STUDIES ON THE PLANT GROWTH REGULATING EFFECT OF PHTHALAMIC ACID AND ITS DERIVATIVES

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Phthalamic acid 및 이의 誘導體의 植物生長調整作用에 미치는 영향에 關한 硏究

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摘 要

植物 生長 調節劑로서 phthalamic acid와 그 誘導體를 使用하여 秋落畓을 改良하기 爲한 豫備 試驗을 實施하여 다음과 같을 結果를 얻었다.

1) 아래 7種의 phthalamic acid 및 그 誘導體를 合成 또는 精製하여 供試藥劑로 하였으며 合成品의 收率은 87~92% 였다. (表 1)

A. Phthalamic acid

B. Phthalanilic acid

C. N-(o-Chlorophenly)-phthalamic acid

D. N-(*p*-Chlorophenyl)-phthalamic acid

E. N-(3, 4-Dichlorophenyl)-phthalamic acid

F. Sodium-N-(*m*-tolyl)-phthalamate (Duraset)

G. Sodium-N-(1-naphthyl)-phthalamate (Alanap)

2) Wheat straight growth test에서 供試藥劑는 10 ppm 및 20 ppm의 濃度에서 Control보다 越等한 生長 促進効果를 보였으며 特히 興味있는 것은 phthalamic acid誘導體가 phthalamic acid自體보다 더 좋은 効果를 보였다. (表 4. 그림 1)

3) 水稻 種子의 發芽 試驗 結果

草長은 phthalamic acid誘導體가 亦是 phthalamic acid 自體보다 좋았으며 其中 Alanap. 3, 4-dichlorophenyl phthalamic acid 및 Duraset가 가장 좋았으나 10~100 ppm 範圍內의 濃度에 거의 影響없었다.(表 5)

根의 生育은 一般的으로 藥劑의 種類에 따라 相異할 뿐 아니라 草長의 生育보다 더욱 藥劑에 敏感하였다. 根長은 phthalamic acid, phthalanilic acid, (p-chlorophenyl)phthalamic acid, 3, 4-dichlorophenyl phthalamic acid가 Control보다 약간 좋은 効果를 보였고 Alanap은 매우 不良하였다.(表 6)

根數는 (p-chlorohhenyl) phthalamic acid만 Control에 比해 優秀하였고 나머지 藥劑는 大體로 根數를 減少시키거나 Control과 비슷한 効果를 보였다.(表 7)

또한 濃度가 增加함에 따라 根數는 減少하였으며 Alanap, (o-chlorophenyl) phthalamic acid는 根端에 甚한 藥害가 일어났다.

4) 剪根試驗에서 根數는 供試藥劑에 依하여 增加되었으나, 50~200 ppm의 濃度에서 별 影響이 없었다.(表 8, 그림 2)

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根長은 濃度가 增加함에 따라 減少되는 傾向이었으나 供試藥劑에는 相異한 効果를 보였다.

5) 以上 結果에서 p-chlorophenyl phthalamic acid 및 3,4-dichlorophenyl phthalamic acid가 水稻用 植物生長調節劑로서 實用的 開發의 可能性을 보았다.

I. INTRODUCTION

Yield increase of some plants by secondary effect of Imidan (phthalimidomethyl-O, O-dimethylphosphorodithioate), an insecticide, has been reported in several countries such as Korea (13) Japan, China, Philippines etc, (7) This effect has been suggested to be attributed not to the phosphate moiety, but to the non-phosphate moiety of Imidan.

Menn et al (9,10) reported that Imidan decomposed to phthalamic acid and other metabolites in plants and animals by ready decarboxylation and hydrolysis,

In our previous studies (8), phthalamic acid showed the highest activity in the wheat straight growth test among Imidan and its possible metabolites.

Meanwhile, Smith et al (6, 15, 16, 23) found that *N*-aryl phthalamic acids and *N*-aryl phthalamides are effective plant growth regulants and phytocides, and their typical growth regulatory responses include the prevention of fruit drop, rooting of cuttings, formation of parthenogenic fruit, and altering leaf shape.

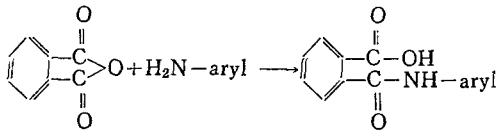
The purpose of this experiment is to improve degraded (Akiochi) paddy field which is characterized by root-decaying phenomena (21) by using phthalamic acid and its derivatives.

II. MATERIALS AND METHODS

1. Synthesis of phthalamic derivatives

A. Methods (2,3,4,14)

The *N*-aryl phthalamic acids were prepared by reacting equal molar amounts of phthalic anhydride and the selected primary monoaryl amine in chloroform at room temperature for about 1 hour according to the following reaction.



The products were recrystallized twice from 95% ethanol and per cent nitrogen of them was analyzed by using Microkjeldahl method. (1)

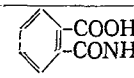
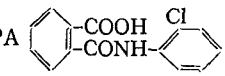
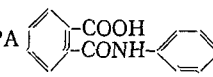
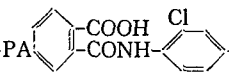
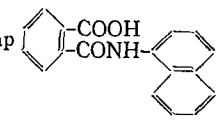
2. The wheat straight growth test

A. Buffer solution; Dissolve 2.6910 g. of potassium phosphate, dibasic and 1.5285 g. of citric acid monohydrate in 1.500m*l* of 2% sucrose solution. (pH 4.6)

Prepare solutions containing 10 ppm and 20 ppm of following chemicals, respectively, with this buffer solution.

B. Test chemicals

Table 1 : Chemicals tested

Chemical Name	Name	Graphic Formula	Source
Phthalamic acid	PA		Kishida Chem Co
Phthalanilic acid	PNA		Synthesized.
N-(*o*-Chlorophenyl) phthalamic acid	*o*-Cl-PA		〃
N-(*p*-Chlorophenyl) phthalamic acid	*p*-Cl-PA		〃
N-(3,4-dichlorophenyl)phthalamic acid	3,4-diCl-PA		〃
Sodium-*N*-(*m*-tolyl) phthalamate	Duraset		Ishihara Ltd.
Sodium-*N*-(1-naphthyl) phthalamate	Alanap		Sankyo Co.

C. Methods;

According to coleoptile straight growth test (5,12), place cylindrical pieces of wheat(Variety; Yungkwang) coleoptile immersed in each prepared solution and measure linear growth length by microcaliper after 20 hours.

3. The germination test of rice seeds

A. Test chemicals;

Control: Prepare 1% ethanol solution and dilute it to the same concentrations as those of test chemicals

Alanap: Dilute 20% Alanap aqueous solution to be 10, 25, 50 and 100 ppm solutions.

Other chemicals: Dissolve 20mg of each chemical in 100 m*l* of 1% ethanol solution to be 200 ppm solution. Dilute and make solutions containing 10, 25, 50 and 100 ppm of each chemical, respectively.

B. Methods;

1) Soak rice seeds (Variety: Jinheung) in 1,000 X solution of Mercron(1.5% PMA) for 6 hours at room temperature, wash three times and again soak them in distilled water for 2 days.

2) Lay 20 seeds in parallel rows, the embryos pointing down in the same direction on two layers of filter papers in Petri dishes previously treated with 5 m*l* of each above sample solution.

3) Grow 20 seeds at 25°C in a incubator in the dark for 8 days.

4) Measure the length of shoot and root, and root number.

4. The rooting test of rice seedling treated with chemicals after root-cutting.

A. Test chemicals;

The same chemicals used in the germination test. Concentrations: 50, 100 and 200 ppm.

B. Semi-irrigated rice nursery method (7, 11, 20)

C. Water culture

1) Container; (22)

400 m*l* brown glass bottle covered with black vinyl paper.

2) Culture solution;

According to Tanaka et al (18) and Tasima et al (19), culture solution was prepared.

Table 2 : Composition of culture solution

Element	Compounds used	Concentration(ppm)
N	NH_4NO_3	40
P_2O_5	$NaH_2PO_4H_2O$	10
K_2O	K_2SO_4	40
CaO	$CaCl_2$	40
MgO	$MgSO_4 \cdot H_2O$	40
Fe_2O_3	$FeCl_3 \cdot 6H_2O$	2
MnO	$MnCl_2$	0.5

3) pH

Adjust pH 5.5-6.0 by 0.1 N NaOH and 0.1 N H_2SO_4 solutions.

D. Methods;

1) Wash and cut off all the visible roots of rice seedlings in the 3 to 4 leaf stage.

2) Transplant in culture solution and support plants with sponge through the center of perforated cork plugs.

3) Spray each seedling with 3 m*l* of each alcoholic solution containing 50, 100 and 200 ppm of a given chemical.

4) Change the culture solutions after 7 days and spray again.

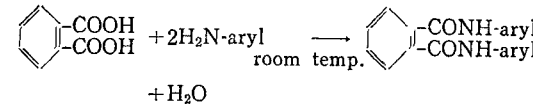
5) Measure the root length and number after 14 days.

III. RESULTS AND DISCUSSION

1. Synthesis of phthalamic acid derivatives.

It has been well known as a good synthetic method to prepare *N*-aryl phthalamic acids from phthalic anhydride and aromatic amines in inert solvent because of its easiness and high yield.

Various inert solvents were reported (3, 5). But chloroform is considered the best one among them as it can not only dissolve more phthalic anhydride than other inert solvents, but also readily remove impure phthalic acid by using its insoluble property in chloroform. Impure phthalic acid combines with monoaryl amine to form *N,N*-diaryl phthalamate as follows:



Reaction temperature should not be raised above 80° C. because the N-aryl phthalamic acids, at hig temperature such as 80 to 180°C. lose water to form the N-aryl phthalamides as follows.

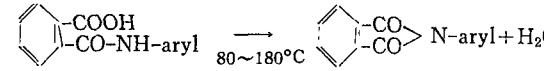


Table 3. The yield, decomposing point and nitrogen content of the treated chemicals.

Name	Yield (%)	Moisture (%)	N% Theoretical	N% Obtained	Decomposing point
PA	—	0.36	8.48	8.08	146-147°C (lit. 147-148°C)
PNA	91.55	0.10	5.81	5.57	169.5-170°C (lit. 170°C)
o-Cl-PA	87.78	0.11	5.08	4.96	145-146°C (lit. 147-148°C)
p-Cl-PA	86.97	0.13	5.08	4.92	180-182°C (lit. 180°C)
3.4-diCl -PA	89.73	0.09	4.52	4.42	195-198°C
Duraset	—	0.25	5.05	4.77	235-238°C

Table 3 indicates that yields of products range from 86.97 to 91.55%, analyzed nitrogen percent of each ethanol-recrystallized chemical is near to theoretical nitrogen percent and their decomoposing points almost coincide with those in previous literature.(2, 3, 4, 14,).

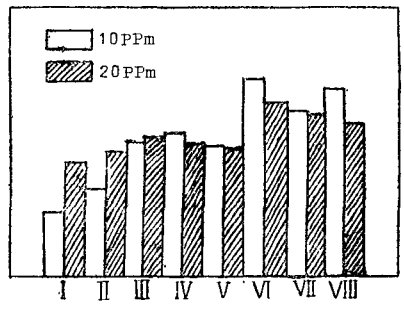
2. The wheat straight growth test

In order to detect and assess plant growth responses induced by treatment with these chemicals the wheat coleopile straight growth test was carried out on excised wheat coleoptile. Linear growth test as laboratory test was carried out on excised wheat coleoptile. Linear growth measurements of cylinederical pieces of wheat coleoptile are shown in Table 4 and Fig. 1.

Table 4. Plant growth regulating effect of phthalamic acid and its derivatives in the wheat straight growth test.

Name	Concentrations 10 ppm mm measure	10 ppm % of control	20 ppm mm measured	20 ppm % of control
I Control	8.01	100	8.01	100
II PA	8.64	107.9	8.33	104.0
III PNA	8.89	108.5	8.73	109.0
IV *o*-Cl-PA	8.90	111.1	8.81	110.0
V *p*-Cl-PA	8.69	108.5	8.83	110.2
VI 3,4-diCl-PA	9.47	118.2	9.22	115.1
VII Duraset	9.20	114.9	9.16	114.4
VIII Alanap	9.55	119.2	9.11	113.7

Fig. 1 : Coparison of wheat coleoptile length



All the chemicals treated show better coleoptile growth response than control and it is interesting that phthalamic acid derivatives are more effective in these responses than phthalamic acid itself which showed the highest growth effect among Imidan and its metabolites in our ealier report(8).

The order of activity is as follows;

Alanap, 3, 4-diCl-PA>Duraset>*o*-Cl-PA > *p*-Cl-PA> PNA>PA>Control.

3. Effect of phthalamic acid and its derivatives on germination of rice seeds.

Table 5, 6 and 7 show growth data of rice seeds germinated on the respective media for 8 days.

On the growth of shoot length, chemicals are highly significant whereas concentrations are not (Table 5).

3, 4-diCl-PA and Alanap show the highest growth responses, o-Cl-PA and Duraset also have considerably high responses, o-Cl-PA and Duraset also have considerably high responses and *p*-Cl-PAndicates a little high effect while PA shows minus significance.

Table 5. Effect of phthalmic acid and its derivatives on shoot length in germination of rice seds.

Chemicals, A	Concentrations, B 10ppm	25ppm	50ppm	100ppm	Mean
Control	3.9	3.3	3.2	3.1	3.4
PA	3.0	3.0	2.9	2.7	2.9
PNA	3.1	3.2	3.3	3.6	3.3
o-Cl-PA	4.3	4.6	4.2	4.7	4.4
p-Cl-PA	3.3	3.7	4.0	4.5	3.9
3, 4-diCl-PA	4.9	4.9	5.4	5.5	5.2
Duraset	4.7	4.7	4.7	4.5	4.7
Alanap	5.5	5.8	5.8	4.8	5.5

Mean	4.1	4.2	4.2	4.2

Figures represent mean values of 3 replication.
A: highly significant
L.S.D.$_{0.05}$=0.34

On the root length, chemicala are as highly effective as they are on the shoot length whereas concentrations show no significance. (Table 6)

Among used chemicals, PA, PNA, *p*-Cl-PA and 3,4-diCl-PA are more significant than control at 5% level. On the other hand, Alanap shows remarkable minus signifibance. *o*-Cl-PA and Duraset haᴬe as high effᵊct as control.

Table 6 : Effect of phalamic acid and its derivatives on root length in germination of rice seed.

Chemicals A	Concentrations, B 10ppm	25ppm	50ppm	100ppm	Mean
Control	5.8	5.8	6.0	5.6	5.8
PA	7.2	6.2	6.3	6.3	6.5
PNA	6.2	7.3	6.4	6.2	6.5
o-Cl-PA	6.3	6.3	6.1	6.0	6.2
p-Cl-PA	5.5	6.3	6.6	6.9	6.3
3.4-diCl-PA	7.0	6.5	6.2	6.4	9.5
Duraset	5.4	6.2	5.6	6.2	5.9
Alanap	4.4	4.5	4.3	4.1	4.3
Mean	6.0	6.1	5.9	5.9	

Figures represent mean values of 3 replication
+: injury
A: highly significant
L.S.D.$_{0.05}$=0.49

Both chemicals and concentrations used are highly significant on the root number.

It has been long known that roots are very sensitive to auxins, but except at very low concentrations, the reponse is one of growth inhibition. (12, 24)

Table 7 contributes to proving the above fact that increased concentrations result in decreased root numbers which mean growth inhibiton.

But root number shows a little deviation from inhibiting effect. For instance. *p*-Cl-PA is more significant than control. PA and 3,4 diCl-PA have as similar effect as control. On the contrary, Alanap and Duraset cause marked inhibiting effect and o-Cl-PA and PNA also show considerable inhibiting effect. Alanap and o-Cl-PA cause severe injury showing chlorosis at the end of root at 100 ppm of each chemical.

Table 7. Effect of phthalamic acid and its derivatives on root number in germination of rice seed.

Chemicals, A	Concentrations, B 10ppm	25ppm	50ppm	100ppm	Mean
Control	4.8	4.4	4.5	4.1	4.4
PA	4.6	3.7	3.8	3.7	4.0
PNA	4.9	4.1	2.7	2.1	3.5
o-Cl-PA	3.9	3.0	3.1	1.5	2.9
p-Cl-PA	5.4	5.7	5.5	5.7	5.6
3,4-diCl-PA	6.9	5.8	5.6	3.4	4.9
Duraset	2.6	2.2	1.0	1.0	1.7
Alanap	1.0	1.0	1.0	1.0	1.0
Mean	4.3	3.7	3.1	2.8	

Figures represent mean values of 3 replication
+: injury
A, B: highly significant
L.S.D.$_{0.05}$=0.05 ……Chemicals(A)
L.S.D.$_{0.05}$=0.04 ……Concentrations(B)

To know the effect of control, a medium only treated with distilled water was prepared. In this test, control showed a little better growth promoting effect than distilled water below 25 ppm. But above 100 ppm, it showed inhibiting effect.

In the germination test, *p*-Cl-PA and 3,4-diCl-PA are the best growth regulants on rice seeds.

4. Effect of phthalamic acid and its derivatives on rooting of rice seedlings

Newly developed root length and number were measured (Table 8, Fig. 2) after 14 days to investigate the capacity of chemilcals to induce rooting responses in cut rice seedings.

The root length of root-excised rice seedlings, in general, has a tendency to be shorter as concentrations increase and it varies considerably among chemicals.

p-Cl-PA and 3,4-diCl-PA indicate remarkable growth promoting effect in the root length below 200 ppm. PA and Alanap also show promoting effect wiinth 50-100 ppm. and their activity decrease above 100 ppm. Duraset is similar to control in activity. But PNA and o-Cl-PA are less active than control.

Concentrations seem to have no close relations with

the root number but all chemicals used increase the root number.

Alanap, o-Cl-PA and Duraset caused injury in the cutting test. This injury included wielding and chlorosis of leaves, and deformation of roots.

Fig. 3 represents typical root injury of Alanap at 100 ppm concentrations.

Out of given chemicals, *p*-Cl-PA and 3,4-diCl-PA are selected as the best growth regulators on rice plant.

Further applicability will be studied to improve degraded paddy field.

Table 8. Effect of phthalamic acid and its derivatives on root growth of root cut rice seedling.

Chemicals	Root Length			Root Number		
	50ppm	100ppm	200ppm	50ppm	100ppm	200ppm
Control	7.8	7.4	7.1	9.3	10.3	9.7
PA	7.7	8.1	6.6	10.7	I2.7	12.7
PNA	7.7	4.3	3.3	11.3	10.3	11.5
o-Cl-PA	8.2	6.0	5.6	13.7	10.0	10.7
p-Cl-PA	8.4	8.2	8.4	11.3	10.0	12.3
3.4-diCl-PA	8.2	8.1	7.6	12.3	11.0	14.3
Duraset	7.7	7.3	7.6	13.3	12.5	12.3
Alanap	8.3	7.6	6.8	11.0	10.7	8.7

Figures: Same as in Table 5.
+: injury

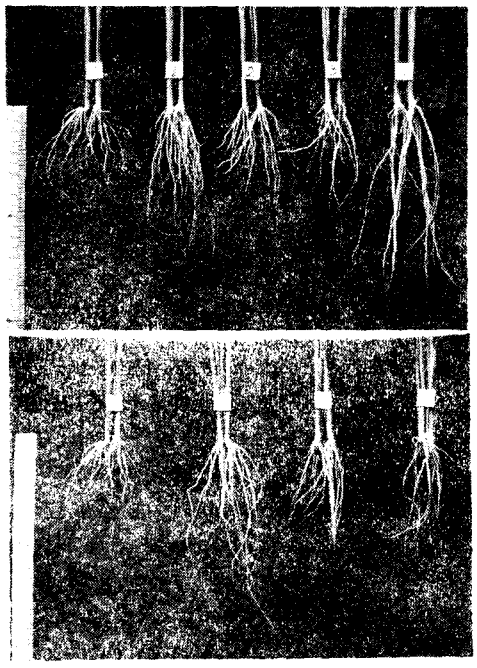


Fig. 2 *Comparison of Root Growth* 14 *days after Root-cutting* (100ppm)

0: Control
1; Phthalamic acid
2; Phthalamilic acid
3; N-(*o*-Chlorophenyl) phthalamic acid
4; N-(*p*-Chlorophenyl) phthalamic acid
5; N-(3,4-Dichlorophenyl) phthalamic acid
6; Sodium-N-(*m*-tolyl) phthalamate (Duraset)
7; Sodium-N-(1-naphthyl) phthalamate (Alanap)

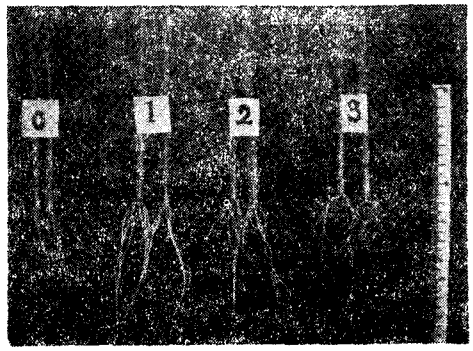


Fig. 3 *Injury caused by Alanap on Root*

0; Control
1; 50 ppm
2; 100 ppm
3; 200 ppm

SUMMARY

This experiment was conducted as a preliminary test

to improve degraded (Akiochi) paddy field by applying phthalamic acid and its derivatives as plant growth regulators

The results are summarized as follows.

1) Following phthalamic acid and its derivatives were synthesized or recrystallized. (Table 1)

A. Phthalamic acid

B. Phthalanilic acid

C. N-(*o*-Chlorophenyl)-phthalamic acid

D. N-(*p*-Chlorophenyl)-phthalamic acid

E. N-(3. 4-Dichlorophenyl)-phthalamic acid

F. Sodium-N-(*m*-tolyl)-phthalamate (Duraset)

G. Sodium-N-(1-naphthyl)-phthalamate (Alanap)

Yield of products ranged from 87 to 92%

2) In the wheat straight growth test, all given chemicals promoted remarkably the growth of wheat coleoptile at concentaarions of 10 ppm. and 20ppm. Especially phthalamic acid derivatives showed better growth effect than phthalamic acid which was the main factor of yield increase among Imidan metabolites. (Table 4, Fig. 1)

3) In the germination test of rice seeds, phthalamic acid derivatives also indicated better growth of shoot length than phthalamic acid itself. Among chemicals used Alanap, N-(3. 4-dichlorophenyl) phthalamic acid and Duraset were the highest ones. Concentrations within 10-100 ppm range didn't affect the shoot length. (Table 5)

In general, root growth varied among used chemicals and was more sensitive than the shoot growth.

The root was a little more elongated in phthalamic acid, phthalanilic acid, N-(*p*-chlorophenyl) phthalamic acid and N-(3. 4-dichlorophenyl) phthlamic acid than in control while it was markedly short in Alanap. (Table 6)

The root number increased only in N-(*p*-chloropheny) phthalamic acid. The remaining chemicals had similar or smaller number of roots than control. (Table 7) Increased concentrations resulted in decreased root numbers. Severe injury at the end of the root was observed in Alanap and N-(o-chlorophenyl) phthalamic acid.

4) In the rooting test, all given chemicals increased the root number while their concentrations had no close relationship with it within 50-200 ppm range. (Table 8, Fig. 2)

The root length tended to be shorter as concentrations increased and it varied considerably among chemicals. Alanap, N-(o-chlorophenyl)phthalamic acid and Duraset caused injury. (Fig. 3)

5) From the above results, N-(*p*-chlorophenyl) phthalamic acid and N-(3, 4-dichlorophenyl) phthalamic acid were selected as promising plant growth regulators on rice plant.

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