

## V. SIMPULAN DAN SARAN

### A. Simpulan

1. Tidak ada perbedaan kecepatan waktu induksi kalus pada medium  $\frac{1}{2}$ MS dan NP yang diinduksi oleh 2,4-D 1 mg/l.
2. Indeks pertumbuhan kalus terbesar diperoleh dari kombinasi hormon IBA 2 mg/l + BAP 2 mg/l, baik pada medium  $\frac{1}{2}$ MS maupun NP.
3. Kadar steviosida terbesar diperoleh dari kombinasi hormon IBA 2 mg/l + BAP 2 mg/l, baik pada medium  $\frac{1}{2}$ MS maupun NP.

### B. Saran

1. Waktu kontak atau konsentrasi larutan fungisida pada metode sterilisasi eksplan perlu ditingkatkan untuk mengurangi kontaminasi yang didominasi oleh jamur.
2. Perlu dilakukan optimalisasi kombinasi ZPT yang digunakan untuk induksi kalus maupun pemeliharaan kalus. Penggunaan auksin tunggal seperti 2,4-D saja sebaiknya tidak dilakukan, melainkan dikombinasi dengan sitokinin.
3. Perlu diteliti ada tidaknya pengaruh dari penambahan prekursor seperti GA<sub>3</sub> untuk meningkatkan produksi steviosida pada kalus.
4. Perlu adanya pengaturan fotoperiod selama inkubasi kultur kalus.

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Lampiran 1. Komposisi Medium MS (*Murashige and Skoog*)  
(Hendaryono dan Wijayani, 1994)

| Komposisi   | Berat (mg/l) | ½ Resep MS |
|---|--------------|------------|
| Makronutrien  |              |            |
| KNO <sub>3</sub>                                    | 1900         | 950        |
| NH <sub>4</sub> NO <sub>3</sub>                     | 1650         | 825        |
| MgSO <sub>4</sub> .7H <sub>2</sub> O                | 370          | 185        |
| KH <sub>2</sub> PO <sub>4</sub>                     | 170          | 85         |
| CaCl <sub>2</sub> .H <sub>2</sub> O                 | 440          | 220        |
| Mikronutrien  |              |            |
| KI  | 0,83         | 0,415      |
| H <sub>3</sub> BO <sub>3</sub>                      | 6,2          | 3,1        |
| MnSO <sub>4</sub> .H <sub>2</sub> O                 | 22,3         | 11,15      |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O                | 8,6          | 4,3        |
| Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O | 0,25         | 0,125      |
| CuSO <sub>4</sub> .5H <sub>2</sub> O                | 0,025        | 0,0125     |
| CoCl <sub>2</sub> .6H <sub>2</sub> O                | 0,025        | 0,0125     |
| Garam Besi  |              |            |
| FeSO <sub>4</sub> .7H <sub>2</sub> O                | 27,8         | 13,9       |
| Na <sub>2</sub> -EDTA.2H <sub>2</sub> O             | 37,3         | 18,65      |
| Senyawa Organik                                     |              |            |
| <i>Thiamine-HCl</i>                                 | 0,1          | -tetap-    |
| <i>Pyridoxine-HCl</i>                               | 0,5          | -tetap-    |
| <i>Nicotinic-acid</i>                               | 0,5          | -tetap-    |
| <i>Glycine</i>                                      | 2            | -tetap-    |
| <i>Myoinositol</i>                                  | 100          | -tetap-    |
| Sumber Karbon                                       |              |            |
| Sukrosa   | 30000        | -tetap-    |
| Pemadat   |              |            |
| Agar  | 8000 – 10000 | -tetap-    |

Lampiran 2. Komposisi Medium NP (*New Phalaenopsis*)  
(Islam dkk., 1998)

| Komposisi  | Berat (mg/l) |
|--|--------------|
| Makronutrien   |              |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>      | 303,9        |
| KH <sub>2</sub> PO <sub>4</sub>                      | 462,7        |
| NH <sub>4</sub> NO <sub>3</sub>                      | 32,0         |
| KNO <sub>3</sub>                                     | 424,6        |
| Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O | 637,6        |
| Mg(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O | 256,4        |
| Mikronutrien   |              |
| MnSO <sub>4</sub> .4H <sub>2</sub> O                 | 11,15        |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O                 | 4,3          |
| H <sub>3</sub> BO <sub>4</sub>                       | 3,1          |
| KI   | 0,415        |
| Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O  | 0,125        |
| CoCl <sub>2</sub> .6H <sub>2</sub> O                 | 0,0125       |
| CuSO <sub>4</sub> .5H <sub>2</sub> O                 | 0,0125       |
| Garam Besi   |              |
| FeSO <sub>4</sub> .7H <sub>2</sub> O                 | 27,8         |
| Na <sub>2</sub> -EDTA.2H <sub>2</sub> O              | 37,3         |
| Senyawa Organik                                      |              |
| <i>Thiamine-HCl</i>                                  | 0,1          |
| <i>Pyridoxine-HCl</i>                                | 0,5          |
| <i>Nicotinic-acid</i>                                | 0,5          |
| <i>Glycine</i>                                       | 2            |
| <i>Myoinositol</i>                                   | 100          |
| Sumber Karbon  |              |
| Sukrosa  | 20000        |
| Pemadat  |              |
| <i>Gelrite</i>                                       | 3000         |

Lampiran 3. Hasil Pengukuran Indeks Pertumbuhan Kalus Eksplan Daun Stevia (%)

| Medium | Ulangan | A       | B       | C       | D       | E       |
|--------|---------|---------|---------|---------|---------|---------|
| ½MS    | 1       | 75,413  | 108,868 | 184,286 | 151,481 | 134,062 |
|        | 2       | 104,505 | 183,871 | 200,000 | 262,655 | 160,848 |
|        | 3       | 106,901 | 206,131 | 219,091 | 274,366 | 214,167 |
|        | 4       | 153,053 | 254,305 | 249,776 | 456,667 | 217,429 |
|        | Rerata  | 109,968 | 188,294 | 213,288 | 286,292 | 181,627 |
| Medium | Ulangan | A       | B       | C       | D       | E       |
| NP     | 1       | 138,350 | 126,455 | 75,912  | 161,598 | 39,467  |
|        | 2       | 190,000 | 204,641 | 90,893  | 227,514 | 76,606  |
|        | 3       | 228,046 | 221,449 | 92,216  | 330,729 | 138,855 |
|        | 4       | 306,667 | 240,417 | 93,764  | 332,097 | 187,258 |
|        | Rerata  | 215,766 | 198,240 | 88,1961 | 262,984 | 110,546 |

Keterangan :

A = 2,4-D 0,5 mg/l + NAA 0,5 mg/l + Kin 1 mg/l

B = NAA 0,1 mg/l + BAP 2 mg/l

C = NAA 2 mg/l

D = IBA 2 mg/l + BAP 2 mg/l

E = 2,4-D 1 mg/l

Lampiran 4. Hasil Pengukuran Berat Kering Kalus (mg) dan  $\lambda$  maksimal steviosida

| Medium | Ulangan | A    | B    | C    | D    | E    |
|--------|---------|------|------|------|------|------|
| ½MS    | 1       | 43,5 | 51,3 | 45,7 | 25,7 | 49,8 |
|        | 2       | 50,7 | 24,3 | 42,4 | 52,2 | 38,8 |
|        | 3       | 54,6 | 25,9 | 22,0 | 38   | 46,6 |
|        | 4       | 25,1 | 38,8 | 53,8 | 22,9 | 53,3 |
|        | Rerata  | 43,5 | 35,1 | 41   | 34,7 | 47,1 |
| Medium | Ulangan | A    | B    | C    | D    | E    |
| NP     | 1       | 42,0 | 22,8 | 36,8 | 45,6 | 37,0 |
|        | 2       | 25,6 | 44,3 | 25,2 | 48,0 | 49,3 |
|        | 3       | 27,4 | 41,4 | 21,6 | 57,2 | 53,2 |
|        | 4       | 33,5 | 36,6 | 39,4 | 54,6 | 15,5 |
|        | Rerata  | 32,1 | 36,3 | 30,8 | 51,4 | 38,8 |

Keterangan :

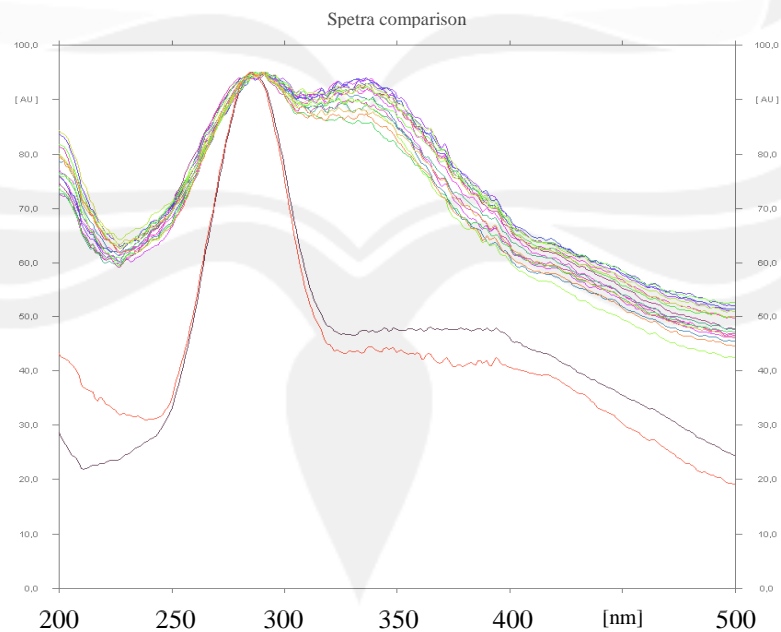
A = 2,4-D 0,5 mg/l + NAA 0,5 mg/l + Kin 1 mg/l

B = NAA 0,1 mg/l + BAP 2 mg/l

C = NAA 2 mg/l

D = IBA 2 mg/l + BAP 2 mg/l

E = 2,4-D 1 mg/l



Gambar 15. Hasil Pengukuran Lamda Maksimal Steviosida

Lampiran 5. Hasil Pengukuran Kadar Steviosida Kalus Eksplan Daun Stevia dan Daun Stevia ( $\mu\text{g}$ )

| Medium           | Ulangan | A      | B      | C      | D      | E      |
|------------------|---------|--------|--------|--------|--------|--------|
| $\frac{1}{2}$ MS | 1       | 14,142 | 22,716 | 6,672  | 16,479 | 14,187 |
|                  | 2       | 9,780  | 7,357  | 0,000* | 6,833  | 4,597  |
|                  | 3       | 12,807 | 8,556  | 16,298 | 20,655 | 4,012  |
|                  | Rerata  | 12,243 | 12,876 | 7,657  | 14,656 | 7,599  |
| Medium           | Ulangan | A      | B      | C      | D      | E      |
| NP               | 1       | 3,826  | 14,233 | 5,196  | 22,323 | 0,642  |
|                  | 2       | 2,828  | 3,755  | 0,000* | 12,979 | 0,128  |
|                  | 3       | 4,546  | 5,302  | 12,989 | 37,953 | 0,491  |
|                  | Rerata  | 3,733  | 7,763  | 6,062  | 24,418 | 0,421  |

Keterangan :

A = 2,4-D 0,5 mg/l + NAA 0,5 mg/l + Kin 1 mg/l

B = NAA 0,1 mg/l + BAP 2 mg/l

C = NAA 2 mg/l

D = IBA 2 mg/l + BAP 2 mg/l

E = 2,4-D 1 mg/l

\* = Luas area kromatogram tidak terdeteksi, diduga kadar steviosida  $<5\mu\text{g}$

| Sampel      | Ulangan | Kadar Steviosida |
|-------------|---------|------------------|
| Daun stevia | 1       | 142,6229         |
|             | 2       | 207,6088         |
|             | 3       | 88,4319          |
|             | Rerata  | 146,221          |

Lampiran 6. Hasil Pengukuran Harga Rf dan AUC Bercak Steviosida

| Sampel             | Ulangan |             |      |             |      |             | Rerata Harga Rf |
|--------------------|---------|-------------|------|-------------|------|-------------|-----------------|
|                    | Rf 1    | Area 1 (AU) | Rf 2 | Area 2 (AU) | Rf 3 | Area 3 (AU) |                 |
| Standar Steviosida | 0,33    | 9211,9      | 0,30 | 10515,2     | 0,30 | 10597,4     | 0,31            |
| ½MS A              | 0,37    | 433         | 0,25 | 346,4       | 0,34 | 406,5       | 0,32            |
| ½MS B              | 0,37    | 603,2       | 0,24 | 298,3       | 0,31 | 322,1       | 0,31            |
| ½MS C              | 0,36    | 284,7       | 0,29 | 87,5        | 0,34 | 475,8       | 0,33            |
| ½MS D              | 0,37    | 479,4       | 0,32 | 287,9       | 0,32 | 562,3       | 0,34            |
| ½MS E              | 0,37    | 433,9       | 0,24 | 243,5       | 0,31 | 231,9       | 0,31            |
| NP A               | 0,37    | 228,2       | 0,24 | 208,4       | 0,31 | 242,5       | 0,31            |
| NP B               | 0,37    | 434,8       | 0,31 | 226,8       | 0,31 | 257,5       | 0,33            |
| NP C               | 0,37    | 255,4       | 0,25 | 132,3       | 0,33 | 410,1       | 0,32            |
| NP D               | 0,36    | 595,4       | 0,32 | 409,9       | 0,33 | 905,7       | 0,34            |
| NP E               | 0,31    | 165         | 0,31 | 154,8       | 0,30 | 162         | 0,31            |
| Daun stevia        | 0,29    | 2983,6      | 0,28 | 4273,7      | 0,27 | 1907,8      | 0,28            |

Keterangan :

A = 2,4-D 0,5 mg/l + NAA 0,5 mg/l + Kin 1 mg/l

B = NAA 0,1 mg/l + BAP 2 mg/l

C = NAA 2 mg/l

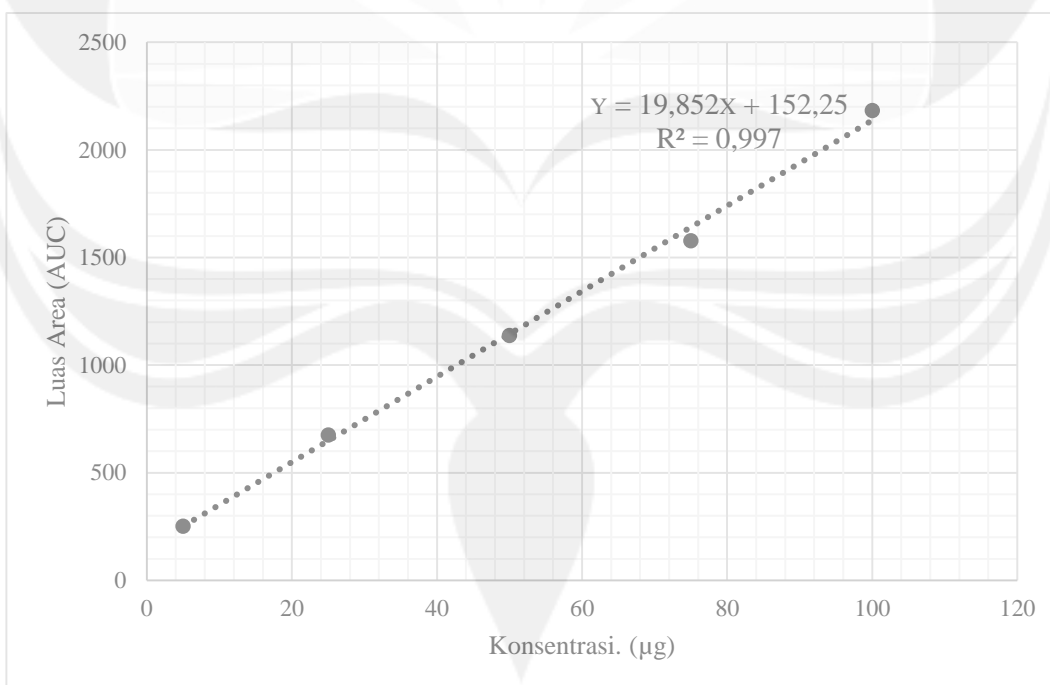
D = IBA 2 mg/l + BAP 2 mg/l

E = 2,4-D 1 mg/l

## Lampiran 7. Kurva Regresi Standar Steviosida

| Konsentrasi | Jalur | Puncak | Posisi Maks | Area      |
|-------------|-------|--------|-------------|-----------|
| 5 µg/ml     | 1     | 1      | 0,29 Rf     | 250,9 AU  |
| 25 µg/ml    | 2     | 1      | 0,27 Rf     | 675,4 AU  |
| 50 µg/ml    | 3     | 1      | 0,27 Rf     | 1137,4 AU |
| 75 µg/ml    | 4     | 1      | 0,27 Rf     | 1577,8 AU |
| 100 µg/ml   | 5     | 1      | 0,27 Rf     | 2182 AU   |

| x          | y             | x <sup>2</sup> | xy            |
|------------|---------------|----------------|---------------|
| 5          | 250,9         | 25             | 1254,5        |
| 25         | 675,4         | 625            | 16885         |
| 50         | 1137,4        | 2500           | 56870         |
| 75         | 1577,8        | 5625           | 118335        |
| 100        | 2182          | 10000          | 218200        |
| <b>255</b> | <b>5823,5</b> | <b>18775</b>   | <b>411545</b> |



Lampiran 8. Uji t parameter Kecepatan Waktu Induksi Kalus Eksplan Daun Stevia

**Uji-T**

**Kelompok Statistik**

|                   | Medium             | N | Rerata  | Std. Deviasi | Std. Kesalahan Rerata |
|-------------------|--------------------|---|---------|--------------|-----------------------|
| Kec_waktu_induksi | ½MS + 2,4-D 1 mg/l | 8 | 10,3750 | 0,51755      | 0,18298               |
|                   | NP + 2,4-D 1 mg/l  | 8 | 10,3750 | 0,51755      | 0,18298               |

Uji Sampel Independen

|                   |                             | Uji Levene untuk Kesamaan Varians |       | Uji t untuk Kesamaan Rerata |    |                |                  |
|-------------------|-----------------------------|-----------------------------------|-------|-----------------------------|----|----------------|------------------|
|                   |                             | F                                 | Sig.  | t                           | DB | Sig. (2-pihak) | Perbedaan Rerata |
| Kec_waktu_induksi | Varians dianggap sama       | 0,000                             | 1,000 | 0,000                       | 14 | 1,000          | 0,00000          |
|                   | Varians tidak dianggap sama |                                   |       | 0,000                       | 14 | 1,000          | 0,00000          |



Lampiran 9. Analisis Varian dan Uji Duncan Parameter Indeks Pertumbuhan (%)  
Kalus Eksplan Daun Stevia

### Analisis Varians Univariat

#### Variabel Terikat : Indeks\_Pertumbuhan

| Sumber                      | Jumlah Kuadrat Tipe III | DB | Rerata Kuadrat | F     | Sig.  |
|-----------------------------|-------------------------|----|----------------|-------|-------|
| Medium                      | 4304,440                | 1  | 4304,440       | 1,025 | 0,319 |
| Kombinasi_Hormon            | 90238,203               | 4  | 22559,551      | 5,372 | 0,002 |
| Medium*<br>Kombinasi_Hormon | 60767,074               | 4  | 15191,769      | 3,617 | 0,016 |
| Total                       | 1658013,162             | 30 |                |       |       |

#### Indeks\_Pertumbuhan

#### Duncan <sup>a,b</sup>

| Kombinasi_Hormon | N | Subset    |           |
|------------------|---|-----------|-----------|
|                  |   | 1         | 2         |
| E                | 8 | 146,08649 |           |
| C                | 8 | 150,74215 |           |
| A                | 8 | 162,86669 |           |
| B                | 8 | 193,26705 |           |
| D                | 8 |           | 274,63838 |
| Sig.             |   | 0,194     | 1,000     |

Rerata untuk kelompok dalam subset yang sama telah ditampilkan.

Berdasarkan rerata yang dihitung.

- a. Rerata ukuran sampel = 8,000.
- b. Alfa = 0,05.

### Interaksi

| Interaksi     | N | Subset    |           |           |
|---------------|---|-----------|-----------|-----------|
|               |   | 1         | 2         | 3         |
| NP*Kom_Hor C  | 4 | 88,19611  |           |           |
| ½MS*Kom_Hor A | 4 | 109,96785 | 109,96785 |           |
| NP*Kom_Hor E  | 4 | 110,54642 | 110,54642 |           |
| ½MS*Kom_Hor E | 4 | 181,62656 | 181,62656 | 181,62656 |
| ½MS*Kom_Hor B | 4 | 188,29373 | 188,29373 | 188,29373 |
| NP*Kom_Hor B  | 4 |           | 198,24037 | 198,24037 |
| ½MS*Kom_Hor C | 4 |           | 213,28819 | 213,28819 |
| NP*Kom_Hor A  | 4 |           | 215,76554 | 215,76554 |
| NP*Kom_Hor D  | 4 |           |           | 262,98445 |
| ½MS*Kom_Hor D | 4 |           |           | 286,29230 |
| Sig           |   | 0,58      | 0,52      | 0,54      |

Rerata untuk kelompok dalam subset yang sama telah ditampilkan.

Berdasarkan rerata yang dihitung.

- a. Rerata ukuran sampel = 4,000.
- b. Alfa = 0,05.

Lampiran 10. Analisis Varian dan Uji Duncan Parameter Kadar Steviosida ( $\mu\text{g}$ ) Kalus Eksplan Daun Stevia

**Analisis Varians Univariat**

**Variabel Terikat : Indeks\_Pertumbuhan**

| Sumber                      | Jumlah Kuadrat Tipe III | DB | Rerata Kuadrat | F     | Sig.  |
|-----------------------------|-------------------------|----|----------------|-------|-------|
| Medium                      | 47,882                  | 1  | 47,882         | 1,035 | 0,321 |
| Kombinasi_Hormon            | 843,141                 | 4  | 210,785        | 4,555 | 0,009 |
| Medium*<br>Kombinasi_Hormon | 324,008                 | 4  | 81,002         | 1,750 | 0,179 |
| Total                       | 4988,219                | 30 |                |       |       |

**Kadar\_ Steviosida**

**Duncan<sup>a,b</sup>**

| Kombinasi_Hormon | N | Subset    |           |
|------------------|---|-----------|-----------|
|                  |   | 1         | 2         |
| E                | 8 | 4,009672  |           |
| C                | 8 | 6,859092  |           |
| A                | 8 | 7,988279  |           |
| B                | 8 | 10,319699 |           |
| D                | 8 |           | 19,537074 |
| Sig.             |   | 0,155     | 1,000     |

Rerata untuk kelompok dalam subset yang sama telah ditampilkan.

Berdasarkan rerata yang dihitung.

- a. Rerata ukuran sampel = 6,000.
- b. Alfa = 0,05.

Lampiran 11. Analisis Korelasi Indeks Pertumbuhan Kalus (%) dan Kadar Steviosida ( $\mu\text{g}$ )

|                       |                  | Indeks<br>Pertumbuhan | Kadar<br>Steviosida |
|-----------------------|------------------|-----------------------|---------------------|
| Indeks<br>Pertumbuhan | Korelasi Pearson | 1                     | 0,568               |
|                       | Sig.(2-pihak)    |                       | 0,087               |
|                       | N                | 10                    | 10                  |
| Kadar<br>Steviosida   | Korelasi Pearson | 0,568                 | 1                   |
|                       | Sig.(2-pihak)    | 0,087                 |                     |
|                       | N                | 10                    | 10                  |