Effects of Some Organic Acids on the Dark Germination of Tobacco Seed

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In our previous experiments^{1,2)}, we found that the rates of gibberellin induced dark germination changed remarkably by the lengthening of the storage period of the tobacco seed, that several inorganic compounds seemed to accelerate the dark germination inducing action of gibberellin in different ways, and that the effect of gibberellin on the seed of short storage length was promoted at two or three pH ranges, i.e. at around pH 2.0 to 2.5, and from pH 4.7 to 5.5 in all the cases of previous experiments, and at one more specific pH range at around 4.0 in the presence of phosphate in the germination bed; in the presence of several inorganic nitrogenous compounds like potassium nitrate, ammonium chloride, the seed also germinated well at around pH 3.0. When the storage length of the seed is long, however, the accelerating effect of gibberellin on the dark germination was increased only at pH 2.0 to 2.5, and decreased at around pH 4.7 to 5.5.

Hashimoto and Yamaki²⁾ and Hashimoto⁴⁾ also reported the dark germination inducing action of ammonium salts of several carboxylic acids. In the present paper, are reported the results of the experiments concerning the effects of ammonium or potassium salts of several organic acids on the dark germination of tobacco seed on the germination beds of various pH: on the one hand those dealing with the effect of these organic acids on the dark germination inducing action of gibberellin, and on the other hand those dealing with the dark germination inducing action of these organic acids themselves.

Material and Methods are same as those in the previous papers^{1,2)}.

Results

Effect of malic acid on the dark germination:

Malic acid was added to the germination bed at final concentration of M/100 in the presence or absence of 10 ppm gibberellin. pH of the germination bed was adjusted with this acid and NH_4OH or KOH , with the intervals of pH 0.2. The storage length of the seed used was 15 and 27 months in the experiments using malic acid and NH_4OH as pH adjusters, and 15 months in the experiments using malic acid and KOH .

The results of the former experiments are shown in Fig. 1 and those of the latter in Fig. 2.

From these figures, the following results are observed.

- 1) In the presence of malic acid and NH_4OH the dark germination of 15 months

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Fig. 1. Effect of malic acid on the dark germination of tobacco seed. pH was adjusted with NH_4OH . —; 15 months stored seed, in presence of 10 ppm gibberellin, ----; 27 months stored seed, in presence of 10 ppm gibberellin,; 15 months stored seed, in absence of gibberellin. No dark germination of 27 months stored seed was observed on the germination beds of varying pH, being adjusted with malic acid and NH_4OH . No dark germination was observed on the control germination bed wetted with distilled water and/or 10 ppm gibberellin.

Fig. 2. Effect of malic acid on the dark germination of tobacco seed. pH was adjusted with KOH. —; 15 months stored seed, in presence of 10 ppm gibberellin,; 15 months stored seed, in absence of gibberellin. No dark germination was observed in the control germination bed wetted with distilled water and/or 10 ppm gibberellin.

stored tobacco seed is induced even in the absence of gibberellin in the pH ranges from pH 3.8 to 8.0. The highest germination rate is observed at around pH 4.8.

2) The dark germination inducing action of the mixture of malic acid and NH_4OH in the absence of gibberellin is not observed on the seed having been stored for 27 months.

3) If the value of germination rate induced by the mixture of malic acid and NH_4OH at each pH is deduced from the value of the germination rate induced by gibberellin and the mixture at the corresponding pH, the accelerating effect of the mixture of malic acid and NH_4OH on gibberellin induced dark germination can be determined (Fig. 1). In case of the seed stored for 15 months, the mixtures of malic acid and NH_4OH accelerate the gibberellin induced dark germination not only in the far acidic pH ranges but also at pH 4.3 and pH 4.9.

4) In case of the seed having been stored for 27 months, the mixture of malic

acid and NH_4OH accelerates remarkably the gibberellin induced dark germination at pH range from 2.0 to 4.0, and above pH 4.5, no acceleration was observed.

5) The mixture of malic acid and KOH does not induce the dark germination; however, it accelerates the gibberellin induced dark germination of tobacco seed of 15 months' storage at acidic pH ranges from pH 2.5 through 4.5.

These phenomena, especially 1) and 5), seem to indicate that for the seed germination, especially the germination at pH around 4.7, at least two groups of factors are essential, one being represented by a substance probably synthesized from malic acid and NH_4OH . The seeds having the storage length of 15 months have still some amount of gibberellin or gibberellin-like substance in them and retain the ability to synthesize the substance belonging to the second group, so they can germinate when malic acid and NH_4OH are added to the germination bed. On the contrary, the seeds having been stored 27 months have used up or destroyed already both groups of the substances, and the ability to synthesize the second group of the substance, so they cannot germinate on the germination bed having only one of these two groups of substances. And even when the substances belonging to both groups are added to the germination bed, the seed of long storage length cannot germinate at the pH range around 4.7 to 5.5; germination in the pH range below 4.0 seemed to depend on some other factors which are common in the tobacco seed.

The mixture of malic acid and KOH, however, has no germination inducing action. But it accelerates the gibberellin induced germination in acidic pH range. This effect seems to be identical with the effect of the mixture of malic acid and NH_4OH and the effect of pH, which are adjusted with inorganic base and acid.

Effect of tartaric acid on dark germination:

Tartaric acid was added to the germination bed with final concentration of M/100 in presence and absence of 10 ppm gibberellin. pH of the germination bed was adjusted with acid and NH_4OH or KOH. The storage length of the seed used was 15 months. The results of such experiments are shown in Figs. 3 and 4.

From these figures, the following results are observed.

Fig. 3. Effect of tartaric acid on the dark germination of tobacco seed. pH was adjusted with NH_4OH . —; 15 months stored seed, in presence of 10 ppm gibberellin,; 15 months stored seed, in absence of gibberellin. No dark germination was observed in the control germination beds.

Fig. 4. Effect of tartaric acid on the dark germination of tobacco seed pH was adjusted with KOH. —; 15 months stored seed, in presence of 10 ppm gibberellin,; 15 months stored seed, in absence of gibberellin. No dark germination was observed on the control germination beds.

1) The mixture of tartaric acid and NH_4OH induces the dark germination in the absence of gibberellin in the pH ranges from 4.5 to 8.0.

2) The highest germination rate is observed at around pH 4.8.

3) The mixture of tartaric acid and NH_4OH accelerates gibberellin induced dark germination of the seed, not only at acidic pH ranges below 4.0, but also at around 4.7.

4) The mixture of tartaric acid and KOH induces no dark germination, but accelerates the gibberellin induced dark germination from far acidic pH ranges up to pH 4.8.

5) At pH 5.5, acceleration is observed in both cases.

From these results, considerations similar to those in case of malic acid can be deduced.

Effect of pyruvic, acetic, citric, α -ketoglutaric, succinic and fumaric acid on the dark germination:

Ammonium or potassium salts of these acids were added to the germination bed with final concentration of M/100, in presence and absence of 10 ppm gibberellin. pH of the germination bed was adjusted to pH 4.5 and 6.0 with these acids and NH_4OH or KOH. The storage length of the seed used was 15–16 months. The results of these experiments are summarized in Table 1.

From the results of the experiments indicated on the table the followings can be deduced.

1) In the presence of NH_4OH in the germination bed, any of these acids, except acetic acid and α -ketoglutaric acid, induce the dark germination remarkably at pH 4.5, but at pH 6.0 this effect is very small.

2) In the case where KOH is used for pH adjustment, these acids have almost no germination inducing activities.

3) In the presence of NH_4OH , the organic acids accelerate the gibberellin induced dark germination of the seed remarkably at pH 4.5 but not so remarkably at pH 6.0.

Table 1. Effects of organic acids on the dark germination of tobacco seed.

The values in the table indicate the germination percentages at the end of 5 day incubation at 25°. The concentration of the acids and gibberellin is M/100 and 10 ppm, respectively.

pH adjuster	NH ₄ OH				KOH			
pH	4.5		6.0		4.5		6.0	
Gibberellin	—	+	—	+	—	+	—	+
Pyruvic	32.7	54.2	0	10.1	0	62.9	0	3.8
Acetic	3.9	34.0	6.5	18.8	0	41.2	0	5.3
Citric	15.7	85.3	8.9	25.5	2.2	76.5	0	7.3
α -ketoglutaric	1.5	47.8	0	2.3	0	71.8	0	19.5
Succinic	16.0	80.0	3.7	17.5	1.4	87.0	0	6.5
Fumaric	10.8	64.1	0	14.9	1.0	77.0	1.0	4.6
Malic	25.8	74.4	3.4	9.1	3.2	76.8	0	8.4
Tartaric	6.3	70.5	6.9	16.7	0	23.3	0	4.2

4) In the presence of KOH, the organic acids accelerate the gibberellin induced dark germination remarkably at pH 4.5 but slightly at pH 6.0.

5) Effectiveness of the germination inducing activities of these organic acids in the presence of NH₄OH are in the following order at pH 4.5: pyruvic>malic>succinic>citric>fumaric>tartaric>acetic> α -ketoglutaric acid.

6) Effectiveness of the acceleration on the gibberellin induced dark germination of these organic acids are in the following order at pH 4.5, in the presence of NH₄OH: citric>succinic>malic>tartaric>fumaric> α -ketoglutaric>pyruvic>acetic acid.

7) Accelerating effect at pH 4.5 on gibberellin induced dark germination of the acids in the presence of KOH is larger than that of the corresponding acid in the presence of NH₄OH.

Discussion

In the experiments using HCl-KOH, HNO₃-KOH, HCl-NH₄OH, H₃PO₄-KOH and H₃PO₄-NH₄OH as pH adjusters, Takahashi *et al.*^{1,2)} reported that these inorganic salts accelerated the gibberellin induced dark germination of tobacco seed in acidic pH ranges, especially at around 2.0-2.5 and 4.7-5.5 and in case of phosphate, one more specific pH range at around 4.0 was observed, and in the presence of a nitrogenous compound, the germination was accelerated at around pH 3.0, too. They also found that the longer the storage period, the smaller the accelerating effect of these salts at pH 4.7-5.5, and that these salts fails to induce dark germination of the seed of short storage length.

In the present experiment, the authors found that, in the presence of ammonia or potassium hydroxyde, several carboxylic acids; pyruvic, acetic, succinic, α -ketoglutaric, fumaric, malic, and tartaric acid accelerated the gibberellin induced dark germination of tobacco seed in acidic pH range, especially at around pH 2.0 to 5.0 in case of the short stored seed, and from pH 2.0 to 4.0 in case of long stored seed.

They also found that in the presence of NH₄OH, these acids, with an exception of α -ketoglutaric acid, had germination inducing activities through a wide range of

pH; e. g., from pH 3.8 to 8.0 in case of malic acid, from pH 4.5 to 8.0 in case of tartaric acid. And other acids induce the dark germination at pH 4.5.

When the seed of short storage length was used as the plant material, malic acid and tartaric acid induced the dark germination mostly around pH 4.8 in the presence of NH_4OH , and these acids accelerated the gibberellin induced dark germination at the same pH. And the facts that other organic acids induced the dark germination and accelerated the gibberellin induced dark germination at pH 4.5 indicate the possibility that other organic acids induce the dark germination mostly at around pH 4.7 and accelerated the gibberellin induced dark germination at the same pH as inorganic salts accelerated the gibberellin induced germination at around pH 4.7.

From the same experimental results an accelerating effect of gibberellin on an organic acid induced dark germination was observed in case of the seed of short storage length.

On the contrary when the seed of long storage length was used as the plant material, none of these organic acids induced the dark germination nor accelerated the gibberellin induced dark germination at around pH 4.7.

These results seem to indicate that for the dark germination of the seed, especially the germination at pH around 4.7, at least two groups of substances are essential, one being represented by a substance probably synthesized from an organic acid and NH_4OH , the other is gibberellin or gibberellin-like substance. The seed having been stored for a short period have some amount of gibberellin or gibberellin-like substance and maintains the ability to synthesize the substance necessary for germination from an organic acid and NH_4OH . The seed having been stored for a long period, however, loses gibberellin-like substance, and the ability to synthesize the substance in question.

Further discussion about the mechanism of gibberellin induced dark germination of tobacco seed is difficult before we find the presence of the substance in question and determine the exact condition of germination and the age of the seed used.

Summary

1) Effects on gibberellin induced dark germination of tobacco seed of pyruvic, acetic, citric, α -ketoglutaric, succinic, fumaric, malic and tartaric acids were examined in presence of NH_4OH or KOH .

2) These acids accelerated the gibberellin action at acidic pH range, especially from pH 2.0 through 4.5. But when the seed of short storage length was used as the material, these acids accelerated the gibberellin induced dark germination at around pH 4.7, too.

3) Effects of these acids on dark germination of tobacco seed were studied in the presence of NH_4OH or KOH .

4) Malic and tartaric acids induced dark germination in a wide pH range in the presence of NH_4OH . The highest germination rate was observed at around pH 4.8.

5) Pyruvic, acetic, citric, succinic and fumaric acids induced the dark germination at pH 4.5 in the presence of NH_4OH . These acids seemed to induce the dark germination mostly at around pH 4.8.

6) The seed of short storage length seemed to have the ability to synthesize some compounds necessary for the induction of dark germination from an organic acid and NH_4OH at around pH 4.8.

References

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摘 要

高橋憲子*・師尾武子*・橋本 徹**・八巻敏雄***: タバコ種子の暗発芽に
対する有機酸の影響

ブライトイエロー種タバコ種子の暗発芽に対する有機酸の影響を M/100 の濃度で発芽床に与えられたピルビン酸、酢酸、クエン酸、 α -ケトグルタル酸、コハク酸、リンゴ酸、酒石酸などの pH を NH_4OH , KOH で pH 0.2 間隔で調整することによって検討した。実験の範囲では酢酸と α -ケトグルタル酸を除き他の酸は NH_4OH で pH を調整された場合に、かなり広い pH 域にわたって暗発芽をひきおこす。その最適 pH は 4.7 附近である。これらの酸はまた、それ自体では発芽を起こさせることのない濃度のジベレリンと共存させられることによって、pH 4.0 より酸性の域で顕著な発芽をひきすが、それとともに pH 4.7 附近でもこうした作用が認められる。また pH 4.7 での有機酸と NH_4OH とによってひきおこされる暗発芽は、貯蔵期間の長い種子では次第に低くなり、ついにはジベレリンを共存させても発芽しなくなる。これらのことからこの pH における暗発芽に注目をした。この pH 域における暗発芽には有機酸と NH_4OH とから合成されると思われる物質と、ジベレリンによって代用される物質とが必要であり、貯蔵期間の短いものではこの両物質ともかなり（暗発芽をおこすには少し不足）の量で含まれており、わずかに補給することにより発芽がおこると推察する。この場合ジベレリン様物質の不足の程度はやや軽微であり、有機酸と NH_4OH が与えられれば、種子中に残存する合成機能が發揮され必要な物質がつくられ、発芽がおこるが、貯蔵期間が長くなると発芽に必要な両物質の不足が大きくなり、また合成能の低下に伴ってジベレリンも補給する必要が生じ、さらには有機酸と NH_4OH およびジベレリンを与えても発芽が起こらなくなることが考えられる。（* 日本女子大学生理学教室，** 武蔵大学生物学教室，*** 東京大学教養学部生物学教室）