

1 **Title: Variation in relaxation of non-photochemical quenching in a soybean nested**
2 **association mapping panel as a potential source for breeding improved photosynthesis**

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22 **Keywords**

23 Non-photochemical quenching, photosynthesis, soybean, breeding

24 **Abstract**

25 Improving the efficiency of crop photosynthesis has the potential to increase yields. Genetic
26 manipulation showed photosynthesis can be improved in Tobacco by speeding up relaxation of
27 photoprotective mechanisms, known as non-photochemical quenching (NPQ), during high to low
28 light transitions. However, it is unclear if natural variation in NPQ relaxation can be exploited in
29 crop breeding programs. To address this issue, we measured NPQ relaxation in the 41 parents of
30 a soybean NAM population in field experiments in Illinois during 2018 and 2019. There was
31 significant variation in amount and rate of fast, energy dependent quenching (qE) between
32 genotypes. However, strong environmental effects led to a lack of correlation between values
33 measured over the two growing season, and low broad-sense heritability estimates (< 0.3). These
34 data suggest that either improvements in screening techniques, or transgenic manipulation, will be
35 required to unlock the potential for improving the efficiency of NPQ relaxation in soybean.

36 Table of Abbreviations

Symbol	Description
F_v/F_m	Maximum potential quantum efficiency of Photosystem II
qE	Fast phase of NPQ relaxation, energy-dependent quenching (< 2 min)
qM	Intermediate phase of NPQ relaxation (2-30 min)
qI	Slow phase of NPQ relaxation (> 30 min)
AqE	Amplitude of qE
AqM	Amplitude of qM
AqI	Amplitude of qI
τ_{qE}	Half-life of qE relaxation
τ_{qM}	Half-life of qM relaxation
Max. NPQ	Maximum recorded value of NPQ
J_{max}	Maximum rate of electron transport

37

38 Introduction

39 Soybean is a major source of vegetable protein worldwide. Improved agronomic practices and
40 intensive breeding efforts led to sustained yield increases of 23.3 kg ha⁻¹ y⁻¹ between 1924 and
41 2012 in the USA (Specht et al., 2014). However, further genetic improvements are required to
42 meet future demands without expanding the area of land under cultivation and causing
43 environmental degradation (Grassini et al., 2013).

44

45 The maximum achievable yield in the absence of abiotic or biotic stress is known as the yield
46 potential, and can be defined as:

47

$$48 Y_p = 0.487 St \times \epsilon_i \times \epsilon_c \times \epsilon_p$$

49

50 Where St is the total solar incident radiation, 48.7 % of which is photosynthetically active (PAR);
51 canopy light interception (ϵ_i) is the amount of solar radiation captured over a growing season; solar
52 conversion efficiency (ϵ_c) is the ability to convert captured solar energy into seed biomass; and
53 harvest index (ϵ_p) is the proportion of above-ground biomass that is partitioned into seed (Zhu et
54 al., 2010).

55

56 Research suggests ϵ_p (Morrison et al., 1999; Koester et al., 2016) and ϵ_i (Dermody et al., 2008;
57 Koester et al., 2014) are close to their theoretical maximum in modern soybean genotypes. In
58 contrast, ϵ_c is calculated to be between 2-4 % (Koester et al., 2014), which is less than half the
59 theoretical maximum (Zhu et al., 2010) making it an attractive target for improvement. Several
60 strategies have been proposed to increase ϵ_c (Zhu et al., 2010; Long et al., 2019) and experiments
61 in *Nicotiana tabacum* have shown it is possible by genetic modification (Lefebvre et al., 2005;
62 Kromdijk et al., 2016; South et al., 2019).

63

64 One strategy to improve ϵ_c focuses on altering photoprotection (Kromdijk et al., 2016).
65 Photodamage can occur when the amount of light absorbed exceeds the rate at which it is used for
66 the fixation of carbon, also known as photochemical quenching. Excess absorbed light energy can
67 be dissipated by non-photochemical quenching (NPQ), reducing the likelihood that damaging
68 reactive oxygen species are formed (Murchie and Lawson, 2013; Ruban, 2016). In a field
69 environment, leaves within a canopy are exposed to instantaneous fluctuations in light caused by

70 intermittent cloud cover and wind induced leaf movements. This requires rapid activation and
71 deactivation of NPQ to adjust to altering light levels and reduce field recombination-induced
72 photodamage (FRIP) caused by $^1\text{O}_2$ production (Davis et al., 2016). However, a delay in relaxing
73 NPQ during high to low light transitions is predicted to result in unnecessary dissipation of energy,
74 reducing the efficiency of photosynthesis (Zhu et al., 2004). To address this, Kromdijk et al. (2016)
75 manipulated the expression of three genes involved in photoprotection, including Zeaxanthin
76 epoxidase (ZEP), Violaxanthin de-epoxidase (VDE) and Photosystem II subunit S (PsbS),
77 showing it is possible to speed up the rate of activation and relaxation of NPQ, leading to improved
78 efficiency of photosynthesis and increased biomass accumulation (Kromdijk et al., 2016).

79
80 NPQ is a collection of processes, including fast, energy dependent quenching (qE)(Krause et al.,
81 1982), medium term processes (qM): zeaxanthin dependent quenching (qZ)(Nilkens et al., 2010)
82 and state transitions (qT)(Quick and Stitt, 1989), and long term quenching: photoinhibition
83 (qI)(Krause, 1988) and photoinhibition independent quenching (qH)(Malnoë et al., 2018).

84
85 However, it is unclear what is an ideal amount of photoprotection, measured as the amplitude of
86 qE and qM, and if it can be optimized, as it is likely to vary between species and environment. Too
87 much photoprotection will unnecessarily dissipate light energy that could be used for
88 photosynthesis, while too little will result in photodamage, reducing photosynthetic efficiency.
89 Therefore the best genotypes will have an empirically determined optimum level of NPQ, relax
90 photoprotection quickly and experience minimal photodamage, thereby maximizing
91 photosynthetic efficiency.

92
93 From a breeding perspective, it is important to assess both the degree of variation available in
94 germplasm and the extent to which this variation is heritable in order to determine whether a trait
95 can be improved through breeding. Previous studies on diverse genotypes in rice (Kasajima et al.,
96 2011; Wang et al., 2017) and arabidopsis (Jung and Niyogi, 2009; Rungrat et al., 2019) have
97 demonstrated the existence of substantial variation in NPQ within species, but focused only on
98 maximum NPQ (Wang et al., 2017) or qE (Jung and Niyogi, 2009; Kasajima et al., 2011; Rungrat
99 et al., 2019) and did not assess heritability.

100

101 Chlorophyll fluorescence was evaluated in two studies with field grown soybean, using canopy
102 reflectance to calculate photochemical reflectance index (PRI), which is a proxy for NPQ (Herritt
103 et al., 2016), and OJIP transients to look at variation in fluorescence kinetics (Herritt et al., 2018).
104 Estimates of the broad sense heritability of traits (H^2), ranged from 69 % for PRI and 36.8 % for
105 dark fluorescence values F_0 , to 0.4 % for energy flux for electron transport per reaction center.
106 However, the individual components of NPQ relaxation was not assessed by Herritt *et al.* (2016,
107 2018). Therefore, the extent to which individual components of NPQ co-vary in natural
108 populations and whether they can be easily selected in breeding programs remains unclear.

109
110 Here, rates of NPQ relaxation in the 41 parents of the soybean nested association mapping (NAM)
111 population (Song et al., 2017; Diers et al., 2018) were measured and a double exponential curve
112 was fit (Dall'Osto et al., 2014) to calculate the amplitude of qE (AqE), qM (AqM), qI (AqI), and
113 the half-life of qE (τ_{qE}) and qM (τ_{qM}) relaxation. This population includes 18 elite breeding
114 genotypes, 15 diverse breeding genotypes that contain exotic diversity, and eight exotically
115 sourced plant introductions. These data were used to test the hypothesis that (1) there is significant
116 diversity in rates of NPQ relaxation between soybean genotypes and (2) individual components of
117 NPQ relaxation vary independently between parents. The heritability of NPQ relaxation
118 components were then calculated to assess the potential for breeding improved solar conversion
119 efficiency.

120 **Methods**

121 **Plants and growth conditions**

122 The 41 parents of the Soybean Nested Association Mapping (NAM) population (Song et al., 2017,
123 Diers et al., 2018) were grown in the field at the Crop Sciences Research and Education Center at
124 the University of Illinois at Urbana-Champaign in 2018 and 2019. Seeds were planted on 23 May
125 2018 and 6 June 2019 in 1.2 meter long single-row plots with a 0.75 m row spacing at a rate of 40
126 seed m⁻¹. The experiment was arranged in a randomized complete block design with five replicates
127 and standard agronomic practices were employed.

128

129 **Meteorological data collection**

130 Meteorological variables were measured every 30-minutes by a weather station at the University
131 of Illinois Energy Farm, approximately 1 km from the Crop Sciences Research and Education
132 Center (latitude 40.062832, longitude -88.198417). Air temperature (Ta, °C) and relative humidity
133 (RH, %) were recorded by a HMP45C probe (Campbell Scientific, Logan, UT, USA), and
134 incoming shortwave radiation (Fsd, W m⁻²) was from a CNR1 radiometer (Kipp & Zonen, The
135 Netherlands), both instruments were installed 4 m above the ground. Rainfall (mm) was obtained
136 from the Illinois Water Survey at 30-minute time step (Illinois State Water Survey, 2020). Ta and
137 RH were used to calculate saturation vapor pressure (e_s) and actual vapor pressure (e_a) for each
138 30-minute period, which were then used to calculate vapor pressure deficit (VPD, kPa) as per
139 Equations 1-3:

$$140 \quad e_s = 0.6106 * \exp\left(\frac{(17.27*Ta)}{(237.3+Ta)}\right) \quad \text{Equation 1}$$

$$141 \quad e_a = \frac{RH * e_s}{100} \quad \text{Equation 2}$$

$$142 \quad VPD = e_s - e_a \quad \text{Equation 3}$$

143

144 Occasional gaps in meteorological data are inevitable when measuring at these time scales, so data
145 gaps were filled where needed following (Isaac et al., 2017), whereby an artificial neural network
146 was used to generate a complete time-series with external data sourced from the University of
147 Illinois Willard Airport weather station (station ID: 725315-94870) and ERA-interim data from
148 the European Centre for Medium Range Forecasts (Dee et al., 2011). In total, less than 5 % of data
149 required gap filling. Daily summary values were then calculated for each variable, with Ta and

150 VPD presented as daily mean, Fsd as daytime-only (i.e. 06:00-18:00) mean, and rainfall as a daily
151 sum. A 7-day rolling mean was calculated to aid with data visualization.

152

153 **Chlorophyll fluorescence analysis**

154 Plants were sampled on the 19th of July 2018 and the 26th July 2019, when at the R1-R3
155 developmental stage. Three 4.8 mm leaf disks were collected from the upper-most mature leaf of
156 each replicate strip in the field using a cork borer (Humboldt H9663; Fisher Scientific 07-865-
157 10B).

158

159 In 2018 leaf disks were placed face down in clear 96 well plates and held in position with a moist
160 sponge cut to the size of a well. Plates were transported back to the laboratory for overnight dark
161 incubation to allow for relaxation of long term NPQ. In 2019 leaf disks sampled in the field were
162 floated on dH₂O in a 24 well plate which was sealed with parafilm for transportation back to the
163 laboratory for ease of manipulation. Leaf disks were then transferred to a square petri dish lined
164 with wet filter paper, and plates sealed with parafilm and wrapped in aluminum foil for overnight
165 incubation.

166

167 Measurements were taken with modulated chlorophyll fluorescence imaging systems, in 2018
168 using a CF imager (Technologica, UK) and in 2019 a FluorCam FC 800-C (PSI Systems, Czech
169 Republic in 2019). Briefly, overnight incubated disks were subjected to 10 min illumination at
170 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light (6500 K) followed by 50 min of darkness. F_m , was determined by
171 applying saturating pulses (4000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light) at 9, 40, 60, 80, 100, 120, 160, 200,
172 240, 300, 360, 420, 480, 540 and 598 s after the actinic light was turned on, and at 1, 2, 4, 6, 8, 10,
173 14, 18, 22, 26, 32, 38, 44 and 50 min after the actinic light was turned off. The background was
174 excluded automatically and NPQ values at each pulse were calculated.

175

176 In 2018, NPQ values were calculated for each time point using FluorImager v2.305 with custom
177 MatLab R0218b scripts (see accession numbers) and in 2019 using the software FluorCam7
178 v.1.2.5.3. NPQ relaxation parameters AqE, AqM, AqI, τqE and τqM were then calculated by fitting
179 a double exponential function to measured NPQ values following shut off of the actinic light,
180 according to Equation 4 (Dall'Osto et al., 2014):

181
$$\phi NPQ = \phi AqI + \phi AqE^{(-\frac{t}{\tau qE})} + \phi AqM^{(-\frac{t}{\tau qM})}$$
 Equation 4

182
183 using the fit function in MatLab R0218b (see accession numbers), where t is the measured
184 fluorescence at a given timepoint. Maximum NPQ values are defined as the maximum value
185 reached during the 10 min illumination.

186
187 **Statistical Analysis**

188 After visual inspection of data distributions, outliers were defined and removed if values were
189 outside 1.5 x the interquartile range (IQR). Statistical significance was determined by one-way
190 ANOVA with a post-hoc Games-Howell test at an alpha level 0.05 using the R package
191 ‘userfriendlyscience 0.7.2’ (Peters, 2018) and custom R-scripts (see accession numbers). Pearson
192 correlations between NPQ relaxation parameters AqE , AqM , AqI , τqE and τqM , and between
193 years, were determined using the R package ‘PerformanceAnalytics 2.0.4’ (Peterson et al., 2020).

194
195 **Broad sense heritability calculation**

196 The mean of technical replicates taken from genotypes in each year was used to estimate the
197 heritability of NPQ relaxation parameters. Variance components were determined using a linear
198 mixed effects model (lmer) function in the R package ‘lme4 1.1-23’ (Bates et al., 2015), with
199 genotype as a random effect and environmental variance estimated from the residuals. Broad-sense
200 heritability was then calculated according to Equation 5:

201
202
$$H = \delta_G^2 / (\delta_G^2 + \delta_E^2)$$
 Equation 5

203
204 Where H^2 is broad sense heritability, δ_G^2 is genotypic variance and δ_E^2 is environmental variance.

205
206 **Accession numbers**

207 Raw chlorophyll fluorescence imager files and scripts are deposited on figshare and can be
208 accessed at <https://doi.org/10.6084/m9.figshare.c.5075717>.

209 **Supplemental Data Files**

210 Supplemental Data 1: NPQ relaxation parameters calculated for the 41 parents of the Soybean

211 Nested Association Mapping panel

212 Supplemental Data 2: ANOVA and Games-Howell posthoc test results comparing NPQ

213 relaxation kinetics between 41 founder lines of Soybean NAM population

214 Supplemental Figure 1: NPQ relaxation kinetics of the 41 parents of the soybean NAM

215 population in 2018.

216 Supplemental Figure 2: NPQ relaxation kinetics of the 41 parents of the soybean NAM population

217 in 2018.

218 Supplemental Figure 3: Pearson correlation between NPQ parameters of the 41 parents of the

219 soybean NAM population in 2018.

220 Supplemental Figure 4: Pearson correlation between NPQ parameters of the 41 parents of the

221 soybean NAM population in 2019.

222 Supplemental Figure 5: Pearson correlation between measured AqE values of 41 parents of the

223 soybean NAM population in 2018 and 2019.

224 Supplemental Figure 6: Pearson correlation between measured AqM values of 41 parents of the

225 soybean NAM population in 2018 and 2019.

226 Supplemental Figure 7: Pearson correlation between measured AqI values of 41 parents of the

227 soybean NAM population in 2018 and 2019.

228 Supplemental Figure 8: Pearson correlation between measured τ qE values of 41 parents of the

229 soybean NAM population in 2018 and 2019.

230 Supplemental Figure 9: Pearson correlation between measured τ qM values of 41 parents of the

231 soybean NAM population in 2018 and 2019.

232 Supplemental Figure 10: Pearson correlation between measured maximum NPQ values of 41

233 parents of the soybean NAM population in 2018 and 2019.

234 **Results**

235 NPQ relaxation was measured for the 41 parents of the soybean NAM population over two
236 growing seasons in Urbana, IL, USA (2018 and 2019), to investigate genotypic and environmental
237 variation. Trends between genotypes were compared between years, rather than measured values,
238 as different chlorophyll fluorescence imaging systems were used (see methods). In both years, the
239 amplitude of NPQ attributable to fast, medium and slow relaxing phases was determined to be qE
240 $> qM > qI$ for all parents (Figure 1 and 2; Supplemental Figure 1 and 2, Supplemental Data 1).

241
242 Here we describe in detail the results for 2019, which was performed with increased technical
243 replicates to allow for discarding of outliers facilitating statistical analysis, but population level
244 trends were similar in 2018 (Supplemental Data 1; Supplemental Figure 1 and 2). In 2019, there
245 was a statistically significant difference in the amplitude of qE (AqE) between parents ($F(40,127)$
246 $= 2.06$, $p=0.0013$), which ranged between 1.57 ± 0.11 (NAM64) and 2.36 ± 0.28 (NAM2)(Figure
247 1). Measured NPQ attributable to qI , AqI ($F(40,127) = 1.54$, $p=0.037$), differed ~ 2 fold between
248 the smallest (0.09 ± 0.05 ; NAM33) and largest (0.021 ± 0.03 ; NAM28) (Figure 1). In terms of
249 relaxation rates, there was a globally statistically significant difference ($F(40,127) = 2.51$,
250 $p=<0.001$) in measured τqE , which varied ~ 1.5 fold between the fastest ($28.2 \pm 3.6s$; NAM64) and
251 slowest ($43.2 \pm 5.4s$; NAM38) parent, but non-significant pairwise comparisons by Games-
252 Howells posthoc test, which may be a result of the relatively low replication. In addition, there was
253 a ~ 2 fold difference in τqM between the fastest (12.7 ± 2.0 min; NAM27) and slowest relaxing
254 (25.0 ± 6.5 min; RC) parents which was not statistically significant ($p>0.05$) (Figure 2;
255 Supplemental Data 2) and there was no statistically significance difference between maximum
256 recorded NPQ values (lowest, 2.13 ± 0.41 , NAM50; highest, 3.42 ± 0.43)(Figure 2). However,
257 taken together, these data indicate there is substantial variation in NPQ parameters within soybean
258 germplasm.

259
260 Comparing trends from both years, there was a significant positive correlation between maximum
261 NPQ and AqE (0.75 , $p<0.001$ in 2018; 0.67 , $p<0.001$ in 2019) (Supplemental Figures 3 and 4).
262 However, there was no correlation between maximum NPQ and AqI (0.08 , $p>0.05$ in 2018; 0.26 ,
263 $p>0.05$ in 2019)(Supplemental Figures 3 and 4), which would be in accordance with the majority

264 of qI being unrelated to photoprotection, being instead generated by photodamage, given the
265 assumption NPQ increases in response to a greater requirement to dissipate excess reducing power.

266

267 While measured parameters fell within in a similar range in both 2018 and 2019 (Figure 1 and 2;
268 Supplemental Figure 1 and 2), parents behaved differently between years, as there was no
269 correlation between the recorded values in 2018 and 2019 (Supplemental Figures 5-10), which is
270 consistent with a strong environmental and genotype by environment effect on NPQ. To
271 investigate this further, we analyzed the variation in climatic conditions over the growing period
272 in 2018 and 2019 taking data from a weather station on a nearby farm (Figure 3). Fluorescence
273 measurements were made on day 201 in 2018 and day 207 in 2019.

274

275 On the day of measurement, total solar radiation and mean air temperature were similar across the
276 two years, at $\sim 45 \text{ MJ m}^{-2}$ and $\sim 23 \text{ }^\circ\text{C}$ respectively, while VPD was at ~ 0.8 in 2019 compared to
277 ~ 0.7 (Figure 3). However, cumulative rainfall in 2018 was approximately double that of 2019
278 reaching 250 mm over June and July, and average air temperature was higher in the early growing
279 season, increasing from $22 \text{ }^\circ\text{C}$ to a high of $26 \text{ }^\circ\text{C}$ over the first 20 days, compared to a decrease in
280 temperature from $23 \text{ }^\circ\text{C}$ to $19 \text{ }^\circ\text{C}$ in 2019 (Figure 3). While there was no clear difference in total
281 incident solar radiation, which is influenced by cloud cover and fluctuated between 30 and 55 MJ
282 m^{-2} in both years (Figure 3), the differences in precipitation and temperature were reflected in
283 periodically higher vapor pressure deficit (VPD) in 2019, which fluctuated between 1.2 and 0.4
284 kPa (Figure 3)

285

286 In order to assess the potential for breeding soybean for improved NPQ characteristics we
287 calculated the heritability of parameters showing significant variation between genotypes
288 including AqE (0.21) and AqI (0.11) using data from 2019 (Table 1).

289 **Discussion**

290 Increasing the rate of NPQ activation and relaxation has been demonstrated as a means to improve
291 photosynthetic efficiency (Kromdijk et al., 2016), and although further research is required to
292 understand potential species-specific differences (Garcia-Molina and Leister, 2020) and the
293 potential effects of reduced ROS production from quicker activation (Davis et al., 2017), targeting
294 NPQ is proposed as a means for crop improvement (Long et al., 2019). The data collected here
295 indicate there is a statistically significant difference in NPQ relaxation rates in the parents of the
296 soybean NAM population, and this study provides the basis for further research to map genomic
297 loci responsible through screening of the wider population (Song et al., 2017; Diers et al., 2018).

298
299 Taking the slowest, fastest and average short term relaxation rates of the NAM parents in 2019,
300 and inputting them into a soybean canopy photosynthesis model suggested the difference between
301 the slowest and average could result in a small but substantial (1.3 %) increase in carbon
302 assimilation on a cloudy day (Wang et al., 2020). However, it remained unclear to what extent this
303 variation in soybean is heritable, information which is required to estimate the ease with which
304 variation in NPQ could be selected for in a conventional breeding program. We therefore sort to
305 address this through analysis of the entire NAM population.

306
307 NPQ is a multigene trait, and is likely to be affected by environmental fluctuations that influence
308 cellular energy status, including temperature, water availability, nutrient availability and pathogen
309 attack. Subsequently, we observed a low (< 0.5) heritability value for NPQ relaxation parameters
310 in the NAM population using current screening approaches in a field environment. This is similar
311 to heritability estimates from OJIP measurements of chlorophyll fluorescence in a larger panel of
312 soybean genotypes (Herritt et al., 2018), and those reported for other photosynthetic traits such as
313 J_{\max} in wheat (0.32; (Driever et al., 2014)).

314
315 In addition, we observed no significant correlation between measured parameters over 2018 and
316 2019, suggesting a strong environmental effect. Meteorological data indicates conditions on the
317 day of sampling were similar, but plants may have been more water limited throughout the duration
318 of the growing season in 2019. Therefore, the environmental cause of variation in NPQ relaxation
319 parameters between 2018 and 2019 could be related to long term adaptation to annual differences

320 in rainfall and temperature, requiring further investigation. However, heritability is also dependent
321 on the presence of large effect alleles within a population. While significant genotypic variation in
322 AqE and AqI was identified in the NAM population, the data also suggest that unlike in some other
323 species, such as rice (Kasajima et al., 2011), there were no large effect alleles present in the
324 population screened.

325
326 Therefore, several approaches could be considered to improve the potential for breeding faster
327 relaxation of NPQ: screening a bigger collection of diverse soybean genotypes to identify potential
328 large effect alleles, growing and measuring genotypes under controlled conditions to reduce
329 environmental variation, increasing replication over multiple environments, and improving
330 statistical methods to reduce variation. One step towards achieving this goal could be coupling
331 large scale screening with open availability of data as reported elsewhere (Kuhlgert et al., 2016).

332
333 Genetic modification provides an alternative means of modulating NPQ (Armbruster et al., 2016;
334 Kromdijk et al., 2016), and to be successful this will depend on both an understanding of the genes
335 regulating NPQ relaxation, and how they influence plant energetics in a fluctuating environment
336 (Kramer and Evans, 2011). While several of the genes involved in fast and medium term relaxation
337 of NPQ have been identified, further work is required to understand which are the key genes and
338 processes underlying long term NPQ relaxation, or qI. Advances in what in addition to new
339 forward genetic screens, such as those that identified the novel slow relaxing phase qH (Malnoë et
340 al., 2018), and refinement of candidate gene lists identified by genome wide association analysis
341 (Rungrat et al., 2019) is required.

342
343 In conclusion, we measured NPQ relaxation in the 41 parents of the soybean NAM population;
344 while there is significant genotypic variation, there was a strong environmental effect resulting in
345 low heritability estimates which imply selection by breeding will be difficult. Therefore given
346 current approaches and information, the most effective means of manipulating NPQ for improved
347 photosynthetic performance in soybean will likely rely on genetic modification.

348 **Acknowledgements**

349 We thank Troy Cary for setting up the soybean NAM population plots, Wanne Kromdijk for help
350 setting up scripts for analysis of NPQ relaxation parameters and Liana Acevedo-Siaca for support
351 with statistics. This work was supported by the project Realizing Increased Photosynthetic
352 Efficiency (RIPE), funded by the Bill and Melinda Gates Foundation (BMGF), Foundation for
353 Food and Agriculture Research (FFAR) and the UK Department for International Development
354 (UKAid) under grant number OPP117215. SJB was supported by Carl R. Woese Institute for
355 Genomic Biology Fellowship and EMDB is supported by an Illinois Distinguished Fellowship.

356

357 **Author contributions**

358 SPL, SJB and BWD designed the study. SJB, EMBD, IC, IF and SC conducted the NPQ
359 measurements, CM conducted the meteorological data collection. SJB and CM performed the data
360 analysis. SJB, SPL, EMDB, CM and BWD wrote the article and all authors provided critical
361 revisions.

362

363 **Conflict of Interest**

364 The authors report no conflicts of interest

365 **Figure Legends**

366

367 **Table 1: Estimates of heritability of NPQ relaxation kinetics AqE, AqI, and τ qE based on**
368 **field data.** Data are shown for 2019, including the genotypic (δ^2_G) and environmental (δ^2_E)
369 variance and the derived broad sense heritability value (H^2) for AqE and AqI. Heritability values
370 are represented to two significant figures.

371

372 **Figure 1: NPQ relaxation kinetics of the 41 parents of the soybean NAM population in 2019.**
373 Box plots representing (a) AqE, (b) AqM, (c) AqI, displaying mean and standard deviation. Data
374 points for the mean of n=3-5 (See Supplemental Data 1 for details) biological replicates are
375 displayed. Colors correspond to genotype groups, including: Reference genotype (grey), EL = elite
376 genotype (orange), BX = breeding genotype with exotic ancestry (blue), PI = plant introductions
377 (magenta). Letters denote significant groupings ($\alpha=0.05$) based on Games-Howell posthoc test.

378

379 **Figure 2: NPQ relaxation kinetics of the 41 parents of the soybean NAM population in**
380 **2019.** Box plots representing (a) τ qE, (b) τ qM, (c) maximum NPQ, displaying mean and
381 standard deviation. Data points for the mean of n=3-5 (See Supplemental Data 1 for details)
382 biological replicates are displayed. Colors correspond to genotype groups, including: Reference
383 genotype (grey), EL = elite genotype (orange), BX = breeding genotype with exotic ancestry
384 (blue), PI = plant introductions (magenta).

385

386 **Figure 3: Climate data for June and July in Champaign-Urbana, Illinois comparing 2018**
387 **and 2019.** Line graphs show cumulative rainfall (mm), air temperature ($^{\circ}\text{C}$), solar radiation (MJ
388 m^{-2}) and VPD (kPa) for 2018 (purple), 2019 (green). Data are presented as 7-day running mean
389 with +/- a 7-day running error shading.

390

Parameter	δ^2_G	δ^2_E	H^2
AqE	1.81E-02	6.94E-02	0.21
AqI	3.33E-04	2.66E-03	0.11

391 **Table 1**

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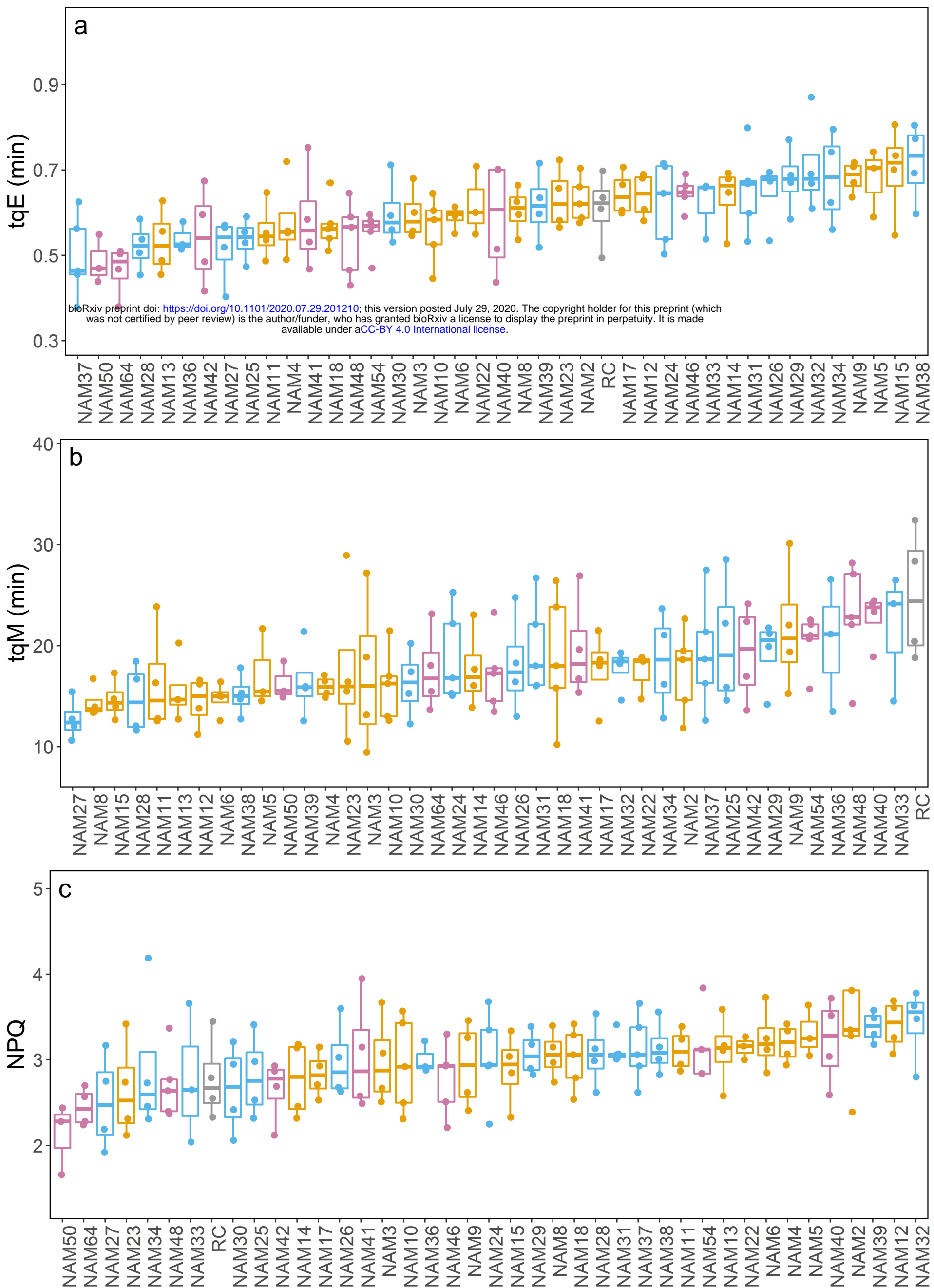


Figure 2

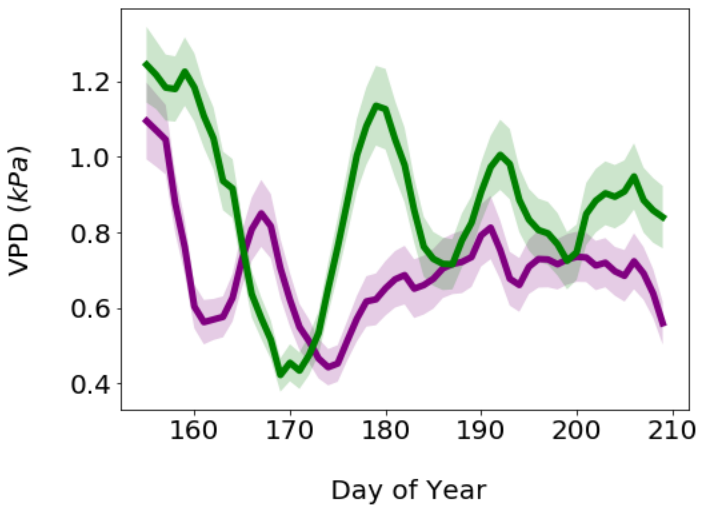
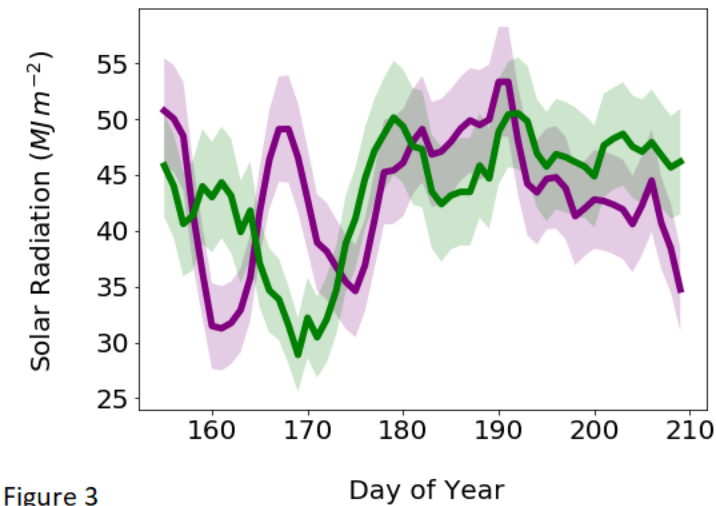
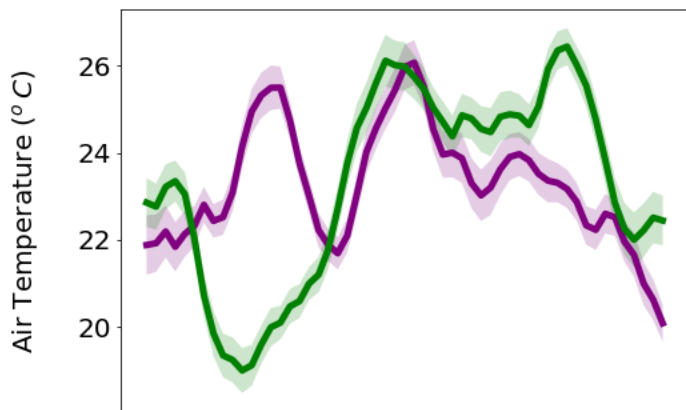
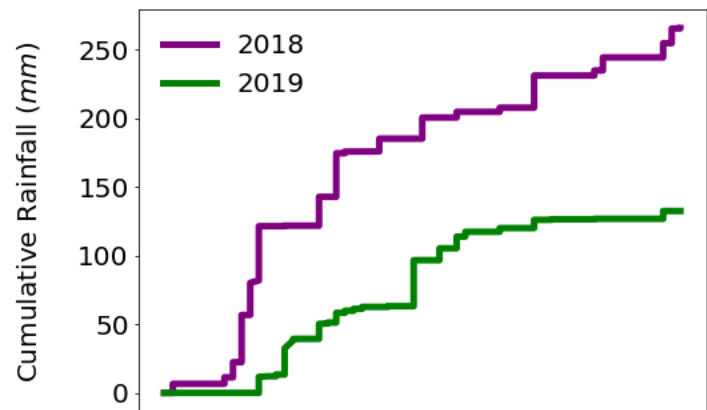


Figure 3