

Abscissic acid (ABA) Hormone Causes Plant and Tissue Growth Inhibition: Prospect for Cancer Cell Growth Inhibition

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Abstract

The experiment was conducted to evaluate the inhibitory effects of abscissic acid (ABA) on the peach shoot and bark phloem tissue growth. The results of the hormonal effects [abscissic acid (ABA)] showed the study on the peach shoot and phloem tissue and prospect of the inhibition of cancer cell division and multiplication. The result presented using peach shoot and bark phloem tissue growth inhibition applying the hormonal application of ABA at different concentrations. The result observed the highest shoot (74.6% at ABA 2000ppm) and bark phloem tissue (100% at ABA 2000ppm) were excessively inhibited. From the results indication, it can be concluded that it is excessively possible to inhibit of the growth of plant shoot and bark tissue by affecting cell division and cell differentiation using ABA and the highest plant tissue growth was inhibited 100% at 2000ppm ABA. So, from our results, it can obviously be prospected that ABA at different concentrations can be effectively inhibited the human cancer cell growth.

Keywords: ABA, plant growth inhibition, cancer cell inhibition.

I. INTRODUCTION

Genetically modified technology can produce dwarf plant using a number of ways such as tissue culture, grafting, to budding methods [1]. T-DNA and hormonal application [2]. It was reported that cultivars of fruit plant are grown as grafted composites as two genotypes [Rootstock (lower portion) and scion (upper)]. It has been stated that it was possible to graft a nontransgenic scion with rootstock of fruit tree before reproductive age [3]. In some species vegetative propagation was induced by the dwarf rootstock or other adventitious rootstock resulting transgenic dwarf flower production. They suggested that double grafting starting with transgenic shoot grafted (dwarf interstock) to a wild type rootstock then grafted again dwarf nontransgenic scion resulting a dwarf transgenic scion between nontransgenic portions.

It has been reported that it was possible to make peach tree greatly dwarfed (small tree size) by using ABA, 500 and 1000ppm, CCC 500 and MH 500ppm applied to the bark phloem tissue when compared to the water control [4]. They also reported that growth inhibitor (ABA) were used by swabbing method with cotton to the bark tissue and found dwarfing effect in bark tissue growth. It had been seen that shoot growth reduced 46% at 1000ppm ABA, and 52% at 1000ppm ccc (Cycocel) and 48% at 2500 Maleic hydrazide (MH)[4].

ABA has shown that the various developmental and physiological processes that affected the growth performance of crop plants [5]. ABA levels increased in tissues subjected to osmotic stress by desiccation, salt, or cold [6]. Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the application of exogenous ABA [7]. Although different sets of ABA-responsive genes exhibited different patterns of developmental and tissue-specific expression, some of them appear to be part of a general reaction to osmotic stress [7]. It has been shown that the response to ABA is Ca²⁺ dependent, suggesting that second messenger signaling mediated ABA action [8]. It has been reported that anticancer properties was supported by using ABA [9]. The objectives of the research were undertaken

1. To investigate the effects of growth inhibition using abscissic acid (ABA) of peach shoot, and bark phloem tissue growth.
2. to support the data for the prospect of cancer cell growth inhibition applying abscissic acid (ABA), hinokitiol and tropolone hormones implementing on human and animal cell.
3. To highlight the data on the cancer cell growth inhibition in further innovative studies.

II. MATERIALS AND METHODS

A. Site

The experiment was carried out in an orchard at Ehime University Experimental Farm located in southern Japan, Matsuyama, Ehime.

1 Plant materials

Two-year-old peach (*Prunus persica*) trees were utilized in the current studies. The trees were spaced at 0.60 m x 1.0 m in a completely randomized design. Weeding and irrigation were maintained well at 7 days interval and insecticides were applied as needed.

2 Treatment setting

Five shoot were maintained per tree to ensure proper growth. There were 3 treatments and 4 replications and a total of 12 trees used in the experiment. Partial ringing was made in the bark tissue by removing a bark ring 2 cm long leaving a connecting bark strip 2 mm width 10 cm above from the ground in the trunk. The treatments were water control (no inhibitor), abscisic acid (ABA) 1000 and 2000 ppm. The treatments were applied on the bark strip tissue at two weeks interval and continued for three months.

3 Data collection

Peach shoot and bark phloem tissue growth inhibition percent were measured.

4 Statistical analysis

Least Significant Differences (LSD) test was employed for the data analysis.

5. Prospective cancer cell culture and hormone treatment

B. Cell culture

HT-29 cells (passage 106) may be used for the cancer cell culture. Between passages 150 and 200 cells can be cultured and passed to RPMI 1640, supplemented with 100 mL/L fetal calf serum and 2 mmol/L glutamine. Antibiotics may be added to the medium using 100,000 U/L penicillin and 100 mg/L streptomycin (Juan et al, 2006).

1 Cell proliferation

In the proliferation assay, HT-29 cells may be seeded at a density of $5 \cdot 10^3$ cells/well onto 24-well cell culture plates and allowed to adhere for 24 h. The media may then be substituted by a fresh culture medium containing different concentrations (lower to higher) [like 10, 20, 30, 50, 100, 500, 1000, 2000ppm] of ABA depending upon the types of the cells. The cells can be allowed to grow for 72 h before total cell count will be determined. Cells can then be analyzed with 1% Triton X-100 in isotonic NaCl, and DNA can be stained

with SYTOX-Green. Cell numbers can be measured using the fluorescence multi-well plate reader (Juan et al 2006).

2 DNA fragmentation.

DNA fragmentation as a late marker of apoptosis can be investigated by staining DNA with Hoechst 33258. HT-29 cells ($3 \cdot 10^4$ cells/well) (Juan et al. 2006).

Hormone	Bark tissue	Shoot growth
Treatment	growth	inhibition
	inhibition (%)	
Water control	0	0
ABA 1000 ppm	50.3b	61.1b
ABA 2000 ppm	100a	90.3a

Table 1. Growth inhibition percent as affected by ABA, hinikitiol and tropolone.

LSD test at 5% Significant difference. Means followed by the common letters are not significantly different at the 5% level by Least Significant different test (LSDT). Mean±SE (n = 4).

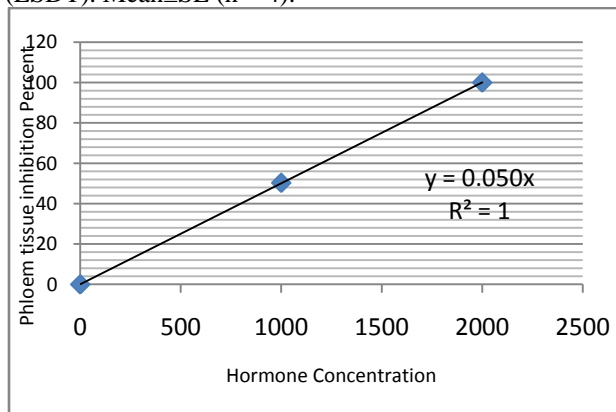


Fig. 1. Correlation between the hormone concentration and inhibition percent of bark phloem tissue. 1= Control, 1000= ABA 1000ppm 2000= ABA 2000ppm.



ABA 2000 PPM (0% healing) Water control (100% healing)

Fig. 2. Bark Phloem Tissue Growth (healing) by Hormone Application and Water Control.

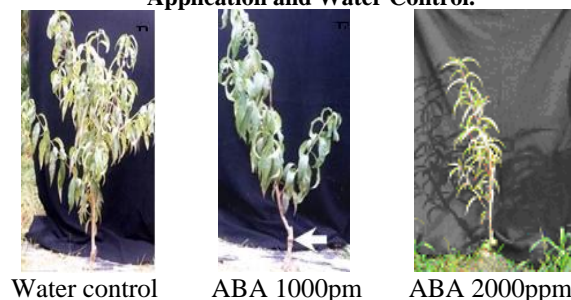


Fig. 3: Peach Dwarf Trees (genetically dwarf) by ABA Hormone.

III. RESULT AND DISCUSSION

From the results, the effects of the growth inhibitors of ascissic acid (ABA) at the concentration 1000 and 2000ppm on the shoot and bark tissue growth inhibition percent have been presented (Table 1). It has been shown that the highest bark phloem tissue was inhibited 100% at ABA 2000ppm (Table 1). Figure 1 shows the strong correlation between the hormone concentration and inhibition percent of bark phloem tissue. ABA 2000ppm showed the highest correlation of the inhibition percent (Figure 1). Figure 2 has explored the image of the excessively growth (healing) of bark tissue inhibition by ABA 2000ppm. In addition, Figure 3 has represented the photograph of the structure of the growth inhibition of shoot and leaves.

From the results it has been exhibited that ABA 2000ppm has showed the highest inhibition of the peach shoot and bark phloem tissue growth. This is might be due to the effectiveness of the given concentration. 100% inhibition was found in these concentration. Cell division and differentiation might not be occurred by these concentration of hormones. That is why organs growth was inhibited 100% by ABA 2000ppm concentration. ABA 1000ppm showed the lower inhibition when compared to the ABA 2000ppm. It might be due to the less effectiveness of the certain concentration. Cell division and differentiation might be occurred in some cases by these concentration of hormones. That is why organs

growth was inhibited 40-74% by ABA 1000ppm concentrations.

Hossain and Mizutani [4] reported that growth of the different organs of peach plant was inhibited by using ABA, 500 and 1000ppm, CCC 500 and MH 500ppm. They also reported that shoot and root growth were inhibited 46% at 1000ppm ABA, and 52% at 1000ppm CCC (Cycocel) and 48% at 2500 Maleic hydrazide (MH). Hossain et al (2007) observed that ABA 1000ppm and 2000ppm reduced the trunk and flower growth.

Marco [10] suggested that salicylic acid, jasmonic acid and ethylene showed 26% of the up-regulated genes were protected plants growth and made abscission. Austin [5] stated that ABA has shown the various developmental and physiological processes that inhibited the growth performance of crop plants. Gonzalo [9] suggested that anticancer properties was exhibited by using ABA hormone. Zhao et al [11] observed that plant stress hormone ABA suppressed the proliferation and induced apoptosis in human cancer cell [12, 13].

IV. CONCLUSION AND RECOMMENDATION

From the results it can be concluded that ABA 2000ppm hormones are the best for the growth inhibition of shoot and phloem tissue. However, ABA 1000ppm have the less effect for the growth inhibition when compared to the ABA 2000ppm. Therefore from our research, though it was applied on the plant samples but also it can be prospected that ABA at different concentrations may be effectively inhibited the human and animal cancer cell growth.

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REFERENCES

- [1]. S. Joseph. Genetically modified seedless fruit. April 23, 2013. <http://www.buzzle.com/articles/genetically-modified-seedless-fruits.html>
- [2]. A.B.M.S. Hossain, F. Mizutani, J.M. Onguso, A.R., AlShereif, H. Yamada. Inhibiting peach-trees growth with Abscisic acid, hinokitiol, and tropolone applied to partially ringed bark strips. Journal of the Horticultural Science and Biotechnology, 82:175-178. 2007

- [3]. S Lev-Yundán and R. Sederoff Grafting for transgenic containment. *Nature Biotechnology*.19,1104. 2001.
- [4]. A.B.M.S. Hossain, F. Mizutani. Growth regulatory effect of cytokinin in peach trees as affected by ringed bark strip by using bioassay in vitro. *Canadian Journal of Pure and Applied Sciences*. 3 (1), 655-659. 2009.
- [5]. R.B. Austin, I.E. Henson, S.A. Quarrie. Abscisic acid and drought resistance in wheat, millet, and rice. In *Drought Resistance in Crops with Emphasis on Rice*, M.R. Vega, ed. (Los Banos, The Philippines: International Rice Research Institute), pp171 -180.1982
- [6]. I.E. Henson. Effects of atmospheric humidity on abscisic acid accumulation and water status in leaves of rice (*Oryza sativa* L.). *Ann. Bot.*1984; 54, 569-582.
- [7]. J. Baker, C. Steele, L. Dure. Sequence and characterization of 6 Lea proteins and their genes from cotton. *Plant Mol. Biol.* 11,277-291. 1988.
- [8]. J.A. Napier J.M. Chapman, M. Black. Calciumdependent induction of novel proteins by abscisic acid in wheat aleurone tissue of different developmental stages. *Planta*, 179. 1989.
- [9]. R.M. Gonzalo. Abscisic acid against cancer. Patent No. US8389024 B2. 2014.
- [10]. Y. Marco. Biological control of bacterial wilt in *Arabidopsis thaliana* involves abscisic acid signaling. *New Phytologist* 194, 1035–1045. 2012.
- [11]. H.W. Zhao et al. Effect of induction of differentiation of TCA813 cells affected by abscisic acid in vitro. *Hua Xi Yi Xue Za Zhi* 25, 508-512. 2007.
- [12]. N. Zhou, Y. Yao, Y. Hongxing, Z. Wei, C. Liang, M. Ying. Abscisic-acid-induced cellular apoptosis and differentiation in glioma via the retinoid acid signaling pathway *Molecular Cancer Biology*. 138(8): 1947-1958. 2016.
- [13]. V.W.C. Livingston *The conquest of cancer: Vaccines and diet*. Franklinwatts, New York.NY. 1984.