quality showed that optimizing culture medium with high myo-inositol, slightly high glycine and low nicotinic acid and thiamine seemed to be suitable to increase plant quality. This response was similar to leaf color but for shoot number and shoot length myo-inositol did not have any effect. Whether applying either any concentration of myo-inositol with low adenine sulfate or any concentration of myo-inositol with high adenine sulfate could increase shoot height.

The validation test and adjusted organic compounds.

To optimize LS organic compounds formulation, there was no significant effect of any factors on overall quality; leaf color and shoot number excepting shoot length. The overall quality was ranged from 1.75 - 2.4 and shoot grown on LS vitamin had 2.0 scores which was quite low compared to

shoots grown on medium supplemented with 80 mg/L adenine sulfate, 122 mg/L myo-inositol, 0.7 mg/L nicotinic acid, 0.7 mg/L thiamine, 2.1 mg/L glycine and 0.3 mg/L pyridoxine (Fig. 2A-B). For shoot number, plants grown on LS organic compounds had lower shoot number compared to those that grown on medium supplemented with 0.2 mg/L adenine sulfate, 122 mg/L myo-inositol, acid 0.7 mg/L nicotinic, 0.7 mg/L thiamine, 2.1 mg/L glycine and 0.3 mg/L pyridoxine (Fig. 3A). Finally, shoot grown on LS organic compounds had lower shoot length compared to shoot grown on medium supplemented with 80 mg/L adenine sulfate, 10 mg/L myo-inositol, 0.1 mg/L nicotinic acid, 1.0 mg/L thiamine, 4.0 mg/L glycine and 1.0 mg/L pyridoxine (Fig. 3B).

Optimizing individual LS organic compounds did not revealed any significant effects on plant growth and multiplication. However, modifying the whole LS organic compounds and adding adenine sulfate did not improve plant growth and multiplication of strawberry (Fig. 4A-B and 5A-B). Plant growth appearance of shoots grown on different optimized media varying organic compound's concentrations were shown in Fig. 6. Although organic compounds are one of components in artificial culture medium, these organic compounds could be synthesized from plant tissues. Nevertheless, there were some studies examined the effects on plant growth. According to the effects of adenine sulfate and other organic compounds on *in vitro* growth and development of *Aloe*, the study showed that adding 160 mg/L adenine sulfate in culture medium did not enhance shoot elongation or multiplication; however, adding 10 mg/L citric acid could increase shoot number (Aggarwal and Barna, 2004). Gerdakaneh *et al*. (2011) studied the effects of different concentrations (0, 50, 100, 150 and 200 mg/L) of three amino acids (proline, alanine and glutamine) on growth and development of three different cultivars of strawberry (Camarosa, Paros and Kurdistan). The results showed that 100 mg/L proline could induce somatic embryogenesis in all cultivars (Gerdakaneh *et al*., 2011). For *in vitro* rooting of Tetra shoots, the different concentrations of Fe-EDDHA and thiamine were tested and the result showed that 150 mg/L Fe-EDDHA and 1.6 mg/L thiamine which higher that concentration in LS organic compounds about four times, using half strength MS and 0.5 mg/L IBA provided highest root number, length, fresh and dry weight (Sadeghi *et al*., 2015). Although in this study, there was significant effect of thiamine, thus, increased this component can provided better growth based on validation test.

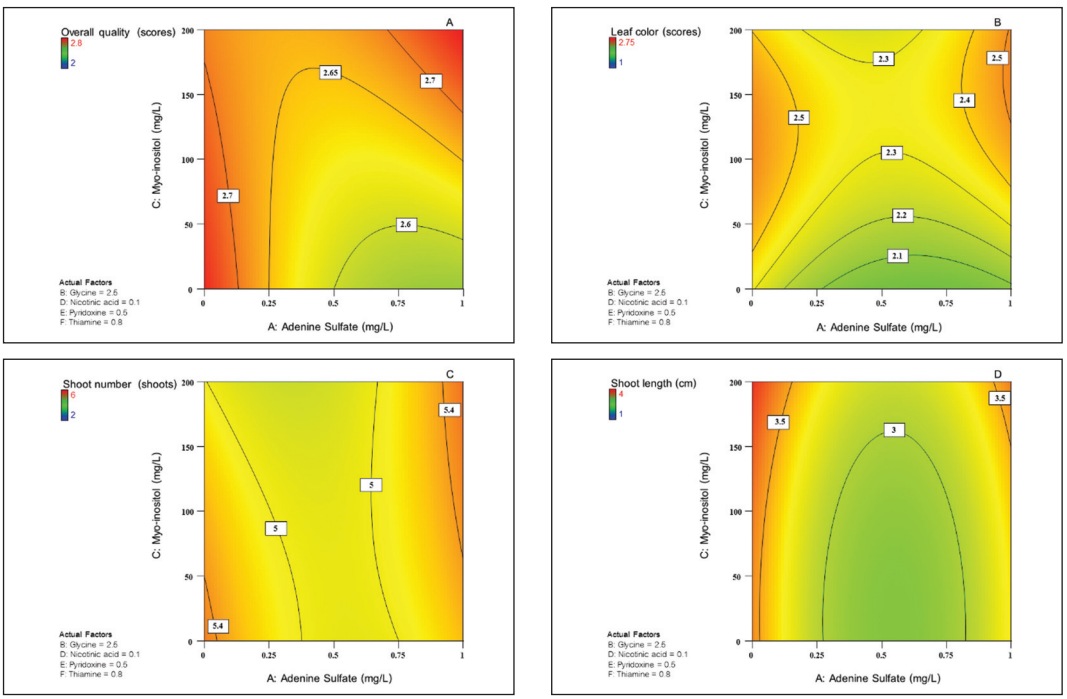

Figure 1 Contour plots showing the effects of organic compounds on shoot responses: (A) overall quality [scored 1 = poor (dark blue) – 3 = good (red)], (B) leaf color [scored 1 = discoloration or yellow (dark blue) – 3 = green (red)], (C) shoot number [low shoot number (dark blue) – high (red)] and (D) shoot length [low shoot length poor (dark blue) – high (red)].

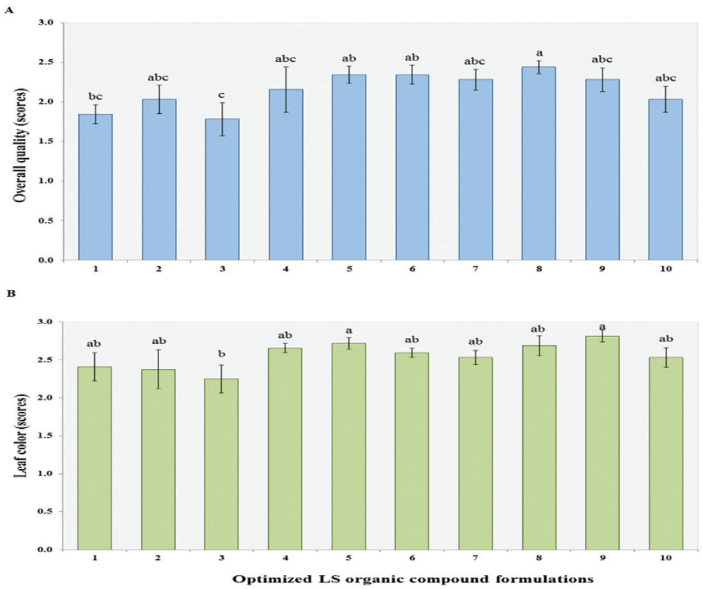

Figure 2 The effects of optimized organic compounds on shoot growth: (A) overall quality and (B) leaf color.

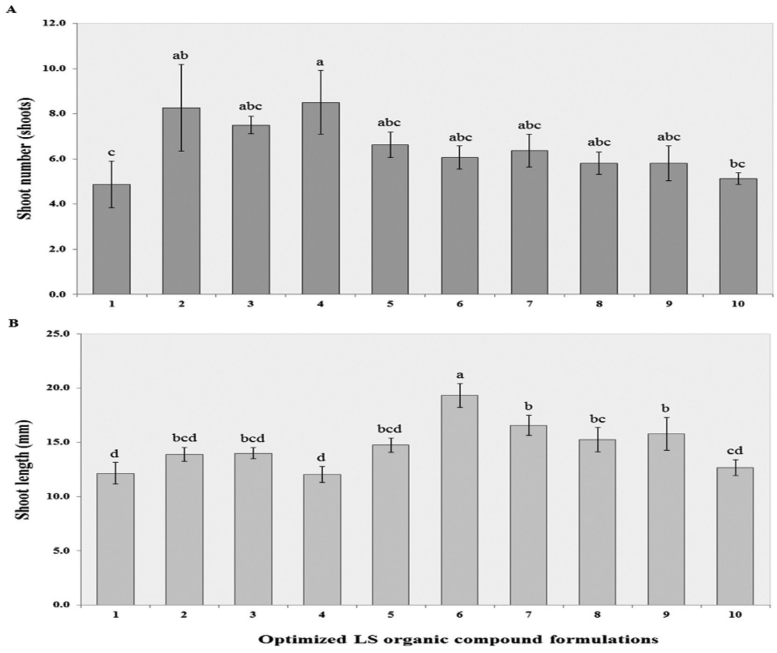

Figure 3 The effects of optimized organic compounds on shoot growth: (A) shoot number and (B) shoot length.

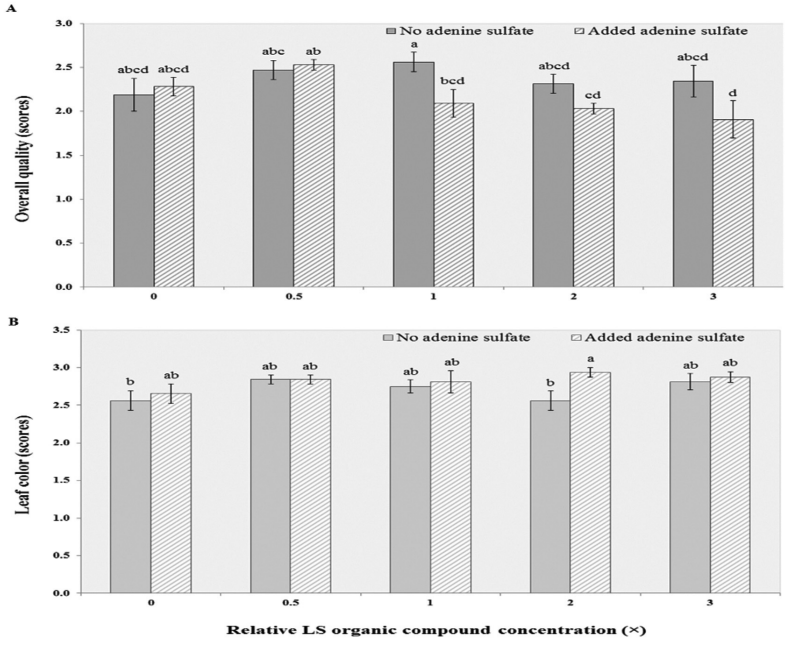

Figure 4 The effects of changed LS organic compounds and adding adenine sulfate on shoot growth: (A) overall quality and (B) leaf color.

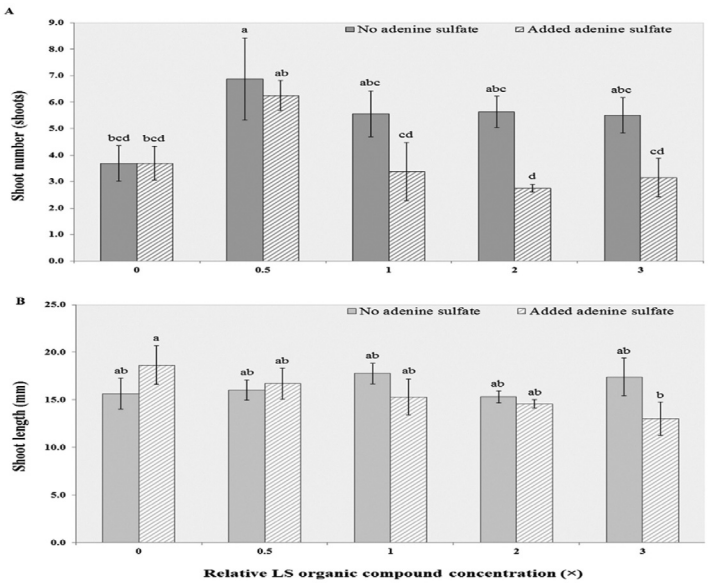

Figure 5 The effects of changed LS organic compounds and adding adenine sulfate on shoot growth: (A) shoot number and (B) shoot length.

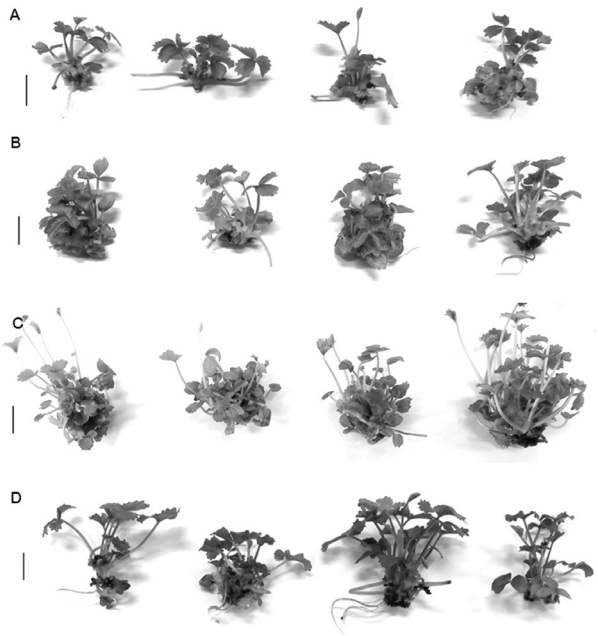

Figure 6 Plant growth appearance of shoots grown on (A) no adenine sulfate with 2.0 mg/L glycine, 100 mg/L myo-inositol, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine and 0.4 mg/L thiamine, (B) 80 mg/L adenine sulfate with 2.0 mg/L glycine, 100 mg/L myo-inositol, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine and 0.4 mg/L thiamine, (C) 0.3 mg/L adenine sulfate with 1.0 mg/L glycine, 150 mg/L myo-inositol, 0.8 mg/L nicotinic acid, 0.5 mg/L pyridoxine and 0.3 mg/L thiamine and (D) 80 mg/L adenine sulfate with no LS organic compounds.

Conclusion

Organic compounds had tiny effects on micropropagated strawberry 'Pharachatan 80' and adding adenine sulfate could slightly improve growth and multiplication. Furthermore, *in vitro* mineral nutrients should be examined for increasing growth and development of strawberry 'Pharachatan 80'.

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