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Assessing Variation in Photosynthetic Performance of Soybean Using MultispeQ Phenotyping

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Summary

Soybean, a globally important crop, requires improved yield potential to meet the rising demand without expanding production areas. Photosynthesis is fundamental to plant growth and a key target for yield enhancement. This study aimed to evaluate the variation in photosynthetic efficiency among 54 elite soybean breeding lines using the non-destructive MultispeQ tool. Field experiment was conducted in 2024 at Lee Farm, Portageville, Missouri. Photosynthetic traits, including SPAD, Light intensity (PAR), PSII efficiency (FvP/FmP), maximum quantum yield (Phi2), non-photochemical quenching (NPQt) and linear electron flow (LEF), were measured at the R2-R3 stage. Significant phenotypic variation was observed across the breeding lines for key traits, such as SPAD (mean 43.3), Phi2 (mean 0.36), and LEF (mean 211

μmol m²/s), highlighting genetic diversity in photosynthetic performance. Correlation analyses revealed positive associations between FvP/FmP and Phi2 (r = 0.47), as well as LEF and PAR (r = 0.80), indicating a strong relationship between PSII efficiency and light-use efficiency. Conversely, NPQt negatively correlated with Phi2 (r = -0.45), illustrating a trade-off between energy dissipation and photosynthetic performance. These findings provide insights into the genetic potential of photosynthetic traits for breeding programs. Future analysis of seed yield will further elucidate the role of these traits in improving soybean yield, supporting targeted breeding efforts to enhance crop performance under varying environmental conditions.

INTRODUCTION

Soybean is one of the world's most important food crops and serves as a crucial source of vegetable protein and oil. To meet the growing global demand for soybean, the rate of yield improvement must double to avoid further expansion of production areas (Zhu et al 2010; Rains 2011). Photosynthesis is the fundamental physiological process driving plant growth and development. Future improvements in crop yield will largely depend on enhancing net photosynthesis and energy transduction efficiency (Ainsworth et al 2008; Parry et al 2011). Leveraging natural variation in photosynthetic capacity offers an opportunity to breed genotypes with enhanced carbon assimilation (Parry et al. 2011; Faralli and Lawson 2020). Multiple studies have shown that improving photosynthetic (PS) efficiency is a promising strategy for increasing soybean yield potential, especially in the context of climate change (Zhu et al 2010; Long et al 2006;). Other than genetic characteristics of the plant (genotype), photosynthesis also depends upon the influence and constraints of environmental parameters including light, temperature, CO_2, moisture etc.

Therefore, it is important to evaluate the variation in photosynthetic performance in natural and breeding populations to utilize this variability to improve seed yield in soybean (Araus et al 2016; Sakoda et al 2016). Traditionally, different methods have been in practice to capture photosynthetic data such as via gas exchange measurements, which measure CO_2 assimilation rates and stomatal characteristics of the leaves. Another major technique involves chlorophyll fluorescence (CF) which provides insights into photosynthetic performance by assessing light energy capture, distribution, and photosystem II (PSII) efficiency in both controlled and field conditions. Screening diverse and elite soybean germplasm for photosynthetic traits is critical for capturing valuable variation and elucidating the genetic basis of PS efficiency (Montez et al 2022; Ort et al 2020; Dhanapal et al 2016).

Non-destructive phenotyping methods are required for effective screening breeding materials in field conditions (Araus et al 2014; Meacham-Hensold et al 2020). Understanding the relationships between photosynthetic traits and seed yield is crucial for leveraging these phenotypic correlations in a breeding program. The first objective of this study was to assess and characterize the variation in multiple PS-traits among soybean breeding lines using a non-destructive phenotyping tool named 'MultispeQ'. In addition, the interrelationships among various photosynthetic traits will be identified and analyzed.

MATERIALS AND METHODS

Fifty-four soybean breeding lines (Maturity group IV late) from advanced yield trials were evaluated in 2024 at Lee Farm, Portageville, Missouri. The experiment was laid out in a randomized complete block design (RCBD) in 2 replications. MultispeQ data collection was done at full flowering (R2-R3) stage on a clear day (between 10.00 AM and 2.00 PM) using upper canopy leaf (**Fig.1**) for different photosynthetic traits including SPAD, Light intensity (PAR), PSII efficiency (FvP/FmP), PSII maximum quantum yield (Phi2), Non-photochemical quenching (NPQt), Linear electron flow (LEF), leaf thickness, leaf angle and leaf temperature. Descriptive statistics (Mean, SD, Min and Max) were calculated for all traits to capture variation within breeding lines. Analysis of variance (ANOVA) were performed to test the significance of differences among lines for each photosynthetic trait. Then, Pearson Correlation coefficients were computed to investigate the interrelationships among measured PS traits, providing insights how these photosynthetic traits interact in our advanced breeding material. The statistical analyses were performed in R-studio software using agricolae, ggplot and dplyr packages (R core team 2022).

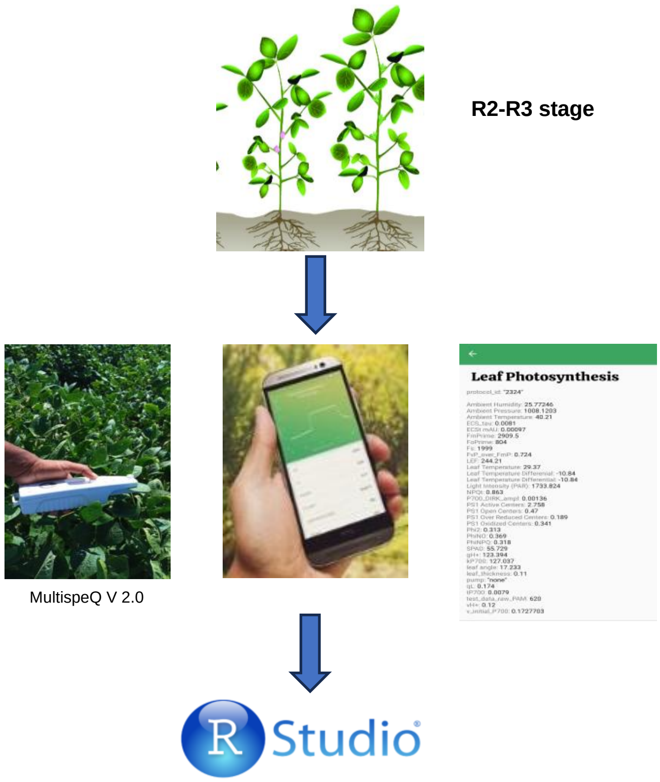

Figure 1. Experimental workflow of photosynthetic data collection and analysis.

RESULTS AND DISCUSSION

To investigate the variation in photosynthetic performance using high throughput non-destructive phenotyping tool MultispeQ 2.0, we used 54 advanced breeding lines (MG IV). Good phenotypic variation was observed in multiple photosynthesis related traits including FvP/FmP (0.70), SPAD (Mean 43.3), Phi2 (mean 0.36) indicating differences in relative chlorophyll content, photochemical efficiency and overall photosynthetic performance of the advance breeding lines. Notably, lines with higher Phi2 and LEF (Mean 211 μ moles/m^2/sec) suggested greater energy conversion efficiency and higher productivity potential. Variation in NPQt (mean 1.10) highlights the differences in heat dissipation mechanisms, with some lines showing enhanced photoprotection than others.

Table. 1. Summary statistics for major PS-traits in elite soybean breeding lines.

TRAIT	MEAN	SD	MIN	MAX
SPAD	43.3	4.37	31.4	53.4
PAR (µ moles/m^2/sec)	1338	366	177	2111
FvP/FmP	0.70	0.04	0.548	0.776
Phi 2	0.36	0.062	0.229	0.546
NPQt	1.10	0.427	0.41	3.02
Leaf thickness (mm)	0.53	0.4	0.04	2.02
Leaf temp (°C)	31.2	1.08	28.2	35.5
LEF (µ moles/m^2/sec)	211	45.7	43.6	324

The variability in key photosynthetic traits in advanced breeding lines is also shown in the density plots (**Fig 2**). The SPAD density plot shows quite wide distribution, indicating a substantial variation ($P < 0.001$) in relative chlorophyll content across soybean breeding lines. The FvP/FmP plot ($P<0.05$) displays relatively narrower distribution, suggesting differences in the maximum quantum efficiency of PSII photochemistry. Leaf thickness also exhibits significant variability ($P < 0.05$), with most lines clustering around thinner leaves but some lines also have much thicker leaves. The Phi2 plot ($P < 0.05$) highlights variation in the quantum yield of PSII, pointing to differences in light energy conversion efficiency. These multiple density plot distributions suggested the diverse photosynthetic efficiencies within the advance breeding lines, which can be valuable for selecting traits linked to improved seed yield and stress tolerance.

We also observed several interesting correlations among multiple photosynthetic traits measured with MultispeQ in elite soybean breeding lines at R2-R3 stage. The strongest positive correlations are observed with LEF and light intensity-PAR i.e. $r = 0.80$, indicating the increased light intensity directly boosts the electron flow, enhancing photosynthetic performance of the plants. Similarly, FvP/FmP is positivity correlated with Phi2 ($r = 0.47$), which suggests that lines with higher quantum yield of PSII exhibit greater PSII efficiency downstream. Also, SPAD positively correlated with FvP/FmP ($r = 0.42$), implying that higher chlorophyll content may support greater PSII efficiency.

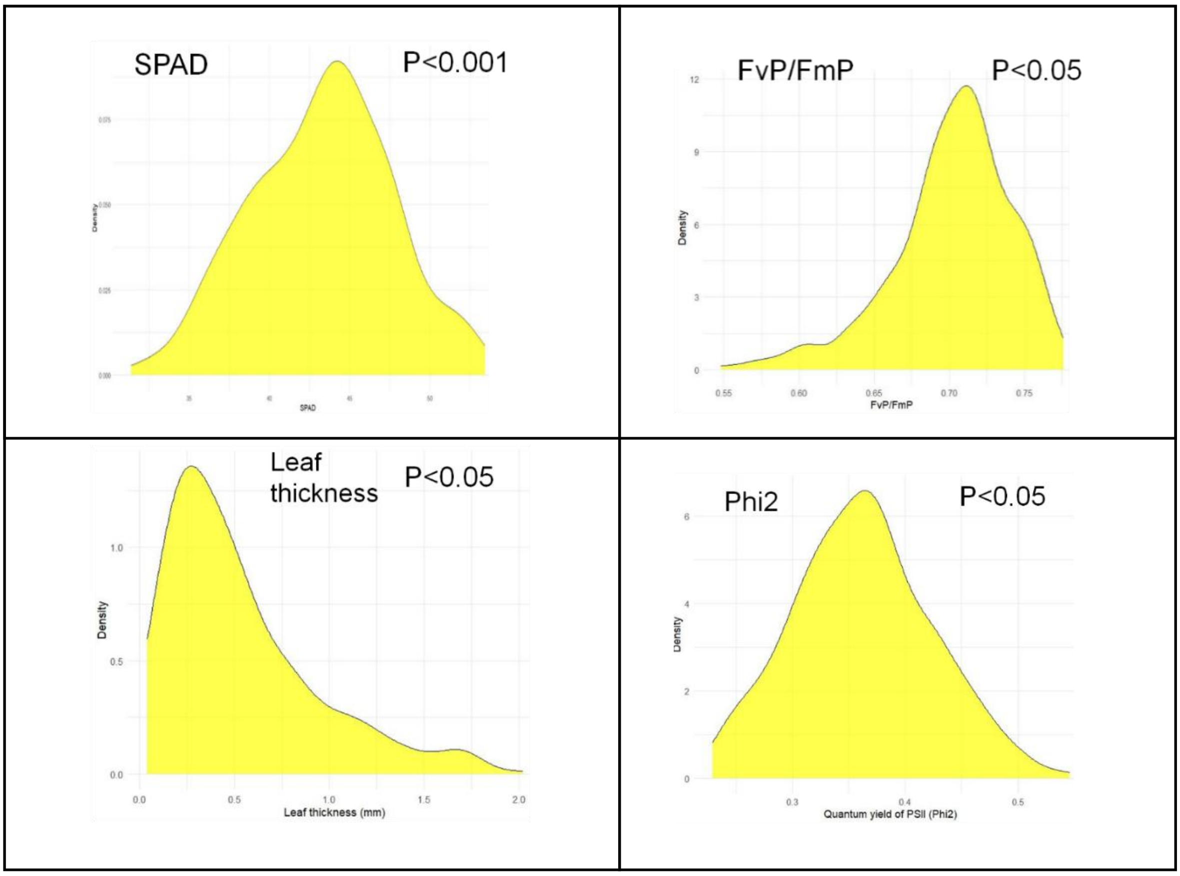

Figure 2. Density plots showing variability for various MultispeQ based traits.

Conversely, NPQt shows a notable negative correlation with Phi2 (r = -0.45), indicating that as energy dissipation through non-photochemical quenching increases, the quantum yield of PSII decreases, reflecting a trade-off between energy use for photosynthesis and dissipation to prevent photo-damage. Leaf thickness has minimal relationships with most of the other traits.

Interestingly, light intensity (PAR) also shows a strong negative correlation with NPQt (r = -0.73), showing that under high light conditions, non-photochemical energy dissipation is reduced. This allows more energy to be directed towards photosynthesis. So, these interrelationships provide insights into how different soybean lines respond to light conditions and regulate photosynthetic efficiency. This information can further be used to correlate with the seed yield performance to select lines with optimal photosynthetic traits for breeding purposes.

Conclusion

In a nutshell, substantial variations were observed for multiple photosynthetic traits in elite soybean materials (Fig.2; Table.1), showing their genetic potential for selection for improvement in relevant traits. Using the MultispeQ v2.0, we successfully found strong and significant correlations (**Fig. 3**) among multiple PS traits like FvP/FmP, NPQt, PAR and SPAD in our breeding materials. In the near future, final seed yield will also be analyzed along with all PS-traits to study multiple correlations, aiming to pinpoint valuable photosynthesis related traits for targeted breeding efforts, ultimately improving soybean yield performance.

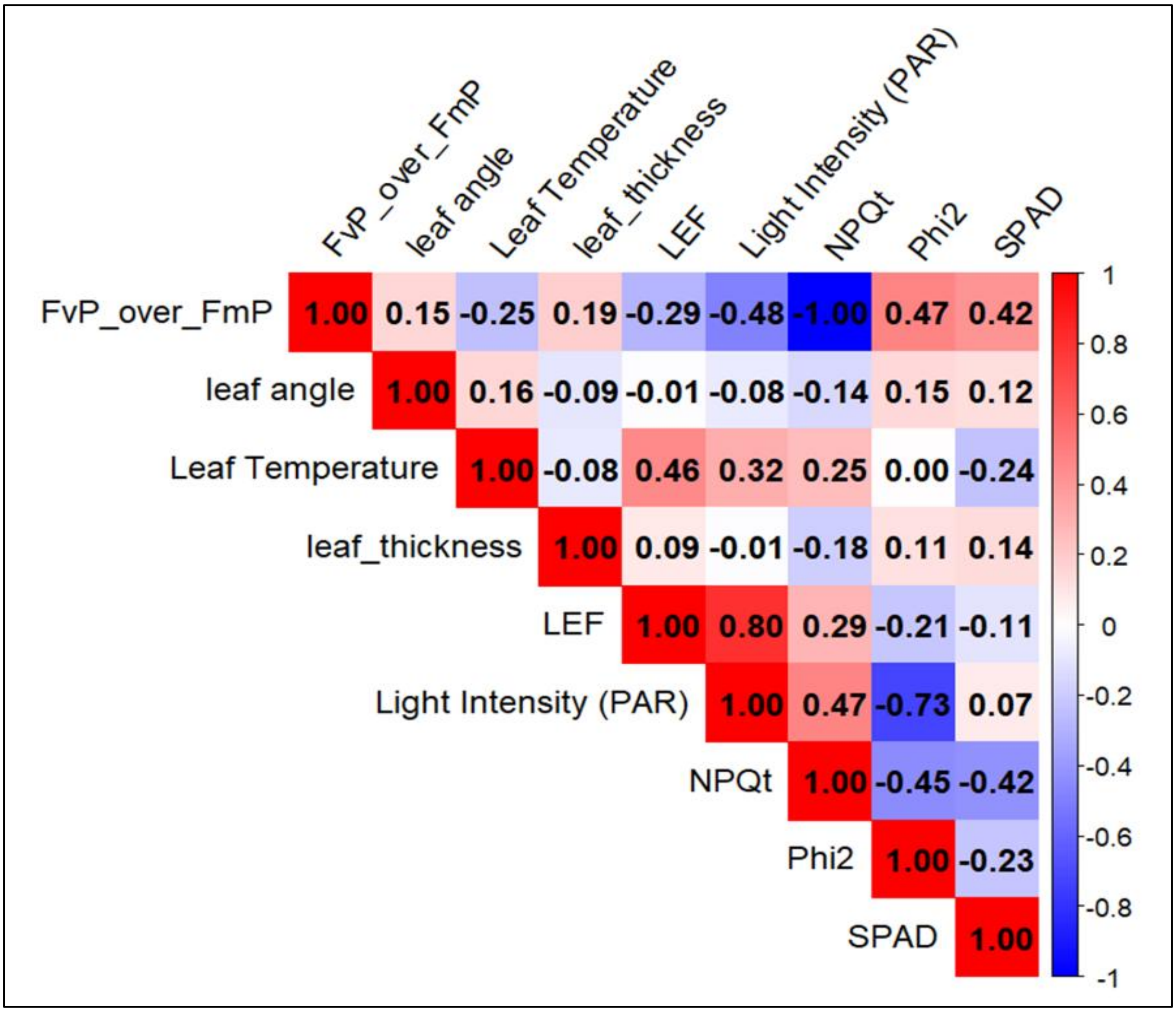

Figure 3. Correlation matrix for PS-traits in elite lines.

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