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Seasonal Change of Growth Regulator Activity in *Panax ginseng* Root*

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人蔘根中 生育調節劑의 季節變化

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Abstract

Activity of endogenous growth regulator in 4th year *Panax ginseng* root was investigated by second leaf sheath test of rice seedling and paper chromatogram of a acidic fraction of methanol extract before (March 28) and after (May 9) emergence of root bud, at the late season (Sept. 4) and after leaf fall (November 11). GA_3 and ABA were used as reference. According to paper and high performance liquid chromatography of samples and authentic growth regulators the presence of indole acetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (cis and trans ABA) was confirmed. These three regulators appeared to consist of major system though the existence of other regulators could not be ruled out. IAA activity seemed little changed through out the seasons. GA activity decreased in the later stages while ABA activity increased.

Introduction

Panax ginseng , the representative medicinal herb in the Orient, is a perennial root commonly grown for six years to harvest. Ginseng plants not only grow slowly but also have dormancy of root bud for overwintering and of seed. Some ginseng roots are sleeping for many years under unfavorable environments. Rapid growing method of ginseng will be very worthwhile and most possible way seems to use growth

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regulators. Growth regulator studies on ginseng root were rare[1-3] and did not succeed probably due to experimental difficulty and relatively weak response. These studies were application of growth regulators. For the growth control of ginseng the understanding of endogenous growth regulator system seems to be important. Studies on eddogenous growth regulators on *P. ginseng* were on seed, fruit[4-7], levaves[7] and hardly found on root. In this study the endogenous growth regulator activity of P. ginseng root was investigated by bioassay at four growth stages.

Materials and Methods

Plant materials. The fourth year ginseng root were taken from the experimental station field at four growth stages before (March 28) and after (May 9) emergence of root bud, at late stage (Sept. 4) and after leaf fall (November 11).

Extraction. The endogenous growth regulator extraction was carried according to Oritani and Yoshida[8]. Fresh root (50-100g) was ground with 80% methanol with Waring blender and extracted for 24 hrs, at 4°C, filtered with Toyo No.6 filter paper. Extraction was two times and pooled. Pigments were eliminated with active charcoal. The extracts were evaporated to 500ml in vacuo below 40°C, adjusted to pH 2.5 with 2N HCl and extracted with ethyl acetate three times. The pooled ethyl acetate layer was washed twice with saturated NaCl solution extracted with 5% sodium bicarbonate solution three times. The sodium bicarbonate solution was adjusted to pH 2.5 with 2N HCl and extracted three times with ethyl acetate. The pooled ethyl acetate was washed with saturated NaCl solution, dehydrated with anhydrous Na_2SO_4, evaporated in vacuo to dryness. The residue (acidic fraction) was dissolved with 2ml absolute methanol and stored at −10°C until use for bioassay. This acidic fraction (200ml) was chromatographed on Whatman No. 1 paper (20 x 25cm) by streaking with solvent system of isopropanol, aqueous ammonia and water (10:1:1 v/v). Paper was cut into 10 strips each eqivalent to 0.1 of Rf, put into a glass vial (dia. 2.5cm, height 4cm) and added with 2ml of distil water.

Bioassay and HPLC. This aqueous extracts were used for bioassay[8,9] by the elongation of second leaf sheath of rice seedling (IR 667, early Tongil) grown for 5 days in a 20-25°C growth chamber after 3 days immersion at 30°C under dark. Standard GA_3 and ABA (sigma) were chromatographed on paper in the above method. Abscisic acid and IAA in sample was identified by high performance liquid chromatography[10,11] with authentic reagents (sigma). HPLC condition for ABA and IAA; Waters Associate Model 240, Column: Bondapak C18, Solvent: 45% MeOH in 0.2N HOAc, Flow rate: 2ml/min, Sensitivity 0.005, Chart speed: cm/min, Detector: UV 254μm.

Results and Discussion

Calibration curve of 2nd rice leaf sheath bioassay for GA_3 and ABA was shown in Fig. 1 A and B. According to paper chromatogram and bioassay authentic GA_3 and ABA were mostly found in Rf 1.6-0.7 and Rf 0.8-0.9 region, respectively. Seasonal change of regulator activity of GA_3 and ABA regions were shown in Fig. 2. The GA activity decreased with growth and did not appeared after leaf fall while ABA activity did not appeared around emergence and increased after the later stage of growth. Growth retarding activity was stronger at Rf 0.9-0.1 region than ABA region (Rf 0.8-0.9) in all season and in Rf 0.7-0.8 region after the late stage of growth as shown in Fig. 3. There were also growth retarding activity in Rf 0.01-0.1 in most stages (Fig. 3). This result is similar to that of seed in the similar method in which growht retarding activity appeared in Rf 0.0-0.2, 0.6-0.7 and 1.0[6] Standard ABA in that case appeared only in Rf 0.6-0.7.

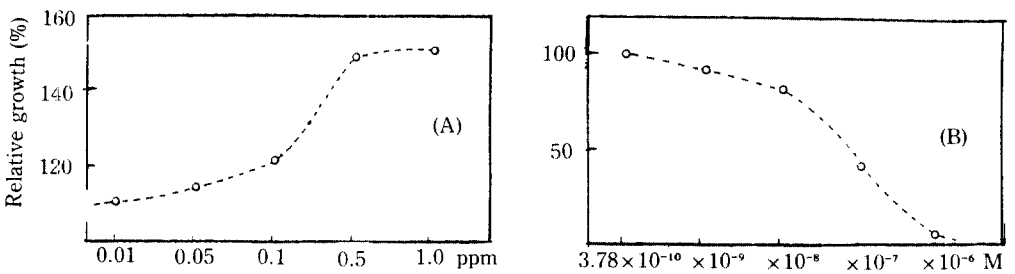

Fig. 1. Calibration curve of GA_3 (A) and ABA (B) for bioassay (rice 2nd leaf sheath test).

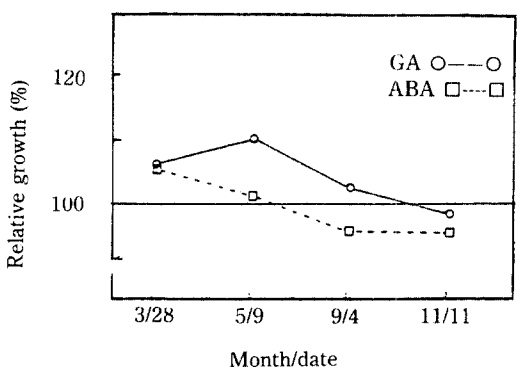

Fig. 2. Seasonal change of GA and ABA activity in bioassay.

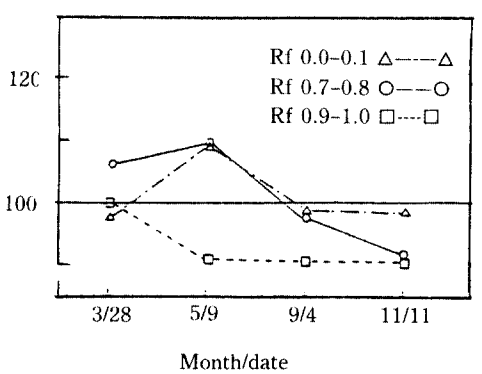

Fig. 3. Seasonal change of growth retarding activity in bioassay.

According to HPLC profile of Rf 0.8-0.9 of sample and cochromatogram with cis-ABA (Fig. 4), and comparion of other's result[10] active substance of Rf 0.8-0.9 could be mostly trans-ABA and partly cis-ABA. Kim and Chang[6] also identified ABA in ginseng seeds by gas chromatography. Other retarding substance in other region is remained unidentified. Identification of strong one of Rf 0.9-1.0 will be especially interesting and important. The fact that growth inhibitors increased continuously in the leaves after flowering[7] may suggest the translocation of inhibitors from the leaves to root in the later stage.

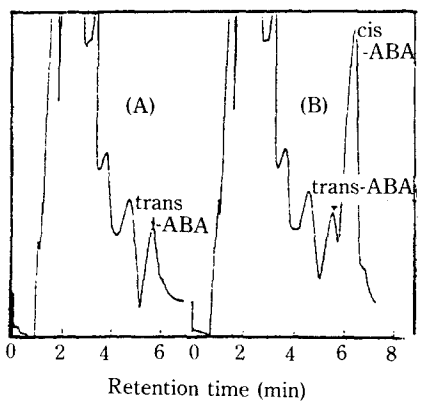

Fig. 4. HPLC profile of Rf 0.8-0.9 fraction (A) and cochromatogram of authentic ABA (B) in *P. ginseng* root.

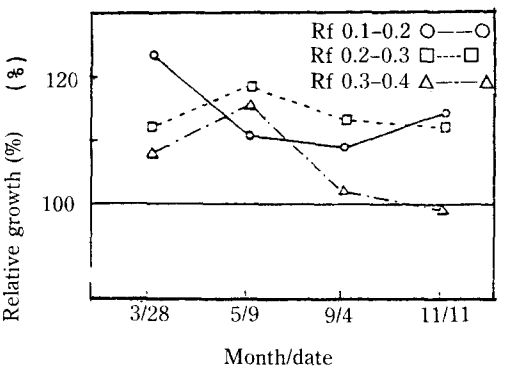

Fig. 5. Growth promoting activity in bioassay of *P. ginseng* root.

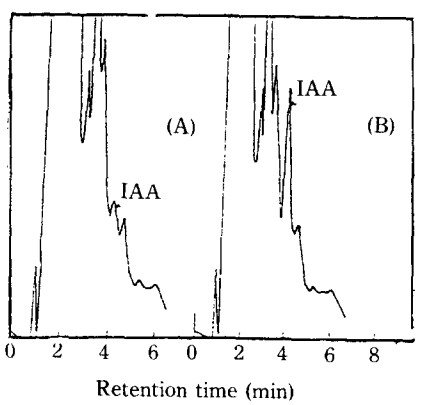

Fig. 6. HPLC profile of Rf 0.2-0.3 fraction (A) cochromatogram of authentic IAA (B) in *P. ginseng* root.

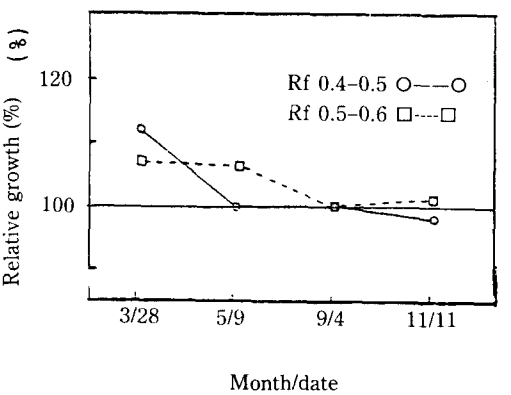

Fig. 7. Growth regulator activity of Rf 0.4-0.6 region in bioassay of *P. ginseng* root.

Growth promoting activity was greater in Rf 0.1-0.4 than that of GA_3 region (Rf 0.6-0.7) as shown in Fig. 5. Growth promoting activity of Rf 0.1-0.3 did not much change during growth but that of Rf 0.3-0.4 decreased greatly in later stages. The presence of IAA in ginseng root was confirmed by HPLC profile of Rf 0.2-0.3 fraction and cochromatogram with authentic IAA (Fig. 6). Hwang and Kim[5] found growth promoting activity in Rf 0.1-0.4 and Rf 0.8-0.9 in ginseng seed in the similar method, and standard IAA activity appeared only in Rf 0.3-0.4 region. They identified IAA in the same region of sample by ga chromatogram. From the above facts it is well postulated that IAA exists in Rf 0.1-0.4 region. The region of Rf 0.4-0.6 was weakest in activity response (Fig. 7) suggesting marginal area though activity on March 28 of Rf 0.4-0.5 was considerablly high. Generally it could be concluded that there are three major regions, IAA (Rf 0.1-0.4), GA (Rf 0.6-0.8) and ABA (0.8-0.1) and the other regions are marginal area, and thus three growth regulators consist of key system of growth regulation. However posibility of the existence of other growth regulators could not be ruled out. Though IAA region seems to be more active than GA region in rice sheath test it may not true to ginseng plant. Grand sum of growth regulator activity was shown in Table 1. Growth promoting activity was greater in the early growth stage while growth retardation appeared to be more meaningful in relation to growth activity of whole plant.

Table 1. Seasonal change of total growth regulator activity (%) in bioassay of *P. ginseng* root

	Mar. 28	May. 9	Sept. 4	Nov. 11
Promotion(P)	98.6	100.9	33.1	35.0
Retardation(R)	3.6	11.3	23.8	38.1
P/R	27.2	9.0	1.39	0.92

要 約

生育時間制(出系前後3월28일과 5월9일, 생육후기 9월4일 및 낙엽후 11월11일)로 4년근 인삼뿌리의 內生 生育調節制 活性을 메타놀 추출 산성획분의 종이크로마토그람과 벼묘의 제이 엽신검정법으로 조사하였다. Gibberellic acid(GA_3)와 Abscisic acid(ABA)표준품을 사용하여 종이 크로마토그라피나 고성능 액체크로마토그라피(HPLC)로 인돌초산(IAA), GA_3 및 ABA(cis 및 trans)의 존재를 알 수 있었다. 이들 세가지 생육조절제가 주역할을 하는 것으로 나타났으나 다른 생육조절제의 존재를 배제하지는 아니하였다. IAA 활성은 생육기간 별로 변하지 않는 것으로 나타났으나 GA 활성은 후기로 갈수록 감소하고 ABA 활성은 증가하였다.

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