Data Article

Data on germination, growth and morphological changes of oil palm (Elaeis guineensis Jacq.) zygotic embryos during in vitro culturing

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Abstract

Oil palm (Elaeis guineensis Jacq.) from being almost unknown crop a mere three decades ago is now the most consumed and the most traded edible oil in the world. It is a highest yielding crop producing on an average 4 to 6 tons of oil per ha per year. Due to its innumerable uses in the food, oleochemicals and biofuel industries, cultivation of oil palm has expanded enormously in recent years. Since oil palm is a perennial monocotyledonous species with a single growing apex, the plant cannot be multiplied vegetatively and the conventional propagation through seed is limited by dormancy. Thus in vitro germination has become the key method for multiplication of elite oil palm genotypes. Although there are several reports on in vitro germination of oil palm, still there is a lack of an efficient & repeatable method. Hence an attempt is made to standardize the suitable culture media for direct germination from mature oil palm zygotic embryos. The data presented here represents the effect of genotypes, pretreatments and culture media on Mean Germination Time, Speed of Germination Index, Shoot Formation Index and Root Formation Index.
1. Data

The present data in Table 1 shows the effect of genotype, pretreatment and culture media and their interactions on mean germination time, the speed of germination index, the speed of shoot formation index and the speed of root formation index taken by oil palm ZEs where between genotype and culture media, no significant difference was noticed for the mean germination time taken by ZEs of oil palm. With respect pretreatment, there was a significant effect. The soaked ZEs recorded more mean germination time (15.63d) than unsoaked ZEs (14.54 d). In three way interactions (between genotype, pretreatment and culture media) the mean time taken for germination of oil palm ZEs showed a
Table 1
Effect of genotypes, pretreatments and culture media on mean germination time (MGT), speed of germination index (SGI), speed of shoot formation index (SSI) and speed of root formation index (SRI).

| Treatments | MGT   | SGI   | SSI   | SRI   |
|------------|-------|-------|-------|-------|
| **Mean of main effects** |       |       |       |       |
| G1         | G1    | 14.62 | 5.63  | 2.11  | 0.21  |
| G2         | G2    | 15.55 | 5.48  | 1.91  | 0.19  |
| Soaked     | P1    | 15.63 | 5.42  | 2.02  | 0.21  |
| Un soaked  | P2    | 14.54 | 5.69  | 2.00  | 0.18  |
| MS         | M1    | 15.14 | 5.09  | 1.52  | 0.00  |
| ½ MS       | M2    | 15.60 | 4.86  | 1.67  | 0.14  |
| MS + AC    | M3    | 15.21 | 5.23  | 2.12  | 0.59  |
| Y3         | M4    | 14.86 | 6.10  | 2.21  | 0.13  |
| N6         | M5    | 14.60 | 6.50  | 2.54  | 0.14  |

| **Mean of two way interactions** |       |       |       |       |
| G1         | Soaked G1P1 | 14.68 | 5.78  | 2.13  | 0.22  |
| G1         | Un soaked G1P2 | 14.55 | 5.48  | 2.09  | 0.21  |
| G2         | Soaked G2P1 | 16.59 | 5.06  | 1.91  | 0.21  |
| G2         | Un soaked G2P2 | 14.52 | 5.90  | 1.91  | 0.16  |
| G1         | MS G1M1 | 15.30 | 4.89  | 1.50  | 0.00  |
| G1         | ½ MS G1M2 | 14.18 | 5.13  | 1.86  | 0.12  |
| G1         | MS + AC G1M3 | 15.52 | 4.99  | 2.12  | 0.60  |
| G1         | Y3 G1M4 | 14.10 | 6.50  | 2.44  | 0.17  |
| G1         | N6 G1M5 | 13.99 | 6.66  | 2.64  | 0.16  |
| G2         | MS G2M1 | 14.99 | 5.29  | 1.54  | 0.00  |
| G2         | ½ MS G2M2 | 17.02 | 4.59  | 1.47  | 0.15  |
| G2         | MS + AC G2M3 | 14.91 | 5.48  | 2.12  | 0.57  |
| G2         | Y3 G2M4 | 15.63 | 5.71  | 1.98  | 0.10  |
| G2         | N6 G2M5 | 15.21 | 6.34  | 2.45  | 0.11  |
| Soaked     | MS P1M1 | 16.52 | 4.70  | 1.44  | 0.00  |
| Soaked     | ½ MS P1M2 | 15.62 | 5.34  | 1.89  | 0.14  |
| Soaked     | MS + AC P1M3 | 16.41 | 4.68  | 1.94  | 0.65  |
| Soaked     | Y3 P1M4 | 15.28 | 5.90  | 2.27  | 0.13  |
| Soaked     | N6 P1M5 | 14.34 | 6.49  | 2.56  | 0.15  |
| Un soaked  | MS P2M1 | 13.76 | 5.48  | 1.59  | 0.00  |
| Un soaked  | ½ MS P2M2 | 15.58 | 4.39  | 1.45  | 0.13  |
| Un soaked  | MS + AC P2M3 | 14.02 | 5.78  | 2.29  | 0.52  |
| Un soaked  | Y3 P2M4 | 14.45 | 6.31  | 2.16  | 0.14  |
| Un soaked  | N6 P2M5 | 14.87 | 6.51  | 2.52  | 0.13  |

| **Mean of three way interactions** |       |       |       |       |
| G1         | Soaked MS T1 (G1P1M1) | 16.17 | 4.87  | 1.50  | 0.00  |
| G1         | Soaked ½ MS T2 (G1P1M2) | 14.85 | 5.80  | 2.18  | 0.12  |
| G1         | Soaked MS + AC T3 (G1P1M3) | 16.85 | 4.01  | 1.64  | 0.60  |
| G1         | Soaked Y3 T4 (G1P1M4) | 13.29 | 6.93  | 2.54  | 0.19  |
| G1         | Soaked N6 T5 (G1P1M5) | 12.25 | 7.30  | 2.80  | 0.17  |
| G1         | Un soaked MS T6 (G1P2M1) | 14.42 | 4.90  | 1.50  | 0.00  |
| G1         | Un soaked ½ MS T7 (G1P2M2) | 13.51 | 4.46  | 1.54  | 0.13  |
| G1         | Un soaked MS + AC T8 (G1P2M3) | 14.19 | 5.97  | 2.59  | 0.60  |
| G1         | Un soaked Y3 T9 (G1P2M4) | 14.90 | 6.07  | 2.35  | 0.14  |
| G1         | Un soaked N6 T10 (G1P2M5) | 15.74 | 6.01  | 2.47  | 0.15  |
| G2         | Soaked MS T11 (G2P1M5) | 16.87 | 4.52  | 1.39  | 0.00  |
| G2         | Soaked ½ MS T12 (G2P1M2) | 16.39 | 4.88  | 1.59  | 0.17  |
| G2         | Soaked MS + AC T13 (G2P1M3) | 15.97 | 5.36  | 2.24  | 0.70  |
| G2         | Soaked Y3 T14 (G2P1M4) | 17.26 | 4.87  | 2.00  | 0.06  |
| G2         | Soaked N6 T15 (G2P1M5) | 16.44 | 5.68  | 2.33  | 0.13  |
| G2         | Un soaked MS T16 (G2P2M1) | 13.10 | 6.05  | 1.68  | 0.00  |
| G2         | Un soaked ½ MS T17 (G2P2M2) | 17.65 | 4.31  | 1.36  | 0.13  |
| G2         | Un soaked MS + AC T18 (G2P2M3) | 13.85 | 5.60  | 2.00  | 0.45  |
| G2         | Un soaked Y3 T19 (G2P2M4) | 14.00 | 6.55  | 1.97  | 0.13  |
| G2         | Un soaked N6 T20 (G2P2M5) | 13.99 | 7.00  | 2.57  | 0.10  |

(continued on next page)
signficant effect (Table 1). The highest mean germination time (17.65 d) was recorded in T16 when compared all other treatment combinations. However, T16 was on par with T14, T11, T3, T15, T12, T3, T13, T10, T9 and T2. The lowest mean germination time (12.25 d) was recorded by T5 which was on par with T16, T4, T7, T18, T20, T19, T7, T6, T2 and T9.

The speed of germination index noticed during culturing of oil palm ZEs did not showed any significant difference between genotype and pretreatments while the culture media showed a significant effect on the speed of germination index. It was faster (6.50) in the ZEs cultured in N6 media followed by Y3 media (6.10) whereas, the speed of germination index was slower (4.86) in \(\frac{1}{2}\) MS which was on par with MS + AC (5.23) and MS (5.09). In three way interactions there was a significant difference observed in speed of germination index. In 20 treatment combinations T5 recorded faster SGI (7.30) which was on par with T20, T4, T19, T9, T10, T7, T2, T15 and T18 whereas the speed of germination index was lowest (4.01) in T3 which was on par with T17, T7, T11, T14, T1, T12, T6, T13, T18 and T15. The present data revealed that the growth of ZEs in 35 days in three different media tested. In the present data, swelling, expansion followed by formation of haustorium leading to the emergence of plumule from the shoot apex was shown.

The speed of shoot formation index showed no significant difference between genotype and pretreatments. The interaction effect of genotype with pretreatments, pretreatments with culture media and genotype with culture media showed non-significant effect for the speed of shoot formation index. Similarly the three way interaction effect between genotype, pretreatments and culture media was also showed non-significant.

The speed of root formation index also followed the same trend as of SSI for the effect of genotype, pretreatments, two way interaction effect (genotype with pretreatments, pretreatments with culture media and genotype with culture media) and three way interaction effect (between genotype, pretreatments and culture media).

### 2. Experimental design, materials and methods

#### 2.1. Treatment details

The experimental design was three factorial treatment combinations of culture media, plant growth regulators and genotypes arranged in a randomized complete block design. The whole set of experiment was repeated twice with 20 embryos per treatment per replication.

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| S.No. | Factors          | Levels                                         |
|-------|------------------|------------------------------------------------|
| 1.    | Genotypes        | 2 levels (G1and G2)                            |
| 2.    | Pretreatments    | 2 levels (Soaked and unsoaked)                 |
| 3.    | Culture media    | 5 levels (MS, \(\frac{1}{2}\) MS, MS + 0.05% AC, Y3 and N6 media) |

Total number of embryos inoculated = 1500 (25 test tubes per treatment x 20 treatments x 3 replications).
The material was from Dura mother palm block in ICAR-IIOPR seed garden where the material belongs to elite dura genotypes. The standardized protocol has been followed for in vitro germination of the zygotic embryos irrespective of genotypes. In which mature oil palm open pollinated fresh fruit bunches of four genotypes were harvested and fruitlets were depericarped using a depericarper in the seed production lab of ICAR-IIOPR, kernels obtained were surface sterilized by adding few drops of Tween-20 and then immersing the kernels in fungicide solution of (1% Carbendazim and 1% Mancozeb) and soaked in distilled water for 5 days, for attaining the required moisture content of the zygotic embryo.

Then the kernels were washed repeatedly with Tween-20 solution (10 drops/100ml v/v) for 15 minutes and washed with running tap water. They were then washed by fungicide solution (1% Carbendazim and 1% Mancozeb). They were then soaked in ethanol for 1 minute and then washed with 20% NaOCl sol for 20 minutes. Then kernels were halved and embryos were sterilized with 20% (v/v) NaOCl for 20 minutes and then washed with sterile double distilled water for three times. Three types of Basal Culture medium [2–4] viz., MS [5] N6 [6] & Y3 [7] were supplemented with 30g/L (W/V) Sucrose, and the pH of the medium was adjusted to 5.8 and added 8.0g/L of Agar (Clerigart™ from HIMEDIA) prior to sterilization at 121 °C for 20 min. Half MS medium was prepared by using half strength of chemicals which were used in MS medium, while MS+0.05% AC medium was prepared by adding 500 mg/l activated charcoal to the full strength MS medium.

2.2. Treatment combinations

After the preparation of explants and culture media, treatment imposition was carried out. The treatments consisted of three factors with 20 treatment combination (2 × 2 × 5) and 25 test tubes (25 × 150mm) per treatment i.e. two genotypes, two pretreatments and five culture media with three replications. Excised embryos were cultured on 10 ml medium in test tubes. All culture tubes with explants were incubated in the dark growth room at a temperature of 25±2 °C for 35 days. The numbers of germinated embryos were recorded. Embryos were considered viable when they had expanded and showed signs of Haustorium formation after 7 days of culture.

| S.No. | Treatments | Combinations              |
|-------|------------|---------------------------|
| 1.    | T1         | G1 + Soaked + MS medium   |
| 2.    | T2         | G1 + Soaked + ½ MS medium |
| 3.    | T3         | G1 + Soaked + MS + AC medium |
| 4.    | T4         | G1 + Soaked + Y3 medium   |
| 5.    | T5         | G1 + Soaked + N6 medium   |
| 6.    | T6         | G1 + Unsoaked + MS medium |
| 7.    | T7         | G1 + Unsoaked + ½ MS medium |
| 8.    | T8         | G1 + Unsoaked + MS + AC medium |
| 9.    | T9         | G1 + Unsoaked + Y3 medium |
| 10.   | T10        | G1 + Unsoaked + N6 medium |
| 11.   | T11        | G2 + Soaked + MS medium   |
| 12.   | T12        | G2 + Soaked + ½ MS medium |
| 13.   | T13        | G2 + Soaked + MS + AC medium |
| 14.   | T14        | G2 + Soaked + Y3 medium   |
| 15.   | T15        | G2 + Soaked + N6 medium   |
| 16.   | T16        | G2 + Unsoaked + MS medium |
| 17.   | T17        | G2 + Unsoaked + ½ MS medium |
| 18.   | T18        | G2 + Unsoaked + MS + AC medium |
| 19.   | T19        | G2 + Unsoaked + Y3 medium |
| 20.   | T20        | G2 + Unsoaked + N6 medium |
2.3. Observations recorded

2.3.1. Mean time taken for germination of oil palm ZEs

Mean germination time (MGT) was calculated according to the equation given by Moradi et al., 2008 [8].

\[
\text{MGT} = \frac{\sum D_n}{\sum n}
\]

Where \( n \) is the number of zygotic embryos, germinated on day \( D \), and \( D \) is the number of days counted from the beginning of germination.

2.3.2. Speed of germination index of oil palm ZEs

This was calculated as described in the Association of Official Seed Analyst (1983) [9] as follow:

\[
\text{SGI} = \frac{\text{Number of germinated zygotic embryos}}{\text{Days of first count}} + \ldots + \frac{\text{Number of germinated zygotic embryos}}{\text{Days of final count}}
\]

2.3.3. Speed of shoot formation index of oil palm ZEs

The speed of shoot formation index (SSI) was obtained from

\[
\text{SSI} = \sum St/t
\]

where \( St \) was number of shoots formed per culture tube on the day \( t \) [10].

2.3.4. Speed of root formation index of oil palm ZEs

The speed of root formation index (SRI) was obtained from

\[
\text{SRI} = \sum Rt/t
\]

Where \( Rt \) was number of roots formed per culture tube on day \( t \) [10].

2.3.5. Speed of shoot and root formation index (SSRI)

The speed of shoot and root formation index (SSRI) was obtained from

\[
\text{SSRI} = \sum Ut/t
\]

Where \( Ut \) was number of shoots and roots formed per culture tube on day \( t \) [10].

2.4. Data analysis

Data were subjected to analysis of variance (ANOVA) and means were compared by F test, at 5% probability, using ICAR-WASP 2.0 programme developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India (http://www.ccari.res.in/wasp2.0/index.php).

The analysis of variance for each character was carried out as indicated below:

| Anova Table |
|-------------|
| Source of variation | df | SS | MSS | F ratio |
| Replications | \((r-1) = (3-1) = 2\) | RSS | RMSS | RMSS/EMSS |
| Factor A | \((a-1) = (2-1) = 1\) | ASS | AMSS | AMSS/EMSS |
The test of significance was carried out by ‘F’ table values given by Fisher and Yates (1963).

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**Conflict of Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104975.
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