

論文の内容の要旨

生産・環境生物学専攻
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論文題目

Physiological analyses of transgenic rice expressing a fungal glutamate dehydrogenase gene

(グルタミン酸脱水素酵素遺伝子を導入したイネの生理学的解析)

The excessive application of nitrogen fertilizer to maximize crop yields causes negative environment effects such as pollution and ecological imbalance. Therefore, there is a need to improve the ability of crop plants to utilize nitrogenous fertilizers by promoting nitrogen uptake, assimilation and metabolism. Recently, rice has been used for feeding livestock as well as for human food. In Japan, forage rice, a new variety for whole-crop silage has been developed. The present thesis shows successful generation of transgenic rice plants expressing *gdhA*, and examines the effects of the introduction of a fungal glutamate dehydrogenase gene (*gdhA*) into forage rice by analyzing the transgenic rice plants in terms of growth, source function, dry matter production, nitrogen contents and nitrogen uptake efficiency.

1. Generation of transgenic rice plants expressing *gdhA* and variety selection

To express introduced *gdhA* constitutively within transgenic rice plants, we used a rice elongation factor-1 beta promoter (EF1 β ; Gene Locus Os04g0118400) and a rice prolamin 10 terminator (P10; Os03g0766000). The resultant EF1 β promoter–*gdhA*–P10 terminator was amplified by PCR and introduced into pSTARA R-4 (Inplanta Innovations, Yokohama, Japan) with substitution of promoter and terminator region of mutated acetolactate synthase (mALS) gene to produce a binary vector for rice transformation. The results of PCR and RT-PCR indicated that *gdhA* was successfully introduced and expressed in the transgenic plants. By the results of pot experiment, introduction of *gdhA* led to a significant increase in the plant height, root length, leaf area, the chlorophyll concentration, relative RuBisCO concentration and soluble protein concentration in two of transgenic lines (TG 7 and TG 10) of Momiroman under both high and low nitrogen treatment conditions. The leaf, stem and root dry weights of transgenic line (TG 30) of Yamahoshi were higher than those of control line in both high and low N plants. Because of all the results of transgenic plants of Leafstar were lower than control line and the poor qualities of the seeds, and previously our research group already reported expression of a *gdhA* in food rice plants (cv. Yamahoshi) (Abiko et al., 2010). So we decided to continue the following research with Momiroman.

2. Effect of *gdhA* expression on GDH activities, dry weight, nitrogen content and nitrogen uptake efficiency at the seedling stage

In order to reduce the influence of factors or substances other than that of nitrogen, hydroponic experiments (NH₄NO₃ as nitrogen source) were carried out from this chapter. We used 360 μ M and 90 μ M nitrogen as high nitrogen (high N) and low nitrogen (low N) treatment. All the transgenic plants used for analyses were checked by a genomic PCR with regard to the introduction of *gdhA*. The 6-week-old seedlings were sampled after 10 and 24 d of nitrogen

supplementation. The GDH activities were higher in TG 7 and TG 10 than in TG 5 regardless of nitrogen conditions. We called the former two ‘high *gdhA*-expressors’ and the latter ‘low *gdhA*-expressor’. Plant growth analysis at the seedling stage revealed that the leaf area and shoot and root dry weights of the high *gdhA*-expressors were higher than those of control plants in both high and low nitrogen conditions. These results suggested that the source ability was enhanced by the *gdhA* introduction. The nitrogen content and nitrogen uptake efficiency of high *gdhA*-expressors were significantly higher than control line under both high and low N treatment conditions.

3. Effect of *gdhA* expression on the Pn, grain weight, nitrogen content and uptake efficiency at the harvest stage

According to some previous reports, transgenic plants expressing fungal or bacterial GDHs that grown in the field produced significantly higher grain weight than did control plants (Ameziane et al., 2000; Abiko et al., 2010; Zhou et al. 2014). To assess the effects of the *gdhA* introduction on the photosynthetic capacity and productivity of forage rice, the control and transgenic rice plants were grown under hydroponics culture conditions. Experiments in chapter 2 suggested that the source ability was enhanced by the *gdhA* introduction. This was supported by the fact that the net photosynthesis rate at the heading stage was higher in transgenic than in control leaves. Furthermore, under both high and low N conditions, the nitrogen contents in the shoots and roots, at seedling and grain-harvest stages, were significantly higher in high *gdhA*-expressors than in control plants, indicating that nitrogen uptake was higher in transgenic than in control plants. At the harvest stage, the high *gdhA*-expressors exhibited greater panicle and spikelet numbers per plant compared with control plants, resulting in higher grain weight under the high N conditions.

4. Effect of *gdhA* expression on the abiotic stress

It has been reported that transgenic plants expressing fungal or bacterial GDH showed improved tolerance to abiotic stresses (Nolte et al., 2004; Lightfoot et al., 2007; Du et al., 2014). Therefore, the introduction of NADP(H)-GDH from microorganisms could improve plant tolerance under stress conditions. To examine the effect of exogenously introduced NADP(H)-GDH on salt and drought stress, we examine the salt and drought tolerance capacity of the transgenic and control plants at the three-leaf stage under 50mM, 100mM, 150mM NaCl and 10% PEG 6000 solution. The NADP(H)-GDH activities were significantly higher for transgenic than control plants under high salt conditions. The dry weights and nitrogen content of high *gdhA*-expressors were significantly higher than control line under all NaCl concentration and 10% PEG treatments conditions.

In conclusion, the present thesis showed that the introduction of a fungal *gdhA* into forage rice could lead to higher source ability, better growth and higher grain weight by enhancing nitrogen uptake efficiency. In addition, *gdhA* expression in forage rice significantly enhanced their tolerance to salt and drought stress compared to control plants.