# The remarkable increasement of maysin and maysin derivatives contents from *Eremochloa ophiuroides* by various stresses

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## 1. Introduction

Centipedegrass belonging to the genus *Eremochloa* (Poaceae) consisted of eight species native to China and Southeast Asia and became one of the most popular lawn grasses in South America [1]. Maysin, flavone *C*-glucoside, in centipedegrass reported antibiotic to resistance to fall armyworm (FAW; *Spodoptera frugiperda*) [2,3].

Until now, maysin found in the maize silk, teosinte, and centipedegrass [4,5]. These plants contained an infinitesimal amount of maysin, in addition, maysin was not able to obtain by chemical synthesis. Therefore, it could be named enough that maysin was the world premium natural compound.

To synthesis maysin *in vivo*, precursors like luteolin, isoorientin, and rhamnoslyisoorientin was required [6].

The investigation on maysin and maysin derivatives have been carried out very limitedly because maysin and maysin derivaeives were not able to synthesize chemically and the rote for obtaining these compounds was very limited. Therefore, the aim of present study was to investigate the methods for obtaining higher yields of maysin and its derivatives from centipedegrass using various stress sources such as gamma-ray, UV-B, and wounding (folding).

## 2. Methods and Results

### *2.1. Fall Armyworm (FAW; Spodoptera frugiperda) Larvae Bioassay*

FAW larvae bioassay was analyzed according to a modified method by Johnson et al. (2002). The green and red leaves of centipedegrass were used for FAW larva bioassay. These samples were placed in petri dishes (15 cm diam. by 1.5 cm deep) on moistened filter paper, and each dish was infested with ten 3- to 4-d-old FAW larvae. Fresh grass clippings were added to each dish in everyday, and moistened the filter paper was changed in everyday. The larvae were allowed to feed for 7 d, and surviving larvae counted for everyday. We performed at least three times for bioassay, independently.

### *2.2. Sample Preparation and Various Treatments of Stresses*

The matured green leaves were folded two third of leaf for wounding stress, and gamma irradiated 100 Gy for 4 h using a cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, ON, Canada) in the Korea Atomic Energy Research Institute (KAERI), and UV-B irradiation was performed daily with a 5 min pulse (5.5 W $m^{-2}$, corresponding to 0.26 KJ UV-B $m^{-2}h^{-1}$) using a UV-B lamp by a modified method by Park et al. (2007). After 20 days, the leaves (0.2 g) were extracted with 100% methanol (2 ml) for 24 h at room temperature in the dark. Samples were filtered by Millex-FG 13 MM filter (0.20 μm, Millipore).

### *2.3. High-performance liquid chromatography (HPLC) analysis for maysin and maysin derivatives*

Chromatography analysis was performed using an Agilent Technologies 1100 series system equipped with UV detector (Agilent Technologies, Palo Alto, CA, USA) and an ODS column (150 × 4.6 mm, 5 μm, YMC-Pack ODS-A, Japan). The mobile phase consisted of 0.1% formic acid (A) and 100% methanol (B). The gradient program was as follow; 0-30 min, 0-50%; 30-60 min, 50-100% (B). The flow rate was performed with 0.5 ml/min and eluted compounds were detected at 360 nm. The sample injected with 20 μl.

### *2.4 Larva Survival Rate*

Larva survival rate was remarkably different in green and red leaves as shown in Fig. 1. While all larvae were survived after 7 days feeding of green leaves, only 40% of larva were after 7 days feeding of red leaves. According to this result, we postulated that red leaves would contain high level of maysin and maysin derivatives that confer greater antibiotic resistance to fall armyworm larvae than found in green leaves. We observed that all samples treated by various stresses such as wounding, UV-B, and gamma ray were converted into red color from green and it took about 20 days after treatments

A

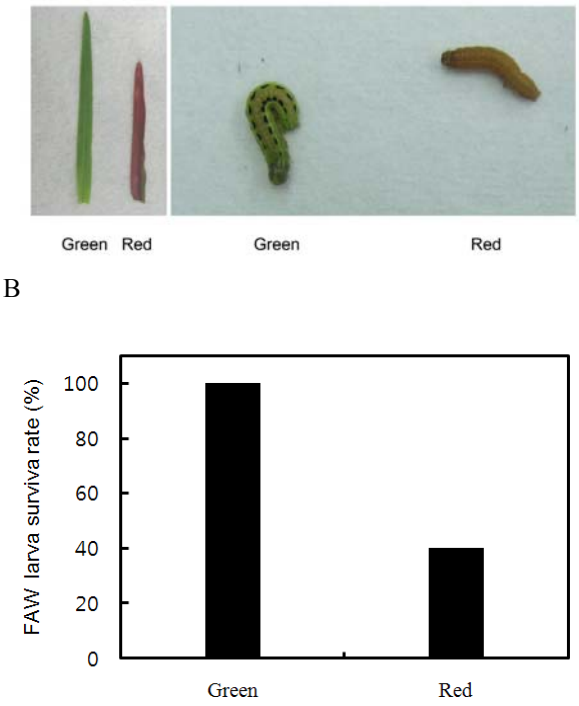


Fig. 1. Bioassay of FAW larvae by different color leaves of centipedegrass. A, Green and red leaves of centipedegrass and pictures of FAW larvae after feeding for 7 days. B, FAW larva survival rate of FAW larvae after feeding for 7 days.

### *2.5 Change of the Matured Green Leaves by Stresses*

The matured green leaves were changed red leaves in nature (2 month) and after treatments about 20 days (Fig. 2).

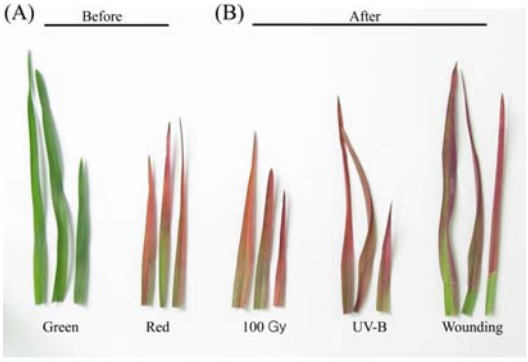


Fig. 2. The color changes of centipedegrass leaves before (A) and after (B) various stress treatments such as gamma ray, UV-B, and wounding for 20 days.

### *2.6 The Contents Changes of Maysin and Maysin Derivatives in Centipedegrass by Various Treatments*

The contents changes of maysin and maysin derivatives in centipedegrass by various treatments were shown in Table 1. The contents of maysin and its derivatives were increased by changing from green leaf to red leaf in natural. The contents of maysin and its derivatives were increased by various treatments compare to in natural.

Table I: The contents maysin and maysin derivatives in centipedegrass by various treatments

| Compounds (μg/ml) | Before | | After | | |
|---|---|---|---|---|---|
| | Green | Red | 100 Gy | UV-B | Wounding |
| Luteolin | 0.75±0.01 | 0.64±0.01 | 0.86±0.01 | 1.00±0.01 | 1.29±0.01 |
| Isoorientin | 9.69±0.38 | 41.72±0.10 | 40.80±0.01 | 91.05±0.45 | 119.50±0.34 |
| Rhamnosylisoorientin (with orientin) | 4.40±0.14 | 15.43±0.01 | 9.06±0.03 | 25.20±0.01 | 37.52±0.04 |
| Derhamnosylmaysin | 12.58±0.01 | 13.86±0.03 | 48.67±0.03 | 58.54±0.01 | 93.65±0.04 |
| Maysin | 9.00±0.05 | 18.87±0.10 | 29.28±0.09 | 22.45±0.05 | 32.57±0.09 |
| Luteolin-6-*C*-boivinopyranoside | 1.51±0.01 | 1.00±0.01 | 2.07±0.02 | 3.45±0.02 | 5.58±0.02 |

## 3. Conclusions

The red leaves have much stronger antibiotic effects than green leaves. Therefore, the aim of stress treatment was making red leaves from green. Green leaves of centipedegrass were effectively turned into a red color by various stresses. According to the results wounding and gamma irradiation were promising methods for increasing the contents of maysin and maysin derivatives.

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