A New Functional Fertilizer Containing 5-Aminolevulinic Acid Promoted Hydroponically-grown Vegetables in the Netherlands

K. Iwai and A. Saito
Koganei Factory, Seiwa Co., Ltd., 262-10 Shiba Kokubunjimachi, 329-0412 Japan

Jeroen van Leeuwen
Pentagrow b.v. Stierenweg 16-4664 TA Lepelstraat The Netherlands

T. Tanaka and Y. Takeuchi
Center for Research on Wild Plants Utsunomiya Univ. 350 Minemachi, Utsunomiyashi 321-8505 Japan

Keywords: 5-ALA, citric acid, NO3, paprika, peat, rockwool, strawberry, sugar, yield

Abstract
5-Aminolevulinic acid (5-ALA) is the key precursor in the biosynthesis of tetrapyrroles such as chlorophylls and hemes. 5-ALA is the unique precursor of tetrapyrroles. Iron element can activate 5-ALA-induced physiological actions. We conducted experiments of a new functional fertilizer including 5-ALA-HCl (0.3%), magnesium nitrate, Fe-DTPA, urea and the other mineral nutrients on the yield of paprika and strawberry fruits. Paprika and strawberry plants were transplanted in a rockwool and a peat respectively, and they were weekly irrigated with a hydroponic solution added with or without the fertilizer (paprika: 500-800 and strawberry: 500 ml ha⁻¹) respectively. The cumulative fruit yield of paprika treated with the fertilizer increased by 4.3 to 9.0% of control. The nutrient solution analysis showed that NO3-N uptake by paprika plants with the fertilizer treatment was increased by 16% of control. The yield increase of paprika may be related partly to NO3-N uptake increase by the fertilizer treatment. The cumulative fruit yield of strawberry during 8 weeks after the treatment increased by 4.8% of control. Size of the fruit slightly decreased, in contrast, percentage of dry matter, sugar and citric acid contents significantly increased.

INTRODUCTION
5-Aminolevulinic acid (5-ALA) is the key precursor in the biosynthesis of tetrapyrroles such as chlorophylls and hemes. 5-ALA is the unique precursor of tetrapyrroles (Beale, 1990), such as chlorophyll a, chlorophyll b, protoheme, heme, and siroheme. They have a magnesium or an iron element at the active center. The enzymes, 5-ALA related metabolites, such as catalase, cytochromes, ascorbate peroxidase, nitrate reductase, nitrite reductase, and sulfite reductase play important roles in plants.

In the redox reactions and electron transport in an enzymes, iron ion may play an essential role with a minimum expenditure of energy. When protoporphyrinIX accumulates highly in plants treated with 5-ALA at a high concentration, magnesium ion may play a role of safener as a metabolic smoothing of protoporphyrinIX. 5-ALA-induced actions can be activated with iron and magnesium elements. The fertilizer, a liquid formulation, containing 5-ALA-HCl (0.3%), magnesium nitrate, urea iron(III)-diethylene triamine pentaacetic acid (Fe-DTPA), and trace elements such as Mn, B, Zn, Cu, and Mo has been used in Japan. 5-ALA is produced with fermentation of photosynthetic bacterium (Rhodobacter sphaeroides).

The fertilizer, exhibits promotive actions such as growth, yield, fruit quality, and salt and cold resistances on crops and vegetables.

MATERIALS AND METHODS
The fertilizer containing 5-ALA was prepared as shown in Table 1. Fe-DTPA was obtained from Chelest Co. (Osaka Japan) as Fe-DTPA-2(NH4) /50% (aq.). 5-ALA was obtained from Cosmo Oil Co. (Tokyo Japan) as 5-ALA-HCl/98%. All other chemicals were purchased from commercial sources and were of reagent-grade or first class industrial-grade.

Four experiments on paprika and one experiment of strawberry were conducted in a greenhouse in the Netherlands in 2003-2004. Paprika (Capsicum annuum ‘Tripoli’, ‘Echo’, ‘Sympathy’) plants were grown in a rockwool. They were weekly irrigated with a...
The analysis was conducted only in one experiment (Lepelstraat 2003).

Strawberry (*Fragaria × ananassa* Duchesne ‘Elsanta’) plants were grown in a peat from first week of January 2004. They were weekly irrigated with a hydroponic solution (Naaldwijk recipe) added with or without the fertilizer (500 ml ha⁻¹) respectively, started in last week of January. All fruits of plants were harvested and their weight were determined. Sufficient ripened fruits of strawberry were weekly analyzed on weight, diameter, volume, dry matter, sugar, citric acid, nitrogen, phosphorous, potassium, magnesium, and other minerals.

RESULTS

The yields of paprika in four experiments were 33.8 kg m⁻² and 31.0 kg m⁻² (Hendrik Ido Ambacht 2003, ‘Tripoli’), 30.2 kg m⁻² and 28.7 kg m⁻² (Lepelstraat 2003, ‘Echo’), 11.0 kg m⁻² and 10.1 kg m⁻² (Ijsselmuiden 2003, ‘Sympathy’, censored data) and 29.0 kg m⁻² and 27.8 kg m⁻² (Lepelstraat 2004, ‘Echo’) with or without the fertilizer treatment respectively. Thus, yield increased by 9.0%, 5.2%, 8.9%, 4.3% of untreated control respectively (Fig. 2). The yield increase was induced through improving root, plant vigor, leaf coloring, number of lateral shoots, fruit size, and specific gravity of the fruit (audit observation). NO₃-N uptake by paprika treated with the fertilizer was increased by 16% of untreated control in June to July (Fig. 3).

The harvest of strawberry fruits started from April 1, 2004. Figure 4 shows weekly compiled fruit yield of strawberry. The cumulative yield during 8 weeks after the treatment was 6.11 kg m⁻² and 5.83 kg m⁻² respectively, with or without the fertilizer treatment, thus, yield increased by 4.8% of untreated control. Averaged across the period of sampling, mean of diameter, volume and fruit weight were 33.6 mm and 33.7 mm, 20.5 ml and 21.5 ml and 19.0g and 19.2g, with or without the fertilizer treatment respectively. These data shows that fruit size was slightly decreased. In contrast, percentages of dry matter with or without the fertilizer treatment were 8.92% and 8.76%. Sugar and citric acid contents also increased (Fig. 5 and 6). Other elements in the fruits such as, nitrogen, phosphorous, potassium, magnesium, and micro minerals were not changed by the fertilizer treatment.

DISCUSSION

In four experiments, the fertilizer promoted fruit yield of paprika. 5-ALA and plant nutrient elements including Fe-DTPA showed synergistic effects in the growth promotion on the weight of creeping bentgrass (*Agrostis palustris* Huds.), radish (*Raphanus sativus* var. *radicula*) and tea (*Camellia sinensis* Kuntze) and the leaf color of creeping bentgrass and tea (Iwai et al., 2003; 2004b). Additionally, 5-ALA and Fe-DTPA combined with urea also showed synergistic effects on node-number and fresh weight of shoots of Japanese lawngrass (*Zoysia japonica* Steud.) and tea (Iwai et al., 2004a; b).

Heme is synthesized from eight molecules of 5-ALA and an iron element. Exogenous 5-ALA stimulated the activity of nitrate reductase in maize leaf segments under the light (Mishra and Srivastava, 1983). The yield increases of paprika may be related partly to NO₃-N uptake increase caused by 5-ALA.

The fertilizer promoted fruit yield of strawberry. The fruit size of strawberry slightly decreased, but percentage of dry matter, sugar and citric acid contents significantly increased by the fertilizer treatment. 5-ALA showed promotive effect on the photosynthesis of manilagrass (*Zoysia matrella* Merr.) (Hotta et al., 2000). Additionally in our several experiments, 5-ALA promoted water uptake of hydroponically-grown plants. The increase of photosynthesis and stomata opening induced by 5-ALA may explain yield and quality increase in strawberry fruit.

ACKNOWLEDGEMENTS

We thanks to Mr. Edwin Stolk, Mr. Sjaak van Spronseen and Mr. Marcel Dings for participants in test growing. We thanks to Dr. Klaas de Jager for consultation in test growing.

Literature Cited

Beale, S.I. 1990. Biosynthesis of the tetrapyrrole pigment precursor δ-aminolevulinic acid
Iwai, K. Zinzai, Y. and Takeuchi, Y. 2001. Effects of 5-aminolevulinic acid and nitrogen-
trace elements mixed fertilizer on dry weight in radish. The 36th annual meeting of the
affects growth of creeping bentgrass. The 32nd annual meeting of the JSTS: p. 134-135.
Iwai, K., Kuramochi, H. and Takeuchi, Y. 2004a. Effects of 5-aminorevulinic acid, iron, and
urea on growth of Japanese lawngrass seedlings. The 33rd annual meeting of the JSTS: p.
68-69.
2004b. Fertilizer containing 5-aminolevulinic acid affects yields and quality of tea. The 48th annual
meeting of the JSTST: p. 44-45.
Mishra, S.N. and Srivastava, H.S. 1983. Stimulation of nitrate reductase activity by delta
aminolevulinic acid in excised maize leaves. Experientia 39: 1118-1120.
Roger, L. and Tsuji, H. 1982. Effect of benzyladenine treatment duration on δ-aminolevulinic
acid accumulation in the dark chlorophyll lay phase abolition, and long-term chlorophyll
production in excised cotyledone of dark-grown cucumber seedlings. Plant Physiol. 69:
663-667.

Tables

Table 1. Ingredient of a new functional fertilizer.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric- N</td>
<td>3.8</td>
</tr>
<tr>
<td>Urea (as N)</td>
<td>5.7</td>
</tr>
<tr>
<td>Water-soluble magnesium (as MgO)</td>
<td>5.7</td>
</tr>
<tr>
<td>Iron-Diethylene triamine pentaacetic acid (as Fe)</td>
<td>0.60</td>
</tr>
<tr>
<td>Water-soluble boron (as B2O3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Water-soluble manganese (as MnO)</td>
<td>0.30</td>
</tr>
<tr>
<td>5-Aminolevulinic acid·HCl</td>
<td>0.30</td>
</tr>
<tr>
<td>Others: Zinc sulphate, Copper sulphate, Disodium Molybdate</td>
<td>trace</td>
</tr>
</tbody>
</table>
Fig. 1. 5-Aminolevulinic acid is the precursor of tetrapyrroles.
Fig. 2. Cumulative yield of paprika (four experiment); weekly treated with 500-800g ha$^{-1}$ of the fertilizer containing 5-ALA (0.3%).

Fig. 3. Drained solutions from the irrigation of paprika; Lepelstraat 2003.

*(Figs. 2 and 3): The week number with Monday the first day of the week. The first Monday of January is the first day of week number 1 (0 to 53).

Fig. 4. Yield of strawberry fruits; weekly treated with 500g ha$^{-1}$ of the fertilizer containing 5-ALA (0.3%).

Fig. 5. Sugar contents of strawberry fruits; weekly treated with 500g ha$^{-1}$ of the fertilizer containing 5-ALA (0.3%)

Fig. 6. Citric acid contents of strawberry fruits; weekly treated with 500g ha$^{-1}$ of the fertilizer containing 5-ALA (0.3%)

A: Hendrik Ido Ambacht  
B: Lepelstraat 2003  
C: Lepelstraat 2004  
D: IJsselmuizen