Response of seed germination and seedling growth of Physalis accession from East Java

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Abstract. Environmental conditions significantly affect response of seed germination. This study aims to determine ex vitro and in vitro seed germination capacity and seedling growth of accession of Physalis sp derived from East Java including Madura Island. Ex vitro germination was carried out in mixed soil media: compost: husk charcoal while in vitro germination was carried out in agar-solidified medium. Ex vitro germinated seeds are generally able to produce 80-100% seedling in all accessions. Accessions from Madura Island have a better growth response compared to accessions from East part of Java Island. Although in vitro germination response was lower, the four accessions from the island of Madura namely A1 (Sumenep), A2 (Sampang), A4 (Sampang) and A5 (Pamekasan) have been able to produce regeneration and multiplication of shoots in vitro. The potential for in vitro growth in Physalis accessions needs to be developed to supply germplasm as a source of natural products in a controlled environment.

1. Introduction
Physalis is a member of the Solanaceae family which grows wild and can be found in the lowlands to an altitude of about 1500 m above sea level. In Java three species of ciplukan have been identified namely P. minima, P. angulata and P. peruviana [1]. The three species are also found in East Java, namely P. minima in Tumpang Malang Regency (473 m asl), P. angulata in the Perhutani forest area (1-600 m asl) and P. peruviana found in UB Forest, Perhutani forest and Bromo Tengger Semeru National Park area (700 - 2300 m asl) [2]. While on the slopes of Mount Kelud, the genus Physalis is often found growing wild on relatively fertile rice fields at an altitude of 400 - 600 m above sea level [3]. In those area, based on the color of the stem and fruit traps the two morphological groups are found. Differences in altitude create differences in temperature, humidity and light intensity as well as soil nutrient content. Therefore, it is necessary to observe the effect of variations in abiotic conditions on the physiology and morphoanatomy of plants accession.

Although known as weeds, in Indonesia Physalis has long been used by people as traditional medicine, such as in the area of Mount Ungaran, Central Java [4], Rambah Samo Subdistrict, Rokan Hulu District [5], and North Sumatra [6]. Many research results showed that this plant extract has potential as an antibacterial [7], immunomodulators [8], antiproliferative, anti-inflammatory [9], and antioxidants [10]. has an antidiabetic effect [11], lowering blood pressure and reducing menopausal symptoms (hypertension, depression and anxiety etc) [12].
In vitro propagation technique is widely used to produce plants in large quantities under controlled conditions. In vitro growth responses are very specific between the same species and even individual plants part. Therefore this study aims to observe the capacity of seed germination and seedling growth ex vitro and in vitro on Physalis accessions from some habitats in East Java.

2. Material and Methods

2.1. Plant materials

Seeds were derived from Physalis accession plants in two regions of East Java (Table 1): 1) Madura island consisted of 5 accessions (A1: SMN-PG (02) # 55, A2: SPG-DC # 65, A3: SPG-GD # 60, A4: SPG-AS # 70, A5: PMK-I # 16) and 2) East part of Java Island also consisted of 5 accessions (TLG-PL (02) # 61, B2: KDR-RM (02) # 33, B3: KDR-NL # 62, B4: MLG-TP # 58, B5: BW-TD # 64).

| No | Accession | Altitude (m asl) | GPS coordinate |
|----|-----------|-----------------|----------------|
| 1  | A1: SMN-PG(02)#55 | 13 | 7° 0' 34.405" LS ; 113° 51' 29.876" BT |
| 2  | A2: SPG-DC#65 | 15 | 7° 11' 38.047" LS ; 113° 14' 48.124" BT |
| 3  | A3: SPG-GD#60 | 15 | 7° 11' 38.047" LS ; 113° 14' 48.124" BT |
| 4  | A4:SPG-AS#70 | 15 | 7° 11' 38.047" LS ; 113° 14' 48.124" BT |
| 5  | A5: PMK-I#16 | 8 | 7° 9' 38.290" LS ; 113° 28' 57.904" BT |
| 6  | B1: TLG-PL(02)#61 | 241,37 | 8° 5' 28.396" LS ; 111° 57' 51.023" BT |
| 7  | B2: KDR-RM(02)#33 | 162,54 | 7° 49' 22.2240" LS ; 112° 0' 42.7104" BT |
| 8  | B3: KDR-NL#62 | 162,54 | 7° 49' 22.2240" LS ; 112° 0' 42.7104" BT |
| 9  | B4: MLG-TP#58 | 498,48 | 7° 59' 2.0688" LS ; 112° 37' 17.0076" BT |
| 10 | B5:BW-TD#64 | 297,79 | 8° 13' 8.4756" LS ; 114° 22' 8.7672" BT |

2.2. Seed germination

2.2.1 Preparation of seed germination medium. Seeds were germinated by ex vitro and in vitro methods. Ex vitro method used compost medium while in vitro methods used compost and agar-solidified water medium. Medium compost for ex vitro germination was mixture of soil:compost:charcoal husk with a ratio of 1: 1: 1. Mixed media about 50 g were put into 100 ml vol plastic glass, then ready to be filled with seeds. This mixed media was one of the media which was also used for in vitro germination after sterilization using autoclave for 15 minutes at 121 C, 1.5 atm. Another media used for germination in vitro was agar-solidified water medium which consisted of 1% commercial agar as solidifying agent dissolved with boiling water. After being put into a culture bottle, the medium is sterilized by autoclave with the conditions as described previously.

2.2.2 Ex vitro seed germination and classification of plant accession. Seeds of each accession that have been washed with water were germinated with four kinds of treatment, namely: 1) seeds were cured in a damp cloth a few days then washed with ash with the addition of a little water, 2) seeds were sown directly in compost media, 3) before sowing in compost media seeds were washed with ash water overnight, and 4) before sowing in compost media seeds were washed with ash with the addition of a little water. Each method was done for 10 replicates. The parameters observed included the speed of germination (days) and the ability of seeds to germinate (%). Subsequently, the plants derived from seedling accession were identified morphologically to determine classification based on determination key in some books of flora identification.

2.2.3. In vitro seed germination and shoot regeneration. The seeds of each accession were sterilized in laminar air flow (LAF) using 20% commercial bleach solution for 15 minutes then rinsed with sterile distilled water for 5 minutes three times. The sterilized seeds were then germinated in vitro on agar or compost medium. If seedling has reached height 2 cm the cotyledonous nodes were excised and
cultured on shoot induction medium (MS + BAP medium 2 mg / L + IAA 0.01 mg / L). Subsequently, the regenerated shoots were subcultured into fresh medium.

3. Result and Discussion
3.1. Ex vitro Seed germination
Four types of seed treatment of 10 accessions of ciplukan plants which will be germinated ex vitro produced varied responses. The ripening treatment of the seeds which aims to facilitate the selection of seeds which actually had raised the radicles does not show a positive response and the seeds do not show any changes for two weeks (treatment 1). All ciplukan accession seeds that were directly sown in compost media and washed with rubbing soaking water also did not show germination response (treatment 2) (Table 2). Treatment of seeds with ash (treatments 3 and 4) resulted in a better germination response reaching 80-100%. Ash water facilitated to eliminate mucus that surrounds the seeds so that the radicles more easily appear.

Table 2. Ex vitro germination response of Physalis plant accession.

| No | Accession       | Germination response (%) on some seed treatment *) | 1 | 2 | 3 | 4 |
|----|----------------|--------------------------------------------------|---|---|---|---|
| 1  | A1: SMN-PG(02)#55 | -                                                | - | - | 100 | 100 |
| 2  | A2: SPG-DC#65    | -                                                | - | - | 100 | 100 |
| 3  | A3: SPG-GD#60    | -                                                | - | - | 100 | 100 |
| 4  | A4:SPG-AS#70     | -                                                | - | - | 100 | 100 |
| 5  | A5: PMK-I#16     | -                                                | - | - | 100 | 80 |
| 6  | B1: TLG-PL(02)#61| -                                                | - | - | 100 | 100 |
| 7  | B2: KDR-RM(02)#33| -                                                | - | - | 80 | 100 |
| 8  | B3: KDR-NL#62    | -                                                | - | - | 100 | 80 |
| 9  | B4: MLG-TP#58    | -                                                | - | - | 100 | 100 |
| 10 | B5:BW-TD#64      | -                                                | - | - | 100 | 100 |

*) sub 2.2.2

Rate of seed germination of each accession varied (Figure 1), but almost all were able to germinate 100%. B3 accession (KDR-NL # 62) needed the longest significant germination time, which was about 17 days. While other accessions showed varying time of germination, ranging from 4.2 - 13 days.
Some accessions of Physalis plants from Madura Island (Sumenep (A1) and Pamekasan (A2-A4)) and the eastern part of Java (Kediri (B2 & B3) and Malang (B4)) showed faster growth compared to other accessions. This can be seen from the height of plants at five weeks after germination that has reached more than 25 cm (Figure 2A). At the same age, the height of plants produced by germination of accessions from Pamekasan (A5) and Tulungagung (B1) was only about 14-16 cm. Accession from Banyuwangi (B5) showed the slowest growth and only lasted until the third week by reaching plant height of about 5.8 cm. Generally the number of leaves increased until the fifth week but accessions A2 and A3 originating from Sumenep produced more leaves (10-12 sheets) compared to other accessions (Figure 2B; Figure 3).

Figure 1. Rate of ex vitro seed germination of Physalis accession.

![Figure 1](image1.png)

| Accession | Seed germination (day) |
|-----------|------------------------|
| A1        | 10.8                   |
| A2        | 6.8                    |
| A3        | 5.8                    |
| A4        | 4.2                    |
| A5        | 4.4                    |
| B1        | 6.8                    |
| B2        | 13                     |
| B3        | 17                     |
| B4        | 5.4                    |
| B5        | 5.2                    |

Figure 2. Plant growth at five weeks after germination. A. Plant height, B. Leaf number.
3.2. In vitro Seed germination

Seed germination of six accessions of Physalis plants germinated in vitro on the agar-solidified media to vary between 4-13 days after culture (Table 3). However, four other accessions namely A3, B1, B3 and B5 have not succeeded in germination. In contrast, the response of in vitro germination on compost media was only demonstrated by accessions of A3 and A4. The ability to regenerate in vitro shoots from cotyledonary nodes explants was demonstrated by all accessions from Madura Island except A3 accessions.

Table 3. Response of seed germination and shoot regeneration in vitro.

| No | Accession | Seed germination (%) | Shoot regeneration in shoot induction media\(^d\) |
|----|-----------|----------------------|--------------------------------------------------|
|    |           | Agar-solidified media | Compost media |                                                  |
| 1  | A1: SMN-PG(02)#55 | 3.33(7)\(^a\) | 0 | + |
| 2  | A2: SPG-DC#65  | 10(7)\(^a\) | 0 | + |
| 3  | A3: SPG-GD#60  | 0 | 100\(^c\) | - |
| 4  | A4:SPG-AS#70  | 18.33(8)\(^a\) | 33.33\(^a\) | + |
| 5  | A5: PMK-1#16  | 5.33(4)\(^a\) | 0 | + |
| 6  | B1: TLG-PL(02)#61 | 0 | 0 | - |
| 7  | B2: KDR-RM(02)#33 | 1.67(13)\(^a,b\) | 0 | - |
| 8  | B3: KDR-NL#62  | 0 | 0 | - |
| 9  | B4: MLG-TP#58  | 1.67(10)\(^a,c\) | 0 | - |
| 10 | B5:BW-TD#64   | 0 | 0 | - |

Note: \(^a\): numbers in parentheses indicate age of germination (days), \(^b\): seeds germinate browning and die, \(^c\): morphologically sprouts are not yet ready to be induced by shoots, + undergoes shoot induction. - not yet experienced shoot induction, \(^d\): from seedling – derived cotyledonous nodes in agar media

Figure 4A-D showed in vitro shoots regenerated from accessions A1, A2, A4 and A5, respectively. Even accession A4 showed good multiplication response of shoots after subcultured into MS medium supplemented with 2 mg/L BAP + 0.01 mg/L IAA (Figure 4E).
Physalis is generally propagated generatively through the use of seeds and vegetatively using in vitro techniques or grafting. Generative propagation by seed is a natural way in which plants are reproduced in their natural environment or in an artificial environment as a result of human intervention [13]. Efforts to grow accessions that originated from wild growth generally begin with a collection of fruits that are sometimes physiologically immature. In fact, the fruit harvested at the right level of maturity will determine the success of the seed germination process. In addition, seed maturity is also a major component of seed quality and a prerequisite for successful germination and emergence of seedlings [14]. Seeds that are harvested at the proper physiological stage have maximum viability and vigor. The seeds with high vigor show a better final appearance compared to seeds with low vigor [15].

Each species has a different seed germination period. *P. angulata* seeds should be collected from fruits with green petals and used immediately after harvesting, because seed germination decreases sharply after 45 days after storage, either in storage chamber or refrigerated rooms [16]. In addition to plant factors, the growing environment influences seed quality during seed formation, and, therefore, has an impact on seed formation, plant growth and productivity.

The results of this study indicate variations in growth responses both ex vitro and in vitro from 10 accessions of Physalis plants originating from Madura Island and East Java. This variation shows the nature of the seed which is influenced by many factors including genetic variation and environmental conditions during seed development. Seeds from different individuals in populations of the same species show variations in the biology of seed germination, including dormancy and response to dormancy-breaking factors [17]. In addition, seeds collected from different locations, years, height gradients and habitats also have the opportunity to show differences in the biology of seed germination because they are strongly influenced by environmental conditions [18].

Germination is considered as the end result of a complex and interactive process that involves a number of physiological, morphological, environmental and cultural factors. Harvest time is the main factor responsible for the physiological maturation level, size and strength of the seed during ripening [19]. Harvesting fruit at the right time will affect the quality of seeds and the percentage of seed germination. Various stages of development including differentiation, maturation, or cell expansion, drying and drying of the seeds are involved during the process before the germination and growth phases. Harvesting Physalis fruit for consumption is done if the petals are yellow because they indicate good fruit quality. Whereas harvesting for seeds production is based on the 'capus' color of the plant, which is when greenish yellow or yellow [20]. The highest percentage of germination was shown in
seeds from fruit aged 56 days after fertilization, which were significantly superior to seeds 63 days and 49 days due [21]. In this study, the low seed germination response might be due to the fruit being harvested when it was not ripe physiologically.

The accession of Physalis plants from Madura Island generally shows a better seed germination response and plant growth than the accession of Physalis plants from East Java Island. In this study the good response of on the accession Physalis A1, A2, A4 and A5 plants in shoot regeneration in vitro provides an opportunity for the development of cultivation techniques in the context of germplasm conservation. For accessions that did not show a germination response can be improved by applying appropriate method that can solve the problem of seed dormancy.

4. Conclusion
The results of this study indicate that both ex vitro and in vitro germination methods are able to induce seed germination of Physalis accession derived from East Java although the percentage of germination is still not optimal. Accession from Madura Island has better capability to regenerate shoot in vitro. The establishment of in vitro system leads to plant production in large quantities.

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References
[1] Backer CA and Brink RCB 1968 Flora of Java vol 3 (Groningen : Wolters-Noordhoff N.V.) pp 464-8
[2] Batoro J and Arumingtyas EL 2018 Morphological Characterization Genus Ciplukan (Physalis Spp.) Family Solanaceae in Malang East Java, Indonesia. Trav. Hum. 81 1905-11
[3] Hadiyanti N, Pardono and Supriyadi 2017 Jurnal Hijau Cendekia 2 71-7
[4] Utami NR, Rahayuninginsih M, Abudullah M and Haka FH 2019 Pro. Sem. Nas. Masy. Biodiv. Indon. 5 205-8
[5] Safitiri S, Yolanda R and Brahmana M 2015 e Journal Mahasiswa Prodi Biologi 1 1-4
[6] Amrul HMZN, Susilo F and Huda MK 2019 IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14 38-42
[7] Januário AH, Filho ER, Pietro RCLR, Kashima S, Sato DN and França SC 2002 Phytother. Res. 16 445-8
[8] Lin YS, Chiang HC, Kan WS, Hone E, Shih SJ and Won MH 1992 Am J Chin Med. 20 233-43
[9] Sun CP, Qiu CY, Yuan T, Nie XF, Sun HX, Zhang Q, Li HX, Ding LQ, Zhao F, Chen LX and Qiu F 2016 J Nat Prod. 79 1586-97
[10] Kusumaningtyas RW, Larly N and Limandha P 2015 Pro. Chem. 14 367-372
[11] Adewoye EO, Oguntola MA and Ige AO 2016 Afr J Med Med Sci. 45 99-108
[12] Lestari B, Permatasari N and Rohman MS 2016 Adv. Pharmacol. Sci. Article ID 2428052. 1-7
[13] Muniz J, Krettschmar AA, Rufato L, Pelizza TR, Rufato AR, TA de Macedo 2014 Cienc. Rural 44 964-970
[14] Perry DA 1982 Sci. Hort. 33 67-75
[15] Johnson RR and Wax LM 1981 Agron J. 73 859-863
[16] Carvalho TC, D’Angelo JWO, Scariot GN, Júnior LAS, Cuquel FL 2014 Pesqui. Agropecu. Trop. 44 357-362
[17] Black M, Bewley JD and Halmer P 2006 The Encyclopedia of Seeds: Science, Technology and Uses (Cambridge: CAB) p 203
[18] Fernández-Pascual E, B Jiménez-Alfaro, J Caujapé-Castells, R Jaén-Molina, TE Diaz 2013 Ann. Bot. 112 937-45
[19] Delouche, JC 1980 Hort. Sci., 15 13-18.
[20] Pellizzaro V, Omura MS, Marinke LS, Furlan FF, Takahashi LSA 2019. Agri Res & Tech: Open Access J. 22 00151-5
[21] Ali A and Singh B 2015 Internat. J. Forestry & Crop Improv 6 100-4