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Enhancement of Growth and Yield of Barley by the Soil Conditioner FFC-ace

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The effects of a unique soil conditioner, FFC-ace, on photosynthesis, transpiration, growth and yield of barley were examined in a field experiment. FFC-ace well-mixed with sandy soil greatly enhanced root and shoot growth, tillering and the number of grains per stock. The total yield in the treated plot increased by about 172 %. The plants grown in the FFC-ace plot were greener and contained a higher level of chlorophyll, compared with the control. Photosynthesis and transpiration, which are tightly linked to productivity were also significantly enhanced at the broad range of photon flux observed in our study. The quality of grain harvested from the FFC-ace plot was similar to the control plot in terms of nutritional and inorganic components. The increased photosynthesis in the FFC-ace treated barley reflects a higher absorption of CO_2 from the atmosphere. It was also noted that the efficiency of water utilization for photosynthesis was significantly greater under the high light intensity in the treated plot. The relationship between application of FFC-ace and absorption of atmospheric CO_2 is discussed. Our investigation provides data showing that application of FFC-ace to soil significantly reduces water requirements for plant growth and yield.

Key words : barley (*Hordeum vulgare* L.), enhanced growth and yield, FFC-ace (soil conditioner), enhanced photosynthesis and transpiration, chemical analysis of grains

Introduction

Of all the environmental cues that challenge the developing plant, the condition of the soil is one of the most important issues. In addition to its key role in plant metabolism, where it drives the process of water and nutrient supply, root growth also acts to regulate plant growth and development. Many researchers have studied the effects of soil amendment on plant growth using diverse materials. The objective was to obtain a higher yield. Examples include the application of chemical fertilizers on wheat and barley[4,5,21), flyash on wheat, rice, maize and mustard[14), plant wastes such as olive mill waste, sawdust and bark waste on various crops[3,6,15,18-20,24), and soil microbes as biofertilizers[5,13). Some amendments increased plant growth while others were ineffective.

According to official reports of the Japanese government, food self-sufficiency declined to 39% in 2007. In order to bring it back to a high level, and to avoid a major shortage of food in the future, basic and applied researches to increase productivity in existing farmlands are urgent and essential rather than the reorganization of the agricultural scale in our country. Because of the limitation of the nation's land, it would be difficult to expand our farmlands further. As emphasized above, soil amendment is one of the best approaches to increase productivity of farmlands without increasing the area farmed. However, large scale soil amendment cannot be practiced, since soil conditions in different parts of the country vary dramatically. Thus it is appropriate to find the best methods for the specific agricultural land which may be acceptable as broadly as possible.

From this point of view, we attempted to find desirable and broadly-acceptable materials to amend soil with the objective of obtaining high productivity. With this goal in mind we tested the use of FFC-ace (manufactured and sold by FFC Japan, Tsu, Japan) (Fig. 1). According to the manufacturer's instruction, FFC

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(the trademark, abbreviation of ferrous ferric chloride) products including FFC-ace were reported to have specific effects on plant, animal and microbial growth. The product has the potential to enhance growth of plants, especially root growth[9], to increase of beneficial soil-microbes, and to stimulate the proliferation and differentiation of cultured keratinocytes and melanocytes in the epidermis of mouse skin[10]. In addition, plants grown using FFC-ace have been shown to acquire tolerance to adverse environments such as disease, high salinity, drought and frost. The farmers who have used this material increase income because the quality and quantity of their products are improved drastically by its application. These include increased yield of cereal grains, high sugar content in vegetables and fruits, and high protein content of beans, corn etc. More than 750 tons of FFC-ace are used at more than 1000 ha farmlands throughout the country each year, its effectiveness has been evaluated through farmers' experience. However, a controlled scientific study of the effectiveness of soil treatment with FFC-ace has not been undertaken. The goal of our study was to evaluate the effect of this soil conditioner on plant growth and yield. In this paper we report significant stimulative effects of FFC-ace on growth and, hence, yield of barley.

Materials and Methods

1. *Field preparation*

The experimental field of the Faculty of Agriculture, Okayama University, was used for this study. The experimental plot (3.3 x 3.3 m^2) consisted of sandy soil in which no crops had been cultivated. Approximately 11 L (9.7 kg) of granular and fragile FFC-ace (Fig. 1) was well mixed with the plot soil (15 cm deep) on Oct 13, 2006 (described as FFC plot hereafter). The control plot was ploughed at 15 cm depth without adding FFC-ace. On Nov 7, 20 L of fermented cow dung (N=1.05%, P=1.13%, K=1.59%, C/N=15.5) (Shoei, Okayama, Japan) and 5 kg of bark compost (Greentop, Kokubu Farm Inc., Fukushima, Japan) were mixed well with soil in both plots. On Nov 13, the soil pH was adjusted to ca 6.5 with 5 kg/plot of the calcium carbonate fertilizer (Leisure Life, Ehime, Japan).

2. *Seeding*

Seeds of barley (*Hordeum vulgare* L., cultivar Kobinkatagi) were soaked in running tap water overnight. Germinating seeds were selected and 33.4 g of the seeds were sown in both plots on Nov 22.

3. *Estimate of chlorophyll contents in leaves*

Relative concentrations of chlorophyll in leaves grown in both plots were measured using SPAD-502 (Konica-Minolta, Japan) on Feb 26, 2007. Twenty five leaves were randomly selected from each plot and the chlorophyll content at the apical, central and basal portions of leaves were measured. The values were expressed as the average readings of 25 leaves x 3 portions.

4. *Measurement of photosynthesis and transpiration rates in field-grown plants*

Photosynthesis and transpiration rates of young leaves were measured on Feb 26, 2007 (96 days after seeding) in the field using a portable-type apparatus for photosynthesis and transpiration (LI-6400, LI-COR, Lincoln, NE, USA).

In the first experiment, the measurement was done using a chamber set for a photon flux at 7 steps (100, 150, 300, 500, 1000, 1500, 2000 μmol m^{-2} s^{-1}) and CO_2 concentration at 400 ppm and temperature at 20 °C. During the measurement CO_2 was supplied from a portable gas cylinder. The measurement was replicated three times each for FFC and the control plots.

In the second experiment, the photon flux in the chamber was adjusted to 150 μmol m^{-2} s^{-1} and the temperature to 20 °C. Instead of supplied CO_2, ambient air at 1.9 m pole height was used. Measurements were repeated four times each for FFC and control plots. Test leaves were placed in the chamber and photosynthesis and transpiration rates were monitored preliminarily for the first 5 min. After a stable rate was confirmed, the analytical data were collected at 5 sec intervals for 5 min. The photosynthesis and transpiration rates were expressed by the CO_2 amount incorporated per 1 m^2 of leaf area per sec (μmol m^{-2} s^{-1}) and by the H_2O amount released from 1 m^2 of leaf area per sec (mmol m^{-2} s^{-1}), respectively.

Water use efficiency for photosynthesis was calculated by dividing transpiration rate by photosynthesis rate at each photon flux level.

5. *Analyses of growth and yield*

On May 22, 2007, ten mature plants were harvested randomly from 4 locations in the center of the plot to avoid the edge effects on growth. For evaluation of the total yield, all of the remaining plants were harvested from each plot. They were air-dried in a greenhouse until June 2. The number of tillers and total weight of grains per stock, lengths of shoot and ear, number of spikelets per ear and weight per 1000 grains were compared between FFC and control plots using 40 stocks (10 stocks x 4 locations) each data were expressed as mean ± standard deviation, followed by statistical analysis using the student *t*-test. The total weight of grains per stock obtained above was added to provide the total yield for each plot.

6. *Chemical analyses of harvested grains*

Chemical components (crude proteins, lipids and nonfibrous carbohydrates, fibers, and minerals) of harvested grains were compared between FFC and the control plots by applying the routine analytic protocol used for livestock feed. Crude protein, crude ash, crude fat were analyzed by AOAC methods[2]. Fibers were estimated as acid-detergent fiber using the method of Van Soest[23]. Crude proteins were assayed by the Kjeldahl method and crude lipids by ether extraction, was analyzed by the acid-alkali insolubility method and mineral contents were determined by atomic absorption spectrometry. The content of nonfibrous carbohydrates including starch was calculated by subtraction of the content of the components above from the initial weight of samples. Because the water content of grains in both plots ranged between 8.3~8.9%, the values of analyzed components were expressed as percentages of dry mass of the grain.

7. *Qualitative and quantitative analysis of inorganic components*

Ground powder of harvested grains was analyzed using the Evans and Krähenbühl[7] method with some modification. The powder samples were weighed into Teflon vessels (0.5g) and treated with 2 ml of 65% nitric acid and 4 ml of 30% hydrogen peroxide (both Wako Pure Chemicals, Japan). They were treated in a microwave oven (Multiwave 3000, Perkin Elmer, Shelton, CT, U.S.A.) at 200-240 °C for 30 min. After cooling, the sample solutions were diluted and the inorganic components were determined by ICP-AES (Optima 5300DV, Perkin Elmer, Shelton, CT, U.S.A.), using wavelengths of 455, 405nm and 493, 409 nm.

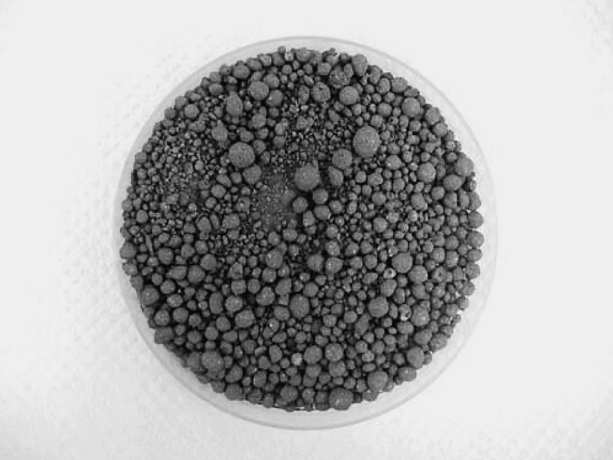

Fig. 1 Granular FFC-ace in a 9 cm Petri dish.

Results

1. *Chlorophyll content, photosynthesis and transpiration of leaves*

Within 10 days after seeding, seedlings emerged both in FFC and control plots and gradually grew during Dec-Feb. As the temperature rose in mid Feb, seedlings in the FFC plot grew more rapidly than those in the control plot : they produced both taller and thicker shoots, more greenish leaves and enhanced tillering (Fig. 2). In Feb 26, 2007, the SPAD value of leaves in the control plot was 37.7±3.6, while that in the FFC plot was 42.4±3.5, indicating that the chlorophyll content was significantly higher in the latter plot ($P<0.05$).

As illustrated in Fig. 3, when photosynthesis was measured in a chamber, with photon fluxes ranging between 100-2000μmol m^{-2} s^{-1} and a fixed CO_2 concentration and temperature, the rate was always significantly greater ($P<0.05$ or 0.01) in leaves of the FFC plot than those in the control plot at photon fluxes higher than 100μmol m^{-2} s^{-1}. The transpiration rates were significantly greater ($P<0.05$ or 0.01) in leaves of FFC than those from control plots at a photon flux range between 150 and 1000μmol m^{-2} s^{-1} (Fig. 4). Interestingly, at the photon flux higher than 1500μmol m^{-2} s^{-1} there were no significant differences ($P>0.05$) in the transpiration rate regardless of the elevated photosynthesis rate (Fig. 3). Similarly, water use efficiency for photosynthesis was significantly greater ($P<0.05$ or 0.01) in leaves of control than FFC plots at the photon flux range between 150 and 500μmol m^{-2} s^{-1}(Fig. 5). Nevertheless, this relationship was reversed in the range between 1500 and 2000μmol m^{-2} s^{-1}.

On the other hand, when the measurements were performed at the fixed photon flux and temperature in the chamber using ambient air, the photosynthesis and transpiration rates in the FFC plot were 5.66±0.49

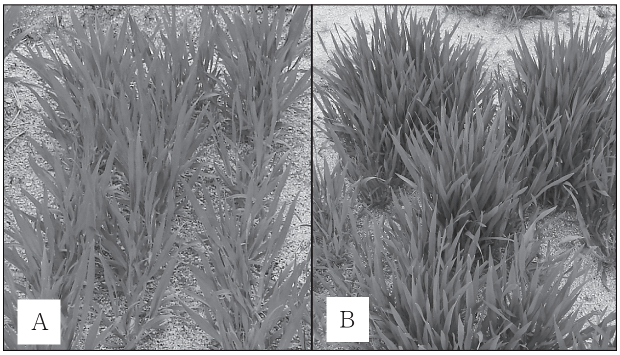

Fig. 2 Growth of barley in a control plot (A) and FFC plot (B) 4 months after seeding. More luxuriant and greener growth in the FFC plot, compared with the control.

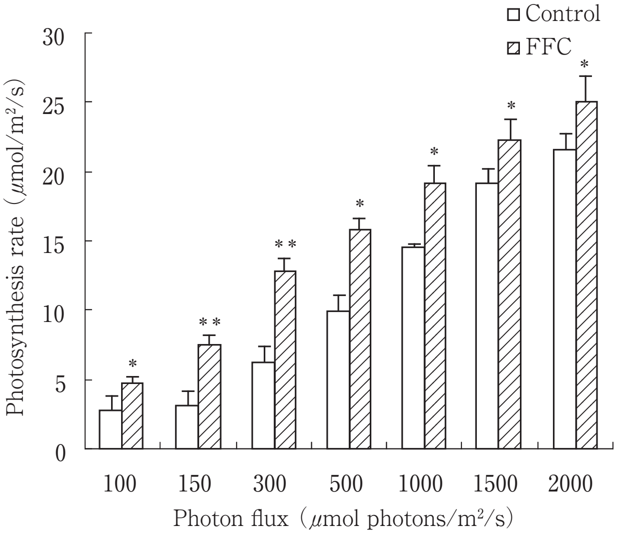

Fig. 3 The photosynthesis rate in leaves of control and FFC plots at photon fluxes ranging from 100 to 2000 μmol m^{-2} s^{-1} and a CO_2 concentration of 400 ppm and a temperature of 20 °C. Note that the rates were always significantly greater in FFC than control plots at all photon flux higher than 100 μmol m^{-2} s^{-1} es examined in this study.

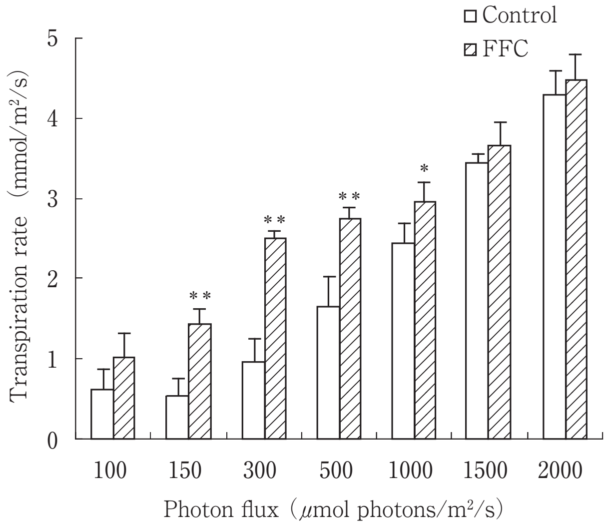

Fig. 4 Transpiration rate in leaves of control and FFC plots at photon fluxes ranging from 100 to 2000 μmol m^{-2} s^{-1}, a CO_2 concentration of 400 ppm and temperature of 20 °C. Note that the rates were significantly greater in FFC than control plots at photon fluxes between 150-1000 μmol m^{-2} s^{-1}. There were no significant differences at photon fluxes higher than 1500 μmol m^{-2} s^{-1}. $^{*}P<0.01$, $^{**}P<0.05$.

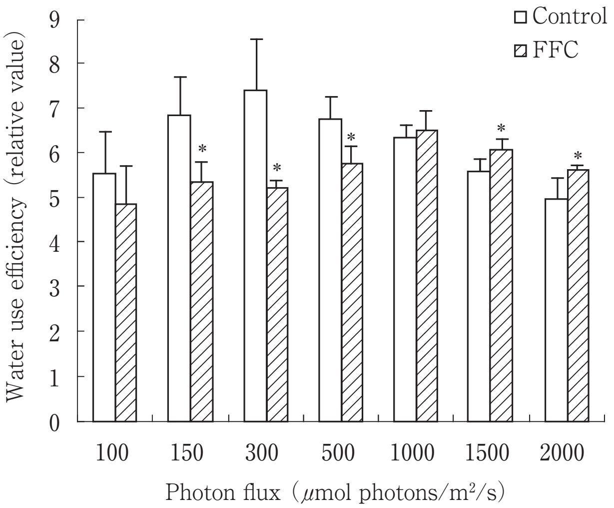

Fig. 5 Water use for photosynthesis in leaves of control and FFC plots. Note that the efficiencies were significantly lower at the photon fluxes between 150-500 μmol m^{-2} s^{-1} but greater at the fluxes higher than 1500 μmol m^{-2} s^{-1} in FFC than control plots $^{*}P<0.01$.

μmol m^{-2} s^{-1} and 1.02 ± 0.10 mmol m^{-2} s^{-1}, respectively, whereas those in the control plot were 4.31 ± 0.44μmol m^{-2} s^{-1} and 0.62 ± 0.11 mmol m^{-2} s^{-1}, respectively. Thus, both rates showed similar tendencies in CO_2 and field air supplied at 150μmol m^{-2} s^{-1} photon flux.

2. *Analyses of growth and yield*

Table 1 summarizes the analytical data for plant growth and yield. The number of tillers was significantly greater ($P<0.01$, almost 2.7 times more) and consequently both the number of grains and total weight of grains per stock were much higher ($P<0.01$) in FFC than control plots. Lengths of shoot and ear and the number of spikelets per ear were again significantly higher ($P<0.05$ and <0.01) in FFC than control plots. This increased growth in the FFC plot led to a yield per plot approximately 1.7 times higher than that in the control plot. The grain weight was a slightly higher in the FFC plot but was not significantly different from that of the control plot.

Table 1 Growth and yield of barley grown in control and FFC plots

	Control plot	FFC plot
No. of tiller	1.5 ± 1.5	4.1 ± 2.1**
Shoot length (cm)	81.7 ± 14.0	92.4 ± 11.9*
Ear length (cm)	3.5 ± 0.8	4.1 ± 0.8**
No. of grains / stock	58.8 ± 33.8	180.7 ± 106.9**
Total weight of grains (g) / stock	1.5 ± 1.1	4.7 ± 2.9**
No. of spikelets / ear	20.0 ± 5.8	23.7 ± 4.2**
Weight of 1,000 grains (g)	23.8 ± 6.6	25.4 ± 3.2
Total yield (g) / plot	1448.1	2504.3

$^{*}P<0.05$, $^{**}P<0.01$

3. *Composition of the grains*

As illustrated in Fig. 6, no significantly different lev-

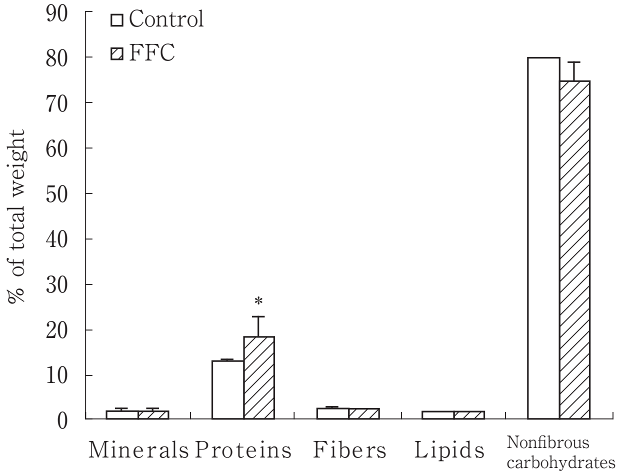

Fig. 6 Quantitative analysis of nutritional components of grains harvested from control and FFC plots. There were no significant differences in most components except protein. $^{*}P<0.05$.

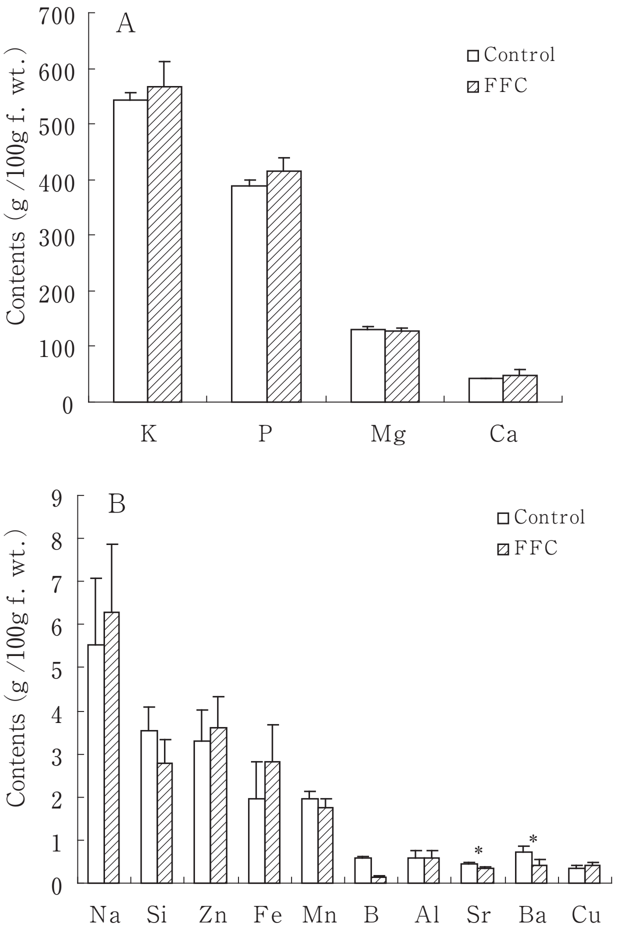

Fig. 7 Quantitative analysis of major (A) and minor (B) inorganic components of grains harvested from control and FFC plots. There were no significant differences in all major and most minor components except Sr and Ba.

els of minerals, fibers and crude lipids in dried grains were observed. They all ranged from 2.11 to 2.55 % in both plots. The percentages of crude protein were 13.18±0.32 and 18.43±4.16, respectively, in the control and FFC plots, showing a significantly higher ($P<0.05$) level in the FFC plot. Nonfibrous carbohydrates, mainly starch were 79.98±0.17 and 74.79±4.25%, respectively, in dry weight in the of control and FFC plots, and were significantly different ($P<0.05$).

Figure 7 shows the analytical data for the inorganic elements in the grain powder. Among the major elements, K, P, Mg and Ca, levels of K and P were relatively higher but there were no significant differences between the plots ($P>0.05$). For the Minor elements, Na, Si, Zn, Fe, Mn, S, Al and Cu, no significant differences were detected. The level of Sr and Ba was a little higher in the control than in the FFC plots ($P<0.05$).

Discussion

As indicated in Table 1, the total yield of grains increased significantly in the FFC plot : almost 1.7 times as high as that of the control plot. Brennan and Jayasena[4] examined the effect of field application of potassium fertilizer on the yield of barley and reported that KCl and K_2SO_4 fertilizers (10–40 kg K/ha) enhanced the yield 1.3~1.4 and 1.2~1.5 fold, respectively. By comparison, FFC-ace demonstrated a greater stimulative effect on growth and yield of barley. This impressive result was caused apparently by enhanced tillering and, hence an increased number of grains per stock. Although numerical analysis of root growth was difficult because too many roots were tangled with fine soil particles, the entire roots in the FFC plot were apparently larger and longer than those in the control plot. Hasegawa et al.[9] proved that the FFC ceramic beads that were manufactured using the same raw materials as those of FFC-ace accelerated root growth of tissue cultured seedlings of mountain laurel. They also confirmed that FFC-ace mixed with pot soil similarly accelerated root growth of the same seedlings. Although the mechanism is still unknown, FFC products seem to have a potential to accelerate root emergence and elongation when mixed in soil.

As illustrated in Fig. 2, the leaves in the FFC plot were greener than those in the control plot. This difference was supported by the SPAD (chlorophyll meter) readings which revealed a higher content of chlorophyll in leaves of the FFC plot. The SPAD value is determined based on the amount of light transmitted by the leaf at two wavelengths in which the absorption of chlorophyll is different, and represents a relative index for the estimation of leaf chlorophyll[11]. The SPAD index is a simple, rapid and non-destructive means of estimating the chlorophyll content of leaves.

Our data showed that the photosynthesis rate was significantly enhanced in the FFC plot when measured at a photon flux higher than 100μmol m^{-2} s^{-1} at a fixed CO_2 supply and temperature (Fig. 3). These data were confirmed in measurements under ambient air condition in the field. Such an elevated level of photosynthetic activity is presumably related to the enhanced growth and greater yield described above. Our results are in agreement with these of Ohsumi et al.[17] who reported that stomatal conductance was an important trait responsible for the genotypic difference in gas diffusion for photosynthesis and transpiration in rice plants. Fischer et al.[8] and Tsunoda and Fukoshima[22] also reported that stomatal conductance and frequency were closely linked with photosynthesis and transpiration and thus yield in wheat and rice, respectively. Therefore, morphological and physiological analyses of the FFC-ace effects on stomatal frequency and conductance need to be investigated further.

The photosynthesis rate was expressed by the amount of CO_2 (μmol m^{-2} s^{-1}) utilized by the plants. For example, approximately 1.5 folds higher CO_2 was absorbed by leaves in FFC than control plots at a photon flux of 300-500μmol m^{-2} s^{-1} (Fig. 3). In other words, the data shown Fig. 3 indicate that a large quantity of CO_2 is absorbed by leaves in the FFC plot. The concentration of CO_2 in the global atmosphere, currently ca 350 ppm, is increasing and is predicted to double by the end of this century[1]. Evidence grows daily of the changing climate and its impact on plants and animals. Plant function is inextricably linked to climate and atmospheric CO_2 concentration. These factors affect the plant's immediate environment and so directly influence physiological processes. An example of this intimate relationship was provided by Aoki et al.[1] who showed that flag-leaf blades of rice grown under high CO_2 accumulated more starch than control leaf blades before heading. Plant growth also influences the local, regional and global climate, through exchange of energy and gases between the plants and the air around them. In this sense, it can be said that agricultural use of FFC-ace has a potential to contribute significantly to reduce atmospheric CO_2 concentrations.

Paralleling the elevated photosynthetic rate, the transpiration rate was also significantly higher in leaves of FFC than control plots at a limited photon flux range between 150 and 1000μmol m^{-2} s^{-1} (Fig. 4). However, there were no significant differences at a photon flux higher than 1500μmol m^{-2} s^{-1}, in spite of significantly higher level of photosynthesis rate in this range (Fig. 3). Data on the efficiency of water use for photosynthesis which was calculated from the data in Figs. 3 and 4 showed significantly a higher level in FFC than control plots at this higher photon flux range. This photon flux level corresponds to that under the intense sunshine, in summer in the temperate zone and almost all dry seasons in the tropical zone. As is well known, more than 1.2 billion people live in areas of physical water scarcity, lacking enough water to meet demand. Moreover, water scarcity, defined in terms of access to and use of water, is a critical constraint to agriculture in many areas of the world. Therefore, FFC-ace may have an invaluable potential in efficient use of agricultural water.

Analytical data in Figs 6 and 7 show that the nutritional and inorganic components were almost the same in the grain harvested from both plots. Only the protein level was significantly higher in plants from the FFC plot. These values reflect physiologically normal growth of the plant in the FFC plot and no absorption of toxic inorganic elements by the plants in this plot.

This paper describes the dramatic effects of FFC-ace on barley growth and yield. FFC-ace enhanced absorption of atmospheric CO_2 through photosynthetic activity. It also elevated efficiency of water use. However, the mechanism of these positive effects increasing agricultural productivity providing and environmental benefits is still unknown. In this study we demonstrated agronomical and physiological effects of FFC-ace. How FFC-ace affects soil structure is an important question that needs to be resolved, because soil structure is a major factor in determining crop yield, it's as Low[16] emphasized. The microbial community in soil also largely affects plant growth[13]. Canbolat et al.[5] examined the effects of mineral and biofertilizers (*Bacillus licheniformis*, *Paenibacillus polymixa* and other bacterial spp.) on many factors concerning barley growth including plant growth promoting rhizobacteria, fungi, seedling growth, soil pH, organic matter content, available P and mineral nitrogen. They concluded that seed inoculation of barley with the biofertilizers significantly increased the population of rhizobacteria and fungi and root and shoot weight by 29.7-43.3%. These data indicate that plant growth is largely influenced by the microbial community through competition, antagonism and/or compensation, as Hyakumachi[12] reviewed. Although the nutritional effects of FFC-ace on plant growth and soil microbes are unknown, a further study of its effects on the microbial community is necessary in order to understand FFC-ace effects.

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土壌改質材 FFC エースによるオオムギの生育と収量の促進効果

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本報は，㈱エフエフシー・ジャパンから販売されている土壌改質材 FFC エース™の作物の生長促進効果について，2006年11月から翌年６月，本学農学部内の実験圃場で実施された，オオムギの生育ならびに収量調査に関する試験結果をとりまとめたものである．実施圃場の砂土壌に FFC エースを所定量混和した区画を設け，オオムギの種子を播種した．なお，対照区は非導入土壌とした．定期的に行った生育調査の結果，FFC エースを導入した土壌では非導入の区画と比べて，生育初期における根の生育が良好となり，地上部における分けつ数の増加とともに穂の生長も旺盛となって，１穂当たりの収穫量（粒数）の著しい増加をもたらした．結果，FFC エース導入区における全収量は非導入区と比べて約1.7倍となった．また，それぞれから収穫したオオムギ粒に含まれる栄養価ならびに無機元素類の量には，FFC エースの導入，非導入によって大きな違いは認められず，導入の効果は収量に大きく反映された．事実，調査期間中に行った測定から，FFC エースを投入した土壌で生育するオオムギ葉は高いクロロフィル量を示しており，光合成が促進されているものと考えられた．実際，播種後４ヶ月目以降，光合成ならびに蒸散速度値を測定した結果，FFC エース導入区で生育したオオムギでは常に高い値を示した．また，FFC エースの導入によって強光条件下における水利用効率が促進された．本報告では，FFC エースの投与と空気中からの二酸化炭素の吸収量との関連について考察するとともに，併せて，FFC エースの土壌への導入によって作物の生育に必要な灌水量を大きく減らすことができる可能性についても言及したい．

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