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Factors Affecting Telang (*Clitoria ternatea* L.) Germination: Systematic Literature Review

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Abstract. *Clitoria ternatea* is a multipurpose plant species. Interest in this plant species has increased recently as its antioxidant properties made it a popular natural food additive. This condition implies that a review study to understand the current development of *C. ternatea* germination biology, especially factors affecting its germination, is essential to help interested stakeholders cope with future demand. Thus, this study aims to use a systematic literature method to provide data on factors affecting *C. ternatea* germination. Data on this topic is acquired from the publication in the Google Scholar database. The publications were screened to acquire relevant publications for the review process. This study found that dormancy, pod maturity and seed age are internal factors affecting *C. ternatea* germination. Meanwhile, external factors such as dormancy alleviation, hormone and PGPR treatment, allelopathic compound, storage, and germination conditions such as storage and substrate temperature also affect the plant species' germination. In conclusion, based on the provided data, *C. ternatea* germination, like the germination of any other plant species, is affected by internal and external factors.

Keywords: butterfly pea; fabaceae; seed; systematic literature review (SLR)

INTRODUCTION

Clitoria ternatea is a legume species belonging to the Fabaceae plant family. This perennial plant is known as butterfly pea or as *telang* in Indonesia. The first description of this plant species was conducted by Linnaeus using plant specimens from Ternate Island, which were then immortalized in its specific name (Oguis et al., 2019). This perennial herbaceous plant has various benefits, from plant fodder to natural food colourant (Oguis et al., 2019; Suarna & Wijaya, 2021). *C. ternatea* flower (Figure 1a) also shows antioxidant activity that adds to its potential benefit (Arsianti et al., 2022; Goh et al., 2021) and has recently made them into a popular beverage (Lakshan et al., 2019). In Indonesia, telang tea is now traded in convenience stores, e-commerce marketplaces, and restaurants (Figure 1b). The plant's increasing popularity implies an effective propagation method for *C. ternatea* must be found to keep up with the demand.

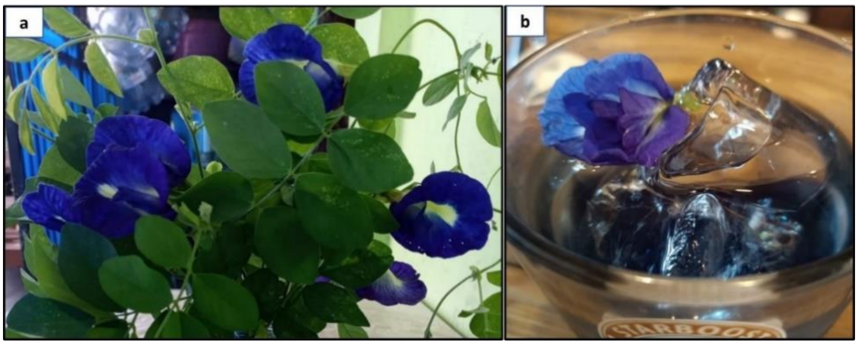

Figure 1. *Clitoria ternatea* Flower (a) and *C. ternatea* flower beverage (b).

Like other legumes, *C. ternatea* is generatively propagated by its seed. Generative propagation depends on the delicate process known as germination to produce seedlings. Germination delicacies are due to the process being highly dependent on external and internal factors. In legumes, external factors such as temperature, drought, and saline stress are reported to affect plant germination (Butler et al., 2014; Chu et al.,

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2022). Meanwhile, internal factors such as seed size and dormancy also affect legume germination (Hill & Auld, 2020; Kuswantoro & Li'aini, 2022). Human intervention, such as dormancy alleviation and hormonal treatments (Kuswantoro & Li'aini, 2022; Mensah et al., 2020), might also affect legume germination in laboratory or agricultural environments. This condition implies that understanding *C. ternatea* germination biology, including factors affecting the process, is essential to finding an effective propagation method.

Clitoria ternatea's wide use, potential, and popularity may entice researchers to study the plant. This condition, added to the fact that germination improvement for the plant is needed, implies that studies on *C. ternatea* germination biology, including its affecting factors, should have already been conducted. However, a review on this topic is still absent to our knowledge. This condition is unfortunate, as a review regarding this topic might enable us to understand and evaluate recent breakthroughs and locate existing knowledge gaps that might be explored for future improvement.

With the background mentioned above, this study aims to conduct a systematic literature review (SLR) study on *C. ternatea* germination studies to determine its affecting factors. SLR is a transparent and reproducible method of scientific evidence synthesis from all relevant studies to answer a particular research question in a topic of interest (Lame, 2019; Siswanto, 2010). SLR has already been conducted in seed biology to analyze the longevity and storage of orthodox seeds (Solberg et al., 2020). This SLR is the first study that employs such a method to provide data on the germination biology of *C. ternatea*, particularly on factors affecting its germination. The data from this SLR study might help future researchers, farmers, and other interested stakeholders develop advanced research to improve the plant propagation method.

METHODS

Information regarding *C. ternatea* propagation is acquired from academic publications on the Google Scholar (GS) database from 2010 to 2023. GS was chosen as it provides an accessible and vast publication database. Publication retrieval was conducted using Publish or Perish (PoP) Software on 6 September 2023 (Harzing, 2007). Word strings are used as a search query for publication retrieval. The word queries are "Clitoria AND ternatea AND germination", "Clitoria AND ternatea AND dormancy", and "Butterfly AND Pea AND Germination". In this study, the retrieved publication is limited to 2010 to 2023. This condition is applied as we want to present the most recent research result in this field. Manual publication acquirement of the publications using the same keywords is also conducted to broaden the literature source. Retrieved publications are then screened for publication type and content. The current study includes only published papers; the thesis and dissertation are excluded. For content screening, two screening stages are conducted: the first is by reading the publication titles and abstract, and the second is by reading its full text. Unrelated publications are then excluded, while relevant publications are included. Included publications are then used in the literature review. The data acquired from the analysis is presented qualitatively.

RESULTS AND DISCUSSION

Publication retrieval during this study used PoP software to retrieve 12 titles. However, after the screening process, two titles were excluded. The reason for exclusion is the title publication type (thesis or dissertation and paper not found). Thus, only ten publications from the PoP software retrieval are included in the SLR of this study. Meanwhile, manual publication retrieval found 11 related publications. As a result, 21 publications are included in this study. A list of included publications in this SLR study is presented in Table 1.

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Table 1. Included publication in this systematic literature review

Author(s)	Publication Title	Publication Year
Morris	Multivariate Analysis of Butterfly Pea (*Clitoria ternatea* L.) Genotypes with Potentially Healthy Nutraceuticals and Uses	2023
Campbell et al.	Substrate Temperature and Seed Scarification on Germination Parameters of Butterfly Pea (*Clitoria ternatea*)	2022
Turnos	Pod Maturity and Seed Germination of Blue Ternate (*Clitoria ternatea* L.)	2021
Md Salleh & Pa'ee	Effect of Various Immersion Time and Water Temperatures on Seed Germination of *Clitoria ternatea* and *Momordica charantia*.	2021
Pincay-Ganchozo et al.	Chemical and biological scarification in the emergence and growth of *Clitoria ternatea*	2021
Campbell et al.	Substrate Type and Temperature on Germination Parameters of Butterfly Pea	2020
Aeron et al.	Endophytic bacteria promote the growth of the medicinal legume *Clitoria ternatea* L. by chemotactic activity	2020
Das et al.	Effect of moisture content and storage temperature on germination and seedling vigor index in *Clitoria ternatea* L.	2019
Suma et al.	Effect of seed treatment and nutrient levels on growth, yield and quality of Shankapushpi (*Clitoria ternatea* L.)	2019
Shuba et al.	Studies on seed quality attributes in shankapushpi (*Clitorea ternatea* L.)	2019
Shobharani & Sundareswaran	Effect of different dormancy breaking treatments on seed germination and seedling growth in Shankhapushpi (*Clitoria Ternatea* L.)	2018
Dhanraj et al.	Effect of plant growth promoting rhizobacteria on growth, yield and quality of shankapushpi (*Clitorea ternatea* L.)" under rainfed situation	2018
Das et al.	Seed germination and seedling vigor index in *Bixa orellana* and *Clitoria ternatea*	2017
Makasana et al.	Effect of seed treatment on germination and flavonoids diversity in accessions of butterfly pea (*Clitoria ternatea*)	2016
Khaksar et al.	Endophytic Bacillus cereus ERBP—*Clitoria ternatea* interactions: Potentials for the enhancement of gaseous formaldehyde removal	2016
Nagar & Meena	Effect of physical and chemical scarification and aging on hardseededness in *Clitoria ternatea*	2015
Quintana et al.	Effect of two plant growth regulators and illumination conditions in the germination of conserved seeds of *Clitoria ternatea*.	2013
Namkeleja et al.	Allelopathic Effect of Aqueous Extract of *Argemone mexicana* L on Germination and Growth of *Brachiaria dictyoneura* L and *Clitoria ternatea* L	2013

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Reino et al.	Combined effect of scarification and temperature on the germination of herbaceous legume seeds	2011
Hawari et al.	Respon Perkecambahan dan Pertumbuhan Bunga Telang (*Clitoria ternatea* L.) Terhadap Asal Benih dan Berbagai Perlakuan Pematahan Dormansi (in *Bahasa Indonesia*)	2011
Reddy et al.	Seed Treatment with GA3 Improves Germination and Seedling Characteristics of Butterfly Pea (*Clitoria Ternatea* L., Family: Fabaceae)	2010

Our study found that plant origin may affect *C. ternatea* germination properties. Included publication in this SLR found that low- and high-altitude *C. ternatea* have different germination values (Hawari et al., 2021). Varied germination percentage value is also found in *C. ternatea* seeds from different countries (Morris, 2023). Plant accessions might have different physical and physiological properties affecting their germination capacity. This condition is because germination is affected not only by an external factor but also by intrinsic factors such as dormancy.

The effect of dormancy and its alleviation method on *C. ternatea* germination is also recorded in publications in this SLR study. Mechanical scarification, alone or followed with water immersion, is mentioned to improve *C. ternatea* germination (Campbell et al., 2022; Md Salleh & Pa'ee, 2021; Nagar & Meena, 2015). Chemical scarification using various concentrations and immersion duration of sulphuric acid (H_2SO_4) is another treatment already used in *C. ternatea* (Makasana et al., 2016; Nagar & Meena, 2015; Pincay-Ganchozo et al., 2021; Reino et al., 2011; Shobharani & Sundareswaran, 2018). Different wet scarification using varied water temperatures has also already been explored for the plant species (Hawari et al., 2021; Nagar & Meena, 2015; Pincay-Ganchozo et al., 2021; Reino et al., 2011; Shobharani & Sundareswaran, 2018). The mention of this treatment to improve *C. ternatea* germination suggests that this species exhibits physical dormancy. Physical dormancy is caused by an imbibition-preventing impermeable layer on the seed or fruit coat that inhibits the seed germination initiation (Hu et al., 2018; Hudson et al., 2015). *C. ternatea* is a legume species with physically dormant seeds (Jayasuriya et al., 2013; Shobharani & Sundareswaran, 2018). The mention of physical dormancy in *C. ternatea* seed is unsurprising as this type of dormancy is widely reported from legume species (Galíndez et al., 2016; Jayasuriya et al., 2013; Rodrigues-Junior et al., 2020).

The mention of various scarification methods in the publications included in this SLR study is unsurprising as researchers continuously explore the most effective treatment. Different scarification methods show different effectiveness on different species. In *C. ternatea*, mechanical scarification followed by 24 hours of immersion in water is reported to improve the plant seed germination compared to control and mechanical scarification alone (Campbell et al., 2022). However, 72 hours of water immersion following mechanical scarification reduces seed germination (Md Salleh & Pa'ee, 2021). Meanwhile, 10-90 minutes of immersion of the plant species seed in concentrated H_2SO_4 improves its germination than the shorter treatment period (Makasana et al., 2016). On the other hand, immersion of the seed for 5 minutes in 200 ml/kg H_2SO_4 solution produces higher germination percentage compared to 5 and 10 minutes immersion of the seeds in 100 ml/kg solution and 10 minutes immersion in 200 ml/kg H_2SO_4 solution (Shobharani & Sundareswaran, 2018). Wet heat treatment is also mentioned to alleviate *C. ternatea*'s physical seed dormancy, and 48 hours of water immersion produced better seed germination than a shorter period, while 4

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minutes of immersion in 80°C water produced better germination than other immersion periods using the same and lower water temperature of 60°C (Makasana et al., 2016; Shobharani & Sundareswaran, 2018; Shuba et al., 2019). Temperature provided by wet heat treatment will soften the *C. ternatea* seed coat and, thus, facilitate water imbibition. Longer immersion duration and higher water temperature in this treatment will provide a more effective scarification effect. However, the duration and temperature should be monitored closely. Different plant accessions or species might have other seed coat properties and, thus, need different temperatures and durations in wet heat treatment.

Other publications in this SLR study indicated that temperature treatment alleviated physiological dormancy, which also occurs in *C. ternatea* (Das et al., 2019; Das et al., 2017). Physiological dormancy happens when the seed is water-permeable and has a fully developed embryo, but its germination must be initiated by environmental cues (Baskin & Baskin, 2022; Wyse & Dickie, 2018). The presence of physiological dormancy in *C. ternatea* is possible, as in legumes, physiological dormancy is also present (Wyse & Dickie, 2018). However, the report on physiological dormancy in *C. ternatea* also implies that combinational dormancy may occur in this species. Combinational dormancy is another type of dormancy in which both physical and physiological dormancy present in the same plant species (Wyse & Dickie, 2018). Despite no research explicitly mentioning the presence of combinational dormancy in *C. ternatea*, this type of dormancy is manifested by the presence of dormant embryos that need temperature treatment to germinate after the seed coat impermeability is removed in numerous legume species (Van Assche & Vandelook, 2010; Wyse & Dickie, 2018).

Dormancy is not the only internal factor affecting *C. ternatea* germination. The included publication shows that pod maturity and seed age affect plant germination (Nagar & Meena, 2015; Turnos, 2021). Pod maturity is closely related to the seed maturity. Previous research shows that different seed maturity stages may produce different germination parameter values in various plant species, including legumes such as *Acacia longifolia* (Riveiro et al., 2020). Meanwhile, the effect of pod maturation on seed germination was found in *Bituminaria* species (Carruggio et al., 2020). In *C. ternatea*, over-mature pods are reported to produce worse germination, while young, green pods can stimulate seed germination as long as they contain mature seeds (Turnos, 2021). The overripe pod may have an ageing seed already undergoing deterioration. Seed loses its vigour and viability during deterioration (Choudhury & Bordolui, 2023). The deterioration process is also why seed aging can affect the germination of *C. ternatea*. Like germination, seed deterioration is affected by internal and external factors (Choudhury & Bordolui, 2023).

Storage conditions also affect *C. ternatea* germination by affecting the seed deterioration rate. Despite its varied storage behaviour, many legume species, including *C. ternatea*, show orthodox storage behaviour (Delahaie et al., 2013; Jayasuriya et al., 2013). Orthodox seed storage behaviour implies that *C. ternatea* seed can be dried and stored at low temperatures to improve its longevity (Kijak & Ratajczak, 2020; Yuniarti & Nurhasybi, 2015). Despite the phenomenon, seed storage conditions, including temperature and moisture content, must be carefully managed as the optimum condition will significantly prolong orthodox seed longevity (Solberg et al., 2020). Publications in this SLR study show that seed moisture content and storage temperature affect *C. ternatea* germination parameters, with 10% moisture content and -20 and 20^{0}C storage temperature as the best storage conditions to maintain the seed germinability (Das et al., 2019; Das et al., 2017). Temperature and moisture content are the two most critical factors for seed longevity in

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storage conditions (Gianella et al., 2022; Nadarajan et al., 2023). Despite its ability to prolong seed survival, too low seed moisture content should be avoided as it might increase seed deterioration rate by enhancing lipid oxidation (Ranganathan & Groot, 2023). On the other hand, high temperatures raise seed chemical oxidation and improve the seed deterioration rate, hampering seed longevity (Ranganathan & Groot, 2023).

Publications included in this SLR study also found that external application of plant growth regulators can affect *C. ternatea* germination. A plant growth regulator is a natural or synthetic compound that modifies plant growth through physiological action (Agboola et al., 2014; Singh et al., 2021). Some phytohormones, such as auxins, cytokinin, gibberellins, ethylene and abscisic acid, are considered plant growth regulators (Agboola et al., 2014). Various phytohormones such as abscisic acid, benzilaminopurin, gibberellin and indole-3-butyric acid are used to improve the germination of different legume species (Carruggio et al., 2020; Solichatun et al., 2016). Included publication of this SLR study shows that phytohormone application improves *C. ternatea* germination with a combination of 1 m g/l of GA_3 and 0.1 mg/l of NAA, which is the recommended concentration (Quintana et al., 2013). Various GA_3 application without combination with other plant hormone is also reported by included publication in this SLR study to affect *C. ternatea* germination (Hawari et al., 2021; Reddy et al., 2010; Shuba et al., 2019). Gibberellin's ability to improve *C. ternatea* germination is unsurprising, as the hormone can also enhance the germination of other legume species. In combination with scarification, exogenous gibberellin application, for example, is reported to improve germination of several physically dormant legume species such as *Mucuna bracteata* and *Cercis siliquastrum* (Grbić et al., 2014; Sari et al., 2014). Meanwhile, the same hormone has also been reported to be able to initiate the germination of intact seeds of two physically dormant legume species, *Sesbania rostata* and *S. sesban* (Mensah et al., 2020). Gibberellin also significantly improve the germination of *D. regia* (Solichatun et al., 2016). Gibberellin improves germination by increasing and enhancing the degradation of germination repressor RGL2 DELLA protein (Lee et al., 2021; Shah et al., 2023). Meanwhile, NAA is reported to improve soybean germination stress conditions by strengthening the seed antioxidant and triacylglycerol mobilization and sucrose transport (Xing et al., 2023).

The allelopathic compound is another factor affecting *C. ternatea* germination in this SLR study. By definition, allelopathy is a direct or indirect chemical compound that mediates interaction between plant species that can positively or negatively affect the recipient plant species (Darmanti et al., 2015; Scavo & Mauromicale, 2021). However, allelopathy is widely defined as plant interaction that reduces the recipient plant's growth and germination. *C. ternatea* germination is reported to be significantly reduced by leaf and seed extract of *Argemone mexicana* (Namkeleja et al., 2013). This condition is not surprising as germination of other legume species can also reduced by the allelopathic effect of different plant species such as *Lantana camara*, *Cyanthillium cinereum*, and *Cyperus rotundus* (Bhattacharya et al., 2020; Darmanti et al., 2015). Despite this condition, previous research also found that *C. ternatea* shows allelopathic activity to inhibit germination and growth parameters of several other plant species (Piyatida & Kato-Noguchi, 2010; Poonpaiboonpipattana et al., 2015). This condition is also possible as other legume species, such as *Crotalaria juncea* and *Gliricidia maculata*, show a germination inhibitory effect on another plant species (da Cruz Silva et al., 2015; Nugroho et al., 2022). In general, allelopathic compounds inhibit plant germination and growth of target plants by increasing the cell reactive oxygen species (ROS) that will lead to oxidative stress and

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cell death (Šoln et al., 2022; Yan et al., 2022). However, some allelopathic compounds, such as acacetin, affect plant growth by influencing the target plant's mitosis rate (Yan et al., 2022), while other allelopathic compound, such as myrigalone, affect target species by affecting its hormonal regulation (Nakabayashi et al., 2022; Oracz et al., 2012).

The included publication in this SLR study found that substrate temperature and type also affect *C. ternatea* germination (Campbell et al., 2020, 2022; Shuba et al., 2019). Substrate type may affect germination as it may affect the substrate water content and, thus, the seed imbibition rate (Santosa et al., 2019). The same condition applies to germination media temperature as varieties in germination media temperature, such as soil, affect the media water content, conductivity and availability (Onwuka, 2016). The effect of different media types and temperatures on water properties is why these two factors can affect *C. ternatea* germination. Previous studies highlight different media types for germination of other legume species, such as *Albizia niopoides* and *Aganope heptaphylla* (Purwantoro, 2016; Silva et al., 2018).

Publication in this study also found that the application of several bacteria strains is also able to affect *C. ternatea* germination (Aeron et al., 2020; Dhanraj et al., 2018; Khaksar et al., 2016; Suma et al., 2019). The application of rhizobacteria to improve plant germination has gained more and more interest in recent years. Thus, this SLR study result that found that bacteria application can affect *C. ternatea* germination is not surprising as the treatment is also reported to affect the germination of several other legume species. Through numerous direct and indirect mechanisms, PGPR promotes plant germination and growth in normal or stressful conditions (Mohanty et al., 2021). This statement corresponds with the included publication in this SLR study, which found that inoculated endophytic bacteria affect *C. ternatea* by chemotactic activity (Aeron et al., 2020). An included publication in this SLR study also mentions that bacteria inoculation improved *C. ternatea* germination under formaldehyde stress (Khaksar et al., 2016).

CONCLUSION

This systematic literature review can document factors affecting *C. ternatea* seed germination. Both intrinsic and extrinsic factors are mentioned to affect this species' germination. Further study is essential to understand *C. ternatea* germination and the affecting factors, such as the possible occurrence of combinational dormancy, the effect and mechanism of plant growth regulator, allelopathic compounds and other external factors, such as storage duration and germination conditions, more thoroughly. However, the current result should benefit future research and breeding efforts of this multifunctional legume species as it covers the most recent and comprehensive knowledge.

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