

Analysis of Endogenous Gibberellic Acid, Abscisic Acid and Ethylene in Cucumber Seed (*Cucumis sativus* L.)

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ABSTRACT

Endogenous GA, ABA and ethylene could be detected in cucumber seed. Cucumber seed lot No. 06 presented high endogenous GAs especially the derivative GA₁, GA₃, GA₉, GA₁₂ and GA₁₅, and seed lots No. 04 obtained high level of GAs, ABA, ACC and ethylene, while seed lot No. 07 contained low GAs, high ABA and ethylene. The exogenous GA₃ could overcome the dormancy problem of cucumber seed with the rate of success depending on the initial quality of seed.

Keywords: gibberellic acid, abscisic acid, ethylene, *Cucumis sativus* L. endogenous dormancy

INTRODUCTION

Endogenous dormancy is commonly found in seeds of many plant species. They be affected by rudimentary embryo, immaturation, unbalance of hormones or environmental changes. The concept of hormone balance theory supposes that induction, maintenance and release of seed dormancy are regulated by simultaneous action of promotor and inhibitor. Khan (1971) concluded that gibberellin must be present for gemination occurrence but abscisic acid could inhibit this process, meanwhile cytokinin, has been reported to allowed GA to induce germination of seed inhibited by exogenous ABA, whereas GA alone are unable to do so.

Generally during seed germination, gibberellic acid (GA₃) is present in the seed. The presence of ABA can directly prevent the translation from mRNA in protein synthesis during the early stage of germination (Ho and Verner, 1976). Jacobsen (1973) reported that GA induced α -amylase synthesis of barley aleurone layer which was inhibited by ABA

and ABA activity is partly removed by the additional of either GA or ethylene.

Ethylene mechanism is known to stimulate seed germination of some species. Peanut seed produced ethylene during germination while Virginia-type seed produced very little during imbibition stage and seed could not germination (Ketring and Morgan, 1969). Many literatures identified 1-amino-cyclopropane-1-carboxylic acid (ACC) occurred during ethylene biosynthesis in plant tissue.

It has been known that ethylene would stimulate germination of certain species of seed such as peanut (Ketring and Morgan, 1969). Many reports also showed that dormant seeds have a lower capacity for ethylene production than non-dormant after ripened-seed. (Yang and Hoffman, 1984). Nevertheless, high temperature and light also involved in increasing the sensitivity of ethylene but only for a period of days. (Yang and Hoffman, 1984; Wang and Adams, 1982).

Bioassay is necessary when the particular GAs and cytokinin content of plant materials were studied. Among the various bioassay for GAs, the micro-drop

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method using GA-deficient mutant of dwarf rice cv. Tan-ginbozu is the most useful method with high degree of sensitivity to a broad spectrum of GAs (Murakami, 1968).

The objective of this study was to analyse the endogenous GAs, ABA and ethylene level of cucumber seed, to determine how endogenous hormones related to germination and dormancy. The response of exogenous GA₃ treatment of germination was observed.

MATERIALS AND METOHDS

1. Four seed lots (No. 04, 06, 07 and 09) of

Thai cucumber cv. Pol-lek were treated with and without exogenous GA₃ 100 ppm to determine the degree of seed dormancy. Germination rate was obtained at 8 days after germination (ISTA, 1985).

2. Extraction, purification and bioassay analysis of endogenous gibberellic acid of cucumber seed.

- 2.1 The flowchart of gibberellic acid extraction and purification procedure was shown in Fig. 1. The extracted sample was separated with ethylacetate by adjusting pH to 2-2.5 having phosphate buffer interval. Purification with PVP and Sep-pak cartridge were necessary as well with HPLC efficient. The HPLC column was Develosil ODS C₅, 1.0 x 15 cm

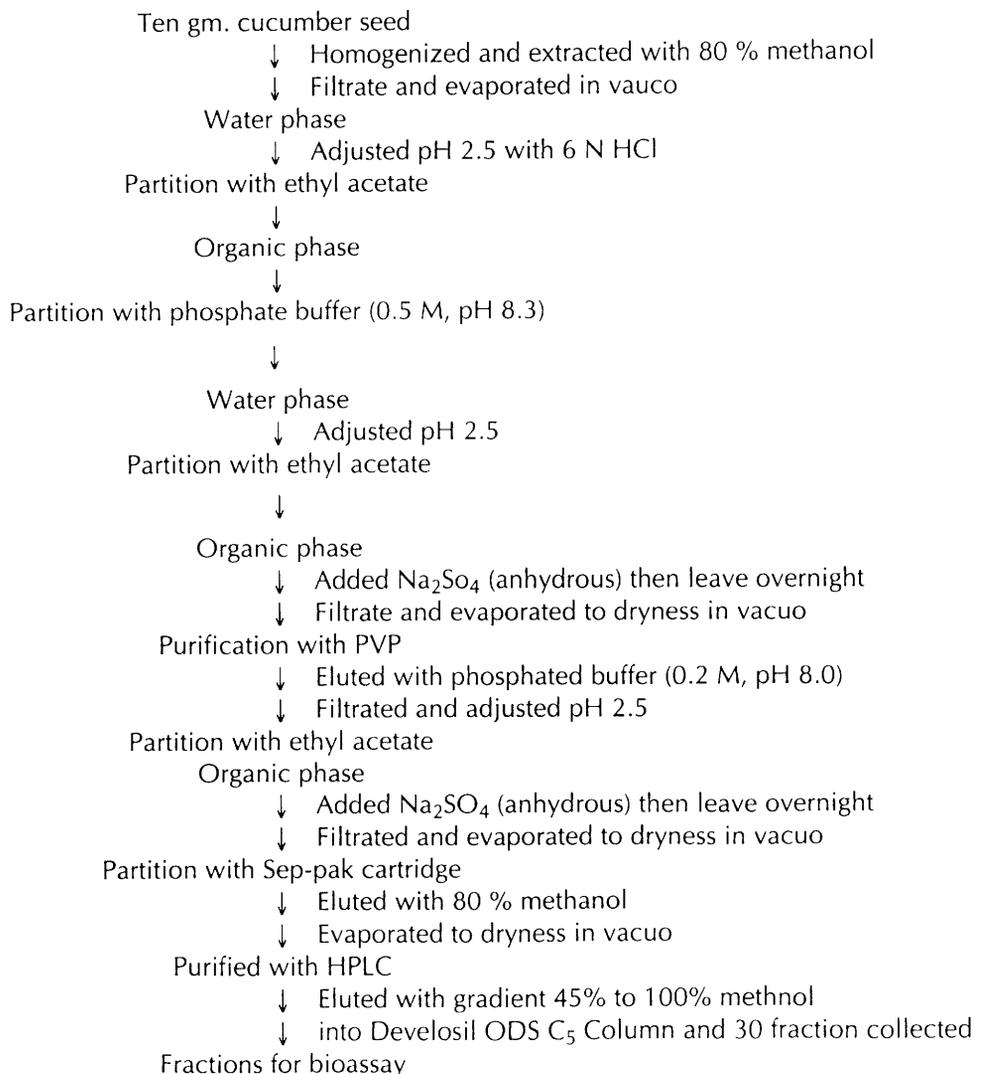


Figure 1 A flowchart of extraction and purification procedures for GAs analysis in cucumber seed.

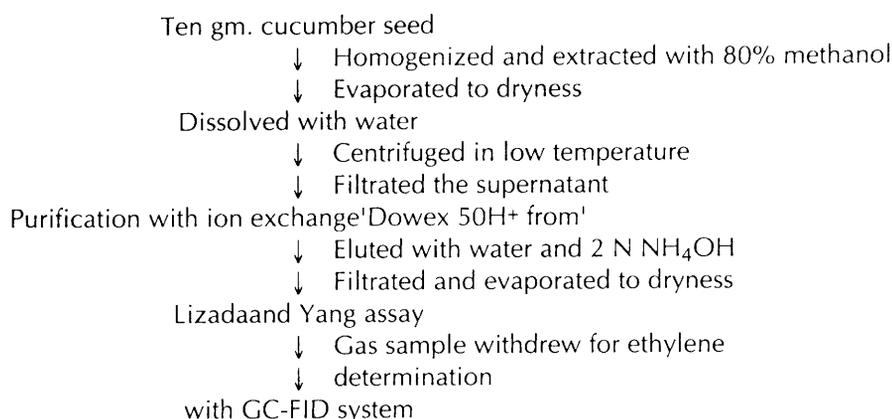


Figure 2 A flowchart of extraction, purification and determination for ethylene analysis in cucumber seed.

with UV. detector adsorbance 206 nm and automatically linear gradient solvent from 45% to 100% (V/V) methanol (contained 0.1% acetic acid). Thirty fractions were collected from the column eluted for further bioassay procedure.

2.2 Modified micro drop method was used with dwarf rice (Nishijima and Katsura, 1989) for GAs activity analysis from each fraction collected.

3. Extraction, purification, methylation and GC analysis of endogenous ABA of cucumber seed.

3.1 The extraction and purification of ABA procedures were the same as GAs analysis.

3.2 Methylation procedure was modified by passing N₂ gas 6 ml.min flow rate to acetone solution. Ethyl cabitol and 60% KOH and mixture solution of acetone : methanol (9:1) dissolved sample were extracted. The N-methyl-N-nitroso-P-toluenesulfonamide (MNSA) solution was injected into the fracible teflon tube while gas passing through. The monitor period was 5-6 min, until sample solution turned yellow to transparent.

3.3 Gas chromatograph - ECD was used for detecting ABA with Silicon AE 18 column resin, 210-220°C and N₂ carrier gas efficient.

4. Extraction purification and GC analysis of endogenous ethylene and ACC from cucumber seed were monitored by the method of Prof. Masaharu Mazuda Okayama University and followed by assay procedure of Lizada and Yang (1979) (Fig. 2). Ethylene was identified with Porapak Q column, H₂ carrier gas at 70-80°C with GC-FID system. The ACC activ-

ity was calculated using the $Y = 0.0903464 + 1.0687115X$.

RESULTS AND DISCUSSION

The application of 100 ppm GA₃ to cucumber seed lots No. 04, 06, 07 and 09 showed that, the germination percentages were increased only in seed lot No. 04, 07 and 09 (Table 1). These evidence was very clear because of the non-treated seeds obtaining very high percentages of abnormal seedlings particularly in seed lot No. 09. However it is assumed that GA₃ helped promoting seed germination (Don, 1979) because those of abnormal seedlings were deformed types which could be germinated later. In the case of seed lot No. 09 which was kept under room conditions for 3 years, the seed quality was quite low GA₃ could only stimulate in some germination process. However, the response was very low due to the dryness and age of seed inresponsive to exogenous GA₃. The seed lot No. 06 which kept under cold condition for 1 year, was still in high quality and showed highest germination and abnormal seedling was not found (Table 1). The endogenous GAs of each fraction was estimated by measuring the leaf sheath length of Tan-ginbozu rice seedling. Generally, the amount of GA₃ and GA₇ are classified as very high activity; GA₁ and GA₄ are higher than GA₉ and GA₂₀ which are non-active group (Grabe and Ropers, 1978; Audus, 1972). As shown in Table 2, the high concentrations of GA₁ and GA₃ were obtained in seed lot No. 04, 06 and 09 but the lowest

Table 1 Germination of treated and non-treated GA₃ cucumber seed.

Seed lot No.	Normal seedling (%)		Abnormal seedling (%)	
	Non-GA ₃	GA ₃	Non-GA ₃	GA ₃
04	58	84	17	2
06	100	100	0	0
07	71	97	20	1
09	33	56	35	18

Table 2 Qualitative grouping of GA-like substances form four cucumber seed lots.

Seed lot	GA ₁ GA ₃ (fr 3-4)	GA ₇₇ (fr 6)	GA ₂₀ (fr 11-13)	GA ₅₃ (fr 17)	GA ₁₉ (fr 18-19)	GA ₇ GA ₄ (fr 20-21)	GA ₂₄ GA ₂₅ (fr 22-25)	GA ₉ GA ₁₅ GA ₁₂ (fr 26-28)	GAs (all fr)
04	0.12-7.8 ^a 111.6-230.2 ^b	0.12 109.30	- -	0.42 134.9	- -	- -	- -	0.18 114.00	8.63 -
06	0.81-6.0 174.4-200.0	0.06 104.00	0.06 104.70	- -	- -	- -	0.06 107.00	0.30 125.60	7.32 -
07	0.54-1.02 144.2-183.7	1.78 172.10	0.30 123.30	0.12 109.30	- -	0.12-1.18 109.3-116.3	0.12-0.42 111.6-134.9	0.12-0.42 116-134.9	3.96 -
09	0.54-6.30 144.2-204.7	- -	0.12-0.18 109.3-114.0	0.12 104.30	0.06 104.70	- -	- -	0.18 118.60	7.58 -

a = The concentrations of GA like substances were detected (ng GA₃ equiv./g dry wt.)

b = Percentage of total GA like substances were based on control, in terms of the second leaf sheath elongation in Tan-ginbozu rice seedling

Table 3 The quantity of *cis* and *trans* methylated ABA analysis in cucumber seed extract by GC-ECD.

Seed lot No.	ABA concentration (µg/g dry wt.)	
	<i>cis</i> form	<i>trans</i> form
04	0.10	0.296
06	0.03	*
07	0.203	0.0074
09	*	0.0071

* Could not be obtained

Table 4 The quantity of ethylene and ACC determination of cucumber seed extracted by GC-FID.

Seed lot No.	ethylene (ppm)	ACC (n mol/g)
04	0.325	19.184
06	0.133	7.399
07	0.818	51.472
09	*	*

* Could not be obtained

germination was in seed lot No. 09 (Table 1). This result showed no relation between GAs and seed dormancy, However the endogenous ABA contents in the seed lot No. 04 and 07 (Table 3) were higher than the other seed lots which might have supported Khan's theory of playing as an inhibitor in seed germination (Khan, 1982). Therefore the dormancy of cucumber seed could be considered as; the presence of inhibitor (ABA), the unbalance of ABA and GAs (Jacobson, 1973) or low seed vigor (Delouche, 1980) etc. On the other hand, the purification procedure might be partially incompleated and the qualified period of the methylation technique of the sample extracted, were not enough which resulted in unclear results. However, dried samples after methylation procedured before gas chromatograph detecting are recommended (Groot *et al.*, 1991).

The endogenous ethylene concentration of cucumber seed was also detected, maximum ACC (51.472 nmol/g) or ethylene (0.818 ppm) concentration was obtained in seed lot No. 07. (Table 4). Again, the result followed the same pattern as GAs and ABA (Table 1, 2, 3) which hardly revealed that ethylene could promote the germination or overcome dormancy problem since all the seed lots were not deep dormant but slow in germination process (Jacobson, 1973).

Although the result did not really support Khan's theory, it could be concluded that ethylene, together with exogenous GA₃ did stimulate germination of cucumber seed lot No. 04 and 07. Both seed lots presented high concentration of all plant hormones but unbalanced, the exogenous GA₃ was possible to make balance.

In this case, ethylene played a permissive role, in balancing with promotor (GA) while ABA content was presented. (Jacobson, 1973). Ethylene was also overcome ABA inhibition of seed (Ketring and Morgan, 1971).

CONCLUSION

The endogenous GAs with derivative GA₁, GA₃, GA₉, GA₁₂ and GA₁₅ were detected in cucumber seed which showed no sign of dormancy (seed lot No.06). High level of ABA was also determined in seed being show in germination (seed lot No.07). Seed with high germination percentages (seed lot No.06) exhibited low level of both ABA and ethylene.

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