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Conference Paper

In Vitro Culture for Developing Plant Varieties Resistant to Extreme Climates and Producing Secondary Metabolites to Support Various Industries

Sutini Sutini[1]*, Didik Utomo Pribadi[1], Veronika Me Alfianti[2]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia
[2]Master of Agrotechnology Study Program, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia

*Corresponding author:
E-mail:
sutini.agro@upnjatim.ac.id

ABSTRACT

Drastic climate changes and the increasing demand for agro-industrial products necessitate innovations in agriculture. This article is based on the premise that *in vitro* culture techniques offer solutions by developing plant varieties that are resilient to drastic environmental changes and capable of producing secondary metabolites beneficial to various industries. This article aims to review research findings related to plant varieties that withstand extreme climatic conditions and produce secondary metabolites through *in vitro* culture techniques, and to evaluate the potential of *in vitro* cultured plants in supporting various industrial sectors. The methodology involves an integrated literature review from various journals, relevant literature, and references, covering plant variety selection, evaluation of plant resilience, and analysis of secondary metabolite production. The results indicate that plant varieties developed through *in vitro* culture show resilience to extreme climatic conditions such as temperature stress, drought, and salinity, and are capable of producing secondary metabolites like alkaloids, flavonoids, and terpenoids more quickly than non-*in vitro* plants. The conclusion is that the implementation of *in vitro* culture can be used to develop plant varieties that are resistant to extreme climates and capable of producing secondary metabolites useful for various industries.

Keywords: Extreme climates, In vitro culture, Plant variety, Secondary metabolites

Introduction

Global climate change has increased the frequency and intensity of extreme climate conditions such as drought, high temperatures, plant diseases, and soil salinity that threaten crop production around the world. To overcome these challenges, the plant varieties development that are resistant to extreme climate conditions is very important. The use of *in vitro* culture in developing plant varieties that are resistant to extreme climates is a promising solution in facing the climate change challenges and the increasing need for industrial raw materials (Karim et al., 2019). Plants developed through *in vitro* culture are not only able to adapt to global climate change, but also produce secondary metabolites that are important for various industries, including pharmaceuticals, cosmetics, agriculture, and resistance to disease attacks. The production of secondary metabolites through *in vitro* culture offers many advantages, including the ability to produce large amounts of bioactive compounds without having to rely on field cultivation. Secondary metabolites, such as alkaloids, flavonoids, and terpenoids produced have high economic value due to their role in the development of medicines, cosmetic products, and natural pesticides. The use

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of *in vitro* culture techniques is not only used for plant resistance to environmental conditions, but also for the sustainability of the important compound production that is the backbone of various global industries (Chandran et al., 2020).

The purpose of this article is to explore *in vitro* cultivation techniques as an effective approach in developing plant varieties that are resistant to extreme climates, disease, and able to increase the secondary metabolites production. The results of this article are expected to support various industries that depend on high-quality plant raw materials.

Material and Methods

Materials are taken from various research literature, journals, and relevant references. The method includes an integrated literature review of various journals, literature, and reference books related to the theme of *in vitro* culture. The theme of *in vitro* culture in this method concerns: In vitro culture cultivation techniques, selection of plant varieties, plant resistance to disease, and analysis of secondary metabolite production and its applications.

In vitro culture cultivation techniques

In vitro culture cultivation techniques include: 1. Young leaf/stem explants are used for callus culture initiation. 2. Medium Murashige and Skoog (MS) is used with the addition of growth regulators such as 2,4-D and kinetin to stimulate callus/suspension growth (Sandal et al., 2001). 3. The culture/suspension is placed in controlled environmental conditions with a temperature of 25°C and 16 hours/day of lighting (Yuliyantika & Sudarti 2021). 4. The callus/suspension formed can be implemented for various purposes, for example, to obtain plant varieties, make plants more resistant to pests-diseases or certain environments, and to produce secondary metabolites.

Plant varieties selection through in vitro culture

Callus formed from *in vitro* culture cultivation techniques is regenerated into shoots by being transferred to media with different compositions of growth regulators to stimulate shoot formation. The formed shoots are acclimatized in stages, and the shoots are transferred to the media for root growth and become plantlets that are ready to be acclimatized (Thomas et al., 2010). The acclimatized plantlets are continued for Genetic and Phenotypic tests to ensure that the produced varieties have superior and stable characteristics. To obtain superior character, selection and evaluation are carried out based on the desired criteria, such as productivity, quality of results, and the ability to adapt well in different environments. The selected varieties are then multiplied in large quantities through *in vitro* culture. After environmental / field tests are completed, the variety can be released and registered as a superior variety.

Plant resistance associated with in vitro culture

Plant resistance associated with *in vitro* culture is important in plant research in the face of disease and drastic environmental changes. For example, *in vitro* culture of peanut somatic embryos increases the resistance to Fusarium sp. wilt disease. The stages of *in vitro* culture for plant resistance include: 1. Induction of Somatic Embryo (ES) and somaclonal variation in plants. 2. *In Vitro* Selection; somatic embryos are tested for resistance to Fusarium sp. using selective media containing Fusarium culture filtrate. 3. Production of Planlets: insensitive somatic embryos are developed into plantlets. 4. Planting and Evaluation: Plantlets are planted to produce R1 and R2 generation plants, which are evaluated for their resistance to Fusarium sp. fungal wilt disease. (Sumarjan & Hemon, 2011).

Analysis of secondary metabolite production through in vitro culture and its applications

Secondary metabolite production through *in vitro* culture is an important technique that can produce bioactive compounds in higher quantities than in vivo. The applications applied include: 1. Optimizing the culture environment that can increase metabolites with high economic value

(Manoj et.al, 2018); 2. Can be applied to the health industry; 3. Optimizing the use of elicitors that can stimulate metabolite production; 4. Can be used to preserve rare plants while producing secondary metabolites; 5. *In vitro* culture that produces high metabolites while also making plants highly resistant to extreme environments.

Results and Discussion

In vitro culture cultivation techniques

In vitro culture cultivation is one of the important techniques in the development of superior plant varieties and sustainable agricultural biotechnology applications that can be the basis for further applications. The results of *in vitro* plant culture techniques can be in the form of biomass, in the form of suspension/callus form (Figure 1).

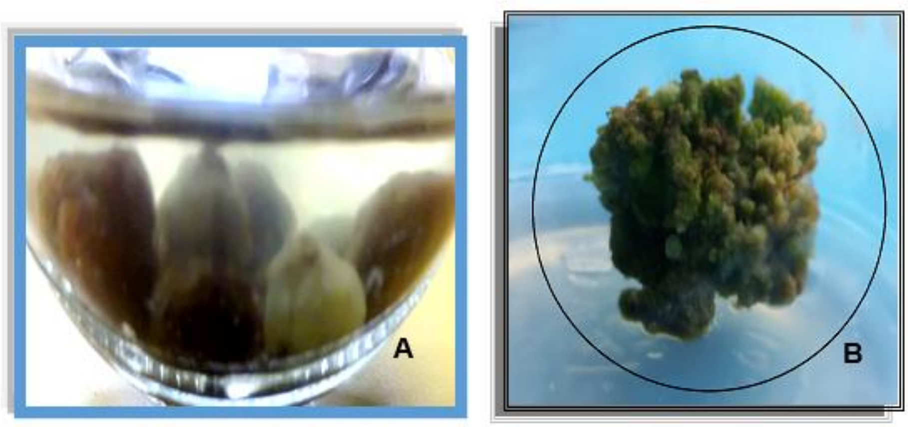

Figure 1. Results of in vitro culture of plants in the form of suspension biomass (A) and callus form (B)

Figure 1-A. Suspension biomass with a softer texture was obtained with a faster time frequency. Figure 1-A. More compact callus biomass, whose formation takes longer than the suspension form. Both forms of biomass can be used as initial raw materials for the development of further materials according to research objectives. For example, in plants that can produce new cultivars that are more tolerant to biotic/abiotic stress (Mirni & Werbrouck, 2018).

Plant selection of varieties through in vitro culture

Plant varieties through *in vitro* culture can be released and registered as superior varieties with the following characteristics: higher productivity, resistance to pests and diseases, increased quality and quantity, plants can grow on various types of land / adaptive. Plant varieties through *in vitro* culture can be released and adapted as superior varieties are listed in Table 1.

Table 1. Plant varieties through *in vitro* culture that are released, adaptive, and superior varieties

No	Name of Plant	Culture in vitro (type)	Adaptive as superior varieties	Reference
1	Hortikultura (Papaya,banana, grapes, pineapple)	callus	Superior varieties	(Krishna et al., 2016)
2	*Nepenthes mirabilis and N. Gracilis*	callus	Varigata varieties	(Damayanti & Roostika, 2015).
3	*Camellia sinensis (*L*). o. kuntze*	planlet	Adaptive genes	(Tapan & Pradeep, 2002).

To be continued…

4	*Cannabis sativa L.*	callus	Superior varieties	(Zarei et al., 2023).
5	*Saccharum officinarum* L.	planlet	Adaptive varieties	(Sumariana & Juswardi, 2022).
6	*Psidium guajava* L.	Callus embryo	Adaptive varieties	(Akhtar, 2005).

Plant resistance associated with in vitro culture

In vitro culture of plants not only has better resistance to diseases and environmental stress, but also has the potential to increase crop yields and product quality, making it an important solution in sustainable agriculture today and in the future. *In vitro* cultured plants that have resistance to diseases and environmental stress are listed in Table 2.

Table 2. Plants from *in vitro* culture have resistance to disease and environmental stress

No	**Name of Plant**	**invitro culture (type)**	**Resistance to disease and environmental stress**	**Reference**
1	*Coffea arabica L.*	Suspense, callus	environmental stress	(Aguilar et al., 2018).
2	*Citrulus lanatus*, cv. Giza 1	Callus	environmental stress – hyperhydration	(Awatef et al., 2012).
3	*Saccharum officinarum* L.	Suspense, callus	Virus free	(Arie, 2016).
4	Malus domestica 'Fuji'	Callus	resistance to an alkaline environment	(Xiaoxin et al., 2017).
5	Citrus	Callus	Saline stress	(Verde et al.,2024).
6	*Anthurium adreanum*	Callus	Vigorous/strong plants	(Rizka et al., 2017).

Analysis of secondary metabolite production through in vitro culture and its applications

Secondary metabolite production through *in vitro* culture is an increasingly important method in plant biotechnology, which allows the production of bioactive compounds in higher quantities than conventional plants. Secondary metabolite production through *in vitro* culture of plants and their bioactive applications are listed in Table 3.

Table 3. Production of secondary metabolites through *in vitro* culture of plants and their bioactive applications

No	**Plant**	**Type of in vitro culture (+ booster)**	**Bioactive applications**	**Secondary metabolites**	**Reference**
1	Phyllanthusamarus	Callus+blue light	anti-hepatitis	flavonoid, terpenoid	(Cardoso et al., 2019)
2	*Typonium flagelliforme Lodd- Blume*	Callus, planlet	Anti-cancer	steroid	(Sitti, 2008).
3	Pimpinella pruatjan Molk	Callus Precursor of mevalonic acid.	afrodisik	stigmasterol	(Roostika et al., 2007)
4	*Eurycoma longifoliaJack.*	Callus	afrodisik	2furancarbox aldehyde	(Iriawatia et al., 2014)

To be continued…

5	*Camellia sinensis L*	Suspense+ precursors Phenylalanin	anti-hepatitis	catechin	(Sutini et al., 2020)
6	*Hordeum vulgare*	Callus+ nanosilver	human diet	polyphenolic	(Marcelina et al., 2019)
7	*Lecythidaceae family*	Suspense +periodization of light	asthma and diarrhea	lycopene	(Behbahani et al., 2019)
8	*Oldenlandia umbellata L*	Callus+ organic, inorganic ZPT	dye	Anthraquinones	(Sivaa et al., 2012)

Conclusion

In vitro culture cultivation has proven to be effective in developing plant varieties that are resistant to extreme climate conditions, disease, and increase the production of secondary metabolites. This *in vitro* culture cultivation has great potential to be applied on a wider scale to support the agricultural, pharmaceutical, cosmetic, and food industries and does not rule out the possibility for other industries that require high-quality and sustainable raw materials.

Conclusion

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