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# FIELD EVALUATION OF MORPHOLOGICALLY CHARACTERISED NORMAL AND OFF-TYPE OIL PALM PLANTLETS REGENERATED VIA SOMATIC EMBRYOGENESIS

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## ABSTRACT

*Somatic embryogenesis is a key technique for oil palm clonal propagation. This study assessed the regenerative capacity of embryoids and monitored the growth of plantlets, classified as normal or off-type, up to fruiting in the field. Young leaf explants were cultured to induce callus, which developed into embryoids and subsequently regenerated into plantlets. Callus induction rate among virescen clones ranged from 5.91%-19.92%, with embryoid induction rate between 6.35% and 31.94%. Regeneration rates varied, and 4.62% of plantlets exhibited off-type traits, including heights of 8-10 cm, stem diameters exceeding 4 mm, dark green pigmentation, grass-like morphology, more than five leaves, and wrinkled leaves. A total of 2,906 seedlings (2,518 normal and 388 off-type) were planted. After five to six years, all plants produced normal fruit without mantling. The findings demonstrate that virescent off-type plantlets, often discarded due to mantling concerns, can develop normal phenotypes – challenging the common culling practice. Retaining off-type plantlets may enhance seedling production efficiency.*

**Keywords:** callus, embryoid, off-type, oil palm fruit, tissue culture.

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## INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a vital crop for food and biofuel production. The widely grown *tenera* fruit form is primarily derived from the nigrescens type, while the virescens type, though nutritionally similar, is less common. These varieties are distinguished by immature fruit colour: Nigrescens fruits are dark purple to black when unripe, turning reddish-purple when ripe, whereas virescens fruits shift from deep green to bright orange at maturity (Rao & Chang, 2021). Seedlings are mainly produced through controlled crosses and clonal propagation via tissue culture (Soh et al., 2011). Somatic embryogenesis, involving callus induction, proliferation, embryo maturation and plantlet regeneration, is critical for large-scale propagation but is prone to variability due to culture conditions, requiring optimised protocols to minimise abnormal plantlet development (Corredoira et al., 2019; Padua et al., 2017). While adjusting growth media can reduce mantled fruit formation, it often delays somatic embryogenesis (Kushairi et al., 2010).

Somaclonal variation, a frequent issue in tissue culture propagation, results from morphological and genetic changes in undifferentiated callus cells (Rival et al., 2013). Stress during *in vitro* culture

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can trigger epigenetic modifications, disrupting normal growth (Bairu et al., 2011). Early signs, such as leaf colour changes and organ abnormalities, often indicate somaclonal variation (Soltabayeva et al., 2021). Research by Martineau et al. (2021) underscores the role of small non-coding RNAs (sncRNAs) in epigenetic regulation, affecting stress memory and acclimation in perennial species. These insights highlight the need for genetic and morphological assessments to ensure the selection of high-quality clonal plantlets.

Clonal propagation, while efficient, raises concerns about mantled fruit development (Correa et al., 2016), necessitating early selection of normal and off-type plantlets (Ernayunita et al., 2019). Traditionally, virescens clones with off-type morphology are discarded due to potential long-term performance issues. DNA hypomethylation of a LINE retrotransposon within the DEFICIENS gene intron, identified as the Bad Karma epiallele, has been linked to homeotic transformation and yield loss in mantled oil palm clones (Ong-Abdullah et al., 2015). This study evaluates the regeneration capacity of such plantlets, tracking their development from *in vitro* culture to field fruiting. Contrary to conventional practices, our findings reveal that off-type virescens clones can achieve normal growth and produce viable fruit under standard agronomic conditions. The potential for spontaneous epigenetic reversion, as observed in some mantled clones, suggests that strict early-stage culling may lead to the unnecessary loss of productive plantlets (Ong-Abdullah et al., 2015). These results offer critical insights for refining oil palm clonal seed production and minimising the loss of potentially productive plantlets.

## MATERIALS AND METHODS

### Location

Plant propagation was conducted from 2013-2016 at Tissue Culture Laboratory, Biotechnology Laboratory-BRIN, Banten, Indonesia. Field trials began in December 2017 and December 2018 at an oil palm plantation in Pangkalan Bun, Central Kalimantan, Indonesia. Observations were recorded in December 2023.

### Clone Material

Young leaf explants from three virescens oil palm clones (VR-1, VR-2, VR-3) were collected from four fronds and cut into 1.5 cm segments. The explants were sterilised with 0.02% sodium hypochlorite for 20 min, immersed in a 0.20% glucose solution and then planted in callus induction media (*Figure 1a-1c*).

### Callus and Embryoid Induction

Young leaf explants were cultured in callus induction medium (modified Murashige & Skoog [MS] + 2,4-Dichlorophenoxyacetic acid 10-50 µM) for 12 months, with biweekly observations of asynchronous callus formation (De Fossard et al., 1974; Yusnita & Hapsoro, 2011). Formed calluses were coded and transferred to embryoid induction medium. The calluses were then cultured in embryoid induction medium (modified MS + 6-Benzylaminopurine [BAP] 5-50 µM) for six months, with weekly observations of embryoid formation (De Fossard et al., 1974; Yusnita & Hapsoro, 2011). Embryoids were coded based on clone and number (e.g., VR-1-01: Clone VR-1, embryoid 01) and subsequently transferred to regeneration media (*Figure 1d-1f*).

### Regeneration and Root Induction

Mature embryoids were cultured in regeneration media (MS basal medium without growth regulators) for eight-week cycles until multiple shoots formed. These shoots were then transferred to root induction media (modified MS + 1-Naphthaleneacetic acid [NAA] 5-10 µM) (De Fossard et al., 1974; Yusnita & Hapsoro, 2011) to develop roots, reaching a height of 10-12 cm with two leaves. Rooted plantlets were subsequently cultured in hormone-free media for eight weeks before acclimatisation (*Figure 1h-1i*).

### Acclimatisation and Field Planting

Off-type plantlets were identified morphologically before acclimatisation based on characteristics such as curled or twisted leaves, terminal inflorescence and grassy shoots (more than five leaves). Selected off-types were labelled for continuous monitoring. Acclimatisation was conducted in a greenhouse for seven days, followed by transfer to polybags containing soil and sand mixture. The plantlets were maintained under shade for 6-8 weeks before being exposed to full sunlight for four weeks and subsequently moved to nursery stages. Growth and development were monitored annually from the nursery through field planting, with performance evaluation based on fruit development, classified as normal or abnormal (*Figure 2*).

### Data Analysis

A completely randomised design with a single factor and four replications was used, with each replication containing 440 young oil palm leaf explants. Callus formation was observed monthly for 12 months, while embryoid formation was

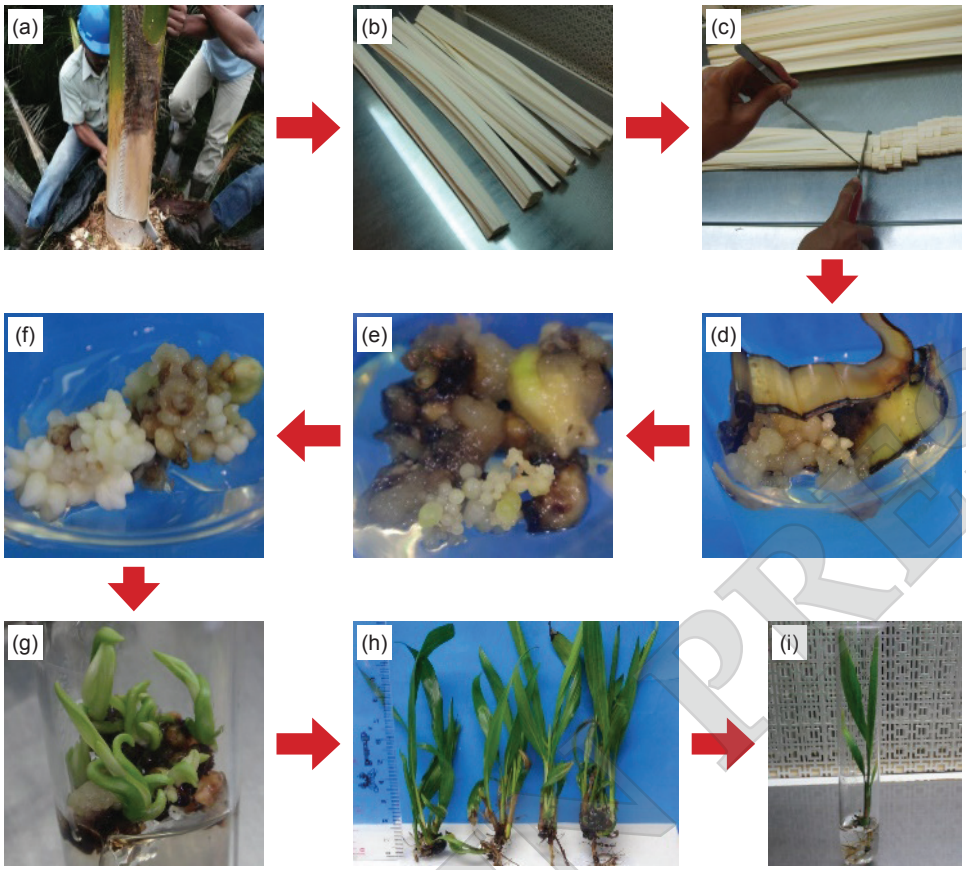


*Figure 1. Key stages of oil palm tissue culture: (a) Ortet sampling, (b) selection of the four youngest fronds for explants, (c) frond segmentation into 20 pieces (1.5 cm each), (d) callus induction, (e) embryogenic callus formation, (f) embryoid induction and maturation, (g) shoot initiation, (h) shoot enlargement and (i) root induction and plantlet formation (Black bar = 1 cm).*

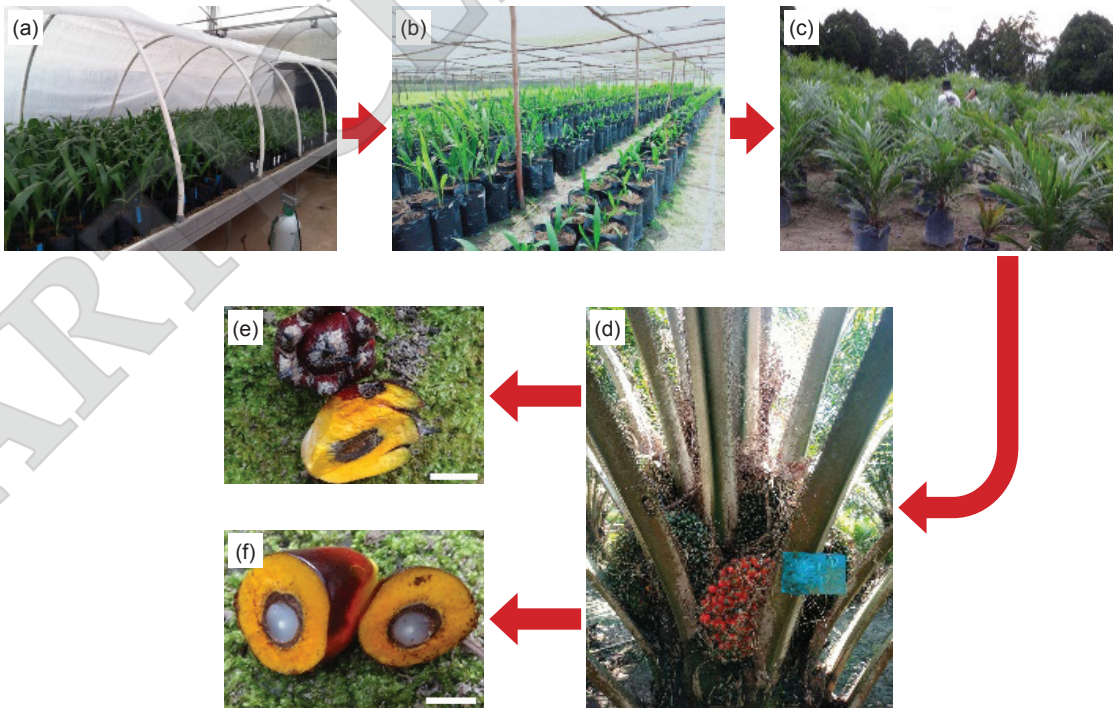


*Figure 2. Advanced stages of oil palm clonal propagation through tissue culture: (a) Acclimatisation, (b) pre-nursery, (c) nursery, (d) 5 year field assessment, (e) abnormal fruit and (f) normal fruit (White bar = 1 cm).*

recorded up to six months. The percentage of callus and embryoid formation was calculated [Equation (1) and (2)]:

$$\text{Callus formation (\%)} = \left( \frac{\text{Total callus}}{\text{Total sterile leaf explants}} \right) \times 100\% \quad (1)$$

$$\text{Embryoid formation (\%)} = \left( \frac{\text{Total embryoids}}{\text{Total callus}} \right) \times 100\% \quad (2)$$

Percentage data were square root-transformed before ANOVA, followed by Tukey's test at 1% and 5% significance levels. Data analysis was conducted using Microsoft Excel. Regeneration was assessed quantitatively (normal and off-type plantlet counts) and qualitatively (fruit morphology).

## RESULTS AND DISCUSSION

### Callus and Embryoid Induction

Young oil palm leaves cultured in callus induction media for 6-12 months showed clone-dependent induction rates. ANOVA indicated no significant differences, with VR-1 (6.50%), VR-2 (19.92%) and VR-3 (5.91%) displaying above- and below-average results, respectively (*Figure 3*). The low efficiency aligns with Soh et al. (2011), who reported ~15.00% callus formation. Weckx et al. (2019) noted lower induction potential in young leaves compared to zygotic embryos and floral explants, with variability linked to growth regulators, genotype and endogenous hormones.

Callus morphology varied, ranging from dense, round structures to elongated, soft forms (*Figure 4*). Induction is influenced by growth regulators, explant origin, media and genotype (Kartika et al., 2019), with colour and texture dependent on growth regulator type and concentration (Kumar et al., 2015).

The isolated callus was cultured on embryoid induction media, with only embryogenic calluses producing multiple embryos. The embryoid induction rates of clones VR-2 (6.35%) and VR-3 (17.39%) were not significantly different from each other, but both were significantly lower than that of VR-1 (31.94%) (*Figure 3*), though all rates were below average. Notably, embryo percentages in this study surpassed those reported by Gomes et al. (2016) and Monteiro et al. (2017) (3.00%-5.00%). Enhanced embryo formation in certain clones was attributed to robust embryogenic callus development, driven by balanced growth regulator application (Al-Oqab et al., 2022). Proper auxin and cytokinin selection is crucial for embryogenic callus formation (Kartika et al., 2019), characterised by dense, round structures (*Figure 4a-4b*). Embryoids from clones VR-1, VR-2 and VR-3 showed uniform size and morphology (*Figure 4c-4e*), though not all exhibited regeneration potential into plantlets (*Table 1*).

### Regeneration Normal and Off-Type Plantlets

The embryoid production from calluses varied significantly among clones VR-1, VR-2, and VR-3, with VR-1 yielding the highest (69 embryoids), followed by VR-3 (16 embryoids) and VR-2 (8 embryoids). Mature embryoids differentiated into plantlets under balanced growth regulators and light conditions (Dou et al., 2017), as light-endogenous hormone interactions influence plant regeneration (Chen et al., 2019). Regeneration success varied: VR-1 achieved 60.87% (42 of 69 embryoids), VR-3 93.75% (15 of 16) and VR-2 100.00% (8 of 8) (*Table 1*). Challenges in oil palm somatic embryogenesis, such as low embryo initiation and regeneration rates (Weckx et al., 2019), align with findings of 5.00% regeneration rates in oil palm (Gomes et al., 2017) and with incomplete shoot-root development in other palms (Gomes et al., 2016).

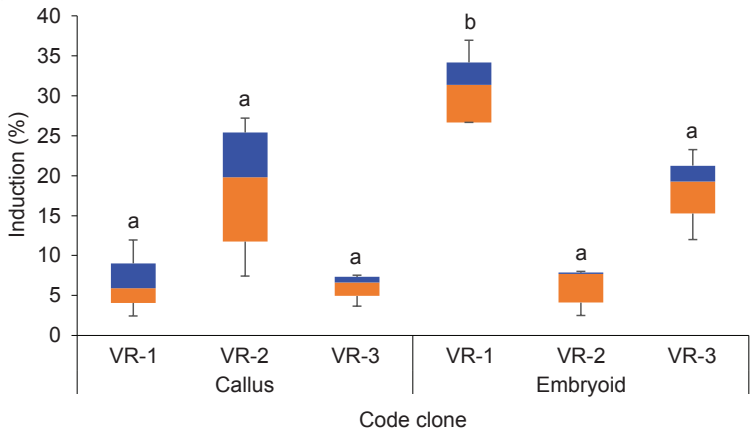


*Figure 3. Callus and embryoid induction percentages from young leaf explants of clones VR-1, VR-2 and VR-3.*

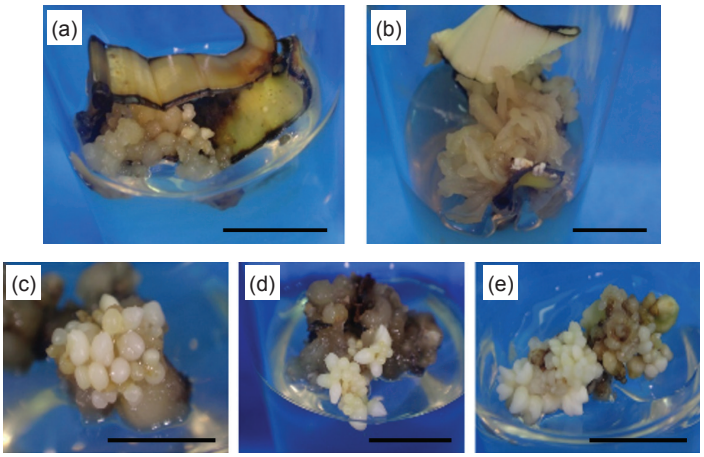


*Figure 4. Callus morphology: (a) Round and solid; (b) elongated and soft. Embryoids from clones (c) VR-1, (d) VR-2 and (e) VR-3 (Black bar = 1 cm).*

**TABLE 1. NUMBER OF OIL PALM EMBRYOIDS CAPABLE OF REGENERATION AND PRODUCING PLANTLETS**

| Clone | Embryo code | Subculture number | Number of regenerated plantlets | |
|---|---|---|---|---|
| | | | Normal | Off-type |
| VR-1 | VR-1-1 | 22 | 290 | 37 |
| | VR-1-2 | 17 | 72 | 2 |
| | VR-1-3 | 12 | 59 | 8 |
| | VR-1-4* | 10 | 0 | 0 |
| | VR-1-5 | 10 | 9 | 0 |
| | VR-1-6 | 21 | 65 | 7 |
| | VR-1-7 | 15 | 29 | 0 |
| | VR-1-8* | 15 | 0 | 0 |
| | VR-1-9 | 22 | 13 | 0 |
| | VR-1-10 | 17 | 2 | 2 |
| | VR-1-11 | 12 | 67 | 0 |
| | VR-1-12* | 17 | 0 | 0 |
| | VR-1-13 | 10 | 510 | 61 |
| | VR-1-14* | 16 | 0 | 0 |
| | VR-1-15 | 21 | 10 | 0 |
| | VR-1-16 | 18 | 10 | 0 |
| | VR-1-17* | 5 | 0 | 0 |
| | VR-1-18* | 8 | 0 | 0 |
| | VR-1-19* | 10 | 0 | 0 |
| | VR-1-20 | 19 | 566 | 41 |
| | VR-1-21* | 8 | 0 | 0 |
| | VR-1-22 | 18 | 5 | 0 |
| | VR-1-23 | 19 | 7 | 0 |
| | VR-1-24* | 10 | 0 | 0 |
| | VR-1-25 | 19 | 1 | 0 |
| | VR-1-26 | 20 | 5 | 0 |
| | VR-1-27 | 18 | 6 | 0 |
| | VR-1-28* | 5 | 0 | 0 |
| | VR-1-29 | 21 | 121 | 0 |
| | VR-1-30 | 19 | 4 | 0 |

**TABLE 1. NUMBER OF OIL PALM EMBRYOIDS CAPABLE OF REGENERATION AND PRODUCING PLANTLETS (continued)**

| Clone | Embryo code | Subculture number | Number of regenerated plantlets | |
|---|---|---|---|---|
| | | | Normal | Off-type |
| VR-1 | VR-1-31 | 15 | 42 | 0 |
| | VR-1-32* | 8 | 0 | 0 |
| | VR-1-33* | 8 | 0 | 0 |
| | VR-1-34* | 7 | 0 | 0 |
| | VR-1-35* | 9 | 0 | 0 |
| | VR-1-36 | 18 | 412 | 76 |
| | VR-1-37 | 17 | 10 | 0 |
| | VR-1-38 | 17 | 21 | 0 |
| | VR-1-39 | 10 | 4 | 0 |
| | VR-1-40 | 12 | 2 | 0 |
| | VR-1-41* | 10 | 0 | 0 |
| | VR-1-42* | 9 | 0 | 0 |
| | VR-1-43* | 9 | 0 | 0 |
| | VR-1-44* | 8 | 0 | 0 |
| | VR-1-45* | 9 | 0 | 0 |
| | VR-1-46 | 20 | 1,130 | 68 |
| | VR-1-47 | 17 | 1 | 0 |
| | VR-1-48* | 12 | 0 | 0 |
| | VR-1-49 | 10 | 16 | 1 |
| | VR-1-50* | 10 | 0 | 0 |
| | VR-1-51 | 17 | 9 | 0 |
| | VR-1-52 | 17 | 10 | 0 |
| | VR-1-53 | 16 | 46 | 1 |
| | VR-1-54* | 11 | 0 | 0 |
| | VR-1-55 | 20 | 170 | 23 |
| | VR-1-56* | 5 | 0 | 0 |
| | VR-1-57 | 21 | 341 | 38 |
| | VR-1-58 | 12 | 5 | 0 |
| | VR-1-59 | 20 | 28 | 1 |
| | VR-1-60* | 6 | 0 | 0 |
| | VR-1-61 | 18 | 1 | 0 |
| | VR-1-62 | 19 | 8 | 7 |
| | VR-1-63 | 17 | 7 | 4 |
| | VR-1-64* | 8 | 0 | 0 |
| | VR-1-65* | 9 | 0 | 0 |
| | VR-1-66 | 19 | 122 | 15 |
| | VR-1-67* | 10 | 0 | 0 |
| | VR-1-68 | 15 | 1 | 0 |
| | VR-1-69 | 16 | 20 | 2 |
| VR-2 | VR-2-1 | 21 | 151 | 14 |
| | VR-2-2 | 14 | 11 | 3 |
| | VR-2-3 | 19 | 287 | 0 |
| | VR-2-4 | 20 | 40 | 14 |
| | VR-2-5 | 18 | 0 | 4 |
| | VR-2-6 | 15 | 3 | 0 |
| | VR-2-7 | 20 | 165 | 0 |
| | VR-2-8 | 19 | 13 | 1 |

**TABLE 1. NUMBER OF OIL PALM EMBRYOIDS CAPABLE OF REGENERATION AND PRODUCING PLANTLETS (continued)**

| Clone | Embryo code | Subculture number | Number of regenerated plantlets | |
|---|---|---|---|---|
| | | | Normal | Off-type |
| VR-3 | VR-3-1 | 19 | 83 | 0 |
| | VR-3-2 | 19 | 24 | 0 |
| | VR-3-3 | 17 | 10 | 0 |
| | VR-3-4 | 17 | 43 | 0 |
| | VR-3-5 | 17 | 16 | 0 |
| | VR-3-6 | 18 | 105 | 0 |
| | VR-3-7 | 22 | 9 | 13 |
| | VR-3-8 | 19 | 12 | 0 |
| | VR-3-9 | 18 | 12 | 0 |
| | VR-3-10 | 19 | 187 | 0 |
| | VR-3-11 | 19 | 140 | 12 |
| | VR-3-12 | 19 | 31 | 0 |
| | VR-3-13 | 15 | 18 | 0 |
| | VR-3-14 | 18 | 18 | 0 |
| | VR-3-15* | 15 | 0 | 0 |
| | VR-3-16 | 18 | 210 | 0 |

| Clone | Total number of embryoids | | Number of regenerated plantlets | |
|---|---|---|---|---|
| | With regeneration ability (Viable) | Without regeneration ability (Non-viable) | Normal | Off-type |
| VR-1 | 42 (60.87%) | 27 (39.13%) | 4,214 (91.45%) | 394 (8.55%) |
| VR-2 | 8 (100.00%) | 0 (0.00%) | 670 (94.90%) | 36 (5.10%) |
| VR-3 | 15 (93.75%) | 1 (6.25%) | 918 (97.35%) | 25 (2.65%) |

Note: *Clone unable to regenerate normal and/or off-type plantlets (number of regenerated plantlet = 0).

Prolonged embryoid proliferation increases the risk of somaclonal variation (Rival et al., 2013), often resulting in off-type plantlets with morphological abnormalities. In this study, oil palm somatic embryogenesis produced both normal and off-type plantlets, though the process faces challenges such as limited explant sources, low embryo initiation and regeneration rates, inefficient callus proliferation and high somaclonal variation risks, which can lead to abnormal fruit phenotypes (Weckx et al., 2019). Normal plantlets predominated, with clone VR-1 yielding 91.45% normal (4,214 plantlets) and 8.55% off-type (394 plantlets), VR-2 producing 94.90% normal (670 plantlets) and 5.10% off-type (36 plantlets), and VR-3 generating 97.35% normal (918 plantlets) and 2.65% off-type (25 plantlets). Off-types, typically discarded due to potential field abnormalities, were identified by deviations from normal traits: Height of 10-12 cm, stem diameter of 2-4 mm, ≥3 leaves, green colouration, normal leaf shape and 1-2 primary roots (*Figure 5a-5d*).

Off-type plantlets were categorised into three morphological types: Off-type-1, characterised by a height of 10-12 cm, stiff stems and leaves, stem diameters of 3-4 mm, dark green pigmentation and 2-3 wrinkled or twisted leaves (*Figure 5e-5h*); off-type-2, exhibiting clustered growth with flowers, normal-shaped leaves, a height of 10-12 cm, green stems and leaves and stem diameters of 2-3 mm (*Figure 5i-5l*); and off-type-3, identified by a height of 8-10 cm, stem diameters exceeding 4 mm, dark green pigmentation, grass-like morphology, more than five leaves and wrinkled leaves (*Figure 5m-5p*). Among 6,257 plantlets, off-type-3 was the most prevalent at 4.62%, while off-type-1 and off-type-2 each accounted for less than 1.00% (*Figure 6*). Further field evaluation is required to assess their contamination risk or potential utility.

The occurrence of off-type plantlets in this study is likely attributed to suboptimal tissue culture conditions, which may induce somaclonal variation. Feher (2015) identifies stress factors such as sterilisation, explant injuries, high growth regulator concentrations, inappropriate media composition and suboptimal growing conditions as key contributors. Bairu et al. (2011) further noted that tissue culture techniques, particularly indirect embryogenesis with uncontrolled callus proliferation, can lead to abnormalities like mantled fruit, influenced by genotype, growth regulator type and concentration and explant source. Effective management of tissue culture procedures, including subculture frequency and duration, is

crucial to minimise off-type occurrences (Rival et al., 2013). In this study, off-type plantlets were monitored through acclimatisation, pre-nursery, nursery and field stages to ensure normal fruit development. Jaligot et al. (2011) reported that generative abnormalities at flowering are typically detected around four years after planting and confirmed in the fifth year.

**Observation of Fruit Morphology and Identification of Off-Type Plantlets**

The selection of oil palm seedlings was conducted sequentially from the laboratory to the nursery, with field planting carried out progressively based on the number of seedlings meeting selection criteria. In the initial phase, 2,518 seedlings from normal plantlets and 388 from off-type plantlets were selected for plantation establishment at the end of 2017, followed by monthly maintenance. By the end of 2023, all seedlings derived from both normal and off-type plantlets of clones VR-1 (*Figure 7a-7b*), VR-2 (*Figure 7c-7d*) and VR-3 (*Figure 7e-7f)* exhibited 100% normal fruit production, with no abnormalities observed. These plants yielded an average of 13-15 fruit bunches per palm, with an average annual bunch weight of 103-106 kg and an average bunch weight of 6.67-7.43 kg (*Table 2*).

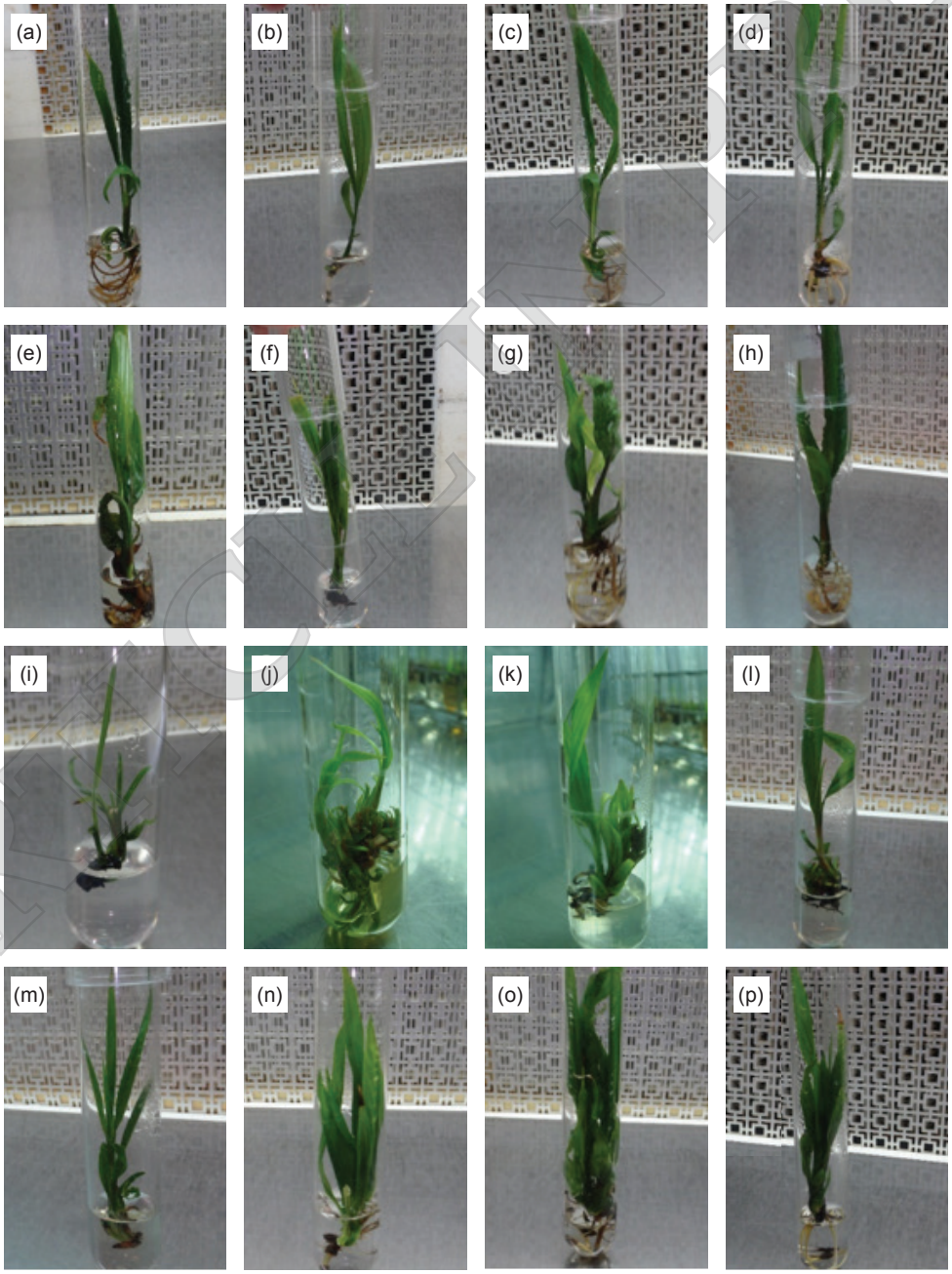


*Figure 5. (a-d) Morphology of normal, (e-h) off-type-1, (i-l) off-type-2 and (m-p) off-type-3 oil palm plantlets.*

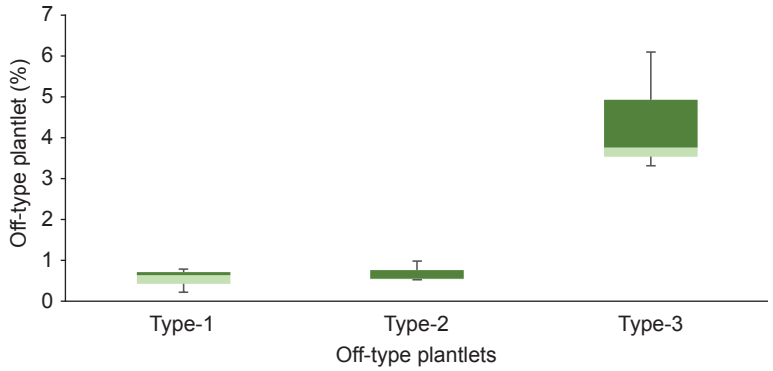


*Figure 6. Off-type plantlets: Type-1, type-2 and type-3.*

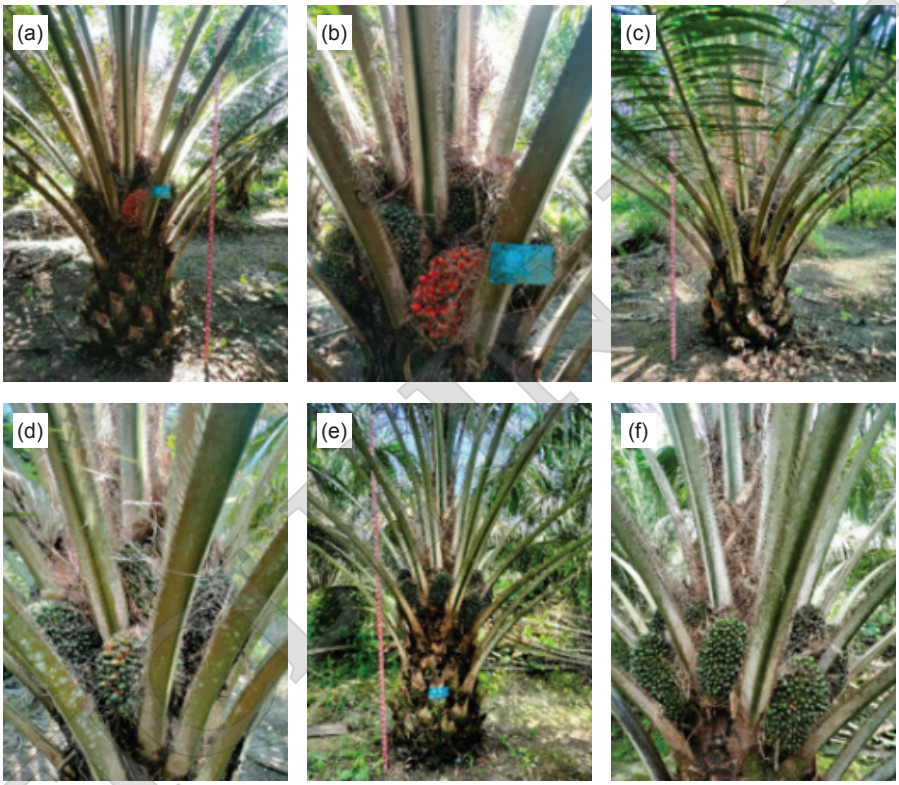


*Figure 7. Normal fruits from oil palm plantations of clones (a, b) VR-1, (c, d) VR-2 and (e, f) VR-3.*

**TABLE 2. FIELD PERFORMANCE OF NORMAL AND OFF-TYPE CLONAL PLANTLETS**

| Field observation | Clone code | | |
|---|---|---|---|
| | VR-1 | VR-2 | VR-3 |
| Normal plantlets planted | 1,802 | 265 | 451 |
| Off-type plantlets planted | 335 | 35 | 18 |
| Fruit morphology | Normal | Normal | Normal |
| Normal fruit (%) | 100 | 100 | 100 |
| Average annual bunches* (palm/yr) | 14.00 ± 8.88 | 15.00 ± 7.71 | 13.00 ± 9.07 |
| Average annual bunch weight* (kg/palm/yr) | 106.25 ± 78.71 | 103.86 ± 71.89 | 105.57 ± 76.19 |
| Average bunch weight* (kg) | 6.90 ± 2.24 | 6.67 ± 2.40 | 7.43 ± 1.68 |

Note: * Values: mean ± standard deviation. Plantlets were planted in December 2017 and December 2018, with observations recorded in December 2023.

The data in *Table 2* demonstrate that all plants derived from embryoids of the same or different clones produced 100.00% normal fruits. This contrasts with findings by Rival et al. (2013), who reported 9.70% mantled fruit (3.70% mild and 6.00% severe abnormalities) in 29,415 clonal plants from 127 parent palms, negatively affecting oil yield. Jaligot et al. (2000) also observed mantled fruit abnormalities in clonal plants from both different and the same parent palms. The off-type plantlets in this study are hypothesised to exhibit epigenetic, rather than genetic, modifications, as they demonstrated normal growth and development during acclimatisation, pre-nursery, nursery and fruiting stages under proper care. Consistent with Weckx et al. (2019), such morphological abnormalities are attributed to somaclonal variations common in tissue culture. Bairu et al. (2011) further emphasise that high-stress conditions in tissue culture can disrupt normal growth, inducing genetic or epigenetic somaclonal variations. When cultivated under identical field conditions with standardised fertilisation and maintenance, all off-type plantlets developed into phenotypically normal plants and produced normal fruit (*Figure 7*), raising the possibility that these variations might have an epigenetic basis and could potentially be reversible under optimal conditions.

This long-term study on three virescen oil palm clones, spanning callus induction, embryoid formation and regeneration, identified plantlets as normal or off-type, yet none produced abnormal or mantled fruit upon field planting. Off-type clones, typically culled, exhibited normal phenotypes in the field, suggesting their potential utility and challenging current culling practices. These findings highlight retaining morphologically off-type plantlets could enhance production without compromising seed quality, providing a valuable reference for *in vitro* selection strategies.

## CONCLUSION

Somatic embryogenesis of three virescen oil palm clones (VR-1, VR-2, VR-3) showed callus induction rates of 5.91%-19.92% and varied embryoid induction rates, with VR-1 achieving the highest (31.94%) compared to VR-2 (6.35%) and VR-3 (17.39%). Among regenerated plantlets, 4.62% exhibited off-type traits. Field cultivation of 2,518 normal and 388 off-type plantlets demonstrated that all produced normal fruit after 5-6 years, indicating off-type plantlets, often discarded, can yield normal fruit, suggesting their retention could improve production efficiency without compromising seed quality.

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