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# Efficacy of Yeast Cell Wall Extract, a Byproduct of Beer Brewing, in Tomato (*Solanum lycopersicum*) Culture

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**Abstract.** The beer brewing process produces surplus yeast, and yeast cell wall extract (YCWE) is an unutilized byproduct of brewing. In this study, we developed a new phosphorus and potassium liquid fertilizer, CW1, containing YCWE, investigated the effects of CW1 application, and observed that CW1 treatment reduced physiological disorders in fruits of tomato (*Solanum lycopersicum*). CW1 spray on leaves of tomato reduced the incidence of blossom-end rot and increased the total yield of fruits by increasing total number of harvested fruits and average fruit weight. There was no significant difference in Brix or acidity. These results suggest that YCWE exerts plant-activating effects and that the application of CW1 is a new means of using a food residual substance effectively in agriculture.

**Keywords:** Yeast Cell Wall Extract, Liquid Fertilizer, Tomato, Blossom-End Rot.

## 1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most popular and valuable vegetables worldwide. There is concern in the agricultural industry that crop productivity will be adversely affected by increases in average temperature. [1], [2] In Japan, the average temperature in spring and summer is increasing with global warming, and increased incidences of physiological disorders and high-temperature stress symptoms such as excess blossom drop and blossom-end rot are being reported. [2] These physiological injuries reduce tomato fruit yield, incurring economic loss to farmers.

The budding yeast *Saccharomyces pastorianus* is a bottom fermentation yeast used in beer brewing. Given that yeast increases in weight by two to three times during the beer fermentation process, the residual yeast contains surplus organic matter. Yeast extract is widely used as a natural seasoning, a dietary supplement, and a microbiological medium. However, yeast cell wall extract (YCWE), comprising mainly polysaccharides such as polymers of glucose (β-glucan) and mannose (mannoproteins), has not been effectively used. A part of YCWE is used as feed additives to livestock or aquaculture, but most YCWE has been discarded presently at many beer breweries. [3]

These β-glucan and mannoproteins are reported as microbe-associated molecular patterns (MAMPs), activating plant metabolism. [4], [5] MAMPs are common conserved structures in microbes among pathogenic, non-pathogenic, and saprophytic microorganisms and they include chitin, ergosterol and bacterial lipopolysaccarides. MAMPs induce plant defence responses which include the generation of reactive oxygen species, reactive nitrogen species, ethylene (ET) and expression of *pathogenesis-related* (*PR*) genes. In addition, MAMPs trigger the activation of calcium-dependent protein kinases and mitogen-activated protein kinase cascades, which lead to transcriptional changes in numerous genes. [6] - [8]

With its primary ingredient YCWE processed by treatment with cell wall-degrading enzymes, Housaku Monogatari (HM) has been developed as a compound fertilizer (http://www.asahi-fh.com/products/wholesale/good-harvest/). Our previous study indicated that HM induced resistance

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(systemic acquired resistance, SAR) and activated systemic resistance signaling pathways, and activated many metabolic pathways such as salicylic acid (SA) and jasmonate (JA)/ET signaling pathways in *Arabidopsis thaliana*. [9]

In this study, we developed a new phosphorus and potassium liquid fertilizer, CW1, containing YCWE with the aim of preparing an agricultural material focused less on resistance than on fertilizer efficacy.

# 2. Materials and Methods

## 2.1. Preparation of CW1

CW1 was produced using a byproduct, yeast cell wall extract (YCWE), prepared from the budding yeast *Saccharomyces pastorianus* in the beer brewing process. The autolysed yeast slurry was collected after beer fermentation and YCWE was obtained by removal of the supernatant following centrifugation. YCWE contains polysaccharides (15–25% β-glucan, 5–15% α-glucan, 10–20% mannan, and 0.5–2% chitin). CW1 was produced by hydrothermal reaction of liquid containing 15% of YCWE, 5% of $P_2O_5$ (derived from phosphoric acid), and 4% of $K_2O$ (derived from potassium hydroxide). As a control for CW1, PK fertilizer was also produced by mixing the same quantities of $P_2O_5$ and $K_2O$. A 2000-fold dilution of CW1 and PK fertilizer was foliar-sprayed on tomato plants every 10 days after transplanting to a glass greenhouse.

## 2.2. Plant Materials

Tomato (cv. Momotaro-Fight, Takii & Co., Kyoto, Japan) plants were grown in a glass greenhouse at the Ehime Prefectural Research Institute of Agriculture, Forestry and Fishery. The growing season was 6 months from the end of January to June 2013. At the beginning of March, young plants were transferred to soil in the greenhouse that contained $N/P_2O_5/K_2O$ 250/170/210 kg $ha^{-1}$ as basal fertilizer. Planting density was 2.2 plants $m^{-2}$. Every plant was pruned to retain typically one vine and also pruned above the sixth floral cluster. After physiological flower drop, every floral cluster was thinned to four fruits to control the number of fruits per plant.

# 3. Results and Discussion

## 3.1. Characteristics of CW1

Table 1 shows the characteristics of CW1. It comprised 75.4% water, 0.68% nitrogen, 5.0% $P_2O_5$, 4.5% $K_2O$, and 2.4% β-glucan. Since YCWE hardly contained fertilizer components, we added phosphoric acid and potassium hydroxide in order to satisfy the Japanese fertilizer regulation. CW1 also contained little total nitrogen and could not be expected to serve as a nitrogen fertilizer. pH was 5.8 and oxidation–reduction potential (ORP) was −200 mV. The colour was dark brown and the aroma was of that of a roasted material such as chocolate or coffee. Although it is difficult to suspend YCWE because of its dissolubility, YCWE in CW1 was decomposed and well suspended due to hydrothermal reaction and it is assumed that this characteristic makes farmers use it easily.

Table 1: Characteristics of CW1

| Assays | Results |
|---|---|
| pH | 5.8 |
| ORP | −200 mV |
| Total nitrogen | 0.68% |
| $P_2O_5$ | 5.0% |
| $K_2O$ | 4.5% |
| Moisture | 75.4% |
| β-glucan | 2.4% |

## 3.2. Plant Growth and Fruit Quality and Yield

To investigate whether CW1 acts as a plant activator in addition to a phosphate and potassium fertilizer, three test plots were established as CW1, PK fertilizer, and water control. Each plot contained 21 tomato plants (seven plants and three replications).

In the early and middle growth stages, there was no significant difference in plant height between CW1, PK fertilizer, and water. However, in the late growth stage, the height of plants sprayed with CW1 became higher than those in the PK and water treatments (Fig. 1). Although nitrogen generally plays a primary role in vegetative stage growth, a 2000-fold dilution of CW1 exerts little or no nitrogen effect. Although differences in root spread were not investigated in this study, our previous study showed that YCWE treatment promoted the root spread of wheat seedlings and of *A. thaliana* (data not shown). CW1 may also promote the uptake of basal nitrogen fertilizer by activating the tomato root system especially in the late growth stage.

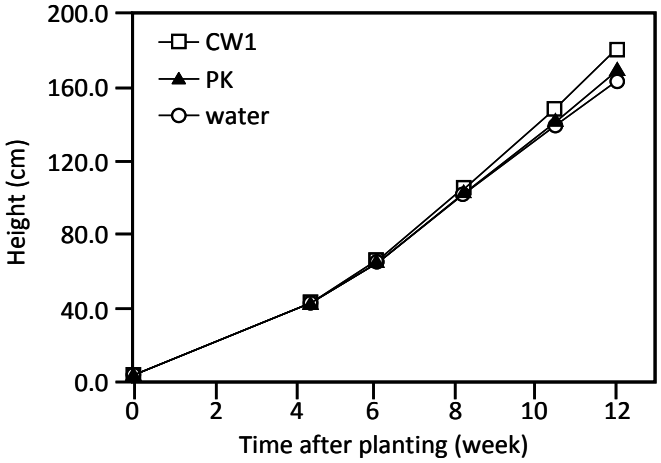


Fig. 1: Height of tomato plants under three fertilization treatments over time

After physiological flower drop, every cluster had 6–7 flowers on an average. CW1 treatment did not affect flower bud formation in tomato (Fig. 2A). Every plant was pinched off above the sixth floral cluster and every cluster was reduced to four fruits for conformity to Ehime prefectural customs. During this test time little plant diseases were observed and there were no significant differences in the incidence rate of diseases between CW1, PK fertilizer and water treatment.

CW1 foliar spray increased fruit yield more than PK fertilizer or water treatment (Fig. 2B). The average yield in CW1 plots was 3.5 kg per plant and this was equivalent to 77 t $ha^{-1}$. We suggest two main reasons for this yield increase. The first was that CW1 increased the number of harvested fruits by reducing the incidence of blossom-end rot, particularly from the first to third floral clusters (Fig. 2C, D). This result indicated that CW1 promoted the absorption of calcium from the soil by promoting the spread of plant roots. It is well known that blossom-end rot is caused by calcium-deficiency. [10]

The second proposed reason for increased fruit yield was that CW1 treatment increased individual fruit weights in every floral cluster (Table 2). The average tomato weight in CW1 plots was 175.5 g and was 1.1–1.2 times that of PK and water. This effect may be generally due to the promotion of photosynthesis, absorption of PK fertilizer, or translocation of photosynthesis products from leaves to fruits. As for photosynthesis, our previous study showed that the photosystem II proteins of Lettuce (*Lactuca sativa*), such as chlorophyll a-binding protein, ATP synthase, and RuBisCO, were induced by treatment of YCWE, so it is suggested that CW1 treatment also promotes the plant photosynthesis and fruit enlargements. [11] There were no significant differences in Brix or acidity of tomato fruits (Table 3). Elucidating the mechanism underlying these effects awaits an analysis of transcriptomic or metabolomic changes triggered by CW1 treatment.

Our results indicate that CW1 exerts plant-activating effects and increases tomato fruit yield. In 2012, beer production around the world was more than 187 million kL and it is conceivable that almost all occurring YCWE are discarded or not used effectively. [12] Our finding suggests that the production and application of CW1 is a new tool of using a food residual substance effectively in agriculture.

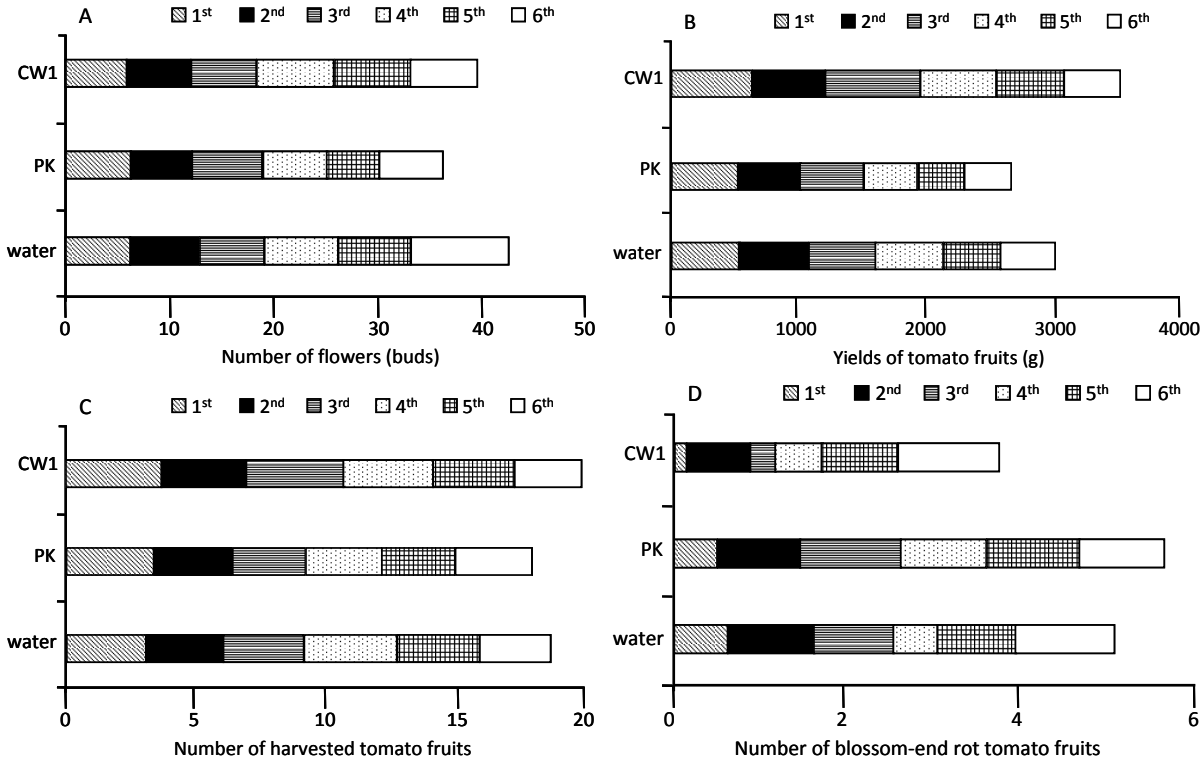


Fig. 2: Effect of CW1 treatments on floral fruits clusters per plant. A: number of flowers (buds) before physiological drop. B: total weight of tomato yield. C: numbers of fruits that could be harvested. D: numbers of blossom-end rot tomatoes.

Table 2: Average tomato weights by floral cluster

| | Fruit weight (g) | | | | | | |
|---|---|---|---|---|---|---|---|
| Floral cluster | 1st | 2nd | 3rd | 4th | 5th | 6th | Total average |
| CW1 | 169.1 | 171.0 | 205.7 | 175.6 | 169.3 | 163.4 | 175.5 |
| PK | 152.8 | 158.2 | 152.5 | 140.4 | 134.4 | 130.8 | 144.8 |
| Water | 156.1 | 167.0 | 157.1 | 146.9 | 145.9 | 126.1 | 149.9 |

Table 3: Average Brix and acidity of tomatoes by floral cluster

| | Brix | | | | | | | Acidity | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Floral cluster | 1st | 2nd | 3rd | 4th | 5th | 6th | Total ave. | 1st | 2nd | 3rd | 4th | 5th | 6th | Total ave. |
| CW1 | 5.2 | 4.6 | 5.8 | 4.9 | 4.6 | 4.8 | 5.0 | 0.44 | 0.43 | 0.42 | 0.38 | 0.30 | 0.40 | 0.39 |
| PK | 5.1 | 4.5 | 5.5 | 4.5 | 5.0 | 4.8 | 4.9 | 0.40 | 0.40 | 0.44 | 0.32 | 0.36 | 0.39 | 0.39 |
| water | 5.0 | 4.2 | 4.7 | 5.3 | 5.2 | 5.3 | 5.0 | 0.42 | 0.39 | 0.39 | 0.39 | 0.41 | 0.49 | 0.41 |

Brix was measured by a refractometer. The acidity of samples were titrated to pH 8.1 using 0.1N NaOH to determine titratable acidity and reported as % citric acid.

## 4. Acknowledgements

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