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EFFECT OF PHLOEMIC STRESS ON CYTOKININ CONTENT IN YOUNG PEACH TREES BY USING SOYBEAN CALLUS BIOASSY

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ABSTRACT

Cytokinin content in roots of peach trees (*Prunus persica* Batsch cv. Hikawahakuho) as affected by phloemic stress (bark ring) was studied. The treatments were applied to the trunk as control (no ringing), partial ringing [(represented by phloemic stress) PR] and complete ringing (CR). Phloem ring was made by peeling out 2 cm length of bark (phloem) from the trunk leaving a connecting 2 mm thickness of phloem strip (a bridge) while complete ringing (CR) left no phloem strip. Trunk diameter was higher in PR and CR treated trees than in un-ringed trees. Trunk diameter was higher above the ring than in below the ring both in PR and CR treated trees. However, it was higher in above the ring in case of PR and CR treated trees than in lower the ring both in PR and CR treated trees. The cytokinin content represented by callus weight was higher in control trees than in PR and CR treated trees. The results showed that cytokinin content decreased in roots of PR and CR treated trees. The results also indicated that growth promoting hormone (cytokinin) in peach roots affected overall plant nutrition and growth inhibiting hormon that led small sized peach trees.

Keywords: Cytokinin, dwarfing, ringing.

[Abbreviations: CR, Complete ring; PR, Partial ring; PVP, Polyvinyl pyrollidone].

INTRODUCTION

Phloem ringing is a horticultural practice used to manipulate tree growth and development, and fruit growth, in a variety of fruit species. Small, compact, dwarfed or size controlled fruit trees provide easier pruning, thinning, spraying and harvesting, high production of high-grade fruit and lower cost of production (Tukey, 1964). The primary factor limiting the use of size controlling rootstocks in stone fruit production was the lack of suitable rootstocks with a wide range of compatibility among cultivars (De Jong *et al*., 2001). Jose (1997) found lower vegetative growth in girdling treatments in relation to control in mango trees.

Arakawa *et al*. (1997) reported that trunk growth of apple trees above girdling was significantly increased and below girdling trunk growth was reduced. Onguso *et al*. (2004) reported that the increase of trunk circumference above girdling might be caused by swelling of trunk with accumulation of carbohydrates. They also stated that girdling blocked the translocation of sucrose from leaves to roots through phloem bundles. The block decreased starch content in root system and accumulated of sucrose in the leaves. Rose and Smith (2001) found that complete girdling of stems killed the plants and partial girdling weakened the plants.

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Mullins (1967) stated that cytokinin successfully stimulated for root growth of young grapes. Antognozzi *et al*. (1993) reported that cytokinin activating compound N_1-(2-chloro-4pyridyl)-N_3-phenylurea (CPPU) increased the transverse diameter, size and fresh weight of olives. Park *et al*. (1997) observed that stem growth of kinetin treated persimmon trees was higher than control trees.

There is not available literature on cytokinin as affected by bark ringing. That is why, this study was undertaken to determine the cytokinin content which occured during senescence as affected by phloemic stress on the trunk represented by girdling.

MATERIALS AND METHODS

Site

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

Plant materials

Two-year-old peach (*Prunus persica* Batsch cv. 'Hikawahakuho') trees grafted on peach seedling stocks (wild form) were used in this experiment in April 2004. The seedling rootstocks were collected from nursery and transplanted to the main field on April 13 2004. The transplantation was done by maintaining pit (hole) method in the main field. The pit (hole) was spaced at 0.60 m x 1.0 m. The tree height was 0.70 m initially.

Weeding was done by maintaining row as required. Granular fertilizers were applied after transplanting at the rate of N, P_2O_5 and K_2O 10g, 10g and 10g per tree respectively. Irrigation was applied once in a week by hose pipe. Insecticide was applied once in a month.

Treatment setting

Treatments were set on June 13, 2004. Phloem ringing [partial ringing (PR)] was done by using a small sharp knife removing a partial ringing 2 cm length to leave a connecting strip (band) 2 mm width (thickness) in the trunk, 20 cm above from the ground. In case of complete ringing (CR) there was no connecting strip (band). There were 3 treatments each with 4 replications used in different trees in the experiment. The treatments were control (no ringing) phloem ring and complete ring. Treatments were repeated in different trees. There were a total 12 (4 x 3) trees used for cytokinin (from roots) analysis.

Sample collection and preparation for cytokinin

Root samples (30 g) were collected on July 11 2004 after one month of treatment setting. Twelve (3 x 4) trees were uprooted and washed. Fresh roots were separated, washed and kept in the Freezer immediately after harvest and used for cytokinin analysis.

Cytokinin analysis

The samples were ground with mixture machine and added with 500 ml of 80% ethanol and kept for 12 h in the refrigerator. After 12 h, samples were filtrated and washed the residue with 500 ml of 80% ethanol and keep for 12 h. The same procedure was repeated 3 times. Total 1500 ml of 80% ethanol extractant was collected after 48 h. Extractant was concentrated in vacuo at 35^0C by using a rotary evaporator and made it to the volume of 100 ml. The P^H was adjusted to 2.5 with 1 N HCl. Polyvinyl pyrrolidone (PVP) 5 g was added with the sample and filtrated. The 30 ml ethyle.acetate was added with the sample. The samples were filtrated with Dowex 50 W X 4 (Mesh) and washed with 100 ml of 80% ethanol. The samples were diluted with 200 ml 5N NH_4OH and cytokinin was separated. Finally the samples were dried in vacuo by Rotary evaporato and 5 ml of 35% ethanol was added with samples and kept in the freezer.

Cytokinin samples were purified by using Column Chromatograpy SephadexLH_2O (2.5 cm x 90 cm) and impurities were separated. The 40 ml of cytokinin samples were taken and repeated 32 times. Samples were dried in vacuo by Rotary evaporato and mixed with 5 ml water and standards of kinetin were prepared at 0, 0.01, 0.05, 0.1 and 0.5 ml concentration.

Soybean callus bioassay preparation by micropropagation

Culture media were prepared by using Miller (1967) Media. First of all macro and micronutrients were measured and put into the cylinder. The α- NAA and kinetin were put into the cylinder and poured macro and micronutrient solutions. Then vitamins were added. The media were adjusted with KOH to 5.8 prior to use. All solutions were heated for 30 min. with electric heater and stirred. They were autoclaved at 15 lb/in at 121^oC for 15 min.

Soybean seeds were obtained from National Institute of Agrobiological Sciences, Ibaraki, Japan. Soybean seeds were collected and washed with tap water followed by rinsing with distilled water in the clean bench. Soybean seeds were surface sterilized for one min. with 70% ethanol, soaked in 1% sodium hypochloride plus a drop of liquid detergent. Then seeds were shaken gently for 20 min. followed by 10 times rinse with distilled water. Seeds were put into culture tube containing 10 ml Miller medium. Culture tubes were placed in a growth chamber under the following conditions: 27^oC, in darkness, RH 70-80%. After 15 days calli were grown.

Culture media were prepared 2^{nd} time as described above. In this step, extracted plant cytokinin from different treatments was used as growth regulator and kinetin was used as standard. Grown calli were cut into different pieces and cultured in different replication of treatments and standard of kinetin. The calli were weighed after 2 weeks of growth. This weight showed the grade of cytokinin conent in different treatments.

RESULTS AND DISCUSSION

The effect of partial and complete ringing on trunk diameter after treatments application was shown in figure 1. In control trees, there was no difference between above and below ringing of trunk diameter. Finally it was higher above ring and lower below the ring in PR and CR than control trees (Table 1). There was a difference in diameter between above and below ringing in case of PR and CR. Trunk diameter was higher in CR trees above ring than in PR trees above ring. However, it was lower in CR trees lower ring than in PR trees lower ring. Cytokinin content represented by callus weight was higher in control trees than partial and complete ringing (Fig. 2). There was a significantly difference of cytokinin represented by callus weight between complete ringing (PR) and partial ringing (PR) as well as control. The lowest callus weight was found in complete ringing trees.

The results showed that trunk growth (diameter) was higher above the ring than below the ring possibly due to bark ringing. It was effective as a dwarfing technique in young peach trees by stress phenomenon using bark (phloem) ringing as a result of producing more ABA content and less cytokinin content after blocking translocation of photosynthates from leaves to roots. Arakawa *et al*. (1997) also reported similar result. They mentioned that trunk growth above girdling was significantly increased and below girdling was reduced in apple trees. Hossain *et al*. (2006, 2007) reported similar

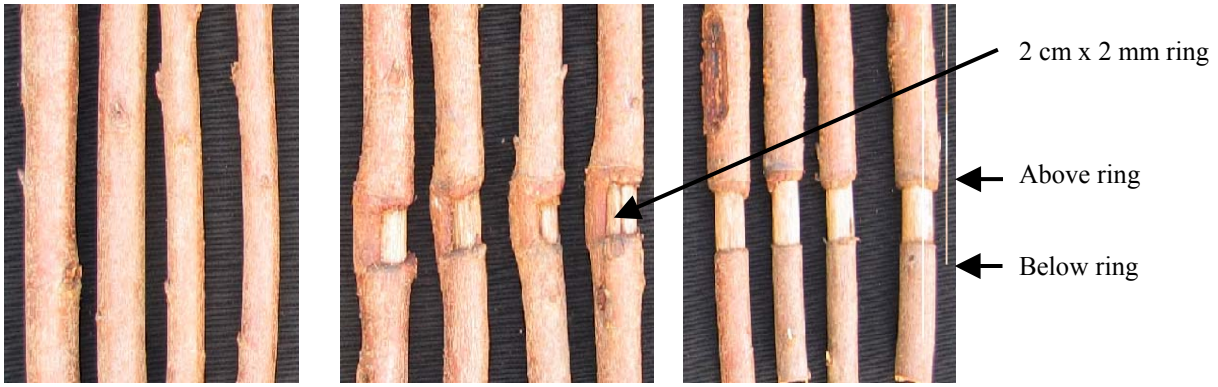

Fig. 1. Photo shows ringing structure and trunkdiameter of peach trees as affected by partial and complete ringing (PR and CR).

result. They stated that trunk circumference was higher above the ring than below the ring in peach trees. Onguso *et al*. (2004) reported that sugar and starch contents were higher above the ringing than below the ringing. The block decreased starch content in root system and accumulated of sucrose in the leaves (Schneider, 1969; Plaut and Reinhold, 1967).

Table 1. The nutrient media (Miller, 1967) were used in soybean callus induction for cytokinin analysis.

Compound	Concentration	
Macronutrients:		
Ammonium nitrate (NH_4NO_3)	1000	(mg/l)
Potassium nitrate (KNO_3)	1000	100
Calcium nitrate {$Ca(NO_3)_2.4H_2O$}	500	-
Magnesium sulphate ($MgSO_4.7H_2O$)	71.5	-
Potassium phosphate (KH_2PO_4)	300	-
Potassium chloride (KCl)	65	-
Na2-EDTA	37.3	-
Iron sulphate ($FeSO_4.7H_2O$)	27.8	-
*From macronutrients 100ml/l was used		
Micronutrients:	-	-
Boric acid (HBO_3)	1.60	(mg/l)
Manganese sulphate ($MnSO_4.4H_2O$)	14.00	-
Zinc sulphate ($ZnSO_4.4H_2O$)	3.80	-
Potassium iodide (KI)	0.75	-
Ammonium molybdate {$(NH_4)_6Mo_7O_{24}.4H_2O$}	0.10	-
Copper nitrate {$Cu(NO_3)_2.3H_2O$}	0.35	-
*From micronutrients 1ml/l was used.		
Vitamins and plant growth regulators:		
Sucrose	30.0	(g/l)
Agar	10.0	-
α-NAA	2.0	(mg/l)
Kinetin	0.50	-
Myo-inositol	100	-
Nicotinic acid	0.5	-
Pyridoxin.HCl	0.2	-
Thiamine.HCl	0.2	-

Table 2. Trunk diameter of peach trees as affected by partial ringing (PR) and complete ringing.

Treatment	Trunk diameter (mm)			
	Initial	Above ring	Below ring	Ratio (A/B)
Control (no ring)	31.0a	32.0b	32.5a	1.01b
Partial ringing	30.5a	34.0a	33.0a	1.03b
Complete ringing	30.5a	34.5a	31.5b	1.09a

Mean in column followed by the same letter is not statistically significant at the 5 % level by DMRT.

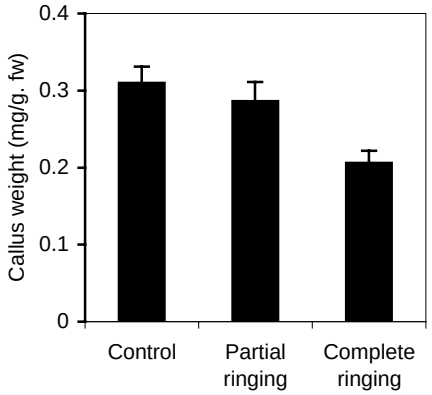

Fig. 2. Cytokinin content in peach root represented by callus weight in different treatments. Bars represented SE (n = 4). Mean in column followed by the same letter is not statistically significant at the 5 % level by DMRT.

Mullins (1967) stated that cytokinin successfully stimulated for root growth of young grapes. Antognozzi *et al*. (1993) reported that cytokinin activating compound N_1-(2-chloro-4pyridyl)-N_3-phenylurea (CPPU) increased the transverse diameter, size and fresh weight of olives. Park *et al*. (1997) observed that stem growth of kinetin treated persimmon trees was higher than control trees. Cytokinin and other plant growth hormones stimulated cell division (cytokinesis) and influenced the pathway of differentiation by stimulating RNA and protein synthesis

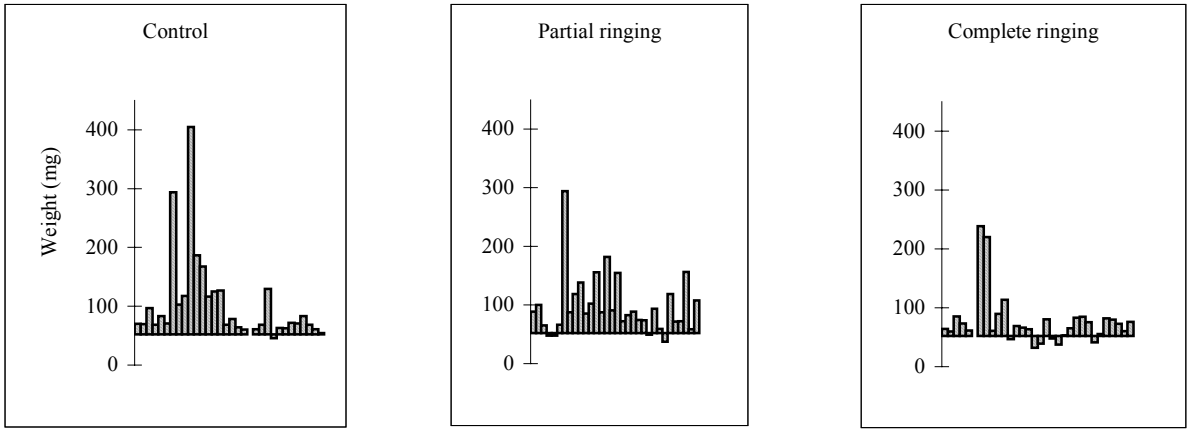

Fig. 3. Callus weight separately of different treatments at 32 times replicated by cytokinin samples.

(Khamis and Holubowicz, 1978). They might accumulate in leaves and cause the stomata to close, reducing transpiration and preventing further water loss. This way they may affect the tree physiological process.

In case of PR and CR trees, sucrose accumulated in the leaves and caused stomata closure resulting ABA increased and cytokinin decreased in leaves, shoots and twigs then it became growth inhibitory effect. A Sudden increase of endogenous ABA has been demonstrated also for several stress phenomena (Wright and Hiron, 1969).

Hossain (2006) found that ABA was higher in PR and CR trees than control. This might be due to more stress (partial ringing) or blocking (complete ringing) of translocation of photosynthates (sugar and starch) from leaves to roots by making restriction of bark ringing in the trunk. In this study, it was found that cytokinin content was lower in PR and CR than in control trees. This might be due to more stress induced by partial ringing (PR) or blocked by complete ringing (CR) of photosynthates translocated from leaves to roots.

CONCLUSION

Our results conclude that PR produced less cytokinin content than trees that have not been ringed (control) by causing nutrient and water stress which can inhibit the trunk growth. As a result, dwarfing is exhibited by whole peach tree. This result can also be effective for other fruit species.

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