Diversity and Mechanism of the Photosynthetic Induction Response among Various Soybean [*Glycine max* (L.) Merr.] Genotypes

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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops in the world, and its dry matter productivity have been the target of the breeding effort for several decades. In the field, fluctuation of the light intensity in the plant canopy is very common phenomenon caused by the scattered clouds and canopy shading. In such condition, the leaf photosynthetic rate does not reach its maximum immediately but gradually approaches toward a maximum value. This phase is called "photosynthetic induction response". The induction response against the strong light potentially limits the carbon assimilation, because the photosynthetic response is generally slower compared with the light fluctuation. Consequently, a faster photosynthetic induction would be a target to improve photosynthetic performance and dry matter productivity of the plant canopy. There are limited studies focused on such the non-steady state photosynthesis of crop species. This study aimed to elucidate the genetic diversity and its physiological mechanism of the photosynthetic induction response among various soybean genotypes. The impact of the observed difference in the induction response on the dry matter production was also evaluated. This information will be useful for improving the photosynthetic performance and the productivity of the soybean in future breeding programs.

2. Evaluation of the genotypic difference and underlying physiological mechanism in the photosynthetic induction response among modern soybean cultivars

The photosynthetic induction response against the sudden increase of light intensity was evaluated among seven soybean cultivars developed in the USA and Japan. The seven soybean cultivars included two Japanese (Tachinagaha: Tc and Fukuyataka: Fy), four US (UA4805: UA, Stressland: St, and 5002T, and LD003309: LD), and a recombinant inbred line derived from a cross between St and Tc (ST053). Four plants of the seven soybean genotypes were sown into 60 × 20 × 20 cm plastic pots, seeds were derived from previous year cultivation in Japan. Two genotypes were sown per pot so in total there were eight plants per pot, with row spacing of 10 × 15 cm. The plants were grown in an air-conditioned greenhouse at the Graduate School of Agriculture, Kyoto University (35°2″ N, 135°47″ E, 65 m altitude) in June 2013 and air temperature was maintained at 28°C. For further analysis of cultivars Tc and UA, experiments were repeated in controlled environment chambers (CMP 6050; Conviron) at the University of Illinois with a photon flux of 1200 μmol m^{-2} s^{-1} at plant height supplied by a mixture of high pressure mercury and sodium lamps, for a 14 hour photoperiod and 25/20°C for day/night temperature, and a maximum leaf to air water vapour pressure deficit of 1.2 kPa. The seven genotypes of soybeans were grown in the experimental field of the Graduate School of Agriculture, Kyoto University for measuring photosynthesis maximum. For all experiments photosynthetic CO_2 and water vapour exchange was measured with an open gas exchange system incorporating infra-red CO_2 and water vapour analyzers, and with the leaf enclosed in a controlled environment cuvette with precisely regulated CO_2 concentration, humidity, temperature and photon flux provided by a mixture of red and blue LEDs (LI-6400, LI-COR Inc., Lincoln, NE, USA). Photosynthesis was initially induced in low light (PPFD = 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to simulate deep shade until a steady-state rate was obtained, and then the light flux was increased to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. To understand the basis of variation in induction rate between cultivars Tc and UA, this experiment was repeated at six different CO_2 concentrations (100, 200, 300, 400, 600, and 800 $\mu mol \cdot mol^{-1}$). This allowed calculation of Vc_{max}. J_{max} and stomatal limitation (Farquhar et al., 1980; Jones, 1985).

There were clear differences in the induction response among these soybean genotypes. Cultivars UA and Fy showed the fastest induction response compared with other 5 cultivars, and cultivar "Tachinagaha" (Tc) showed the slowest induction response. As an indicator of the kinetics of the induction response, the photosynthetic rate (P_n) at 600s after starting the strong light illumination ranged from 13.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in Tc to 23.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in UA. Combining the measurement of induction response with the CO_2 concentration control, the physiological mechanisms underlying the observed difference in the induction responses were analyzed. Major part of the induction kinetics was not explained by the stomatal opening, but by the in vivo activity of the carbon fixation by Rubisco (Vc_{max}). The Vc_{max} at 600s after starting the strong light illumination was 70.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in UA, while that of Tc was only 26.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. There was no clear relationship between the kinetics of the induction response and the maximum photosynthetic rate under the steady state (P_{max}).

3. The diversity of the photosynthetic induction response among the 37 parental lines of Soybean Nested Association Mapping (SoyNAM) population

Evaluation of the difference of induction response among a larger collection of soybean germplasm was conducted using the parental lines of SoyNAM (Soybean Nested Association Mapping) population. The 37 SoyNAM parents are derived from various countries, and consists of 17 high-yielding lines, 11 diverse-ancestry germplasms, 8 drought-resistant lines, and a hub parent (IA3023 (RC)). The 37 SoyNAM lines were sown into 18 cm diameter × 14 cm tall plastic pots with four replications. One plant was sown per pot. Unsterilized alluvial sandy loam soil (Fluvic Endoaquept)-vermiculite mix (1:1) was used as the growing medium, and liquid fertilizer (HYPONeX, Japan) was applied with 1,000 × dilution after cotyledon expansion and again on expansion of the second trifoliate. Treatments in all experiments were designed to ensure that plants would not be nutrient limited. The seeds were planted in pots, which were placed beside the experimental field at the Graduate School of Agriculture, Kyoto University on June 3, 2014 under natural conditions. The four representative lines were selected based on the results of the first experiment, and again grown in September 2014 at the greenhouse for the additional measurements. In the green house, the relative humidity (~70%) and temperature (26°C) were regulated by an air conditioner. All of the 37 SoyNAM parents were also seeded in the experimental field for measuring maximum photosynthesis. Photosynthetic measurement method was similar to the previous experiment (Chapter 2) with a shorter duration of high light for 5 min. The measurement was conducted for 37 genotypes at the vegetative (V4) stage, and it was repeated at the seed filling (R5) stage for 20 of 37 randomly selected genotypes.

The cumulative CO_2 fixation (CCF) during the induction response for 300 s was calculated by the following equation:

$$\mathrm{CCF} = \int_{0}^{300} \boldsymbol{Pn}\,(\boldsymbol{t})\ \boldsymbol{dt}$$

where $P_n\ (t)$ is the temporal photosynthetic rate recorded every 10 s during the induction response.

There was a large variation of the induction response among 37 SoyNAM parents. The cumulative CO_2 fixation (CCF) during the first 5 min of high light exposure was varied from 0.81 mmol CO_2 m^{-2} $5min^{-1}$ for NAM12 to 5.5 mmol CO_2 m^{-2} $5min^{-1}$ for NAM23 at the vegetative stage. The observed differences of the induction response among 37 lines were consistent between the vegetative and the reproductive stages. There was no correlation between CCF and P_{max} measured in the field condition, suggesting the independent regulation of the steady and non-steady state photosynthesis among soybean genotypes. Nevertheless, the present study suggests that the photosynthetic induction response under non steady light conditions is a link to leaf photosynthesis with biomass production in the soybean. It encourages the future cultivation of soybean with a greater P_{max} and a faster induction response. In conclusion, the present study reveals the large genetic diversity of the photosynthetic induction response with a sudden increase in the light intensity in 37 soybean genotypes. The observed difference was stable throughout the growth season. Genetic improvement of the photosynthetic induction response should be enhanced, to increase the photosynthetic capacity for greater biomass productivity in soybean.

4. The impact of the photosynthetic induction response on the dry matter productivity among soybeans with extremely fast and slow induction response

The high and low light intensity was applied to NAM12 and 23 in every 30 min with 14 h photoperiod along with the control that received a constant light intensity. The growth chamber at the

University of Illinois and the LED greenhouse at Kyoto were used for this experiment. Light treatment was set at constant and fluctuating light (CL and FL). In both places, the FL was set with the ON-OFF of the light at every 30 minutes with the total light exposure during daytime being 7 hours for both places. In the CL the light intensity was set constant a 1200 PPFD for growth chamber and 1000 PPFD for the LED greenhouse, photoperiod was 14 hour day. Photosynthetic measurement was conducted at the vegetative stage (V4/V5, 4^{th} and 5^{th} trifoliate) on the central trifoliate of the uppermost expanded leaf for 6 hours fluctuation light intensity using tracking PAR function. Data was recorded every 20 s. Photosynthetic capacity was measured at 26°C with a relative humidity of 70%; CO_2 concentration of the chamber was set at 400 $\mu mol \cdot mol^{-1}$.

At 31 and 20 d after seeding for Illinois and Kyoto respectively, aboveground dry matter (DM) of NAM 23 was significantly greater than that of NAM 12 under both of the fluctuating and constant light treatments. On average of the two experiments, continuous measurement of the leaf photosynthetic rate for 6 hours showed that the CCF of NAM 23 reached 179 mmol CO_2 m^{-2} s^{-1} $6h^{-1}$, while that of NAM 12 was 123 mmol CO_2 m^{-2} s^{-1} $6h^{-1}$. Thus I propose that the photosynthetic induction response could be involved in the observed genotypic difference in DM under the fluctuating light intensity. It might be difficult to find the direct connection between the photosynthetic induction response and the DM productivity in the field. With the significant difference in CCF between NAM23 and NAM12, however, it can be stated that a faster induction response would contribute to the carbon fixation in a single leaf level. Further research is needed on this point. As a whole, the significant difference of CCF was observed between NAM23 and NAM12 under the FL light condition. The limiting factor of the induction response was suggested to be caused by the difference in g_s as well as the biochemical process.

5. General discussion and conclusion

This study demonstrated the diversity of the photosynthetic induction response among various soybean genotypes. To the best of my knowledge, this is the first report showing such a clear variation of the induction response among genotypes of a single crop species. The observed difference was attributable to the activation speed of the carboxylation by Rubisco. The biochemical and genetic mechanisms which determines the speed of photosynthetic induction response, however, remains to be explored. The potential impact of the photosynthetic induction response on the dry matter production was also suggested. Through these findings, the kinetics of the photosynthetic induction response was proposed as the important breeding target to achieve a greater dry matter productivity in soybean.