

Elicitor Effect of Chitosan on In Vitro Culture of Different Explants of *Physalis* Accessions From East Java

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ABSTRACT

Chitosan is a natural molecule derived from arthropods and fungi that can stimulate many physiological responses in plants. Chitosan had also shown positive effect on in vitro shoot regeneration. Therefore, this research aims to observe the response of shoot growth of in vitro *Physalis* plant accessions obtained from several areas in Madura Island and East Java on chitosan effect. Two types of explants shoot tips and in vitro nodal explants for each *Physalis* plant accession were cultured on elicitation medium (MS basal medium supplemented with BAP 2 mg / l and IAA 0.05 mg / l) containing chitosan elicitor (0, 75, 125 mg / l). The number and height of shoots were observed weekly for six weeks. The results showed that both types of explants from all accessions were able to regenerate and multiply shoots however shoot tip explants showed better growth. A2 accessions from Madura Sampang was able to regenerate shoots in high a number than other accessions on chitosan concentration of 125 mg / l. These results provide a future prospect to study synthesis of secondary metabolites in *Physalis* accession induced by chitosan elicitors.

Keywords: *accession, chitosan, elicitors, Madura, Physalis*

1. INTRODUCTION

The genus *Physalis* is one of the largest genera of the Solanaceae family which is not only used as food but also used as traditional medicine [1]. The medicinal benefits of *Physalis* plants are due to various biological and pharmacological activities associated with the presence of flavonoids, phenyl propanoids, alkaloids, physical, withanolides, and other bioactive compounds [2]. Withanolides, a class of lactone steroids, has been known for its antitumor, and anti-inflammatory properties [(3).

Until now, several *Physalis* accessions have been identified from various regions in Indonesia, such as the Surakarta Residency (18 accessions) [4], West Sumatra (31 accessions) [5], East Java, Central Java, and West Java [6]. Each accession can describe the diversity that exists in a certain plant population related to environmental conditions.

Plants that are sessile have a self-defense strategy involving the biosynthesis of secondary compounds. Therefore, the synthesis of secondary compounds is strongly influenced by the environment. However, plant secondary metabolites that are synthesized at certain physiological and developmental stages are also produced in very small amounts, namely less than 1% dry weight [7]. This is a weakness in the use of medicinal ingredients which must be available in large quantities. In vitro culture system is an alternative to produce secondary metabolites because the organs that are grown in vitro can be stimulated to produce secondary compounds in higher level and sometimes have different profiles with mother plants.

Production of secondary compounds in vitro have been frequently induced by elicitation methods [8-11]. Secondary metabolites in plants act as communication signals in response to unfavourable conditions such as pathogenic infections and environmental stresses [12].

Physalis angulata seeds soaked in chitosan elicitor were able to regenerate shoots and showed good in vitro growth. In addition, the levels of withanolide compounds produced by in vitro shoots are also higher than shoots treated with PEG elicitors [13]. This study aims to determine the elicitor effect of chitosan on in vitro shoot growth of different types of explants from several *Physalis* accessions collected from Madura Island and several regions in East Java.

2. MATERIALS AND METHODS

2.1. Plant material

Accessions of *Physalis* plants obtained from Sumenep (A1), Sampang (A2 and A4), and Pamekasan (A5) in Madura Island and from Tulung Agung (B1), Kediri (B3) and Banyuwangi (B5) were the collections of Dr. Budi Waluyo (Department of Agronomy, Brawijaya University).

2.2. Preparation of explant source

Sterilization was initiated by shaking the *Physalis* seeds for 15 minutes in a 20% commercial bleach solution. Then the seeds are rinsed three times with sterile distilled water for 5 minutes each [14]. Subsequently, the germination of sterile seeds were undertaken in water medium solidified with 10 g/l agar without adding nutrients or growth regulators. Two-week old in vitro seedling was ready to be used as a source of explants for shoot induction.

2.3 Shoot induction

Cotyledon nodes explants were put on shoot induction medium (MS + BAP 2 mg / l + IAA 0.05 mg / l). The resulting adventitious shoots were subcultured at 4 weeks of culture to fresh medium for multiplication. Shoots from the third subculture were subcultured to MS0 medium before transferring to elicitation medium.

2.4 Elicitation treatment

Two types of explants, namely shoot tips and nodal explants, were used to see the effect of chitosan elicitor. The shoot tip was cut from the top of the first node. Node explants were taken from the first node after removing the petiole and shoot tips. Each explant was cultured on the elicitation medium, namely MS + BAP 2 mg / l + IAA 0.05 mg / l + chitosan (0, 75, 125 mg / l). The number of shoot and shoot height were observed every week for 6 weeks.

3. RESULT AND DISCUSSION

3.1. The effect of elicitors on shoot number

Both explant types, shoots and nodes in vitro from seven *Physalis* plant accessions (A1, A2, A4, A5, B1, B3 and B5) showed the ability to induce shoot on MS + BAP medium 2 mg / l + IAA 0.05 mg / l. The resulting adventitious shoots were still able to grow in the medium added with 75 and 125 mg/l chitosan elicitor. However, each type of explant showed a different response. In general, at four weeks of culture, shoot tip explants showed faster growth than nodal explants either in control medium or in elicitation medium supplemented with chitosan. The regenerated shoots are numerous and larger in size (Figure 1). On the other hand, nodal explant produces shoots with smaller leaf size. Leaf area was not included in the observed parameters due to technical constraints for measuring objects that were still in the in vitro flask.

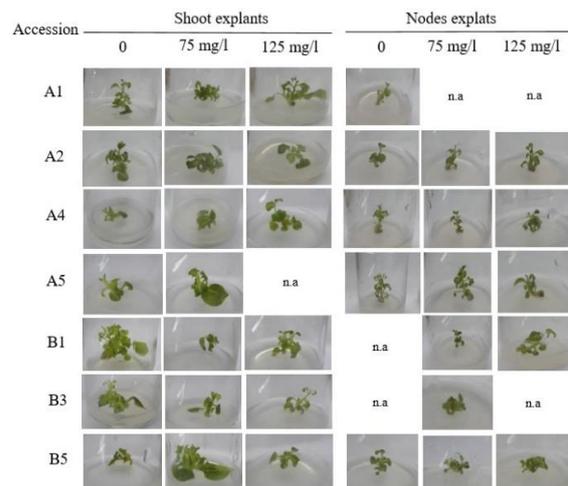


Figure 1 Shoots regeneration of several accessions of *P. angulata* on four weeks of elicitation medium. A1: Sumenep, A2 and A4: Sampang, A5: Pamekasan, B1: Tulung Agung, B3: Kediri and B5: Banyuwangi. Chitosan concentration: 0, 75 and 125 mg/l. n.a = not available data.

Shoot and nodal explants from accessions A1 and A2 produced more shoots than the other five accessions (Figure 2). The highest average number of shoots was 3.75 ± 1.50 produced by A1 accession because of elicitation treatment with 75 mg / L chitosan. The number of shoots decreased to 1.75 ± 0.50 mg / l when the elicitor were increased to 125 mg / l. Whereas A2 accession on 125 mg / l chitosan elicitation treatment was still able to produce the highest average number of shoots, namely 3.33 ± 1.15 . The number of shoots produced by the other

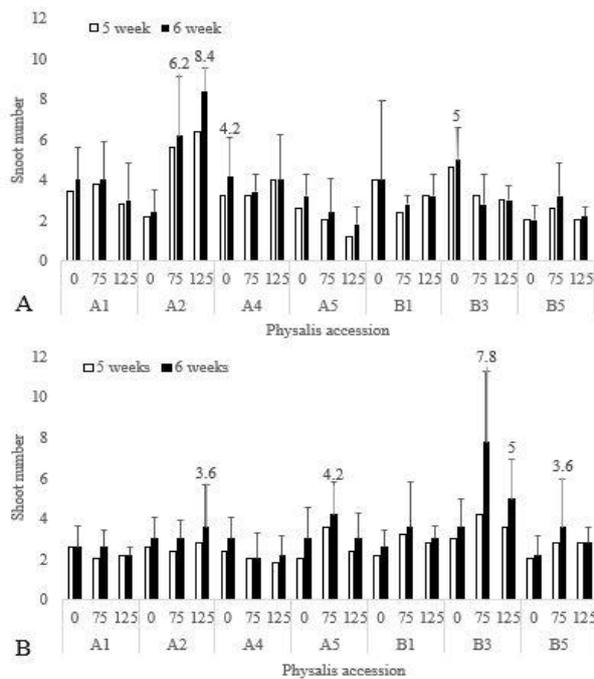


Figure 2. Effect of elicitor chitosan on in vitro shoot number of seven *Physalis* accession at 5 and 6 weeks after culture. A. Shoot explant, B. Nodal explants. A1: Sumenep, A2 and A4: Sampang, A5: Pamekasan, B1: Tulung agung, B3: Kediri dan B5: Banyuwangi; Chitosan concentration: 0, 75 and 125 mg/l.

two accessions (A4 and A5) obtained from Madura Island until the fourth week of culture had not reached three. Node explants in all accessions showed lower growth capability. Until the fourth week of culture all accessions only produced less than two shoots.

Sixth week after culture, the average number of shoots produced by the nodal explants tended to be less, ranging from 2.2 ± 0.98 to 7.8 ± 3.48 (Figure 2). Whereas the number of shoots produced by shoot tip explants ranged from 1.8 ± 0.83 - 8.4 ± 1.14 . The existence of chitosan elicitor did not always reduce the number of shoots produced. Shoot explants from A2 accession and node explant from B3 accession produced shoots with the highest average number of chitosan concentrations of 125 and 75 mg / l, respectively.

3.2. Effect of elicitors on shoot height

The shoot height response to chitosan elicitor varied with the origin of accession and type of explant. Shoot height produced by shoot tip explants that produced by node explants. Accession A2 from Sampang, Madura still showed the best shoot height

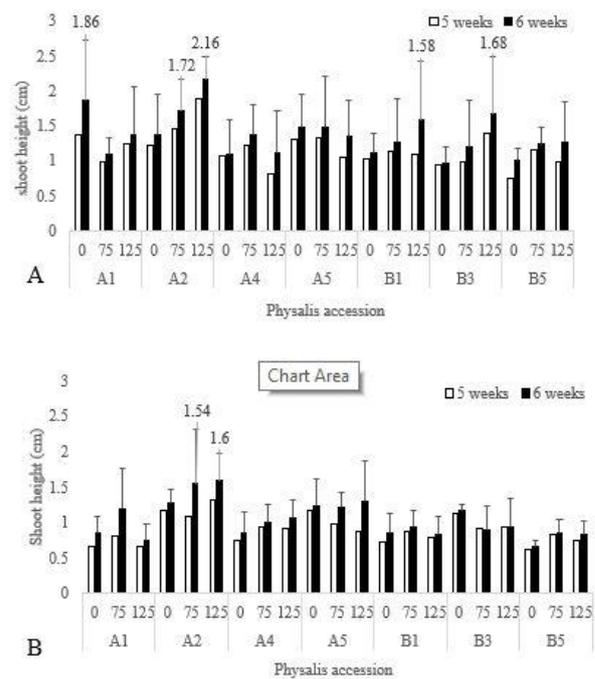


Figure 3. Effect of elicitor chitosan on in vitro shoot height of seven *Physalis* accession at 5 and 6 weeks after culture. A. Shoot explant, B. Nodal explants. A1: Sumenep, A2 and A4: Sampang, A5: Pamekasan, B1: Tulung agung, B3: Kediri dan B5: Banyuwangi; Chitosan concentration: 0, 75 and 125 mg/l.

growth on both shoot tip explants (2.16 ± 0.32 cm) and node explants (1.6 ± 0.37 cm). Other accessions, although many showed a positive response to chitosan elicitor, but the height was not more than 1.5 cm (Figure 3).

The growth response both quantitatively and qualitatively showed that all *Physalis* plant accessions in vitro were still able to grow well on the medium with the addition of chitosan elicitor to a concentration of 125 mg / l. Shoot explants are apical shoots which have a growing tip so that the mineral nutrients and exogenous growth regulators supplemented into the medium are immediately used to continue their growth. Apparently, nodal explants still need time to induce the emergence of axillary shoots so that in the same culture age, the resulting shoots are less and still small compared to shoots produced by shoot tip explants.

Chitosan is an elicitor which derived from the component of fungal cell walls [15]. The positive effect of chitosan on in vitro propagation has been proved in some medicinal plants [16][17][18]. Elicitors are chemical substances which has positive effect on in vitro shoot growth [19]. The positive

effect is also thought to be due to the combination of BAP cytokinins and chitosan elicitors [20].

4. CONCLUSION

All plant accessions of *Physalis* could regenerate and multiply shoots in vitro both without and with the addition of chitosan elicitor. Accession A2 obtained from Sampang, Madura Island resulted in better shoot growth than other accessions. The ability to grow in the elicitation medium provides an opportunity to identify the quantitative and qualitative profiles of secondary compounds.

AUTHORS' CONTRIBUTIONS

BW contributed to the initiation of the *Physalis* plant accession seed collection in the field. JB conducted field research, analyzed data, and compiled a draft publication. RM configures a research project. RM also read, reviewed, and contributed to the finalization of the manuscript.

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