Hybridization and In Vitro Seed Germination of a Commercial Hybrid Oncidium Orchid in Indonesia

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Abstract. Oncidium is the one of orchid family that is not native Indonesia. In the development of Oncidium breeding are widely encountered difficulties, especially when using advanced commercially hybrid varieties. Hybridization and seed germination of Oncidium is still not established yet. The objectives of this study were to obtain population of hybridization and to identify the best medium culture of seed germination and plantlet development of a commercial Oncidium Orchid in Indonesia. The observations were made at a percentage of the success of crosses, seed maturity, the length of the protocorm formation, the effect of media type and sucrose concentration. In this study, the percentage of the cross-success of Oncidium was 15.7% (85 capsules) from 566 crossing and 6.18% (35 capsules) germinated. The fruit harvest age of Oncidium crosses varies between 165 days to 245 days after crossing. The duration of protocorm germination varies between 17-82 days after spreading seed. Protocorms were growth well and fast on medium Tsuchiya supplemented with 1 mg L⁻¹ BAP and 0.25 mg L⁻¹ NAA. and plantlet formation were promoted on the same based media Tsuchiya but supplemented using 0.5 mgL⁻¹ BAP and 0.1 mg L⁻¹ NAA. The optimized protocol required about 24–30 weeks from the spread seed to the plantlet formation. Seedling were grown at size 5-7 cm immediately planted as pot community. The time of acclimatization varies between 6-8 months after the last subculture. while individual time ranges between 4-5 months after acclimatization. Hopefully, the current study will assist with future development of Oncidium Orchid breeding in Indonesia.

1. Introduction
A wide range of attractive sympodial orchids of the genus Oncidium (Orchidaceae) has become economically important, mainly for use in cut-flower and potted-plant industries [1]. Oncidium alliances are also emphasized by the orchid industry for both domestic and foreign markets in the world [2]. Oncidium have been preferred the second after genus Dendrobium by Indonesian community. Oncidium has been favoured as a collection of ornamental plants, and the most important is used as filler or filler flower arrangement. Various species of Oncidium and its group have very wide colour variations, but the species that were widely cultivated in Indonesia are only in yellow colour. The most famous type of Oncidium in Indonesia as cut flowers were very limited in type, such as Oncidium Golden Shower, Oncidium Goldiana, Oncidium Sweet Sugar, and Oncidium Gower Ramsey who the one of the top commercial cut flower in the orchid industry [3]. Many of Orchids Oncidium were imported and adapted in Indonesia by hobbyist, growers, and nurseries with a lot of type and colour.
Breeding of Oncidium was rarely done in Indonesia because the material as a genetic source was very limited, but thus did not cover the possibility of Oncidium development. Breeding opportunities are still open enough especially to acquire new varieties that have superior properties as cut flowers as well as collectible, flowering, and the most important is appropriate and adaptive to the tropical Indonesian environment. Activities of Oncidium breeding, as one of the breeding activities in the ornamental research is usually conducted through sexual hybridization.

The main factors that affect the success of Oncidium breeding through sexual hybridization are the seed germination and seedling development. However, the experience of Oncidium embryo culture has not been owned. Therefore, an Oncidium embryo culture technique needs to be researched. Generally, Orchid seed germination has been developed, but its success was heavily influenced by varieties of factors [4]. Seed maturity media composition and plant growth regulator were influenced seed germination [5, 6, 7]. In addition, the carbon source in the form of carbohydrate was another essential component in the culture medium, which can also influence the growth of the plants [6].

The aims of this research was to establish efficient in vitro seed germination and seedling development techniques for Oncidium, based on investigation of a range of plant growth regulators (PGRs) for maximizing germination and seedling development incubated on defined basal nutrient media.

2. Materials and Method

2.1. Hybridization
Crossing activities were carried out through four stage process i.e maintenance of the parent plant, pollination, formation seeds, picking up the seeds in fruit and seed collecting. Pollination was obtained at two days after the flowers bloom or the first week until the flowers perfectly bloom. The time range between pollination and fertilization on Oncidium orchids was ranges from three to nine months, and then seed maturity and germination time were observed on every crossing.

2.2. The effect of media on seed germination
Success rate of Oncidium crosses would be obtained when ripening capsules physiologically reached. Seed harvesting took several months after pollination and carried out after the capsules have been riped, which characterized by the color that begins to yellow and those about four months to seven months after pollination depending on the genotype. Sterilization Oncidium seed was conducted by cleaning the surface of the fruit capsule with alcohol then burned for around 5 seconds. Then the capsule was split longitudinal to planted the seeds on the medium. Three basal germination media used were (Murashige and Skoog (MS), Vacin & Went (VW) and Tsuchiya supplemented with plant growth regulator substance 1 mg L\(^{-1}\) BAP + 0.25 mgL\(^{-1}\) NAA, or 1 mgL\(^{-1}\) TIBA + 0.25 mgL\(^{-1}\) NAA. In addition, seeds were also planted in media containing carbon source of sucrose 0%; 5%;10% and 20% on MS media without growing regulator substances. Incubation of seed germination was carried out in a bright room with a temperature of 25\(^{\circ}\) C. After germinated, protocorms were sub cultured in the same media until the age of 3 months. The enlargement plantlets into seedling were immediately carried out by the 2nd or 3rd subculture in order to optimum plantlet growth. This plantlet enlargement subculture needed 4-6 months depending on the genotype, until the plantlet reaches height of 6-8 cm in the bottle. Rooting would be growing simultaneously when seedlings developed. Seedling enlargement was carried out on VW media + 0.5 mgL\(^{-1}\)BAP + 0.1 mg/L\(^{-1}\) NAA + 0.5 grL\(^{-1}\) charcoal + 75 grL\(^{-1}\)banana + 100 mlL\(^{-1}\) coconut water.

2.3. Data Analysis
The experiment was designed in Completely Randomized Design and all mean values were determinate by analysis of variance using program SAS version 6.12 and the treatments were compared by Duncan Multiple Range Test (DMRT).
3. Results
3.1. Hybridization
Hybridization of *Oncidium* were conducted from 2013 to 2016, and continued from 2018 to 2019. Number of crosses had been made over a 6 years reaches 556 crosses, but the successful fruit were only about 85 pieces or average 15.29% (table 1). In the first three years, the results of the crossing had been quite numerous but in the fourth year onwards greatly reduced from the percentage of success. Parental qualities had been decreased after three years making it was difficult to flower because the physical condition of the plant became to deteriorate.

Table 1. The success of the *Oncidium* hybridization during years 2013-2019

| No | Years | Number of Crosses | Number of fruit | Percentage (%) |
|----|-------|-------------------|-----------------|----------------|
| 1  | 2013  | 194               | 29              | 14.94          |
| 2  | 2014  | 138               | 14              | 10.14          |
| 3  | 2015  | 128               | 25              | 19.53          |
| 4  | 2016  | 54                | 7               | 12.96          |
| 5  | 2018  | 21                | 4               | 19.04          |
| 6  | 2019  | 21                | 5               | 23.81          |
|    | Total | 556               | 85              | 15.29          |

Figure 1. Parents of *Oncidium* were succeed for crossing in 2013-2019 i.e (a) Odorous Princess yH Tweenstar. (b) Onc. Pagan Love Song. (c) Onc. Belliara Diana Dunn. (d) Onc. Margaret Holm. (e) Odcm Wildcat purple feary. (f) Odontoglossum grande. (g) Onc. Linda Isler. (h) Onc. Space Race Coco. (i) Onc. Yellow King. (j) Brassia. (k) Wils. Garden Afternoon. (l) Wils. Calico Gem. (m) Mtdm Pupukea Sunset x Mtdm Cleos Pride ‘Hama’. (n) Miltonia sp (o) Onc. Spot Berry. (p) Colm. Wildcat Bobcat (q) Wils. Firecrkcer Red Star (r) Onc. Kolibri. (s) Onc. lanceanum. (t) Wils. Tropic Breeze. (u) Mtdm. Issaku nagata. (v) Alcr. Sunday Muffin.
Figure 1 shows twenty two parental has been used on hybridization and succeed to produce germinated seed through protocorm formation. The twenty two’s were Onc. Odorous Princess yH Tweenstar, Onc. Pagan Love Song, Onc. Belliara Diana Dunn, Onc. Margareth Holm, Odcm Wildcat Purple Fear, Odontoglossum Grande, Onc. Linda Isler, Onc. Space Race Coco, Onc. Yellow King, Brasia. Wils.Garden Afternoon, Wils. Calico Gem, Mtdm Pupukea Sunset x Mtdm Cleos Pride ‘Hama’, Miltonia sp. Onc. Spot Berry, Colm. Wilcat Bobcat, Wils. Firecricker Red Star, Onc. Kolibr, Onc. Lanceanum, Wils. Tropic Breeze, Mtdm. Issaku nagata, Alc. Sunday Muffin.

The ripening capsules obtained after pollination were varies about 165-273 days and succeed to germinated within 17-82 days. A crossing between Mtdm. Issaku Nagata as female parent and Onc. Space Race Coco as male parental was obtained with the shortest harvest time at 165 days while Colm.Wildcat Garfield and Onc. Yellow King or Onc. Space race Coco was in 80 days longer. Similarly, selfing of Odontoglossum Grande took an additional 108 days longer than the Mtdm. Issaku Nagata and Onc. Space Race Coco crossing. Selfing between Odbrs. Kenneth Bivins Santa Barbara also took in 233 days which was 68 days longer than the shortest time of all crosses.

Initiation of germination on the process of seed germination was occurred at 17 days on Mtdm. Issaku Nagata with Onc. Odoraos Princess crossing. It was the shortest time for process germination initiation and 82 days occurs on Onc. Margareth Holm with Onc. Wildcat Red Star. A total of 19 series of Oncidium crosses were responded to the formation of protocorm for less than 41 days, which was a half of the longest germination of 82 days, and 12 series of crosses were responded to the formation of protocorm over 41 days (table 2). On average, ripening fruit all Oncidium crosses were occurred in the 189 days and begin to form protocorm within 39 days.

Table 2. Seed maturation and protocorm formation of Oncidium hybridization result

| No | Hybridization Code | Female Parental | Male Parental | Seed Maturity (days) | Protocorm Formation (days) |
|----|-------------------|-----------------|---------------|----------------------|-----------------------------|
| 1. | O 92              | Mtdm. Pupukea Sunset x Mtdm. Cleos Pride Hama | Onc. Yellow King | 195                  | 67                          |
| 2. | O 97              | Mtdm. Issaku Nagata | Onc. Yellow King | 149                  | 60                          |
| 3. | O 105             | Onc. Margareth Holm | Onc. wildcat Red Star | 207                  | 82                          |
| 4. | O 127             | Onc. Linda Isler | Onc. Princess yH Tween Star | 203                  | 44                          |
| 5. | O 138             | Mtdm. Pupukea Sunset x Mtdm. Cleos Pride Hama | Onc. Princess yH Tween Star | 195                  | 56                          |
| 6. | O 175             | Onc. Space Race Coco | Onc. Linda Isler | 196                  | 65                          |
| 7. | O 183             | Mtdm. Issaku Nagata | Onc. Space Race Coco | 165                  | 57                          |
| 8. | O 185             | Onc. Space Race Coco | Onc. Yellow King | 230                  | 25                          |
| 9. | O 259             | Onc. Diana Dawn | Colm. Wildcat Purple | 208                  | 27                          |
| 10. | O 262           | Colm. Wildcat Garfield | Onc. Yellow King | 245                  | 33                          |
| 11. | O 263             | Colm. Wildcat Garfield | Onc. Space Race Coco | 245                  | 33                          |
| 12. | O 334             | Belliara Diana Dunn | Onc. Yellow King | 182                  | 34                          |
| 13. | O 336             | Mtdm. Pupukea Sunset/Cleo’s Pride Hama | Onc. Yellow King | 181                  | 34                          |
| 14. | O 333             | Onc. Pagan Love Song | Onc. maculatum | 210                  | 52                          |
| 15. | O 327             | Wils. Tropic Breeze | Onc. maculatum | 240                  | 45                          |
| 16. | O 429             | Odbrs. Kenneth Bivins Santa Barbara | Odbrs. Kenneth Bivins Santa Barbara | 233                  | 40                          |
| 17. | O 447             | Wils. Calico Gem | Wils. Firecricker Red Star | 188                  | 37                          |
18. O 434 *Mtdm. Pupukea Sunset/CPH* Onc. Yellow King 203 25
19. O 451 *Colm. Wilcat Bobcat* Wils. Firecrecker Red 190 21
20. O 479 *Onc. Linda Isler* Brazzia 187 46
21. O 536 *Alcra Sunday Muffin* Alcr. Steng Wen Spot 193 31
22. O 543 *Anc. Jungle Monarch* Aler. Sunday Muffin 205 35
23. O 541 *Onc. Margareth holm* Aler. Sunday Muffin 167 24
24. O 592 *Onc. Spot Berry* Onc. Odoraos princess 168 24
25. O 595 *Onc. Linda Isler* Aler Sunday Muffin 195 30
26. O 593 *Alcra Sunday muffin* Onc. Kolibri 177 28
27. O 642 *Wils. Tropic Breeze* Wils. Tropic Breeze 230 42
28. O 643 *Onc. lanceanum* Onc. lanceanum 235 46
29. O 646 *Miltonia sp* Miltonia sp 186 34
30. O 647 *Odontoglossum Grande* Odontoglossum Grande 273 21
31. O 657 *Mtdm. Issaku Nagata* Onc. Odoraos Princess 185 17

Average 186 39

3.2. The effect of media on seed germination

Almost all capsules fruit of *Oncidium* was green color with set of seed white color (fig.2a & 2b). Within 8 weeks, protocorm developed from seed (fig.2c). Process initiation germination would be followed by development of protocorm which was cultured on several media ½ MS, VW and Tsuchiya supplemented with plant growth regulator and also sucrose. In the most of tested media, within 8 weeks showed the formation of green light protocorm became plantlets, indicating differentiation and germination. A comparison of three basal media showed that basal media Tsuchiya was most suitable for *Oncidium* seed germination, followed by basal media ½ MS then VW. Media without plant growth regulator (PGR) were not responded both of embryo and shoot differentiation. The data revealed the lowest number of embryo differentiation and shoot formation on media Tsuchiya, ½ MS or VW. The highest number of seed germination was observed in Tsuchiya media supplemented with 1 mgL⁻¹ BAP and 0.25 mgL⁻¹ NAA (P2) (table 3) that indicated with maximum number of shoots formed 67.8, even though only 48.3 embryo differentiated, totally germinated 116.1 was the highest number.

The effect of combine TIBA and BAP, or BAP and NAA was observed on the basis of number of embryo differentiated and shoot per vessel too. The result revealed that after 8 weeks of inoculation, trend number of shoot per culture vessel was highest (67.8) in P2 medium, (51.4) in P4, and (27.6) in P6 on the fortified with BAP and NAA. Also, the highest value was (58.9) in P1, (45.3) in P3 and (25.3) in P5 on the fortified with TIBA and BAP (table 3). On the other hand, trend of the differentiation of embryo was not consistence that maximum number on media using PGR BAP and NAA but it occurred on media combined TIBA and BAP.

**Table 3.** The effect of media with different concentration of plant growth regulator on seed germination and embryo development of *Oncidium*. 8 weeks after inoculation

| Media | Phases of Embryo Development |
|-------|------------------------------|
|       | No. of Differentiated Embryo | No. of Shoot | Total Germinated |
| P1 (Tsuchiya +1 mg L⁻¹ TIBA+ 0.25 mg L⁻¹ BAP) | 52.5±2.4a | 58.9±1.8b | 111.4±2.1a |
| P2 (Tsuchiya +1 mg L⁻¹ BAP + 0.25 mg L⁻¹ NAA) | 48.3±2.8b | 67.8±2.7a | 116.1±2.9a |
| P3 (½ MS + 1 mg L⁻¹ TIBA +0.25 mg L⁻¹ BAP) | 16.8±2.0d | 45.3±2.0d | 62.1±2.2c |
| P4 (½ MS + 1 mg L⁻¹ BAP +0.25 mg L⁻¹ NAA) | 27.2±2.0c | 51.4±1.5c | 78.6±1.9b |
| P5 (VW+ 1 mg L⁻¹ TIBA +0.25 mg L⁻¹ BAP) | 14.8±1.3de | 25.3±2.0e | 40.1±2.0d |
| P6 (VW + 1 mg L⁻¹ BAP + 0.25 mg L⁻¹ NAA) | 46.3±1.1bc | 27.6±1.5e | 73.9±1.6bc |
P7 (Tsuchiya without PGR)       7.3±2.0e  7.7±2.0f  15.0±2.1e
P8 (1/2 MS without PGR)         5.6±1.2ef   4.6±1.2fg  10.2±1.4f
P9 (Vaccine & Went without PGR) 3.1±0.8f  0.6±0.2g  3.7±1.3g

The mean ± SD followed by different letters in the same column indicate significant difference according to Duncan’s test at a significance level $\alpha = 0.05$.

**Figure 2.** Development of seed to individual resulted by hybridization (a) capsule contain seed, (b) seed spreading in media, (c) protocorm like bodies, (d)& (e) plantlet/seedling on media . (f) compot, (g) individual plants using bamboo litter.

**Figure 3.** Four weeks germination seed using several concentration of sucrose (a) 0%. (b) 5%. (c) 10%. (d) 20%. on Media Tsuchiya.

Treatment of sucrose concentration was observed also in the early step of embryo culture. In four weeks germination, seed were indicated green light colour and showed different quantity for each treatment (fig.3). Media with sucrose at concentration 0% and 5% were effected small amount of embryo developed but media with sucrose 10% and 20% were influenced more embryos growing. At the table was described that embryos were planting in media without sucrose for 8 weeks were still grew but it was very poor amount on both embryo differentiated and shoot (Table 4). Media using sucrose 10% and 20% gave almost same effect toward number of embryo differentiated and number of shoots. Embryo differentiated was 21.8 on 10% sucrose and 20.3 on sucrose 20% as well as number of shoot was 24.4 on 10% sucrose and 27.4 on 20% sucrose. Therefore, use of sucrose 10% and 20% could be concluded that would give the same effect toward seed germination of *Oncidium*. All plantlet resulted from germination were continued to develop using media Vaccine & Went supplemented with coconut water and banana homogenate (fig.3d-e). Acclimatization were also successfully applied in this study using bamboo litter or pine wood (fig.3e-f).
Table 4. The effect of sucrose with different concentration on seed germination and embryo development of *Oncidium* at Tsuchiya medium containing 1 mg L\(^{-1}\) BAP + 0.25 mg L\(^{-1}\) NAA for 8 weeks after planting

| Sucrose concentration (gram/L) | Number of Differentiated Embryos | Number of shoots | Total Germinated |
|-------------------------------|----------------------------------|------------------|-----------------|
| 0                             | 11.5±1.4b                        | 3.6±1.2d         | 15.1±1.6c       |
| 5                             | 12.3±2.0b                        | 8.5±1.6c         | 20.8±1.9b       |
| 10                            | 21.8±2.1a                        | 24.3±2.0ab       | 46.1±2.3a       |
| 20                            | 20.3±1.4a                        | 27.4±1.5a        | 47.7±1.5a       |

The mean ± SD followed by different letters in the same column indicate significant difference according to Duncan’s test at a significance level \(\alpha = 0.05\)

3.3. Discussion

Conventional hybridization had potential to contribute to form population of *Oncidium* in Indonesia, even the parental used were not assigned as pure individual species because of multiple crosses from parental had been done before. Further, conventional hybridization is still interesting to explore because hybridization contributed to enhance floral variability, genome size and reproductive success. Hybridization could also contribute to an increase in morphological diversity of the populations as a result of segregation and recombination between the parental genomes [8].

In this research, we were conducting a lot of intergeneric and interspecific crosses but not always produced viable seed. Many factors were inhibited the success of crosses. Reproductive barrier was apparently very strong in *Oncidium* orchid. Some individual parental had infertile pollen or infertile pistil. Several crosses when using Onc. Yellow King acted as pollen donors were produced viable seed but no one when using Onc. Yellow King acted as pistil donors were succeeding to produce viable seed (table1). Phenomenon of decreased crossing ability of individuals parental caused by plant physical condition were less healthy. Climate change factors was affected phenology of many species [9, 10], this might cause the *Oncidium* as well. No flowering, flower earlier or later were caused by environmental change. Based on plant maintenance experience, *Oncidium* plant still grewed optimally in three years but after the condition was not healthy. Hence, the successes of crosses were decreased. Ploidy level also caused the failure of hybridization [11]. Many of *Oncidium* commercial in Indonesia were high level ploidy, which usually caused infertility when used for parental crossing.

All of capsules *Oncidium* that resulted from crossing in this research, only thirty one one were germinated. Germination ability was determined by the maturity of the fruit, nutrient media composition and addition of PGRs [12, 13, 14] as well as in our research. Media Tsuchiya, ½ MS and Vaccine & Went were chosen because the difference contain of macro and micro elements. Media MS had richer contents macro and micro elements but lower vitamins [15] and a lot of research using MS medium. In the experiment, seed germination was successfully established which was similar to the previous reports in *Phalaenopsis* [16, 17], *Paphiopedilum* [18], *Spathoglottis* [19], and *Cymbidium* [20]. Effect synergistic of BAP and NAA or TIBA and BAP were observed and the result revealed that combination BAP and NAA more suitable for seed germination on *Oncidium* than TIBA and BAP. Combination of plant growth regulator BAP and NAA has been reported previously succeed in Orchid [21]. Plant growth regulator TIBA and BAP were used in the experiment could not be increased seed germination. TIBA has been reported that use high concentration would be inhibiting formation of development embryo [22]. The result of sucrose concentration experiment was the best on concentration 10 % and 20%, both concentration affected embryo differentiation and plantlet growth. In addition, carbohydrate might supplied energy to the platlets, especially when they were not ready to photosynthesize their own food during initial stage of tissue culture [23, 24]. Carbon source could be in the form of simple or complex sugars, however, sucrose was the most commonly used in plant tissue culture medium. An appropriate type and concentration of sugar were needed in medium to promote in vitro seed germination [25].
4. Conclusion
A study of hybridization of Oncidium was evaluated conventionally in Indonesia. In vitro culture of Oncidium was also conducted to evaluate several media and sucrose concentrations on seed germination to form complete seedling. Percentage of crossing succeeded was determinate very low but it was useful to construct many hybrid populations. Media Tsuchiya +1 mgL\(^{-1}\) BAP + 0.25 mgL\(^{-1}\) NAA were the most suitable to germinate the Oncidium seed. Moreover, treatment sucrose 20% was also the best concentration on the media to germinate seed.

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