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# Amino Acid Nutrition on Growth and Reproduction of Potato Dry Rot *Fusarium roseum* 'Sambucinum' Variants

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# 감자 乾腐 *Fusarium roseum* 'Sambucinum' 變異體의 菌絲生長 및 分生胞子 形成에 미치는 아미노酸 營養

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**Abstract:** The effects of amino acids on mycerial growth and sporulation of the decreased and increased pathogenicity variants of potato dry rot *Fusarium roseum* 'Sambucinum' were as following. The increased pathogenicity variants Ic52 and Ic116 showed the trend of greater mycerial growth, marking up to 67mg on leucine, than the decreased pathogenicity variants Dc14 and Dc91, and produced markedly low conidia below $25\times10^4$/ml or no sporulation. On methionine all the variants and the wild type showed the lowest growth recording 14mg to 20mg with no sporulation. Cystine similarly as glysine supported the poor growth 30mg to 19mg of the variants Dc14 and Dc91, respectively, and did not support the sporulation. The former amino acid like asparagine supported the growth of the variants Ic52 and Ic116 marking 47mg and 36mg, respectively. On all the eleven amino acids the variant Dc14 showed the trend of more mycerial growth and sporulation than the variant Dc91 which marked the exceptionally high sporulation $195\times10^4$/ml on glutamic acid.

## Introduction

Potato tuber dry rot *Fusarium roseum* 'Sambucinum' produced its decreased and increased variants(Lee 1979). Since the decay of crop means utilization of crop component substances by the decay organism (Tossoun et. al. 1960), the variants in increased or decreased pathogenicity must have their nutritional requirement different from the wild type's.

Although plant disease development by a pathogen is involved with complex interaction, it is generally the form of nitrogen available to the pathogen that affects the disease severity or resistance(Hendrix & Tossoun 1964; Nash & Snyder 1960; Tossoun *et al.* 1959). More mycelium of *F. roseum* was produced on a medium containing methionine than on that containing any other compound, but spore production was very low in the medium containing methionine (Jones & Woltz 1969, Leach & Webb 1981). Arginine deficient mutant showed no growth on the unsupplemented basal medium but methionine deficient mutant showed a limited growth on it(Tuveson & Garber 1959). The identification of various amino acids in the cells of *F. solani* f. *phaseoli* bean root rot organism(McAnelly 1959) suggests that these

substances are synthesized in the cells of pathogen from the nutrient compound obtained from host plant.

The growth of tomato wilt fungus *F. oxysporum* f. *lycepersici* increased as glucose concentration increased in the amino acid medium and some amino acid medium failed to support any growth in the abscence of carbon(Cook & Schroth 1965; Jones & Woltz 1969). Since potato tuber contains diverse forms of carbon and nitrogen including most of the amino acids availalbe to plant pathogens(Schwimmer & Burr 1967), this investigation aimed to elucidate some relationship that may exist between altered pathogenicity of the variants of *F. roseum* 'Sambucinum' and their mycelial growth and reproduction by various amino acids as nitrogen source.

## Materials and Methods

**The basal medium:** The basal medium lacking nitrogen used in this investigation consisted of 1g $KH_2PO_4$, 0.5g $MgSO_4 \cdot 7H_2O$, 0.5g KCl, 0.01g $FeSO_4 \cdot 7H_2O$, and 15g sucrose per liter distilled water. The pH was adjusted to approximately 5.8 and was not changed appreciably by autoclaving. The amino acids used as 0.33g per liter equivalent nitrogen were 2.099g DL-alanine, 1.768g L-asparagine, 3.136g L-aspartic acid, 2.829g L-cystine, 3.466g L-glutamic acid, 3.090 L-leucine, 2.152g L-lysine HCl, 3.151g L-methionine, 2.476g L-serine, 2.406g L-tryptophane, and 1.768g glycine. Seven point five mililiter aliquots of the media were pipetted in 1.8cm×18cm sterilized test tubes, stopered with cotton plugs, and then autoclaved at 105°C for 15 minutes.

**Four variants:** Four variants of the two increased pathogenicity, Ic52 and Ic116, and the two decreased pathogenicity, Dc14 and Dc91, of *F. roseum* 'Sambucinum' were separately cultured on potato sucrose agar in petri dishes of nine centimenter diameter at 25±1°C for seven days. Each culture was added with 15ml of sterilized distilled water enough to cover the culture surface and scratched with a specula. The mycelia together with conidia that came into water suspension were transferred to the test tubes. They were stirred with a blender made by utilizing a shvor for about five minutes until the mycelia were broken into fine fragments of approximately conidial size, passed through a few layers of gauge, and collected in a vial. They were washed three times by centrifuging at 3,000 rpm for 15 minutes, decanting supernatant, and filling the water again. The washed conidia and mycelial fragments in mixed condition were transferred to 150ml frask and then the concentration was adjusted to approximately $2\times10^4$ml by adding sterilized distilled water. Around 2,000 conidia and mycelial fragments were added by adding 0.1ml of the inoculum suspension to the 7.5ml liquid medium in each of 1.8cm×18cm test tubes. They were stoppered with cotton plugs and incubated at 25±1°C for nine days in the condition of 12-hours dark and light alternation before measuring mycerial growth and counting conidial number.

**Mycelial growth:** The contents of each test tube were decanted on a thin 5cm×5cm rectangular cloth that was weighed in advance. After dropping the solution, the cloth carrying the wet mycelia were dried at 35°C for 24 hours and then weighed. The mycelial dry weight was obtained by subtracting the empty cloth weight from the mycelial dry weight with the cloth.

**Number of conidia:** The cultures in the test tubes were killed by placing the test tubes containing the cultures in boilling water for about 30 minutes to fix the conidia formation plus mycelial growth. The culture in each test tube was stirred vigorously with a miniature blender made utilizing a shaver for about 30 seconds so that conidia were detached from the mycelia. Ringfuls of the stirred liquid cultures were taken for count of conidia in a haemocytometer in three replication.

## Results

**Mycelial growth:** All the four variants as well as wild type of the potato dry rot *F. roseum* 'Sambucinum' showed no substantial growth marking only a few miligrams on the basal medium with no amino acid as nitrogen source (Fig. 1). They made least

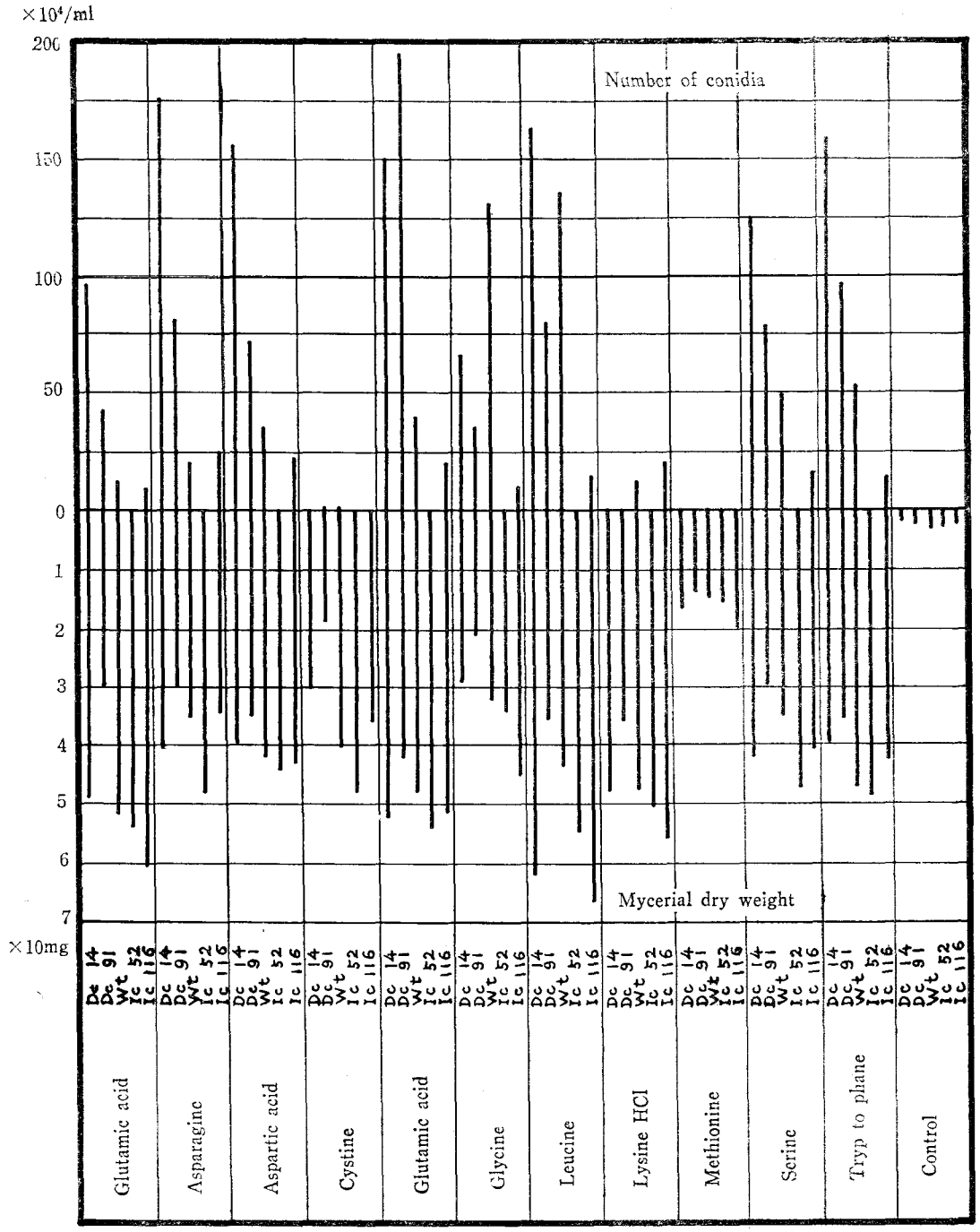


**Fig. 1.** Effect of eleven amino acids on the mycelial growth and sporulation of potato dry rot *Fusarium roseum* 'Sambucinum' variants: Dc14 and Dc91; variants in decreased pathogenicity. Ic52 and Ic116; variants in increased pathogenicity. Wt; their parental wild type.

growth marking less than 20 milligrams on methionine. The decreased pathogenicity variant Dc14 marked the mycelial dry weight 62mg on leucine followed by on glutamic acid, alanine, and lysine • HCl weighing between 52mg and 47mg. The growth on serine, tryptophane, asparagine, and aspartic acid marked similar weight between 40mg and 42mg. The growth was less than 30mg on cystine and glycine. The decreased pathogenicity variant Dc91 showed the mycelial dry weight 42mg on glutamic acid followed by around 35mg on aspartic acid, leucine, lysine, and tryptophane. The growth was 30mg on alanine, asparagine, and serine. The increased pathogenicity variant Ic52 recorded more than 50mg on leucine, alanine, glutamic acid, and lysine HCl while recorded 35mg on glycine. The growth weight was between 44mg and 50mg on tryptophane, serine, asparagine, cystine, and aspartic acid. The increased pathogenicity variant Ic116 grew best on leucine marking 67mg followed by 61mg, 56mg, and 51mg on alanine, lysine HCl, and glutamic acid, respectively. The growth was between 40mg and 45mg on serine, glycine, tryptophane, and aspartic acid while it was around 35mg on asparagine and cystine. The growth of the wild type was 52mg on alanine followed by in decreasing order 48mg on both tryptophane and glutamic acid; and on leucine, aspartic acid, and cystine down to 40mg.

**Number of conidia:** All the four variants and wild type produced no conidia at all on the basal medium with no amino acid as nitrogen source, and on the medium added with methionine(Fig. 1). On cystine there were little conidia formed regardless of the variants or wild type. The decreased pathogenicity variant Dc14 produced its highest number of conidia $175\times10^4$/ml on asparagine followed by 167×, 160×, 155×, and $150\times10^4$/ml on leucine, tryptophane, aspartic acid, and glutamic acid, repectively. The conidial number produced on serine, alanine, and glycine was 125×, 95×, and $65\times10^4$/ml, respectively. No conidia were produced on lysine HCl. The decreased pathogenicity variant Dc91 produced the highest number $195\times10^4$/ml of conidia on glutamic acid among the all cultures and treatments and $97\times10^4$/ml on tryptophane. The number produced on serine, leucine, and asparagine was around $80\times10^4$ml followed by 72×, 42×, and $35\times10^4$/ml on aspartic acid, alanine, and glycine, respectively. On lysine HCl no conidia were produced at all same as on methionine. The increased pathogenicity variant Ic52 produced no conidia regardless of the kind of amino acids used in this investigation. The variant Ic116 of increased pathogenicity produced between 25× and $20\times10^4$/ml on asparagine, aspartic acid, glutamic acid and lysine HCl while produced around $15\times10^4$/ml on serine, tryptophane, and leucine. The number produced on glycine and alanine was approximately $10\times10^4$/ml. The number of conidia produced with the wild type was 135× and $130\times10^4$/ml on leucine and glycine, respectively, followed by 55× and $50\times10^4$/ml on tryptophane and serine. The number on glutamic acid and aspartic acid was 40× and $35\times10^4$/ml followed by $12\times10^4$/ml on both alanine and lysine HCl.

## Discussion

Control measures of potato tuber decay may not remain same when causal pathogen varied in pathogenicity. The decreased and increased pathogenicity variants(Lee 1979) of potato dry rot *F. roseum* 'Sambucinum' differred in their growth response by the kind of amino acids used in this investigation, implying that the utilization of these compound in the potato tuber by the variants are different from that by their parental wild type.

As the cells of virulent pathogens were found to contain smaller amount of free amino acids McAnelly (1959) reasoned in his research with *F. solani* f. *phaseoli* that aspartic acid, glutamic acid, glycine, and alanine contents to be less than those of avirulent or weak pathogens. In the present investigation, the highest mycelial growth of the increased pathogenicity variant Ic116 was supported by leucine whereas methionine supported the lowest mycelial growth of the decreased pathogenicity variant Dc91.

Since the free amino acids in the fungal cells were

not examined in this study, no relation can be reasoned between the difference in pathogenicity and the amino acids in the potato dry rot fungus. Although most of the amino acids used in this experiment supported the fungal growth of the increased pathogenicity variants more than the decreased pathogenicity variants it can not be considered that the cells of strongly virulent variants contain less or more amino acids that were absorbed from the outside source. The amino acids absorbed from the outside sources are known to be utilized in the synthesis of the other component substances beginning with protein. The analysis of the components in the potato dry rot fungus may lead to elucidate the relationship that may exist between the degree of pathogenicity, content of amino acids in the fungal cells, and the amount of those compound absorbed from the potato tuber.

Methionine and cystine were the least effective to support the four variants and the parental wild type. These compounds in the potato tuber are considered to be the last that can be utilized by the fungus decaying the tuber. However, the present result does not accord with that obtained by Jones and Woltz(1969, 1971) who disclosed that *F. oxysporum* f. *lycopersici* grew well on methionine. This difference must be due to the difference of the fungal species or race in combination with the host crop. Methionine did not directly inhibit the pathogen growth or the infection process but prevented the symptom expression(Papavizas & Davey 1963).

The sporulation of the potato dry rot fungus was much more affected than the mycelial growth by the variants as well as by the kind of amino acids used in the present investigation. This result accords with Hendrix and Tossoun (1964) who thought that the kind and the quantity of amino acids available to pathogenic fungi govern the fungal sporulation. Glutamic acid was the nitrogen source that supported the greatest number of conidia to none in the present investigation. Cystine and methionine completely suppressed the sporulation regardless of the variants or their parental wild type while the increased pathogenicity variant Ic52 did not sporulated at all by any of the eleven amino acids. The sporulation of potato dry rot *F. roseum* 'Sambucinum' appears to be dependent on the amino acids other than methionine and cystine. As the efforts have been paid to attain basic knowledge for development of control measure of crop diseases by use of different nitrogen sources including amino acids(McAnelly 1959; Tossoun et al. 1960), the present investigation also indicates the necessity of further research in this field.

## 摘 要

감자 乾腐病菌 *Fusarium roseum* 'Sambucinum'의 病原性 減少 및 增加 變異體의 菌絲生長과 分生胞子 形成에 미치는 各種 아미노酸의 效果를 試驗한 結果는 다음과 같았다.

病原性 增加 變異體 Ic52 및 Ic116은 病原性 減少 變異體 Dc14 및 Dc91보다 모든 供試 아미노酸에서 菌絲生長이 더큰 傾向을 보여 leucine에서 最高 67mg까지 生長하였으며 分生胞子 形成은 $25\times10^4$/ml 이하로 減少 또는 完全히 消失하였다.

Methionine은 變異體 및 野生型에 對하여 14~20mg의 가장 적은 菌絲生長을 보였으며 分生胞子는 全然 形成시키지 않았다. Cystine은 病原性 減少 變異體 Dc14 및 Dc91의 菌絲生長 30mg 및 19mg를 보여 asparagine과 같이 中程度였고 分生胞子 形成에는 效果가 없었다.

모든 供試 아미노酸에서 病原性 減少 變異體 Dc14의 菌絲 生長은 Dc91보다 큰 傾向을 보였으며 分生胞子 形成도 Dc91에서 最高 $195\times10^4$/ml를 보인 glutamic acid를 除外한 아미노酸에서 Dc14 顯著하게 많았으며 lysine HCl은 이들 兩變異體의 分生胞子를 形成시키지 않았다.

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