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Tissue Culture of Oil Palm (Elaeis Guineensis Jacq): Techniques and Applications in Genetic Breeding

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ABSTRACT: Oil palm (Elaeis guineensis Jacq.) has become a leading plantation crop in Indonesia because it can provide employment opportunities and contribute to the country's foreign exchange earnings. The productivity of palm oil always experiences fluctuations. Over the past four years, the highest productivity of palm oil was in 2020, amounting to 48,296.30 tons/ha. Efforts to increase palm oil productivity need to be continuously pursued considering the high consumer demand for oil. One of the efforts to increase palm oil productivity is to cultivate superior seed varieties. The type of superior seedlings can be obtained from tissue culture. The research method used is a qualitative descriptive method with a literature study technique. The purpose of this research is to understand the tissue culture process, the inhibiting and supporting factors of tissue culture, the advantages and disadvantages of tissue culture, and the opportunities and challenges of palm oil tissue culture. The research results show that tissue culture affects palm oil production because the seedlings produced through tissue culture have superior traits, and the supporting factors of the tissue culture technique are the type of media and the addition of appropriate growth regulators (ZPT).

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INTRODUCTION

Oil palm (Elaeis Guineensia Jacq) is one of the leading plantation crops in Indonesia. Oil palm has become one of the unique types of plants because almost all of its parts can be utilized. The main product of oil palm is its oil, which can be processed into various products, both food and non-food. Palm oil has a high oil content. The flesh of the fruit (mesocarp) contains about 45-50% oil, while the kernel contains 47% oil. Palm oil has become the prima donna as a producer of vegetable oil with the highest productivity, requiring the least planting area (Pratiwi dkk., 2020). Oil palm trees have one vegetative meristem bud that produces an unbranched trunk and 40 to 50 leaves. The oil palm leaves are equipped with inflorescence meristems in the axils, which then develop into fruits, with a quantity that can reach up to 2,000 fruits. The source of palm oil is extracted from the inner core. About 90% of palm oil is utilized in the food industry, such as raw materials for margarine, shortening, biscuits, ice cream, salad dressings, and mayonnaise. Palm oil has become the world's most important vegetable oil, accounting for 40% of total vegetable oil production (Weckx et al., 2019). Based on the Central Statistics Agency (BPS) in 2023, the productivity of palm oil in 2021 was 46,223.30 tons/ha, in 2022 the productivity of palm oil reached 46,819.70 tons/ha, and in 2023 the productivity of palm oil was 46,986.10 tons/ha. This figure is lower compared to the productivity of palm oil in 2020, which was 48,296.90 tons/ha.

The increase in palm oil productivity needs to be carried out considering the rising global demand for oil. One of the efforts that can be made to increase palm oil productivity is by cultivating superior seedlings. The characteristics of high-quality oil palm seedlings needed include high productivity, resistance to ganoderma, and greater tolerance to abiotic stress (Setyorini dan Nanik. 2019). Oil palm nursery can be conducted both generatively and vegetatively. Generative nursery produces plant types with a high level of diversity, and the generative plant propagation technique is considered less effective.

According to Ernayunita et al. (2017), vegetative propagation of oil palm plants offers greater advantages because it is more uniform vegetatively, and the yield is higher, by 20% to 30%, compared to generative propagation techniques. One of the vegetative plant propagation techniques that can be performed is the tissue culture technique. Tissue culture is a vegetative propagation technique that involves growing part of the plant's cells or tissues in artificial media under aseptic, controlled, and sterile conditions. Tissue culture has several advantages, including a rapid and uniform multiplication rate of superior plant materials. With tissue culture techniques, existing plant materials can be improved into new plant materials with superior characteristics, such as

high oil production and quality, good, slow growth, and disease resistance with uniform traits, and it is expected that palm oil productivity will be high. In addition, tissue culture also opens up opportunities for the production of new plant materials through genetic engineering, selection of plants resistant to abiotic stress such as drought and salinity, production of haploid plants through anther culture, selection of disease-resistant plants, and as an effort for the conservation of germplasm for old and sick plants (Pratiwi dkk. 2020).

The advantages of tissue culture are its ability to produce seeds en masse in a short period, uniformly, with traits identical to the parent, and a short non-productive period. However, the challenge encountered in performing tissue culture of oil palm is the low percentage of shoots producing roots (Yunita dkk. 2016). Factors supporting the success of oil palm tissue culture include the type of explant used, the culture medium, and the type of growth regulator added. The most effective explant used in oil palm tissue culture is young leaves (Karyanti dkk. 2019).

The purpose of this research is to understand the stages of oil palm tissue culture, to identify challenges and opportunities in oil palm tissue culture, the advantages and disadvantages of oil palm tissue culture, as well as the supporting and inhibiting factors of the oil palm tissue culture technique.

METHOD

The method used in this research is a qualitative descriptive method with a literature study technique. Where the author seeks supporting theories and relevant research data related to the research title through accredited journals. The advantage of the qualitative method is that data is taken from real-world events, explaining problems and their solutions, being flexible, and resulting from a deep understanding of a phenomenon.

RESULT AND DISCUSSION

Stages of Oil Palm Tissue Culture

The stages of oil palm tissue culture according to (Ernayunita et al. 2017) are as follows:

1. **Orthotic fitting**

   Ortet is a selected plant that will be used as an explant. The explants of oil palm that can be utilized are the stem, leaves, seeds, and flower tissues. The use of shoot explants has become more dominant because they can be obtained in large quantities, and the condition of the explants is more sterile. The criteria for selecting explants to be cultured are that they come from high-yielding plants, have good oil quality, exhibit rapid growth rates, are free from pest and disease attacks, and are free from crown disease. (penyakit abnormalitas kelapa sawit).

2. **Tissue Culture in the Laboratory**

   a. **Kalus**

      The initial stage of tissue culture is the planting of explants for callus initiation. The explants are planted in a callus initiation medium containing the hormone 2,4 D. The first callus that appears is called primary callus, and then the callus is propagated, resulting in secondary callus.

   b. **Somatic Embryos**

      Somatic embryos are initiated from secondary callus. The formation of callus and somatic embryos is key in oil palm tissue culture because it is at this phase that plant propagation can be carried out. The characteristics of a good embryo, which is viable, are marked by a yellowish-green color and a firm texture.

   c. **Pupus**

      Pupus is the first leaf that appears from the somatic embryo. Pupus is initiated using pupus initiation media, which will then be induced for root formation. The criteria for cuttings with good growth are fresh green color and a stem length of 5 cm.

   d. **Planlet**

      Pups that have been initiated to root will produce complete plants. The shoot that has rooted is called a plantlet. The root initiation stage to become a planlet takes 3 months. Planlets with a height of 5 cm and already rooted are ready to be acclimatized. Planlets that will be acclimatized are first selected according to the expected criteria.

3. **Planlet Acclimatization**

   Acclimatization is the stage of adaptation of plantlets from the laboratory environment with controlled conditions (temperature, humidity, light intensity) to the uncontrolled field conditions. The stages of acclimatization include washing the planlet roots from the medium to prevent them from sticking and soaking the planlets in a fungicide solution. The plantlets are planted in an acclimatization medium consisting of a mixture of sand, soil, and compost in a ratio of 10:3:1, and the plantlets are covered.

Supporting and Hindering Factors of Oil Palm Network Culture

The media used in tissue culture techniques from the callus induction phase to the embryo maturation process is solid media. Induction and development of callus are influenced by the composition of the medium, vitamins, amino acids, and growth regulators (auksin dan sitokinin). The addition of complex organic compounds (young coconut water and fruit or vegetable extracts) can be used as an alternative medium for the induction of embryogenic callus to reduce the level of abnormalities in oil palm plants derived from tissue culture. (Setyorini dan Nanik, 2019). One of the inhibiting factors in the tissue culture technique for oil palm is the low percentage of plantlets that can form perfect roots. The success of plants in acclimatization is significantly influenced by a good root pattern. The ability of tissue-cultured oil palm plants to adapt to the environment will be optimal when the root pattern is perfect (Yunita dkk, 2016). The right type of media and the balanced addition of plant growth regulators will affect the results of oil palm tissue culture.

Based on the research results from Riyadi and Sumaryono (2015), it is stated that in vitro root induction is effective when using auxin growth regulators, either singly or in combination. Unrooted oil palm plantlets can be induced in vitro in liquid medium using a combination of auxins NAA and IBA. The best auxin combination is NAA 10 µM and IBA 20 µM, which results in an average root induction percentage of 73.3%. The treatment with NAA and IBA also positively affects the growth of the upper part of the oil palm plantlets, including plantlet height, number of leaves, and shoot diameter.

Abnormalities are one of the vulnerabilities that can occur in tissue-cultured oil palm plants. About 10% to 40% of tissue-cultured oil palm plants show abnormalities in the reproductive organs, namely flowers and fruits. In the process of abnormality, one or more primordial anthers change into additional soft carpels and develop into mantle fruits. The worst consequence of abnormality is the failure to form fruit because the fruit bunches are filled with male flowers or heavy mantle fruits, leading to a loss of production. The causes of abnormalities in oil palm plants can be genetic, disturbances in gene expression caused by phytohormones, callus structure, duration of subculture and callus age, selection pressure used, type of explant used, ploidy level of the explant source, and callus proliferation rate (Mathius dkk, 2001).

According to Azahra and Emayunita (2023), the efforts to minimize abnormalities in oil palm clones are as follows:

a. Selection of Types and Concentrations of Plant Growth Regulators

The plant growth regulators (PGRs) commonly used for oil palm tissue culture are auxins and cytokinins. The application of plant growth regulators (PGRs) must be balanced to prevent the phenomenon of clonal abnormalities. At the organogenesis stage, the application of auxins and cytokinins should be balanced, while the somatic embryogenesis process requires more auxins to trigger callus induction and embryo multiplication. The types of synthetic auxins commonly used in oil palm tissue culture are 2,4-dichlorophenoxyacetic acid (2,4-D), picloram (4-amino-3,5,6-tricholorpicolinic acid), naphthaleneacetic acid (NAA), benzyl amino purine (BAP), 2-isopentenyl adenine (2-ip), and kinetin. The application of 2,4-D at a concentration of 50 mg/L in the culture is capable of producing the highest embryogenic callus of oil palm with cells that are isodiametric in shape and yellowish-brown in color. For embryo multiplication, a lighter plant growth regulator (PGR) with a low concentration is needed. In the embryo multiplication stage, NAA-type PGR is usually used. The research conducted shows that the application of NAA at a low concentration of 0.5 mg/L provides a good response in the multiplication of oil palm embryos. PGR with a low dose causes a low stimulation level, with low toxicity as well.

b. Reducing Culture Cycles and ZPT Exposure Time

The appropriate protocol for oil palm tissue culture is that the subculture period is conducted every 2 to 4 months, with a maximum of six subcultures at the callus stage, six at the embryo stage, four at the plantula stage, four at the pupus stage, and three at the root induction stage. To minimize the possibility of abnormalities in oil palm clones, the subculture process should ideally be limited to 15 times in one production cycle. The acceptable time for cultured plants to reduce the percentage of abnormalities to 0% is by limiting the exposure time to plant growth regulators (PGR) in a culture medium to a maximum of 171 days.

c. Improvement of the Culture System

The Temporary Immersion System (TIS) has become one of the tissue culture improvement systems that can be applied. TIS has become one of the bioreactor-based propagation methods used to optimize a bioreactor that employs the temporary immersion system. The mechanism of the TIS operation is that the lower container is pressurized with sterile air, and then the liquid medium is pumped into the upper container so that the medium can rise to the upper container to submerge the explants for a specific duration and interval of immersion, which can be adjusted using an autonic double timer.

d. DNA Analysis

DNA analysis aims to identify the identity and genetic stability of tissue culture material. DNA analysis is commonly referred to as DNA fingerprinting. In the process of DNA fingerprinting analysis, the use of DNA markers is

involved. There are several DNA marker techniques that have been used in plant DNA fingerprinting analysis, including Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), and Single Nucleotide Polymorphism (SNP). The selection of DNA markers to be used is determined based on the analysis objectives, the availability of resources, and the desired accuracy. Differences in DNA profiles of the same material at different culture stages can indicate genetic changes in the culture material, which may be caused by the influence of the tissue culture process. DNA markers can identify genetic changes, making them useful in studying the causes of abnormalities in oil palm clones.

e. Monitoring Clonal Diversity in the Field

The purpose of conducting field monitoring is to determine the condition of oil palm clones resulting from tissue culture after the acclimatization or adaptation process. The observation results can serve as data or information to evaluate tissue culture techniques and select more appropriate methods and media to minimize abnormalities in oil palm clones. The procedure for monitoring the performance of clones in the field is to calculate the percentage of clone abnormalities obtained from individual observations of all types of abnormalities present in the garden as early as possible. The abnormalities that occur in the clones are temporary and permanent, influenced by the severity of the abnormalities. Abnormalities that are temporary and can recover, such as the appearance of light mantle fruit and heavy mantle fruit. Abnormalities that are permanent and will not recover, such as the appearance of abortive fruit and also plants with upright growing canopies (erect). Rahmadi and Ernayunita (2015) stated that abnormalities in the form of light mantle fruit can recover 100% to normal fruit within a period of 3 to 4 years in the field.

f. Use of Traceable Database

The use of the database referred to here is the assembly of a software system that can recapitulate all forms of the origin of the produced oil palm clones. Starting from the identity of the ortet used, the duration of the incubation period, the interval of subculture, the number of subcultures, the development at each stage of tissue culture, the number of abnormal plants at each stage, and other information. With the existence of this database, the background of the abnormal oil palm clones can be traced, and from this data, the causes of the abnormalities in the oil palm clones can be studied, allowing for improvements in the tissue culture procedures in the future to reduce the percentage of abnormalities that appear in the field oil palm clones.

Opportunities and Challenges of Palm Oil Tissue Culture

Tissue culture is a vegetative plant propagation technique that has a high percentage of producing quality seedlings. Quality seedlings will affect the production and productivity of oil palm as a major oil-producing plantation commodity in Indonesia and even the world. Tissue culture in its application faces challenges and has opportunities in line with the prospects of this commodity.

The challenges and opportunities of oil palm tissue culture are outlined by Pratiwi et al. (2020) as follows: the challenges of oil palm tissue culture include pollination issues; clonal plants tend to have a higher sex ratio than hybrid plants, requiring a larger supply of pollen; management of uniformity levels through statistics and other tools; plant disease problems such as Fusarium wilt, Ganoderma, and bud rot; abnormalities; and process efficiency in tissue culture. The opportunities from the application of oil palm tissue culture techniques include the technology of tissue culture producing superior plant materials, the availability of genetic material as an abundant source of explants, and supporting human resources and facilities.

Advantages and Disadvantages of Oil Palm Tissue Culture

The advantages of tissue culture-derived oil palm clones are: they possess superior traits, are identical to the parent plant, and are free from pest and disease attacks. The weaknesses of oil palm clones produced through tissue culture are: low efficiency of somatic embryogenesis, mantle abnormalities, and phenotypic mantle abnormalities (Weckx et.all, 2019). The advantages of using tissue culture-derived oil palm clones include fresh fruit bunch production reaching 20% to 40% higher than generatively propagated seedlings, with oil production per hectare being 30% higher. Tissue culture-derived plants are resistant to pest and disease attacks, allow for the propagation of superior plant types with identical traits, and enable or facilitate genetic engineering. The weaknesses of applying tissue culture techniques include the low capacity of human resources, the low efficiency of activities from start to finish, the success of producing clones from tissue culture depending on the breeding program applied, selective selection of ortets, low efficiency of the somatic embryogenesis process, and strict quality control requirements at every tissue culture procedure (Hapsoro dan Yusnita, 2016).

CONCLUTION

Palm oil has become one of the plantation crops as a producer of oil for both national and international needs. 95% of palm oil is used in the food industry, and palm oil has become the main vegetable oil in the world with a percentage of 40%. The world's demand for palm oil necessitates efforts to increase the production and productivity of this commodity. The productivity of palm

oil from 2021 to 2023 is still lower than the productivity in 2020. Efforts to increase palm oil production and productivity are carried out by cultivating superior seeds. Tissue culture is a vegetative plant propagation technique that can produce high-quality seedlings. Efforts to increase the production and productivity of oil palm plants can be achieved when the seedlings planted are of superior quality. In the application of tissue culture, it is necessary to consider the type of medium used and the dosage of plant growth regulators applied. Tissue culture has become one of the solutions to increase the production and productivity of oil palm by providing superior seedlings for cultivation.

REFERENCES

1. Azahra, Ernayunita. 2023. Efforts to Minimize Abnormalities in Oil Palm Clones. *Warta PPKS*, 28(1): 55-62.
2. Ernayunita, Rahmadi, Yeni. 2017. Propagation of Superior Oil Palm Planting Materials Using Tissue Culture Techniques at PKKS. *Warta PKKS*, 21(4): 8-14.
3. Hapsoro, Yusnita. 2016. *Tissue Culture for Clonal Propagation of Oil Palm (Elaeis Guenennsis Jacq).* Anugrah Utama Raharja: Bandar Lampung.
4. Mathius, N.T., Bangun, S.I.I., Maria, B. 2001. Analysis of Abnormalities in Oil Palm Plants (Elaeis Guenennsis Jacq) Resulting from Tissue Culture Using Random Amplified Polymorphic DNA Technique (RAPD). *Menara Perkebunan*, 69(2): 58-70.
5. Pratiwi, Wening, Supena, Retno, Yarna. 2020. Oil Palm Network Culture: Challenges and Opportunities. *Warta PKKS*, 25(1): 1-10.
6. Riyadi, Sumaryono. 2015. In Vitro Root Formation of Oil Palm Planlets (Elaeis Guenennsis Jacq) in Liquid Medium with Auxin Addition. *Menara Perkebunan*, 1(1): 19-24.
7. Setyorini, Nanik. 2019. Induction of Embryogenic Callus in Oil Palm on MS Medium with the Addition of 2,4-D and Young Coconut Water. *Agroista*, 3(1): 93-98.
8. Weckx, Dark Inze, M. Ludo. 2019. Tissue Culture of Oil Palm: Finding the Balance Between Mass Propagation and Somaclonal Variation. *Warta PKKS*, 25(1): 11-20.
9. Yunita, Marisa, Purnamaningsih, Lestari, Utami. 2016. Induction of Oil Palm (Elaeis Guenennsis Jacq) Roots and Shoots In Vitro and Ex Vitro. *Jurnal Littri*, 22(1): 37-42.