

POD NUMBER AND PHOTOSYNTHESIS AS PHYSIOLOGICAL SELECTION CRITERIA IN SOYBEAN (*Glycine max* L. Merrill) BREEDING FOR HIGH YIELD

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ABSTRACT

Field studies were conducted in two years using 638 F2 and 1185 F3 lines of selected 16 F1 and 15 F2 parent lines (≥ 80 pods plant⁻¹) to evaluate pod number and CO₂ exchange rate (CER) as selection criteria. Pod and seed number, and seed weight of individual lines were observed during harvesting time, and CER of randomly selected 32 F2 and 30 F3 lines was measured at initial seed filling stage. The selection of F2 lines based on pod number to generate F3 lines increased the average of seed yield by 39%, and pod number by 77% in F3 lines compared with F2 lines. A close relationships was found between seed weight and pod or seed number per plant. Net CER responded sensitively to a reduction of light in a short-term and showed 78% of F2 lines and all F3 lines with maximum CER (Pmax) $\geq 20 \mu\text{molCO}_2.\text{m}^{-2}.\text{s}^{-1}$. The ratio of pod number per plant and Pmax varied between lines and were used to group lines resulting in close relationships between Pmax and pod number. It is concluded that the use of pod number and CER (Pmax) as selection criteria offers an alternative approach in soybean breeding for high yield.

Keywords: CO₂ exchange rate (CER), *Glycine max* L. Merrill, model, pod number, soybean

INTRODUCTION

A possibility for Indonesia to be self-sufficient in soybean (*Glycine max* L. Merrill), as planned by the government, would rely mainly on an effort to increase the productivity of soybean at the field level due to the limited area suitably available for soybean extension. An increase in the productivity to approach $>4.0 \text{ t ha}^{-1}$ is required to secure the program of self-sufficiency. The

potential yield of soybean was estimated to be around $7.0\text{-}8.0 \text{ t ha}^{-1}$ (Specht *et al.*, 1999; Sinclair, 1999). From a long term research, $6000\text{-}7000 \text{ kg ha}^{-1}$ were found from individual lines in a favorable environment at Wooster, US (Cooper, 2003). The highest yield of soybean at research level (favorable conditions) reached $>8 \text{ t ha}^{-1}$ in Australia (Cooper, 2003).

The productivity of soybean in Indonesia increased at a rate of $16.1 \text{ kg year}^{-1}$ in the last 27 years (1981-2008) and reached, on national average, $<1.3 \text{ t ha}^{-1}$ in 2010 (Central Bureau of Statistics, 2010). The potential yield of high-yielding varieties released during that period increased from 1.5 to $2.4\text{-}3.4 \text{ t ha}^{-1}$ at a rate of $47.6 \text{ kg year}^{-1}$. The low realization rate of the genetic improvement in potential yields in the fields (33.8%) reflects the extent of difficulty in the optimization of soybean growth conditions. This relates partly to economic return that soybean is not competitive with other crops and treated as a secondary crop. It is thus of great importance to direct soybean breeding to a much higher potential which seems unlikely in Indonesia even with an effective growing duration of 80 days and a day length of 12 hours which are often considered responsible for a low productivity of soybean in Indonesia compared with others in the subtropics. Varieties with a net CO₂ exchange rate (CER) of $20 \mu\text{mol CO}_2.\text{m}^{-2}.\text{s}^{-1}$ at light saturation (Pmax) and a quantum efficiency of $\epsilon = 0.05 \mu\text{mol CO}_2.\mu\text{mol quanta}^{-1}$ would produce $\geq 4 \text{ t ha}^{-1}$ of seed yields with a daily average light of about $800 \mu\text{mol quanta}.\text{m}^{-2}.\text{s}^{-1}$ based on a simulation modeling (Sinclair, 1991; Driessen and Konijn, 1992).

The breeding of soybean in Indonesia for high yield, based on seed yield as a selection criterion, seems difficult to further increase the potential yield. This method of selection limits the

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opportunity of best lines or genotypes to be selected in term of high pod number and photosynthetic rate. This is based on the low heritability of seed yield in general at the early filial generations in combination with a high rate of interaction between genetic and environmental factors (Toledo *et al.*, 2000). The approach of genetic engineering at molecular level is a powerful way of generating genetic diversity that would complement classical breeding. It is, however, hard to expect in a short-term due to the complexity of genes and gene interaction involved in yield formation (Sinclair *et al.*, 2004). Other problems relate to uncertainty on many aspects of gene transfer such as the site of insertion in the genome and the expression of transgenes (Gepts, 2002).

The integration of physiological parameters into the breeding program is an alternative that has received considerable attention (Jackson *et al.*, 1996; Kumudini, 2002; Cooper, 2003; Sinclair, 2004; VanToai and Specht, 2004; Reynolds and Trethowan, 2007; Gillbert *et al.*, 2011). VanToai and Specht (2004) approached physiological traits through the model of Sinclair (1993) showing harvest index and biomass or RUE (radiation use efficiency) as alternative selection criteria. Liu *et al.* (2005) concluded that biomass production and leaf area as the key of future yield increase. The direct and indirect involvement of biomass as a selection criterion is impractical for huge number of lines. Other approaches to reach a high potential yield (i.e. $Q_0 = 4 \text{ t ha}^{-1}$) as proposed in the present study are called pod approach and photosynthesis approach. The pod approach, based on pod number (N), is supported by evidence showing a close relationship between pod number and seed yield in soybean studies involving genotypes, locations, light treatments (Arshad *et al.*, 2006; Malik *et al.*, 2006; Yasari *et al.*, 2009). The use of pod number as a parameter of indirect selection for seed yield was suggested (Pandini *et al.*, 2002). The photosynthesis approach is based on findings suggesting that the supply of photosynthate is major factor determining directly the number of pods and seed yield (Schou *et al.*, 1978; Egli, 1993; Board and Tan, 1995; Jiang and Egli, 1995).

The essence of both physiological parameters as selection criteria is the evidence of pod number, as the primary determinant of seed yield, directly dependent upon the rate of photosynthesis (Egli, 2005). A two-year study was

conducted to evaluate pod number and photosynthetic rate (CER) as selection criteria in soybean breeding for high yield. The ratio of pod number/CER was also explored as an additional physiological trait to represent sink/source ratio. The latter parameter was based on a proposition of source/sink ratio as a likely more robust trait than photosynthate supply alone for further improvement of soybean yield (Kumudini, 2002).

MATERIALS AND METHODS

Growth and Measurements

Field studies were conducted in two years near Malang, East Java, Indonesia ($8^{\circ}7' \text{ S}$, $112^{\circ}32' \text{ E}$, 330 m asl) using 638 F2 and 1185 F3 lines derived from seeds of selected 16 F1 and 15 F2 parent lines ($\geq 80 \text{ pods plant}^{-1}$). The F1 lines were the results of reciprocal crosses between var. Argomulyo and var. Brawijaya. The soil at the location was classified as Typic Tropudalf with clay loam – clay texture, pH (H_2O) = 7.1, 0.09% N total, 8.28 mg kg^{-1} P, and 0.12 me K 100 g^{-1} soil. Seeds of the selected F1 and F2 parent lines were sown manually on 1st June and 30th December 2008 to generate F2 and F3 lines respectively at plant spacing of 25 cm x 25 cm by placing one seed per hole of about 5 cm depth made with a wood stick. Var. Anjasmoro and var. Willis, both high-yielding varieties, were involved as a reference in 2008 and 2008-2009 study respectively. Fertilizers (25 kg N, 15 kg P and 25 Kha^{-1}) were applied at sowing in holes adjacent to seed holes, and an additional N (25 kg N ha^{-1}) was applied on day 21 after sowing. Experimental plots were irrigated when rainfall was not sufficient to meet water requirement of plant.

At initial seed filling stage (around 60 days after sowing), two plants of each plant origins were randomly selected with a total of 32 lines (F2), 30 lines (F3) and 2 plants (varieties) for the measurement of net CO_2 exchange rate (CER), and the concentration internal (C_i) and ambient CO_2 (C_a). A portable photosynthesis system (LI-6400, LI-COR Inc.) was used for the measurement in an open system. The measurement was made on the third fully expanded leaves of the selected plants at different levels of PPFD (photosynthetic photon flux density) using the light source of the instrument (6400-02B LED type). The instrument was set automatically for successive measurements from high to low PPFD (1500, 1000, 500,

300, 200, 100, 50 and 0 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with a duration of ± 100 seconds for each measurement. Temperature, relative humidity, CO_2 concentration, and flow rate of sampler air was $30.7 \pm 2.6^\circ\text{C}$ (standard deviation), $47.0 \pm 16.8\%$, $386.2 \pm 10.3 \mu\text{mol}\cdot\text{mol}^{-1}$ and 500.1 ± 0.4 respectively. The number of pods and seeds were recorded for each of lines and varieties after harvest (85 days after sowing), and seed weight of individual lines and plants was recorded after sun drying for about a week with a final seed water content of around 12%.

Data Analysis

Data were analyzed statistically using two-way analysis of variance with one factor (Sokal and Rohlf, 1969), frequency distribution, chi-square (χ^2) and correlation in addition to basic analyses. In some analyses, lines or data were grouped for the same origins (F1 and F2 lines) or other bases. This approach was used in the analysis of variance to explore genetic variation between groups which were compared with the varieties involved.

The response of net photosynthesis or CER (CO_2 exchange rate) to light (PPFD) was analyzed with an asymptotic exponential model (Boote and Loomis, 1991) as follows:

$$P = P_{\max} \left[1 - e^{(-\varepsilon \cdot \text{PPFD} / P_{\max})} \right] \quad (1)$$

where P = CER ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), P_{\max} = maximum CER, and ε = quantum efficiency ($\mu\text{mol CO}_2$ per $\mu\text{mol photon}$).

The dependency of seed yield per plant on pod number per plant and the rate of photosynthesis (CER) was explored from the relationship between pod number and photosynthesis with the following model:

$$N = N_{\max} \left(1 - e^{-\mu P} \right) \quad (2)$$

where N = observed or estimated pod number per plant, N_{\max} = maximum pod number per plant, and μ = a constant reflecting pod number per plant per unit CER at P_{\max} .

RESULTS AND DISCUSSION

RESULTS

Genetic Variation

A high variation, with coefficient of variations (CV) > 50%, was found in total seed

weight (TSW) and total pod number (TPN) per plant between F2 and F3 lines (Table 1). Var. Anjasmoro (a recommended variety involved in the F2 study), and var. Wilis (a widely cultivated variety involved in the F3 study), also showed a high variation in the TSW and TPN (CV > 50%). The variation of seed weight per pod (SWP), calculated from TSW/TPN, was less either in F2 and F3 (CV \leq 32.6%) or the two varieties (CV \leq 30.8%).

When lines of the same origins were grouped, which brought about a comparable number of sample plants between lines and varieties, the variation of TSW and TPN was much lower between plants of var. Anjasmoro, attributable to environmental factors, than between lines (F2) for most groups. Significant effects of plant origins ($p < 0.001$) were found on TSW, TPN and SWP (Table 2). This suggests that the variation of the observed parameters between plants was due partly to genetic factor. The frequency distribution of plants in classes of TSW or TPN was not significantly different from normality ($P > 0.05$) either in var. Anjasmoro ($n = 40$) or var. Wilis ($n = 74$) based on χ^2 test (Table 1). In turn, the frequency distribution of F2 and F3 lines departed significantly from normality ($P < 0.01$) and skewed to the right (Figure 1 and Table 1) suggesting the role of polygenic influence on TPN and seed yield. The percentage of F2 plants with TPN > 100 pods plant⁻¹ (0.16%) suggests > 4 genes (allelic pairs) likely involved in the pod formation and development.

Pods and Seeds

The use of selected F2 lines to generate F3 lines based on pod number (TPN \geq 80 pods plant⁻¹) increased, on average, TPN of F3 lines by > 80% compared with that of F2 lines. This could be associated to an increase in the number of lines producing more pods reflecting selection effectiveness. High-podding plants (TPN \geq 80 pods plant⁻¹) was 2.5% of total observed F2 lines and increased to 22.0% in F3 lines which were far above that found in the involved varieties (Table 1 and Figure 1). The number of unfilled pods, increasing with the filial generation, was relatively low (3% in F2 and 7% in F3).

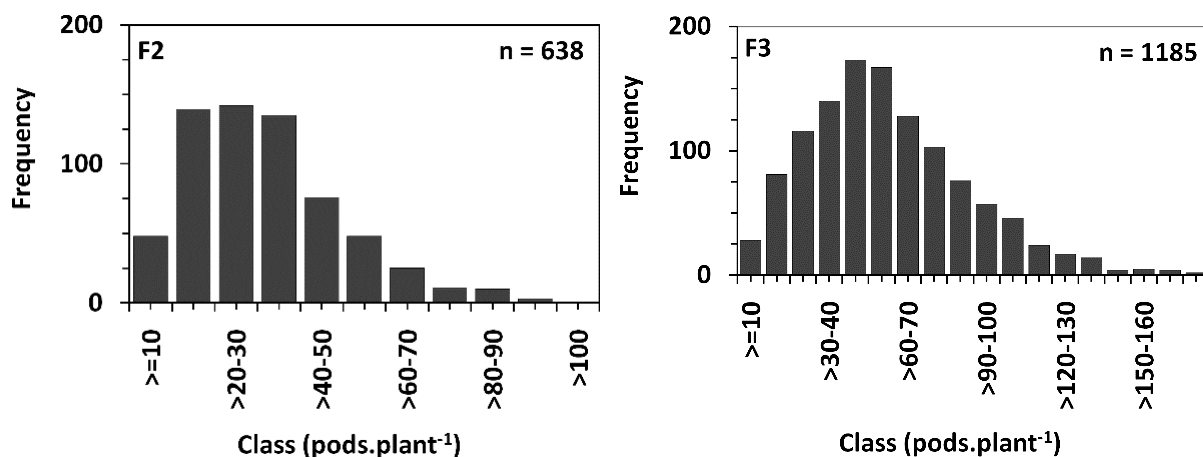


Figure 1. Frequency distribution of 638 F2 and 1185 F3 lines in classes of pod number per plant

Table 1. The range and standard deviation (sd) of TSW (g seed plant⁻¹), TPN (pods plant⁻¹) and SWP (g seed pod⁻¹) of F2 and F3 lines and varieties (AJM and WLS)

| Observations | Year 1 | | | Year 2 | |
|------------------|----------|--------|---------------------|---------|--------------------|
| | F2 | AJM | F3 | WLS | |
| TSW | Min | 0.1 | 0.5 | 2.0 | |
| | Max | 26.5 | 10.0 | 34.0 | |
| | Mean | 7.8 | 4.3 | 11.4 | |
| | sd | 4.9 | 2.5 | 6.4 | |
| | χ^2 | 90.0** | 12.72 ^{ns} | 79.1** | 4.8 ^{ns} |
| TPN | Min | 2.0 | 3.0 | 8.0 | |
| | Max | 108.0 | 33.0 | 163.0 | |
| | Mean | 32.6 | 12.8 | 54.4 | |
| | sd | 18.1 | 7.6 | 29.9 | |
| | χ^2 | 98.6** | 1.34 ^{ns} | 161.7** | 13.4 ^{ns} |
| SWP | Min | 0.050 | 0.207 | 0.137 | |
| | Max | 0.650 | 0.600 | 0.309 | |
| | Mean | 0.236 | 0.334 | 0.211 | |
| | sd | 0.077 | 0.103 | 0.033 | |
| | χ^2 | 58.8** | 25.1** | 353.9** | 47.6** |
| Number of plants | | 638 | 40 | 1185 | 74 |

Remarks: χ^2 = Chi-square, AJM = var. Anjasmoro & WLS = var. Willis, ** significant ($p < 0.001$), and ns = not significant

Table 2. Analysis of variance for TSW, TPN and SWP

| Source of Variation | df | Mean squares | | |
|---------------------|------|--------------|---------|-------|
| | | TSW | TPN | SWP |
| F2 Lines | | | | |
| Between Groups | 16 | 175.5 | 3,385.3 | 0.107 |
| Within Groups | 661 | 19.8 | 260.8 | 0.004 |
| F3 Lines | | | | |
| Between Groups | 15 | 68.4 | 6,331.4 | 0.088 |
| Within Groups | 1242 | 33.1 | 878.0 | 0.002 |

Estimates of narrow sense heritability (h^2), based on the average TSW or TPN of all lines (F3 and F2) and selected F2 lines used to generate F3 plants, was 0.30 for TSW and 0.55 for TPN (Table 1). When F3 lines were separated into groups based on the same F2 origins, and h^2 calculated accordingly, h^2 varied considerably between groups and closely related to F3/F2 ratio in TSW or TPN (Figure 2). A relatively high h^2 of TPN ($h^2 \geq 0.5$) was found in some groups in contrast to h^2 of TSW which was relatively low ($h^2 \leq 0.35$) for all groups in exception of one group. This implies that the number of pods per plant seems to be better than seed weight per plant as a parameter in the selection of soybean progenies in early generations.

The relatively low unfilled pods led very close relationships between TPN and TSN (total seed number per plant), and between TPN and TSW in F2 or F3 lines (Figure 3). Better relationships were found between TPN and TSW when lines were analyzed separately for each group of the same origins (Table 3). An allometric model ($y = ax^b$; $y = \text{TSW}$, $x = \text{TPN}$ and a and $b = \text{constants}$) used to analyze the relationships was able to accommodate the effect of TPN on SWP ($a/x^{(1-b)}$). Values of

parameter 'a' and 'b' were found to vary between groups with most groups (84%) of either F2 or F3 lines showing 'b' ≥ 1.0 . This suggests that SWP of lines selected on the basis of pod number would not be reduced at high TPN.

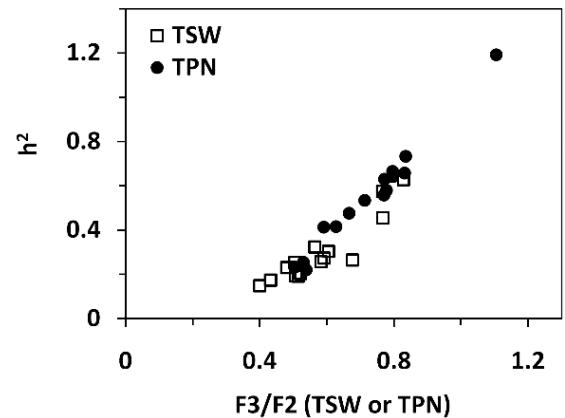


Figure 2. Estimates of narrow sense heritability (h^2) of total seed weight (TSW) and total pod number (TPN) per plant in relation to the ratio of F3/F2 in TSW and TPN

Table 3. Values of a, b and R^2 and number of samples (n) from TSW and TPN relationships ($y = aX^b$; $y = \text{TSW}$ and $x = \text{TPN}$) in F2 and F3 lines

| Groups | a | b | R^2 | n | Groups | a | b | R^2 | n |
|--------|------|-----|-------|----|--------|------|-----|-------|----|
| F2/1 | 0.21 | 1.0 | 0.913 | 33 | F3/1 | 0.23 | 1.0 | 0.843 | 79 |
| F2/2 | 0.12 | 1.2 | 0.896 | 27 | F3/2 | 0.14 | 1.0 | 0.898 | 90 |
| F2/3 | 0.19 | 1.1 | 0.773 | 38 | F3/3 | 0.39 | 0.8 | 0.764 | 82 |
| F2/4 | 0.16 | 1.1 | 0.933 | 32 | F3/4 | 0.28 | 0.9 | 0.883 | 81 |
| F2/5 | 0.06 | 1.4 | 0.876 | 36 | F3/5 | 0.15 | 1.0 | 0.862 | 48 |
| F2/6 | 0.09 | 1.2 | 0.777 | 46 | F3/6 | 0.14 | 1.1 | 0.921 | 84 |
| F2/7 | 0.13 | 1.2 | 0.897 | 37 | F3/7 | 0.16 | 1.0 | 0.916 | 80 |
| F2/8 | 0.21 | 1.1 | 0.643 | 34 | F3/8 | 0.09 | 1.1 | 0.912 | 82 |
| F2/9 | 0.18 | 1.1 | 0.928 | 41 | F3/9 | 0.16 | 1.1 | 0.883 | 81 |
| F2/10 | 0.21 | 1.0 | 0.771 | 46 | F3/10 | 0.12 | 1.1 | 0.932 | 80 |
| F2/11 | 0.09 | 1.2 | 0.875 | 68 | F3/11 | 0.19 | 1.0 | 0.849 | 82 |
| F2/12 | 0.08 | 1.3 | 0.746 | 41 | F3/12 | 0.11 | 1.1 | 0.957 | 80 |
| F2/13 | 0.19 | 1.1 | 0.827 | 35 | F3/13 | 0.09 | 1.1 | 0.938 | 82 |
| F2/14 | 0.21 | 1.0 | 0.728 | 36 | F3/14 | 0.15 | 1.1 | 0.883 | 82 |
| F2/15 | 0.19 | 1.1 | 0.843 | 41 | F3/15 | 0.07 | 1.3 | 0.900 | 72 |
| F2/16 | 0.35 | 0.8 | 0.699 | 47 | WLS | 0.22 | 1.0 | 0.942 | 74 |
| AJM | 0.28 | 1.1 | 0.840 | 40 | | | | | |

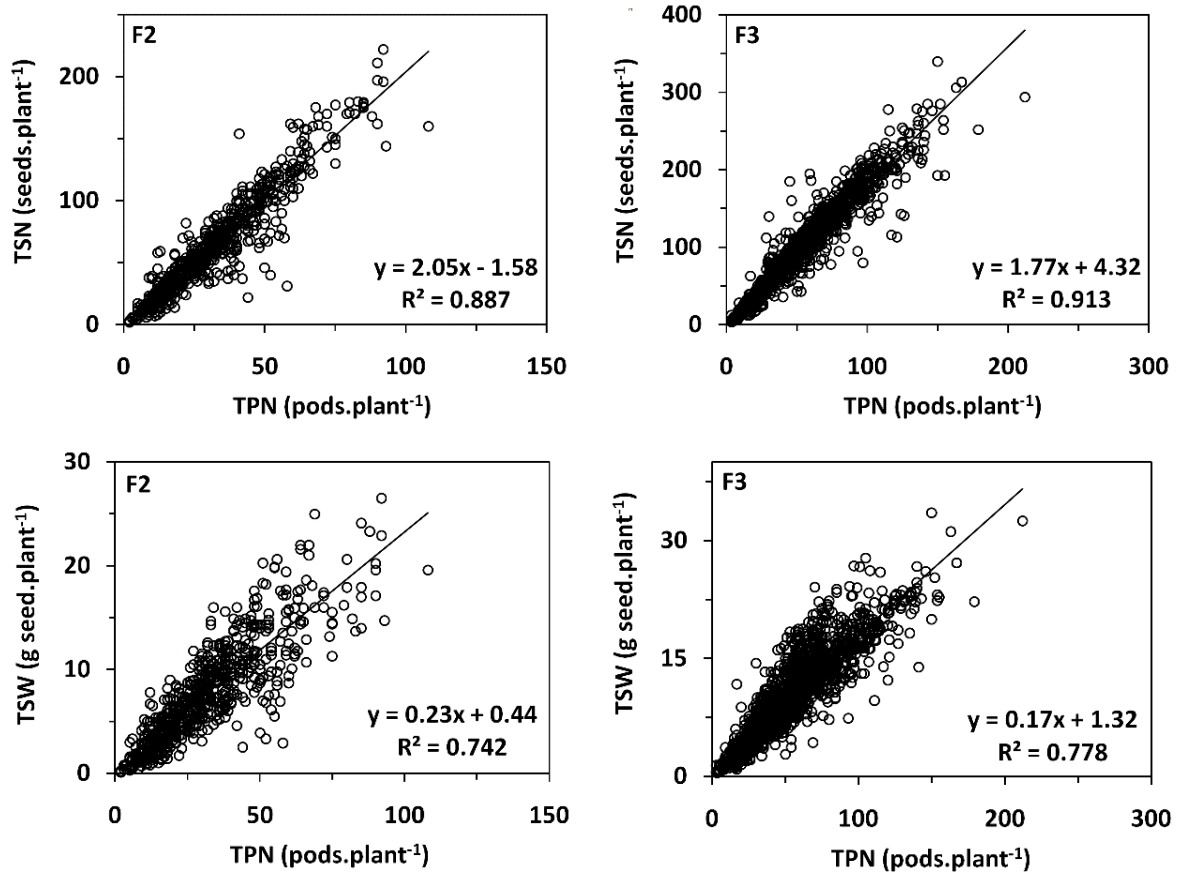


Figure 3. Total seed number (TSN) and total sees weight (TSW) in relation to total pod number (TPN) in F2 and F3 lines. Each symbol represents individual lines

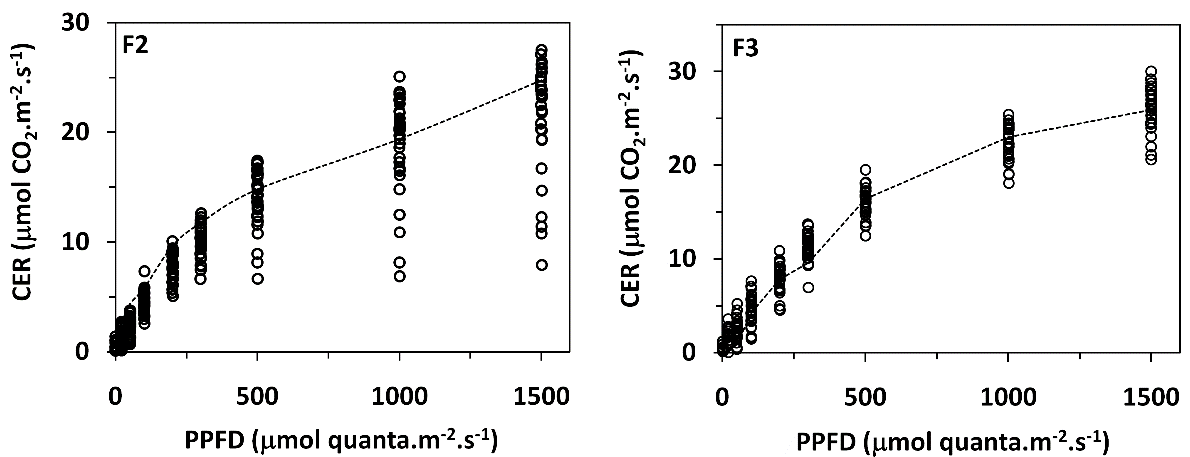


Figure 4. The response of net CO₂ exchange rates (CER) of leaves to changes in photosynthetic photon flux density (PPFD) in 32 F2 and 30 F3 lines randomly selected for the measurement of CER started at high PPFD. CER of var. Anjasmoro and Willis are shown (dashed lines)

Table 4. The estimated values of Pmax ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and ϵ [$\mu\text{mol CO}_2\cdot(\mu\text{mol photon})^{-1}$] for selected F2 lines and var. Anjasmoro (AJM)

| Line | Pmax | ϵ | R ² | Line | Pmax | ϵ | R ² |
|------|------|------------|----------------|------|------|------------|----------------|
| 1. | 15.1 | 0.045 | 0.972 | 18. | 29.5 | 0.055 | 0.989 |
| 2. | 26.5 | 0.045 | 0.987 | 19. | 27.2 | 0.055 | 0.997 |
| 3. | 13.0 | 0.057 | 0.983 | 20. | 20.5 | 0.059 | 0.991 |
| 4. | 23.3 | 0.042 | 0.988 | 21. | 29.0 | 0.046 | 0.992 |
| 5. | 29.5 | 0.049 | 0.997 | 22. | 25.5 | 0.054 | 0.978 |
| 6. | 26.0 | 0.054 | 0.988 | 23. | 24.7 | 0.038 | 0.965 |
| 7. | 12.7 | 0.058 | 0.995 | 24. | 27.2 | 0.050 | 0.990 |
| 8. | 30.0 | 0.067 | 0.996 | 25. | 22.9 | 0.037 | 0.982 |
| 9. | 20.0 | 0.045 | 0.992 | 26. | 26.3 | 0.050 | 0.996 |
| 10. | 25.0 | 0.050 | 0.991 | 27. | 23.3 | 0.042 | 0.991 |
| 11. | 11.3 | 0.045 | 0.985 | 28. | 23.3 | 0.052 | 0.991 |
| 12. | 8.1 | 0.047 | 0.967 | 29. | 28.3 | 0.043 | 0.995 |
| 13. | 26.0 | 0.058 | 0.994 | 30. | 17.6 | 0.054 | 0.996 |
| 14. | 29.0 | 0.068 | 0.994 | 31. | 27.0 | 0.047 | 0.994 |
| 15. | 28.5 | 0.060 | 0.989 | 32. | 8.3 | 0.071 | 0.928 |
| 16. | 26.8 | 0.051 | 0.997 | AJM1 | 28.0 | 0.039 | 0.946 |
| 17. | 24.7 | 0.046 | 0.991 | AJM2 | 26.0 | 0.051 | 0.975 |

Remarks: R² = coefficient determination

Table 5. The estimated values of Pmax and ϵ for selected F3 lines and var. Willis (WLS)

| Line | Pmax | ϵ | R ² | Line | Pmax | ϵ | R ² |
|------|------|------------|----------------|------|------|------------|----------------|
| 1. | 28.0 | 0.053 | 0.998 | 17. | 33.5 | 0.0402 | 0.984 |
| 2. | 28.0 | 0.053 | 0.998 | 18. | 30.4 | 0.0517 | 0.997 |
| 3. | 33.0 | 0.056 | 0.990 | 19. | 29.0 | 0.0551 | 0.999 |
| 4. | 27.0 | 0.059 | 0.996 | 20. | 31.8 | 0.0445 | 0.996 |
| 5. | 30.4 | 0.040 | 0.994 | 21. | 23.9 | 0.0669 | 0.979 |
| 6. | 33.9 | 0.051 | 0.978 | 22. | 31.5 | 0.0567 | 0.995 |
| 7. | 33.9 | 0.054 | 0.987 | 23. | 29.5 | 0.0354 | 0.987 |
| 8. | 26.1 | 0.050 | 0.999 | 24. | 26.2 | 0.0419 | 0.993 |
| 9. | 22.6 | 0.043 | 0.986 | 25. | 26.3 | 0.0631 | 0.992 |
| 10. | 26.4 | 0.066 | 0.998 | 26. | 27.2 | 0.0626 | 0.991 |
| 11. | 34.5 | 0.052 | 0.985 | 27. | 33.1 | 0.0430 | 0.999 |
| 12. | 30.7 | 0.052 | 0.998 | 28. | 25.7 | 0.0540 | 0.992 |
| 13. | 30.9 | 0.049 | 0.967 | 29. | 29.4 | 0.0412 | 0.987 |
| 14. | 31.4 | 0.057 | 0.996 | 30. | 32.5 | 0.0488 | 0.996 |
| 15. | 34.0 | 0.0442 | 0.993 | WLS1 | 26.9 | 0.0560 | 0.999 |
| 16. | 34.0 | 0.0442 | 0.995 | WLS2 | 32.3 | 0.0520 | 0.998 |

Net CO₂ Exchange Rates

Net CO₂ exchange rate (CER) of leaves showed a sensitive response to a reduction in light from 1500 to 0 PPFD ($\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in a short-term that took generally <20 minutes for nine measurements per plant (Figure 4). F2 lines showed a considerable variation in CER under high light with a difference of almost 300% between the highest and the lowest CER under 1500 PPFD which was reduced to <50% in F3 lines. Several F3 lines were significantly better than var. Willis in CER based on χ^2 test ($p < 0.05$ - < 0.01). All lines and varieties showed the same

pattern of CER in response to light and reached light saturation around 1500 PPFD.

An asymptotic exponential model, characterized by the parameter Pmax (maximum CER) and ϵ ($\mu\text{mol CO}_2\cdot\mu\text{mol quanta}^{-1}$), was sufficiently good to capture the response of CER to light increases ($R^2 = 0.9$). A considerable variation was found in Pmax and ϵ between lines with 78% of F2 lines and all F3 lines showing $\text{Pmax} \geq 20 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Table 4 and Table 5). There was even 53% of F3 lines with $\text{Pmax} \geq 30 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ implying a possibility to select lines having potentially a high seed yield.

However, neither TSW nor TPN showed a close relation to Pmax independent of filial generation (Figure 5). On average, there was a tendency of

TSW or TPN to increase with an increase in Pmax based on data grouped in classes of Pmax (Figure 5).

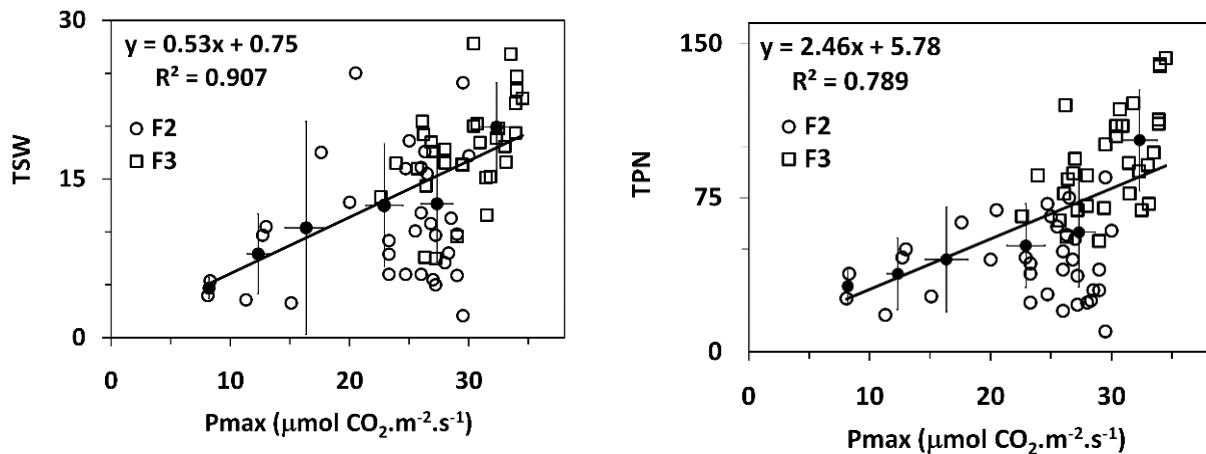


Figure 5. Relationship between TSW and Pmax, and TPN and Pmax for all observed F2 and F3 lines (open symbols), and for the average of classes (closed symbols and lines) based on Pmax. Vertical and horizontal bars are standard deviation (sd)

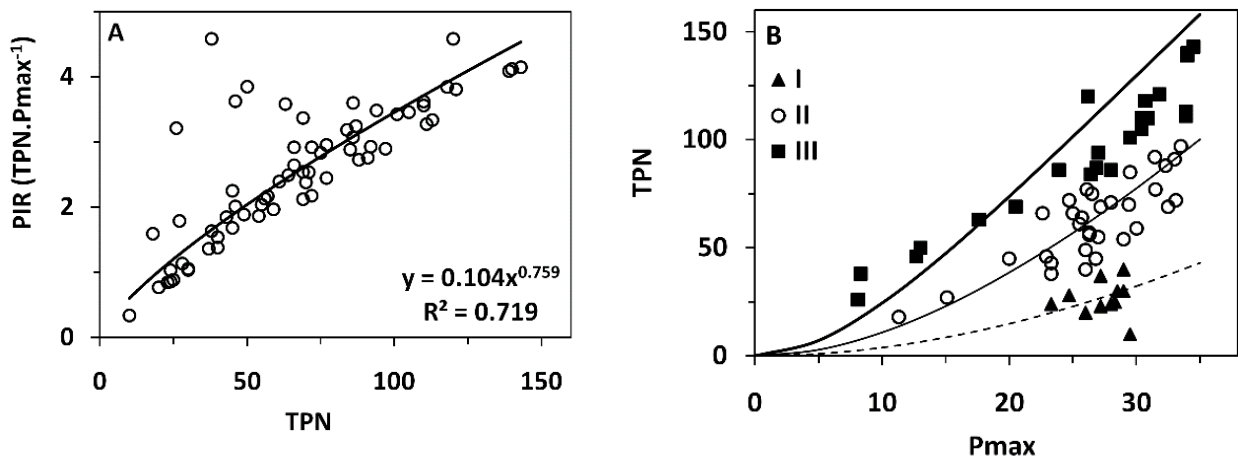


Figure 6. Relationship between PIR and TPN for all selected F2 and F3 lines (A), and TPN and Pmax for each of groups (I, II and III) (B). The groups were based on classes of PIR [$I < 1.5, 1.5 \leq II < 3.0$ and $III \geq 3.0$ pods plant⁻¹.($\mu\text{mol CO}_2\text{.m}^{-2}\text{.s}^{-1}$)⁻¹]. The graphs (B) were generated by eq(2) with Nmax replaced by $\eta\text{.Pmax}$ [$\eta = \text{maximum PIR} = 5$ pods plant⁻¹.($\mu\text{mol CO}_2\text{.m}^{-2}\text{.s}^{-1}$)⁻¹] and $\mu = -0.0081$ (dashed line), -0.0243 (thin line), and -0.0668 (thick line) which was successively the μ average of each group

PIR Model

The relation of TPN to CER is mediated by processes determining the number of pods produced with the existing rate of photosynthesis which could be represented by TPN/CER. This ratio, called as photosynthate interest rate (PIR) in pod formation, varied in a range of 0.3-4.6 pods.plant⁻¹.CER⁻¹ between lines (Figure 6) that explained the lack of close relationship between Pmax and TSW or TPN. When the data of TPN

and Pmax were grouped in classes of PIR (I<1.5, 1.5≤II<3.0, and III≥3.0), a clear relationship was found between TPN and Pmax particularly for two classes (II and III) covering >80% of the data (Figure 6). The relationship could be described by eq (2) using an average μ value for each group of the PIR classes, generated from the same equation, and $\eta \cdot P_{max}$ to replace Nmax where $\eta = 5$ is the maximum PIR (Figure 6).

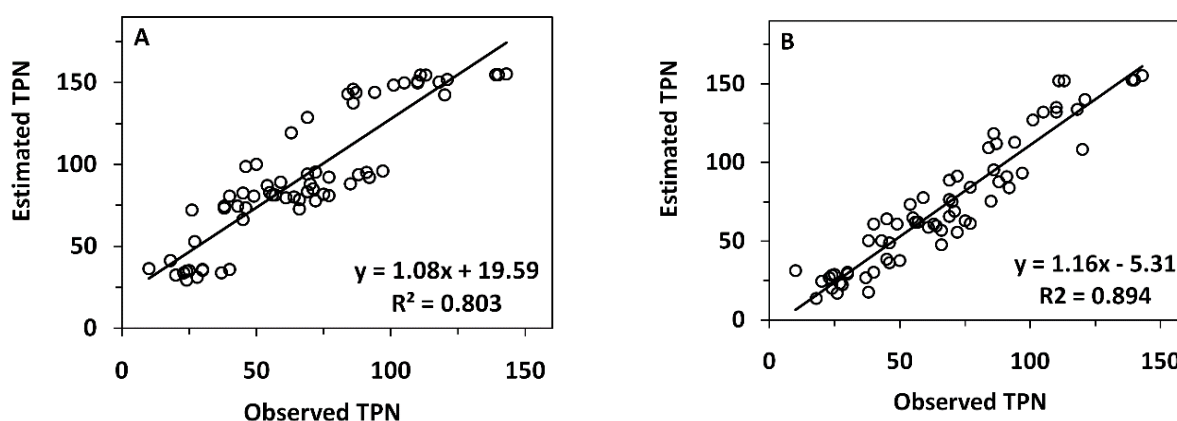


Figure 7. Relationship between estimated and observed TPN. The estimated TPN was obtained from eq (2) with (A) a constant value of $N_{max} = \eta \times 34.5 = 173$ pods plant⁻¹ where 34.5 = the highest Pmax, and (B) $N_{max} = \eta \times \text{observed } P_{max}$. The value of $\mu = -0.0081, -0.0243$ and -0.0668 for I, II and III class of PIR (similar to Figure 6). RMSE = 17.9 (A) and 14.2 (B)

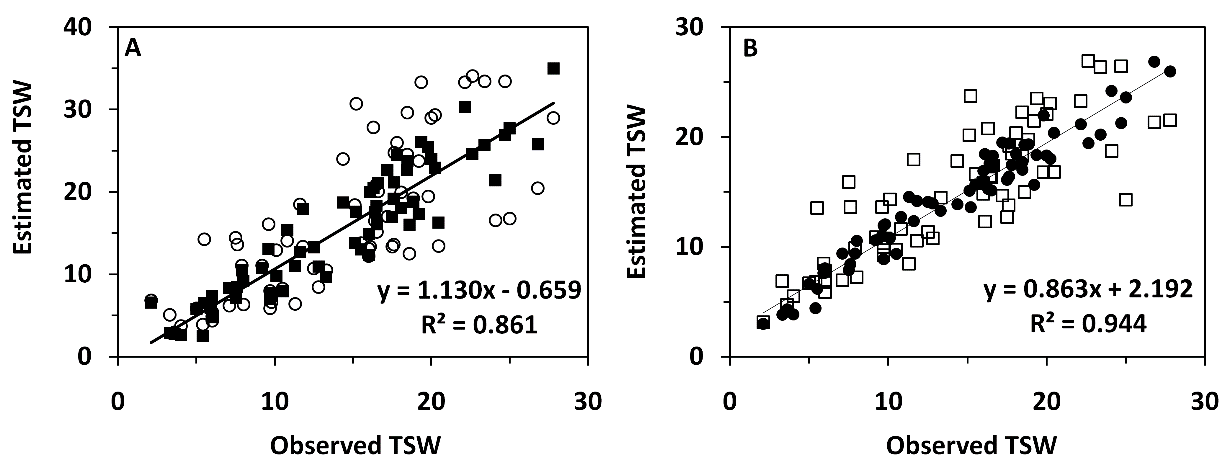


Figure 8. Relationship between estimated and observed TSW. The estimated TSW (A) was obtained from eq (3) with a constant average of $q = 0.22$ g pod⁻¹ (open symbols), and observed values of q (closed symbols), and the estimated TSW (B) was generated by eq (4) with $k = 1.32, \eta = 5$, and a constant value of $q = 0.22$ (open symbols; $R^2 = 0.704$) or observed values of q (closed symbols). RMSE = 5.6 and 3.0 (A), and 3.5 and 1.6 (B) for open and closed symbols respectively

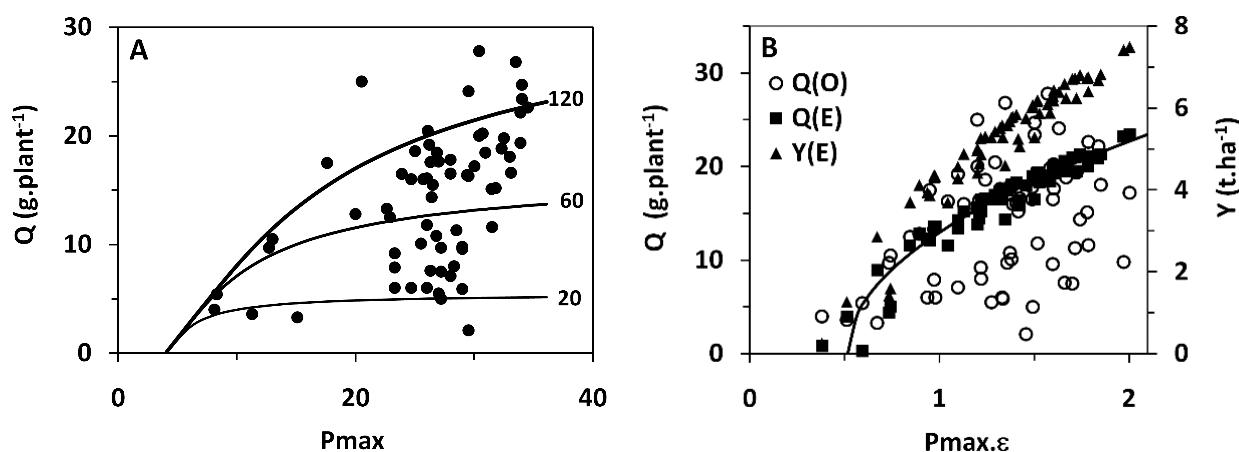


Figure 9. Observed (round symbols) and estimated (lines) seed weight (Q) in relation to Pmax (A) and observed Q (open symbols) and estimated potential yields (closed symbols) in relation to Pmax.ε (B). The lines on Figure A were based on eq(4) for 30, 60 and 120 pods plant⁻¹ as indicated in the figure. The estimated potential yields [Q(E) or Y(E)] in Fig. B were obtained from a simulation modeling based on observed Pmax and ε in the present study

The estimated values of pod number (N) with eq (2), based on the observed values of Pmax and an average μ value, were closely related to observed values of TPN (Figure 7A). A better relationship was found when Nmax was replaced by η.Pmax (Figure 7B).

If this relationship is multiplied with SWP, estimates of TSW would be obtained as shown in the following equation:

$$Q = q\eta P_{\max} \left(1 - e^{-\mu P_{\max}} \right) \quad (3)$$

Where: Q = TSW (g seed plant⁻¹), and q = SWP (g seed.pod⁻¹).

After further analysis, the generated μ values from eq (2) could be approached with N/(-η/1.5*Pmax²) leading to the following equation:

$$Q = q\eta P_{\max} \left(1 - e^{-kN/P_{\max}} \right) \quad (4)$$

Where: k = -1.5/η = 0.3. Estimates of seed yield (Q) with eq(4) using η = 5 and an average q or observed q were closely related to the observed Q (Figure 8).

The dependence of seed yield on photosynthesis associated with pod number mediated by PIR = TPN.CER⁻¹ was thus demonstrated by the PIR model (Figure 9A). This led to an inference that PIR is an important

physiological parameter to consider in the selection of soybean lines as indicated by the component of η and N/Pmax in the PIR model. To figure out the value of pod number, Pmax, and PIR required for a targeted potential yield, the estimation of potential yields in the study location with the applied plant population (320,000 plants ha⁻¹) was made with simulation model (Sinclair, 1991; Driessen and Konijn, 1992). The simulated potential yields, based on the observed Pmax and ε, were in the range of observed seed yield, and closely related to the product of Pmax. ε (Figure 9B) that could be simplified with the following equation:

$$Y^2 = 35.5P_{\max} \cdot \varepsilon - 18.3 \text{ or } P_{\max} = \frac{Y^2 + 18.3}{35.5\varepsilon} \quad (5)$$

With a targeted potential yield of Y = 4 t ha⁻¹ or Q = 3.125Y, then Pmax = 19.3 μmolCO₂.m⁻².s⁻¹ (with ε = 0.05). The substitution of this Pmax value and others (Q = 3.125*4 = 12.5 g plant⁻¹, k = 1.32, η = 5 and q = 0.22) into eq (4) results in N = 57 pods plant⁻¹. Thus, an alternative to develop high-yielding varieties (Y ≥ 4 t ha⁻¹) is to select lines based on Pmax ≥ 20 μmolCO₂.m⁻².s⁻¹ and N ≥ 60 pods plant⁻¹ which may require the involvement of PIR ≥ 3 (Figure 10).

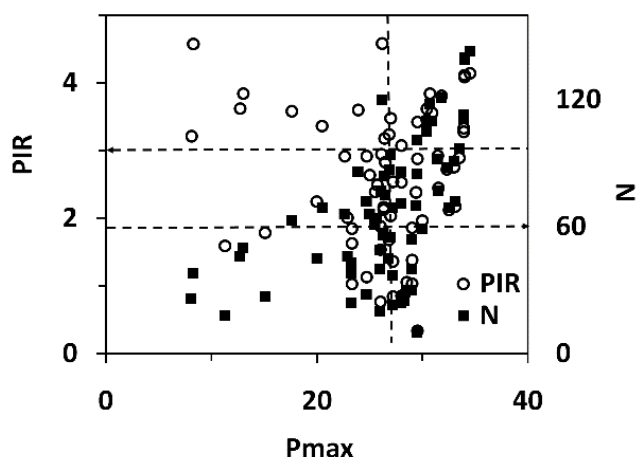


Figure 10. The illustration of line selection based on Pmax ($\geq 20 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), pod number (N or TPN ≥ 60 pods plant $^{-1}$) and PIR [≥ 3 pods plant $^{-1}\cdot(\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1})^{-1}$]. Dashed vertical and horizontal lines indicate the minimum Pmax, PIR and TPN required for a high potential yield (4 t ha $^{-1}$)

DISCUSSION

One of the most striking characteristics of soybean is the high variation of pod number between plants grown at the same area as found in the present study not only in F2 and F3 lines but also in the two involved varieties. This would position pod number as a dominant physiological parameter determining seed yield particularly with its proximity to seed yield in the hierarchical process of yield formation. This is consistent with the conclusion on the variation of soybean yield attributed much to the number of pods or seeds (Egli, 2005). Other prominent results in the present study include the narrow sense heritability (h^2) which was, on average, much higher for TPN (55%) than for TSW (30%) and closely related to the ratio of F3/F2 in TPN and TSW respectively.

The value of h^2 for TSW was comparable with that found for soybean grain yield (29%) by Toledo *et al.* (2000) working with six crosses in 17 environments. An increase in the average seed yield (39%) and pod number (77%) of F3 lines compared with F2 lines was found following the selection of F2 lines based on pod number to generate F3 lines. The evidence, in addition to a close relationship between TSW and TPN,

confirms pod number as an important, indirect selection criterion in breeding of soybean for high yield as proposed previously (Oz *et al.*, 2009; Iqbal *et al.*, 2010; Sedghi *et al.*, 2010).

The pod number needs to be combined with a photosynthetic parameter as selection criteria to minimize pod development to be limited by photosynthate supply in lines with high pod number in particular. The use of photosynthesis alone as a selection criterion was ineffective to increase yield (Kumudini, 2002; Sinclair, 2004). The inclusion of photosynthesis was suggested by the high variation of SWP between lines in the present study. The growth of soybean seeds was influenced by the supply of photosynthate and limited at a low rate of photosynthesis (Egli *et al.*, 1985; Egli and Bruening, 2001). Other studies involving light treatments and plant densities showed the dependence of seed yield on photosynthate supply (Egli and Bruening, 2005; Liu *et al.* 2010).

The study of cultivars released during 56 years (1950-2006) in China showed that an increase in seed number with years of released cultivars, as the main determinant of seed yield, was associated with an increase in photosynthetic rate (Jin *et al.*, 2010).

The rate of photosynthesis (CER) in the present study varied considerably between plants and was comparable to those observed previously (Campbell *et al.*, 1988). The ratio of TPN/CER at Pmax, called PIR (photosynthate interest rate) in pod formation, was found to be an important factor in the relationship between Pmax and TPN or TSW. After taking this into consideration, close relationships were then found between Pmax and TPN ($R^2 = 0.803$ and 0.894) that led to the development of PIR model. The estimated TSW as a function of N, Pmax and PIR with the PIR model was highly correlated to the observed TSW ($R^2 = 0.944$). This supports the inclusion of photosynthetic rate and PIR as selection parameters in addition to pod number. Sedghi and Amanpour-Balaneji (2010) concluded pod number and pre-flowering net photosynthesis as the best selection criteria in soybean for grain yield. Harrison *et al.* (1981) succeeded to use CAP (canopy apparent photosynthesis) as a selection parameter to increase yield of soybean lines, and to select the best lines (F $_3$ -derived lines). The use of PIR as a selection criteria has never been reported previously, but in line with a conclusion that source/sink ratio is an important trait for

further genetic improvement in soybean yield (Kumudini, 2002).

In conclusion, the use of pod number as a selection parameter in soybean breeding for high yield is confirmed in the present study. The inclusion of photosynthesis (Pmax) would complement the number of pods to increase a chance of best lines to be selected. The integration of pod number and Pmax as determinants of seed yield in the PIR model suggests PIR as an important parameter to consider in the selection of soybean lines. As a general guide line, the selection of lines with $TPN \geq 60$ pods plant⁻¹, $P_{max} \geq 20 \mu\text{molCO}_2\text{.m}^{-2}\text{.s}^{-1}$ and $PIR \geq 3$ pods plant⁻¹ ($\mu\text{molCO}_2\text{.m}^{-2}\text{.s}^{-1}$)⁻¹ would be expected to result in promising lines with potential yields ≥ 4 t ha⁻¹. In 1980, Harrison *et al.* (1981) put forward that the application of selection based on CAP to increase seed yield was dependent upon the development of technology to measure CAP of soybean in the fields. With the availability of handy equipments to enable measure CER in the fields at present, CER and PIR in addition to pod number would be easily applied and may reduce the cost and time of selection with a proper management compared with the selection based on seed yield.

CONCLUSIONS

A close relationship was found between seed weight and pod or seed number per plant. The estimated coefficient of narrow sense heritability, on average, was 30% for seed weight and 55% for pod number, and closely related to the ratio of F3/F2 in seed weight and pod number. Net CER responded sensitively to a reduction of light in a short-term and showed 78% of F2 lines and all F3 lines with maximum CER (P_{max}) $\geq 20 \mu\text{mol.CO}_2\text{.m}^{-2}\text{.s}^{-1}$. Neither pod number nor seed yield showed a close, direct relation to Pmax. The ratio of pod number per plant and Pmax, reflecting photosynthate interest rate (PIR) in pod formation, varied between lines and used to group lines resulting in close relationships between Pmax and pod number. A PIR model was developed to integrate the complementary role of pod number and photosynthesis (Pmax) in seed yield indicating PIR as an important parameter. The use of pod number, CER (Pmax) and PIR as selection criteria offers an alternative approach in

soybean breeding for high yield. The selection of lines with $TPN \geq 60$ pods.plant⁻¹, $P_{max} \geq 20 \mu\text{mol CO}_2\text{.m}^{-2}\text{.s}^{-1}$ and $PIR \geq 3$ pods plant⁻¹ ($\mu\text{mol CO}_2\text{.m}^{-2}\text{.s}^{-1}$)⁻¹ is suggested as a guide line to obtain lines with potential yields ≥ 4 t.ha⁻¹.

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