



GROWTH INHIBITION OF PEACH STEM, LEAVES AND PHLOEM TISSUES USING ABA, TROPOLONE AND HINOKITOLIOL HORMONES AND ITS PROSPECT AS GROWTH INHIBITOR OF CANCER CELL

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Abstract

The study was carried out to investigate the inhibitory effects of abscissic acid (ABA), hinokitiol and tropolone on the stem, shoot, leaves, bark phloem tissue and root growth of peach plant. The results of the different hormonal effects exhibited the innovative study on the different organs of peach plant and prospect of the inhibition of cancer cell division and multiplication (growth by abscissic acid (ABA), hinokitiol and tropolone hormone). The data were presented utilizing peach stem, shoot, leaves, bark phloem tissue and root growth inhibition coming after the hormonal application of ABA, hinokitiol and tropolone at different concentrations. The Data observed the highest shoot (74.6% at tropolone 500ppm), bark phloem tissue (100% at tropolone 500ppm and ABA 2000ppm), stem growth (99%), leaves growth (100%) and root growth (78.3% at tropolone 500ppm) were excessively inhibited. From the data indication, it can be concluded that it is excessively possible to inhibit of the growth of plant stem, shoot, leaves, root and bark tissue by affecting cell division and cell differentiation using ABA, hinokitiol and tropolone and the highest plant tissue growth was inhibited 100% at 500ppm tropolone and 2000ppm ABA. So, from our data, it can obviously be prospected that ABA, hinokitiol and tropolone at different concentrations can be effectively inhibited the human cancer cell growth.

Keywords: ABA, hinokitiol, tropolone, plant growth inhibition, cancer cell inhibition

Introduction

Nowadays Plant biotechnologist can produce dwarf plant using a number of ways such as tissue culture, grafting, to budding methods (Joseph 2013), T-DNA and hormonal application (Hossain et al 2007). It was reported that cultivars of fruit plant are grown as grafted composites as two genotypes [Rootstock (lower portion) and scion (upper)]. Lev-Yadun and Sederoff (2001) stated that it was possible to graft a nontransgenic scion with rootstock of fruit tree before reproductive age. 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.

Hossain and Mizutani (2009) observed an experiment to know the genetically dwarf of peach tree by growth inhibition hormone (ABA, CC and MH). It is reported that by this study, it is 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. 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). Almost same proportion of root growth was reduced in the case of same concentrations of ABA, CCC and MH.

Marco (2012) reported that salicylic acid, jasmonic acid and ethylene played a role in the establishment of this resistance. Microarray analysis showed that 26% of the up-regulated genes in protected plants were involved in the biosynthesis and signalling of abscissic acid (ABA) and made abscission. In addition 21% of these genes are constitutively expressed in the irregular xylem cellulose synthase mutants (irx), which presented a high level of resistance to *R. solanacearum*. In addition, ABA has shown that the various developmental and physiological processes that affected the growth performance of crop plants (Austin et al., 1982). ABA levels increased in tissues subjected to osmotic stress by desiccation, salt, or cold (Henson, 1984). Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the application of exogenous ABA (Singh et al., 1987). 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 (Baker et al., 1988). It has been shown that the response to ABA is Ca^{2+} dependent, suggesting that second messenger signaling mediated ABA action (Napier et al., 1989). It has been reported that anticancer properties was supported by using ABA (Gonzalo, 2014). Zhao et al (2007) observed that plant stress hormone ABA suppressed the proliferation and induced apoptosis in human cancer cell (Livingston, 1984). Fingrut et al. (2002) reported that sodium salicylate, jasmonic acid and methyl jasmonate suppressed the proliferation or caused apoptosis in certain mammal cancer cell.

The objectives of the research were undertaken

1. to investigate the effects of growth inhibition using abscissic acid (ABA), hinokitiol and tropolone of peach stem, shoot, leaves, bark phloem tissue and root 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.

Materials and Methods

Site

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

Plant materials

One-year-old peach (*Prunus persica*) trees was 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.

Treatment setting

Five shoot were maintained per tree to ensure proper growth. There were 12 treatments and 4 replications and a total of 48 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), abscissic acid (ABA) 1000 and 2000 ppm, hinokitiol 500 ppm and tropolone 500 ppm. The treatments were applied on the bark strip tissue at two weeks interval and continued for four months.

Data collection

Peach stem growth inhibition, shoot, leaves, bark phloem tissue and root growth inhibition percent were measured.

Statistical analysis

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

Prospective cancer cell culture and hormone treatment

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).

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 or higher) [like 10, 20, 30, 50, 100, 500, 1000, 2000ppm] of ABA, honokitiol and tropolone 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).

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).

Result and Discussion

From the results, the effects of the growth inhibitors of hinokitiol and tropolone and abscissic acid (ABA) at the concentration of 500 and 1000, 2000ppm on the stem growth, shoot, bark tissue leaves and root growth inhibition percent have been presented (Table 1). It has been shown that maximum stem growth was inhibited 99% at

tropolone 500ppm followed by 95% at ABA 2000ppm (Table 1). The highest bark phloem tissue was inhibited 100% at tropolone 500ppm and ABA 2000ppm (Table 1). The highest root growth inhibition was 78.3% at tropolone 500ppm when compare to the ABA and hinokitiol (Table 1). The shoot growth was higher in tropolone 500ppm and ABA 2000ppm than in ABA 100ppm and hinokitiol 500ppm (Table 1). It can be seen from the result that leaves growth inhibition was 100% in tropolone 500ppm and ABA 2000ppm followed by 72.2 and 50.2% in Hinokitiol 500ppm and ABA 1000ppm respectively. Figure 1 shows the strong correlation between the hormone concentration and inhibition percent of bark phloem tissue. ABA 2000ppm and tropolone 500ppm 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 stem, shoot, leaves, bark phloem tissue and root.

From the results it has been exhibited that ABA 2000ppm and tropolone 500ppm has showed the highest inhibition of the peach stem, shoot, leaves, bark phloem tissue and root 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 and tropolone 500ppm concentration. ABA 1000ppm and hinokitiol 500ppm showed the lower inhibition when compared to the ABA 2000ppm and tropolone 500ppm. 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 and hinokitiol 500ppm concentrations.

Hossain and Mizutani (2009) 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, tropolone and hinokitiol 500ppm reduced the trunk and flower growth.

Marco (2012) suggested that salicylic acid, jasmonic acid and ethylene showed 26% of the up-regulated genes were protected plants growth and made abscission. Austin et al., (1982) stated that ABA has shown the various developmental and physiological processes that inhibited the growth performance of crop plants. Gonzalo, (2014) suggested that anticancer properties was exhibited by using ABA hormone. Zhao et al (2007) observed that plant stress hormone ABA suppressed the proliferation and induced apoptosis in human cancer cell (Jhou et al. 2016; Livingston, 1984).

Conclusion and Recommendation

From the results it can be concluded that tropolone 500ppm and ABA 2000ppm hormones are the best for the growth inhibition of stem, shoot, leaves, phloem tissue and root. However, honokitiol 500ppm and ABA 1000ppm have the less effect for the growth inhibition when compared to the tropolone 500ppm and ABA 2000ppm. Therefore from our research though it was applied on the plant samples but also it can be prospected that ABA, hinokitiol and troponle at different concentrations may be effectively inhibited the human cancer cell growth.

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Annexure

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

Hormone Treatment	Stem growth inhibition (%)	Bark tissue growth inhibition (%)	Root growth inhibition (%)	Shoot growth inhibition	Leaves growth inhibition
Water control	0	0	0	0	0
ABA 1000 ppm	40.0c	50.3c	43.4c	61.1c	50.2c
ABA 2000 ppm	95.0a	100a	73.4a	90.3a	100.0a
Hinokitiol 500ppm	67.0b	60.2b	53.5b	74.2b	72.2b
Tropolone 500ppm	99.0a	100a	78.3a	84.6a	100.0a

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).

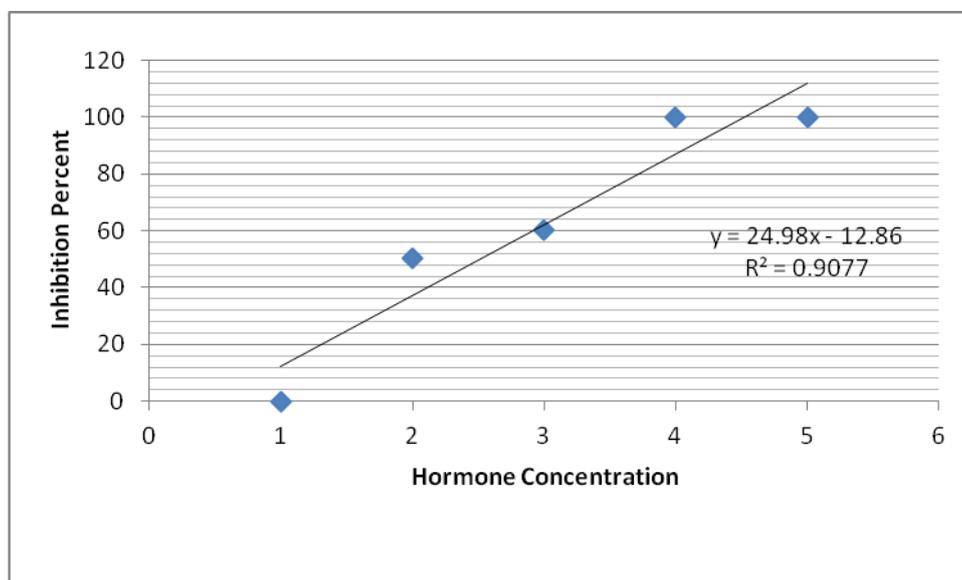


Figure 1. Correlation between the hormone concentration and inhibition percent of bark phloem tissue. 1= Control, 2= Tropolone 500ppm, 3= ABA 2000ppm, 4= Hinokitiol 500ppm, 5= ABA 1000ppm.

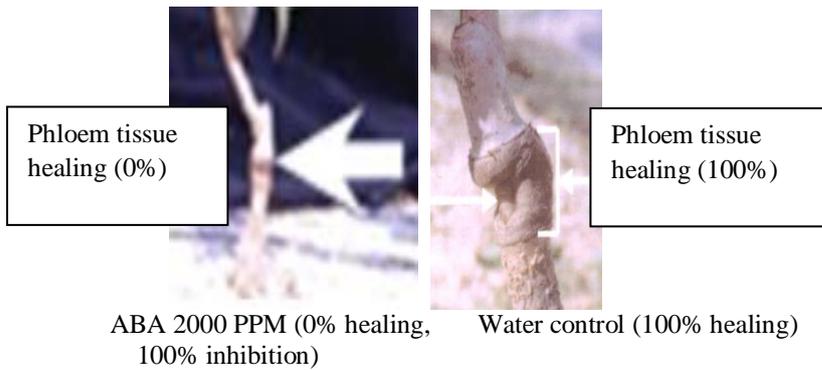


Figure 2. Bark phloem tissue growth (healing) by hormone application and water control

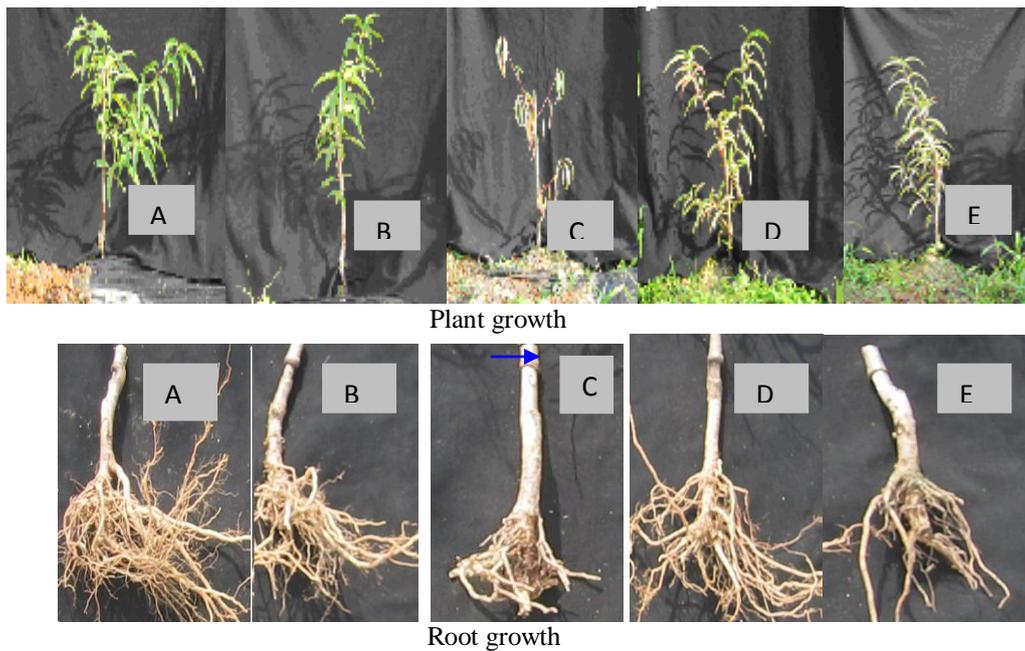


Figure 3. Inhibition of Peach plant growth by ABA, honokitiol and troponole hormone. (Different growth of the same age of tree [1 year tree].)