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Photosynthetic variation for climate-resilient crops: photosynthetic responses to fluctuating light and chilling in tomato

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Abstract

An increase in global demand for crop-based products necessitates an increased crop yield. Optimizing photosynthesis, which is sensitive to environmental fluctuations, offers a promising strategy to improve crop yield and resilience. Photosynthetic responses often lag behind changes in irradiance, resulting in the loss of potential carbon gain. Additionally, global warming is accompanied by unexpected chilling spells, further affecting photosynthesis. Thus, developing chilling-resilient crops and optimizing photosynthetic responses to fluctuating light is critical. This can be achieved by identifying genetic markers associated with desirable photosynthetic traits in plant populations. However, the combined effect of chilling and photosynthetic responses to fluctuating light remains unexplored, and there is a lack in populations designed to explore these responses. Thus, exploration needs to be done in pre-existing populations where there is phenotypic variation in photosynthetic responses and in how chilling affects these responses within parental lines. This study examined the variation in photosynthetic responses of the parental lines of a Multi-parent-Advanced-Generation Inter-Cross (MAGIC) population of tomato (*Solanum lycopersicum*) under fluctuating light and suboptimal temperatures. Photosynthetic responses to step increases and step decreases in irradiance were measured using modulated chlorophyll fluorescence and the effect of lowered temperature (14°C) on these responses was investigated. The results showed variation in the kinetics of the response of quantum efficiency of PSII (Φ_{PSII}) to step changes in irradiance under control and chilling conditions. Chilling had a minimal effect on the photosynthetic responses of some parental lines, indicating resilience to chilling. These findings highlight the potential of exploring genetic components to breed climate-resilient crops.

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1 | INTRODUCTION

Current crop production is insufficient to ensure food security. A growing population combined with an increasing per capita demand for food and other crop-based products warrants an increase in crop production just to maintain the current levels of food insecurity. While crop improvements resulting from systematic interventions in crop management, resource allocation in crop plants, and higher-yielding varieties have led to higher crop production (Miflin 2000; Grassini et al., 2013), novel interventions are needed to address the potential increase in demand in the coming years (OECD et al., 2022). The role of photosynthesis in improving crop yield is one such area that has not been extensively utilised, though numerous studies have been conducted that demonstrate the potential for crop yield improvement via modifications to photosynthetic properties (Kromdijk et al., 2016; Glowacka et al., 2018; Acevedo-Siaca et al., 2020; Simkin et al., 2019). The reasons why photosynthesis has been relatively under-utilised lie with its inherent physiological and genetic complexity, difficulties in adequately phenotyping the trait, and uncertainty over the causal link between improved photosynthesis and increased yield. The photosynthetic apparatus consists of a large number of processes that potentially limit photosynthesis and naturally occurring variations in photosynthesis that are usually associated with trade-offs, many of which are strongly environmentally driven. This complicates understanding the impact of modified photosynthesis on yield and understanding why modified photosynthesis does not always improve yield. Nonetheless, identifying genetic factors is essential if we are to improve photosynthesis and ultimately improve crop production (Zhu et al., 2010; Ort et al., 2015). Identifying such genetic factors can be achieved by associating phenotypes with genomic regions, called Quantitative Trait Loci (QTLs), using a population of plants with a known genetic composition. This has been widely used to develop crop lines with desirable traits (Fernie et al., 2006; Kumar et al., 2017). A prerequisite for such mapping is the existence of variation in the desired trait (phenotype) due to underlying genetic variation (Flood et al., 2011). Variation may naturally occur in a plant population, it may arise via spontaneous mutation or by deliberate mutagenesis, or it may originate via transgenesis and cisgenesis (Commission 2023). A plant population consists of distinct genotypes for which there is a path allowing introgression of a trait from one individual to another by conventional breeding techniques and may include individuals of more than one species. In addition, if the genetic basis of useful variations has been identified at the genomic level, then that variation may be introduced into a genotype via conventional breeding, cisgenesis, transgenesis or targeted mutagenesis. Variation of this kind for photosynthetic traits can help identify genetic regions that underpin specific photosynthetic traits, which can then aid in improving crop production. Variation in photosynthesis due to genetic differences has already been demonstrated. These include variations in the Rubisco small subunit diversity, chlorophyll content, flag leaf photosynthesis rates, and cold tolerance to photosynthesis (Flood et al., 2011; Lawson et al., 2012; Faralli and Lawson 2020). The identification of genetic components of photosynthesis-related traits has been demonstrated by Prinzenberg et al., (2020), Gao et al., (2024), Yoshiyama et al., (2024), and others.

Photosynthesis is highly responsive to the environment over a range of time scales. These responses encompass a range of processes that result in the adjustment of the operation of photosynthesis to optimise its activity and efficiency in terms of light, nutrients, water and biomass (Kaiser et al., 2015). Light is one of the most variable environmental factors influencing photosynthesis, with its intensity and spectral quality fluctuating across different time scales. Irradiance can vary on the millisecond scale due to the fluttering of leaves within the canopy (windflecks), on the second-to-minute scale due to cloud movement (sunflecks), and on the hourly-to-monthly scale due to the Earth's rotation and its position relative to the sun (Slattery et al., 2018). These fluctuations affect different parts of the photosynthetic apparatus. For example, rapid fluctuations (on the millisecond to second scales) influence processes like electron transport, proton transport, and non-photochemical quenching (NPQ) and especially the qE component of NPQ, while slower fluctuations (on the minutes-to-hours scale) affect processes such as RuBisCo activation and stomatal opening (Kaiser et al., 2018a; Long et al., 2022). Temporally, photosynthetic responses often lag behind changes in irradiance, which can lead to a loss in productivity compared to a scenario where photosynthesis closely, or even perfectly tracks irradiance changes. Therefore, minimizing this delay is important for the optimization of photosynthesis, enhancing photosynthetic productivity and increasing plant yield. Exploring variation in photosynthetic responses to irradiance can help identify genetic factors that optimize these responses.

In addition to light, temperature also influences photosynthesis, particularly in its response to changes in irradiance. Climate change is often associated with increased temperatures, but it can also bring periods of lowered temperatures. These periods of lowered temperatures can occur due to meteorological phenomena that push cold Arctic air southward, or due to changes in ocean currents that bring cold air (Singh et al., 2016; Cohen et al., 2018; Kretschmer et al., 2018). Unexpected cold spells can significantly affect the growth and yield of major crops, especially fruits and vegetables, which are more vulnerable to low temperatures (Soualiou et al., 2022). For example, cold spells in April 2021 and December 2023 across mainland Europe had a major effect on grape and fruit yields, while winter crops like wheat and rapeseed were less impacted (Baruth, 2021; Tarnavsky, 2023). Lower temperatures reduce CO_2 assimilation rates, reduce the activity of Calvin-Benson-Bassham cycle enzymes like FBPases and SBPases, and impair carbohydrate metabolism, leading to end-product limitations in plants, leading to decreased crop yield (Allen and Ort 2001). Low temperatures can also slow photosynthetic responses to step change irradiance, further increasing the potential for productivity loss (Soualiou et al., 2022). However, the combined effects of fluctuating light and low temperatures on photosynthesis have not been thoroughly studied. Therefore, understanding how cold affects photosynthetic responses to irradiance is crucial for ‘climate-proofing’ crops. Investigating variations in photosynthetic responses to irradiance under cold conditions can help identify those genetic factors needed to improve climate resilience.

A challenge in exploring the variations in photosynthetic responses to changes in irradiance lies in acquiring and screening populations to identify desirable traits. Traditionally, populations have been created based on parents chosen for particular traits like increased water use efficiency, pollen viability and plant height, but populations designed to explore photosynthetic traits are less common. There have been numerous studies on photosynthetic induction from a dark-adapted state, or its relaxation from a light-adapted state following the transfer to darkness (Sassenrath-Cole and Pearcy 1992, 1994; Sassenrath-Cole et al., 1994; Kaiser et al., 2018b, 2020), and reviews have summarized the key findings (Kaiser et al., 2015, 2018a; Long et al., 2022). Nonetheless, much remains to be understood about how different components of the photosynthetic machinery respond to fluctuating light spanning different time scales. This makes it challenging to generate populations that can effectively explore variations in photosynthetic responses. In contrast, cold tolerance has been much more extensively studied, allowing easier access to populations with phenotypic diversity for crops like maize, chickpea, and rice (Srinivasan et al., 1998, Yi et al., 2021, Li et al., 2022). However, the effect of lowered temperatures on the speed of photosynthetic responses to irradiance changes remains largely unexplored, complicating the process of generating populations with sufficient phenotypic variation. As things stand, therefore, exploring natural variations in photosynthetic responses under fluctuating light and low temperatures must be carried out within existing populations.

To ensure sufficient phenotypic variations in pre-existing populations, it is essential to have variation in both photosynthetic responses to irradiance and the effects of low temperature on these responses among the parental lines of the population. Tomato, a commercially relevant and fast-growing crop, is known for its sensitivity to chilling temperatures. Multiple studies have reported how cold affects tomato's photosynthetic machinery, including changes in stomatal opening, delays in RuBisCO activation, an inability to activate Calvin-Benson-Bassham (CBB) cycle enzyme intermediates, and photodamage caused by reactive oxygen species (ROS) production (Allen and Ort 2001; Soualiou et al., 2022). However, these studies have not directly investigated the effect of low temperatures on the rate of photosynthetic responses to step changes in irradiance. Indirectly, though, they suggest that, given with enough genetic variation, there may be variations in photosynthetic responses to irradiance under lowered temperatures. Furthermore, numerous studies have described the chilling tolerance of wild tomato relatives and how breeding interventions introduce cold tolerance into cultivated tomatoes (Venema et al., 2005). The cultivated tomato has also undergone extensive breeding during domestication, which likely provided genetic adaptations to a range of temperatures and environments (Razifard et al., 2020). This suggests that cultivated tomatoes may harbour genetic markers related to chilling tolerance. Therefore, we aim to investigate phenotypic diversity in photosynthetic responses and their reactions to chilling temperatures across four parental lines of a Multi-parent Advanced Generation Inter-Cross (MAGIC) tomato population. Two of these lines are known for their heat tolerance, though primarily for pollen viability rather than photosynthetic traits. Photosynthetic responses to changing irradiances were measured by monitoring the quantum efficiency of photosynthesis to step increases and decreases in irradiance. These measurements were then repeated under lowered temperatures to investigate phenotypic diversity under chilling conditions.

2 | MATERIALS AND METHODS

2.1 | Plant material and growing conditions

In this study, four parental lines of a Multi-parent Advanced Generation Inter-Cross (MAGIC) population were cultivated under controlled climate conditions at the Netherlands Plant Eco-phenotyping Centre (NPEC, Wageningen). The genotypes used in the study were Nagcarlan (P1), North Carolina heatset-1 (P2), Delfo parent 1 (P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker) (MM) The cultivar Nagcarlan (LA2661) is heat tolerant, producing more pollen under long-term moderate heat. NCHS-1 (LA3847) is a country race with medium heat tolerance that produces a high pollen count (Xu et al., 2017). F1 hybrids Delfo Parents 1 and 2 (BASF, Nunhems, The Netherlands) are pest-resistant. Moneymaker was used as a control genotype because it exhibits stable traits under different environmental conditions and in different years.

Seeds were germinated on 4 cm diameter rockwool plugs (Grodan, Roermond, the Netherlands) by keeping the plugs in the dark at 21°C for three days. The seedlings were then transferred to the rockwool blocks (10 × 10 × 6.5 cm), three days after sowing (DAS). An in-house nutrient solution, Tomato 2.0 (Supplementary S.5) was used to irrigate the plants twice a week. All genotypes were grown in five replicates with a day length of 16 hours (from 7:00 h to 23:00 h), a light intensity of 400 μmol m^{-2} s^{-1}, day/night temperatures of 21/18°C, and a relative humidity of 75%. The day/night temperatures were lowered to a day temperature of 14°C and a night temperature of 11°C 19 days after sowing (19 DAS). The plants were grown under chilling conditions (14°C) for six days before the temperatures were restored to 21°C and 18°C for day and night on day 25 after sowing. To account for environmental and positional variations, a randomized complete block design was implemented with four blocks and five replicates. However, considering genotype P1 was represented by only three healthy plants after 15 DAS, only three replicates per genotypes were considered for this study.

The growth room was equipped with a PlantScreen™ Robotic XYZ System (Photon Systems Instrument) which is a robotic arm designed for plant cultivation and growth monitoring. The imaging head (comprising a camera and LEDs) can be moved laterally to repeatedly image specific group of plants, and vertically to ensure the camera is kept at fixed distance from the top of the plant canopy (https://plantphenotyping.com/products/plantscreen-robotic-xyz-system/#details; Sorrentino et al., 2021). The growth chamber is equipped with LED illumination, including natural daylight simulations, imaging equipment for the visible light spectrum (RGB), and chlorophyll fluorescence imaging (https://growth-chambers.com/products/growth-capsule-gc/#details; Sorrentino et al., 2021). For more details of the imaging head and chamber refer to the Supplementary Information (S.2). The phenotyping imaging head was maintained at a height of 400 mm over the highest leaf and this height was adjusted, by moving the imaging head vertically mechanically using the PlantScreen™ Robotic XYZ software, as the plants grew. Consequently, the camera consistently maintained the same relative distance to the tomato plant's top regardless of the plant's growth stage, guaranteeing consistent image acquisition throughout the course of the experiment.

2.2 | Phenotyping for photosynthetic responses

For the control experiment (13 DAS – 18 DAS), chlorophyll fluorescence was measured with an automated camera system that moved over the plants. Pre-dawn Fv/Fm measurements using saturating light flashes (8000 μmol m^{-2} s^{-1}, for 800 ms) were taken before the lights went on at 5:30 am. For steady-state photosynthesis measurement, Φ_{PSII} at 400 μmol m^{-2} s^{-1} (growth light irradiance) was measured both 1 hour after the light turned on ('dawn Φ_{PSII}') and 1 hour before it turned off ('dusk Φ_{PSII}'). The dawn and dusk ΦPSII measurements were used to monitor how Φ_{PSII} changed from the start to the end of the day. Φ_{PSII} was used to monitor photosynthesis for two main reasons: (1) Φ_{PSII} is a major determinant of linear electron transport (2) Φ_{PSII} can be measured by imaging chlorophyll fluorescence with saturating light flashes, allowing for a non-contact, non-destructive measurement of fast changes in photosynthesis with high spatial and temporal resolutions.

To measure the changes in photosynthesis during a step change in irradiance, the irradiance incident on the plants were controlled via the Robotic XYZ chlorophyll fluorescence imaging head. The Chlorophyll imaging head was used to equilibrate the plants for 2 minutes at 400 μmol m^{-2} s^{-1}, during which Φ_{PSII} was monitored every 30 seconds. The light intensity in the camera head was dropped to 200 μmol m^{-2} s^{-1} and the measurements of Φ_{PSII} were carried out simultaneously for 10 minutes, the time taken for Fm′ to reach steady state (Supplementary information S1.2). To capture the fast changes in Φ_{PSII} with changes in irradiance, saturating light flashes were applied as follows: flashes for every 5 s for a total of 25 s, followed by flashes for every 10 s for the next 40 s. From pilot runs, change in Φ_{PSII} slows down after 1 min, thus, flashes were then applied every 30 s for the next minute and finally flashes were applied every 120 min for the final 8 minutes (S1.2). After 10 minutes, the light intensity was increased to 600 μmol m^{-2} s^{-1} and the Φ_{PSII} measurements were repeated as described above. The same protocol was repeated for all genotypes under chilling (19 DAS-24 DAS) and recovery (25 DAS – 30 DAS) conditions. The application of saturating pulses at that frequency could be disruptive for the underlying change in photosynthesis. However, the data reported by Ramakers et al., (2025) has suggested that the effect of the frequency of flashes on NPQ is non-significant (low resolution and high resolution; as described in the paper). Additionally, to capture the transient change in Φ_{PSII} with good resolution, multiple saturating flashes spaced together were necessary.

2.3 | Calculations

2.3.1 | Photosynthetic parameters

Maximum quantum efficiency of PSII,

$$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm} \tag{1}$$

Where, Fm is maximum fluorescence when all reaction centres are closed, in the dark Fo is minimum fluorescence when all reaction centres are open, in the dark.

Quantum efficiency of electron transport through PSII,

$$\Phi_{PSII} = \frac{Fm' - F}{Fm'} \tag{2}$$

Where, Fm′ is maximum fluorescence when all reaction centres are closed, in actinic light F is steady-state fluorescence obtained, in actinic light.

2.3.2 | Fitting of Φ_{PSII} kinetics to step change in irradiance

The curves were fit (Origin 2024, OriginLab Corporation) using the equation,

$$y = y_0 + A\left(1 - e^{-kt}\right) \tag{3}$$

Where, y_0 is the baseline value.

A is the amplitude of responsek is the rate constant.

2.4 | Statistical analysis

Repeated measures ANOVA with Bonferroni and Tukey HSD post-hoc tests were carried out for all timeseries data to identify whether there was an effect of chilling on these measurements (IBM SPSS Statistics for Windows, Version 28.0). The threshold used was $p < 0.05$.

One way ANOVA with Fischer's LSD correction (Origin 2024, OriginLab Corporation) was used to compare the fit parameters during step increase and step decrease. The threshold used was $p < 0.05$.

3 | RESULTS

3.1 | Influence of chilling on the maximum quantum yield of PSII photochemistry, Fv/Fm

The maximum dark-adapted quantum efficiency of PSII photochemistry (Fv/Fm) was measured to check whether there is any photoinhibition of or incomplete relaxation of slowly relaxing

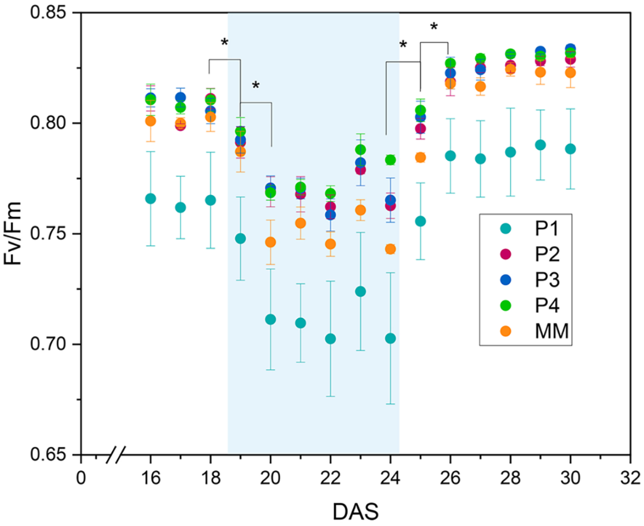

FIGURE 1 Time course plot illustrating maximum quantum efficiency, Fv/Fm, for all genotypes, measured each day after sowing (DAS). Fv/Fm measured 1 h before the lights were switched on. The chilling treatment started at 19 DAS and lasted till 24 DAS (including). The days with chilling exposure are indicated in light blue. Each point indicates the mean ($n = 3$) Φ_{PSII} and the error bar indicates standard error. Nagcarlan (P1), North Carolina heatset-1 (NCHS-1) (P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM). * indicates the time points $p < 0.05$ Bonferroni corrected, Repeated Measures ANOVA.

photoprotective mechanisms of Photosystem II (PSII; Demmig-Adams and Adams III 2006), which can be caused by stress (Krause and Somersalo, 1989). At the control temperature regime (21°C day/18°C night), the daily average Fv/Fm of all genotypes ranged from 0.76 to 0.81(16 DAS to 18 DAS; Figure 1). All genotypes except P1 had an average Fv/Fm above 0.8, indicating the absence of any slowly reversible loss of qE, photodamage, or non-photodynamic chilling-induced damage. P1 did have a lower average Fv/Fm ($\sim$0.76). After the temperature was lowered to 14°C (19 DAS), the average Fv/Fm of all genotypes (measured after 5.5 h of chilling in the dark) dropped slightly (Figure 1; 19 DAS). The average Fv/Fm of P1, P2 and MM dropped by 2.2% (0.76 to 0.74, 0.81 to 0.79 and 0.80 to 0.78 respectively) while P3 and P4 saw a drop in Fv/Fm of 1.6% (0.8 to 0.79 and 0.81 to 0.8 respectively) compared with the last day of control (18 DAS). With prolonged exposure to chilling, the Fv/Fm of all genotypes dropped further (Figure 1; 24 DAS). The average Fv/Fm for P1 dropped the most, from 0.76 to 0.70 ($\sim$8%), followed by MM, which had a drop in Fv/Fm of 7.4% (0.8 to 0.74). P2 and P3 experienced a drop in Fv/Fm of $\sim$5% (0.81 to 0.76,0.8 to 0.76 respectively) while P4 showed the lowest drop of 3.3% (0.81 to 78). This suggests that the Fv/Fm of P4 is the least sensitive to chilling while the Fv/Fm of P1 is most sensitive to chilling. When the temperature was restored to 21°C (25 DAS), the Fv/Fm of all genotypes increased (Figure 1; 25 DAS). At the end of the recovery period (Figure 1; 30 DAS), P2,P3 and P4 had an Fv/Fm of around 0.83, whilst MM and P1 had Fv/Fm·s of 0.82 and 0.78 respectively, indicating that in our case the effect of chilling on the Fv/Fm was completely reversible.

3.2 | Response of the quantum efficiency of PSII photochemistry (Φ_{PSII}) to chilling exposure and recovery

Under control temperatures (21°C), P1 had the lowest dawn Φ_{PSII} (0.63) while all other genotypes exhibited similar quantum efficiencies (0.67; Figure 2 A, 16-18DAS). After subjecting all genotypes to chilling (beginning at 19 DAS) the dawn Φ_{PSII} decreased (Figure 2 A, B). The average dawn Φ_{PSII} of MM decreased the most from 0.68 to 0.6 (11.1%), followed by P1 and P2 (10%; 0.64 to 0.57 and 0.66 to 0.6, respectively), while that of P3 and P4 only decreased by 5.6% (0.67 to 0.63) and 6.6% (0.66 to 0.62), respectively (Figure 2; 19 DAS). The dawn Φ_{PSII} for all genotypes decreased further with prolonged chilling (24 DAS; Figure 2A). When compared to the average dawn Φ_{PSII} on the last day of control (18 DAS) the Φ_{PSII} of P1 decreased the most ($\sim$ 14%; 0.64 to 0.54) while the Φ_{PSII} of the other genotypes decreased by $\sim$9–10%. Following the restoration of the temperature to 21°C (25 DAS), compared to the 18 DAS, the dawn Φ_{PSII} of P1 (measured 8 h after the temperature was restored) recovered from 0.54 to 0.61 (11%), and all the other genotypes recovered around (8%; 25 DAS Figure 2A). The dawn Φ_{PSII} of all genotypes had recovered to pre-chilling levels on the 26th DAS and remained there until the end of the experiment.

For the dusk Φ_{PSII} the trend in the decrease of Φ_{PSII} due to chilling (21 h at 14°C; 19 DAS) mirrored the decrease observed in the dawn Φ_{PSII} (Figure 2B, 19 DAS). The dusk Φ_{PSII} of MM and P1 decreased the most followed by P2 and then P3 and P4. In contrast, the decrease in dusk Φ_{PSII}, compared to the last day of control, at the end of the chilling treatment (Figure 2 B; 24 DAS) was less severe when compared to the decrease in dawn Φ_{PSII} ($\sim$6–8% reduction compared to >9% reduction). During the 'recovery' phase the dusk Φ_{PSII} recovered to pre-chilling levels (Figure 2B; 25–30 DAS). The dusk Φ_{PSII} of P1 recovered the most (13%; 0.55 to 0.63) while the other genotypes experienced an 8–9% recovery after 5.5 hours in 21°C (25 DAS; Figure 2B). The dusk Φ_{PSII}, much like the dawn Φ_{PSII}, recovered to pre-chilling levels on the 26th DAS.

3.3 | Short-term response of photosynthesis to a step increase and step decrease in irradiance

Photosynthetic responses lag behind changes in irradiance. The delay between the response and the change in irradiance results in a loss in photosynthetic light use efficiency. The physiological factors that determine the rate of the photosynthetic response to an increase in irradiance is different from those factors that influence the response to a decrease in irradiance (Kaiser et al., 2018a). This difference implies that the different genotypes can potentially respond differently to an increase and decrease in irradiance, implying a phenotypic

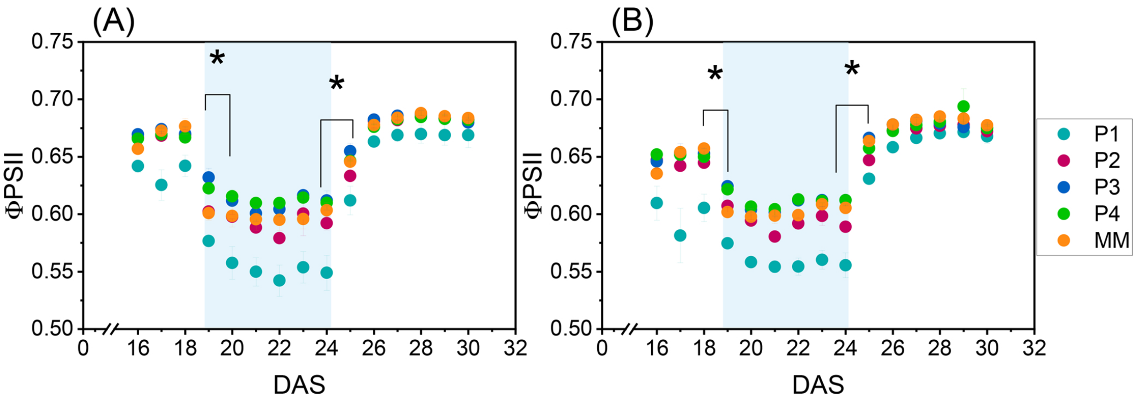

FIGURE 2 Time course plot illustrating quantum efficiency, Φ_{PSII}, for all genotypes, measured each day after sowing (DAS). (A) Φ_{PSII} measured 1 h after the lights were turned on (Dawn), measured at 400 μmol m^{-2} s^{-1}. (B) Φ_{PSII} measured 1 h before the lights were switched off (Dusk), measured at 400 μmol m^{-2} s^{-1}. The chilling treatment started at 19 DAS and lasted till 24 DAS (including). The days with chilling exposure are indicated in light blue. Each point indicates the mean ($n = 3$) Φ_{PSII} and the error bar indicates standard error. Nagcarlan (P1), North Carolina heatset-1 (NCHS-1) (P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM). * indicates the time points $p < 0.05$ Bonferroni corrected, Repeated Measures ANOVA.

variation in photosynthetic light use efficiency. Therefore, the responses of photosynthesis to an increase and decrease in irradiance were determined separately. Φ_{PSII} was used to monitor changes in photosynthesis to both the step increase and the step decrease in irradiance. The initial and final irradiance values for both the step increase and step decrease were selected based first on the desire to use irradiances that span a large change in CO_2 assimilation of the control genotype, MM, as concluded from an irradiance response curve of assimilation (Supplementary Figure S1). Second, they were selected on the need to begin the phenotyping procedure using the growth irradiance in order to make the procedure as time-efficient as possible. For the step decrease, a transition from 400 μmol m^{-2} s^{-1} (growth irradiance) to 200 μmol m^{-2} s^{-1} was selected, while for the step increase, a transition from 200 μmol m^{-2} s^{-1} to 600 μmol m^{-2} s^{-1} was adopted.

During the transition from 400 μmol m^{-2} s^{-1} to a lower light intensity of 200 μmol m^{-2} s^{-1}, Φ_{PSII} of all genotypes increased rapidly but then slowed down to eventually reach a new steady state within 200 s (Figure 3A). During the transition from 200 μmol m^{-2} s^{-1} to 600 μmol m^{-2} s^{-1}, Φ_{PSII} decreased immediately and slowly recovered to a new steady state (Figure 3B). To parameterize the response of photosynthesis to the short-term change in irradiance, the time course of the change in Φ_{PSII} was fitted with a mono-exponential function (equation 3; see calculations, S.3.1 and S.3.2). The estimated parameters are summarized in Table 1. In the case of the photosynthetic response to a step increase in irradiance, the time course change in Φ_{PSII} was fitted to the maximum of the overshoot (Figure 3D). The first-order rate constant (k), probably best considered to be pseudo-first order, is used as a measure of the speed of response. The sum of Φ_{PSII} measured before the change in irradiance (y0) and the estimated amplitude of change in Φ_{PSII} due to change in irradiance (A) reflects the steady-state Φ_{PSII} attained at the final irradiance (y0 + A). Based on the estimated first-order rate constant (k), P2 seems to respond fastest during the transition from high to low irradiance, while all the other genotypes seem to respond equally fast (Figure 3C, Table 1). On the other hand, during the step increase in light intensity, most genotypes showed similar rates of response, except for MM, which had the lowest rate constant (Figure 3D, Table 1). When compared to the other genotypes P1 had the lowest steady-state Φ_{PSII} (0.59) after a step decrease in irradiance (Figure 3A, C). Similarly, P1 also had the lowest steady-state Φ_{PSII} after the step increase (0.49) in irradiance (Figure 3B, D). Interestingly, the genotype that had attained the lowest steady-state Φ_{PSII} following both step increase and step decrease in irradiance was the same (P1); no such correlation was observed with the rate constants. This could imply that the factors affecting rate constants might be different from those influencing steady state photosynthetic rates.

3.4 | Short-term response of photosynthesis to a step increase and step decrease in irradiance under low-temperature conditions

The temperature at which the plants were grown was dropped by 7°C, from 21°C to 14°C (00:00 h; 19 DAS), and the time course change of Φ_{PSII} to step increase and step decrease in irradiance was monitored. The time-course response of Φ_{PSII} were fitted using equation 3 (see calculations; S.3.3 and S.3.4) and the estimated parameters are summarized in Table 2. The fitting procedure were exactly the same as used during the fitting of the responses under control conditions. The kinetics of all genotypes to a step decrease (Figure 4A, C) and step increase (Figure 4B, D) in irradiance are shown in Figure 4. As was observed under control conditions, during a step decrease in irradiance, Φ_{PSII} of all genotypes, initially, increased rapidly after which the rate of increase slowed down to eventually reach a new steady state (Figure 4A). During the step increase in irradiance, the Φ_{PSII} of all genotypes first rapidly decreased and slowly recovered to reach a new steady state (Figure 4B). For both the step increase and step decrease to irradiance, the relative variation in average Φ_{PSII} values

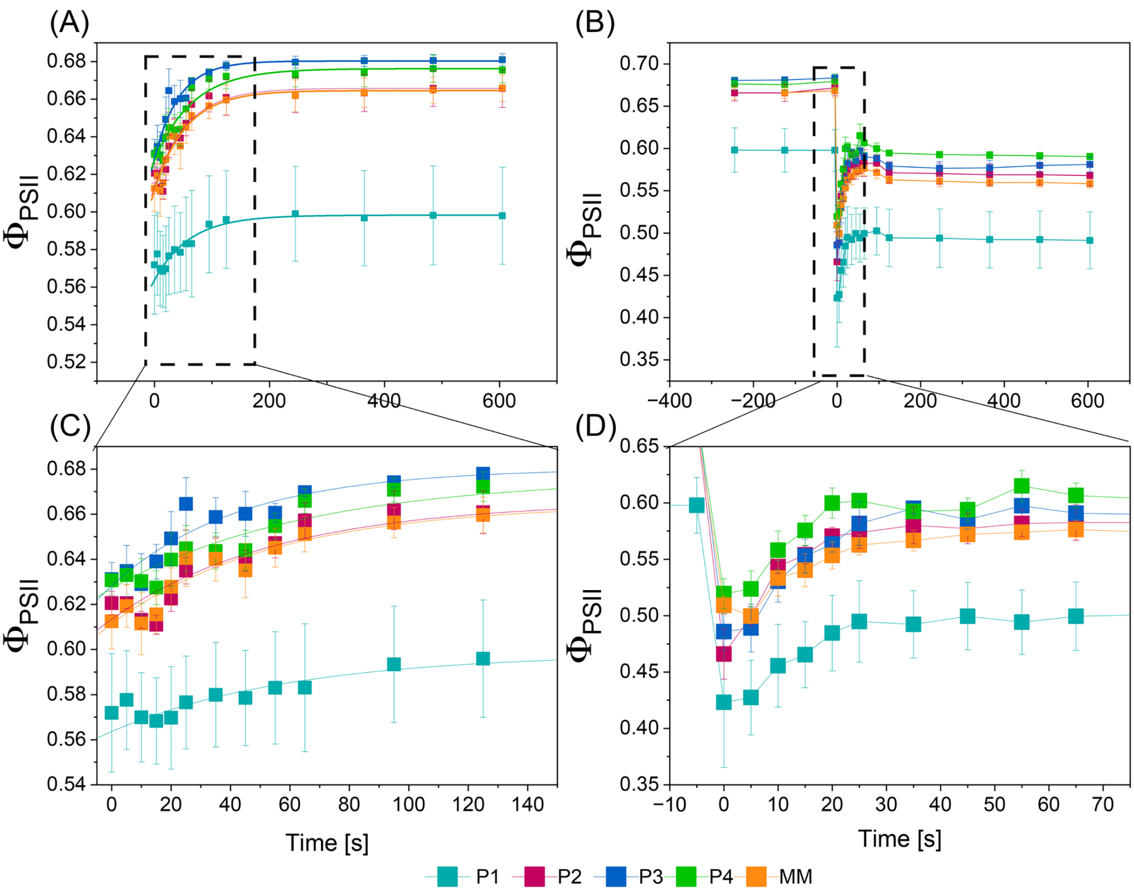

FIGURE 3 Time course plot illustrating kinetics of Φ_{PSII} for all genotypes (18 DAS) following a step change in irradiance at 21°C. (A) The kinetics for a step decrease in irradiance from 400 μmol m^{-2} s^{-1} to 200 μmol m^{-2} s^{-1}. Solid lines indicate the fitted curves. (B) The kinetics for a step increase in irradiance from 200 μmol m^{-2} s^{-1} to 600 μmol m^{-2} s^{-1}. Solid lines are the linear interpolations between each point. (C) Enlarged plot of initial change to step decrease in irradiance (indicated with dashed lines in A). (D) Enlarged plot of initial change to step increase in irradiance (indicated with dashed lines in B). Each point shows a mean value (*n* = 3) and error bars indicate standard error. Nagcarlan (P1), North Carolina Heatset-1 (NCHS-1; P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM).

TABLE 1 Summary of fit parameters for both step increase and step decrease to irradiance at 21°C. The fit parameters are obtained from equation 3 (refer to Calculations). The fit parameters are rate constants (k) and steady state reached after the step increase or step decrease (SS). The standard error (± SE) of the fits (n = 3) is provided. * indicates the values that are statistically significant p < 0.05 post-hoc Fischer's LSD, One-way ANOVA. Nagcarlan (P1), North Carolina heatset-1 (NCHS-1; P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymake; MM).

	Step Decrease		Step Increase	
Genotype	k (s^{-1})	SS (Φ_{PSII})	k (s^{-1})	SS (Φ_{PSII})
P1	0.018 ± 0.002	0.59 ± 0.002*	0.10 ± 0.01	0.49 ± 0.02*
P2	0.031 ± 0.005*	0.66 ± 0.004	0.10 ± 0.019	0.58 ± 0.01
P3	0.021 ± 0.005	0.68 ± 0.004*	0.09 ± 0.021	0.59 ± 0.02
P4	0.023 ± 0.004	0.66 ± 0.005	0.11 ± 0.02	0.59 ± 0.006
MM	0.020 ± 0.004	0.66 ± 0.005	0.06 ± 0.01*	0.57 ± 0.008

were larger under chilling conditions (Figure 4) than under control conditions. After 12 hours of chilling the rate of response of Φ_{PSII} diminished further and steady state Φ_{PSII} decreased (Figure 4, Table 2). Compared to control conditions, the rate constants of all genotypes for the response of Φ_{PSII} to a step decrease in irradiance under chilling conditions decreased slightly (Figure 4 C). In contrast to the response to an irradiance decrease, the rate constants of all genotypes of the response to step increase in irradiance decreased

TABLE 2 Summary of fit parameters for both step increase and step decrease to irradiance at 14°C. The fit parameters are obtained from Equation 3 (refer Calculations). The fit parameters are rate constants (k) and steady state reached after the step increase or step decrease (SS). The standard error (± SE) of the fits ($n = 3$) is provided. * indicates the values that are statistically significant $p < 0.05$ post-hoc Fischer's LSD, One-way ANOVA. Nagcarlan (P1), North Carolina heatset-1 (NCHS-1; P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM).

	Step Decrease		Step Increase	
Genotype	**k (s^{-1})**	**SS (Φ_{PSII})**	**k (s^{-1})**	**SS (Φ_{PSII})**
P1	0.016 ± 0.0026	0.58 ± 0.004	0.067 ± 0.01	0.5 ± 0.02
P2	0.017 ± 0.003	0.63 ± 0.003	0.074 ± 0.014	0.55 ± 0.03
P3	0.014 ± 0.003	0.65 ± 0.005	0.072 ± 0.016	0.56 ± 0.03
P4	0.013 ± 0.002	0.66 ± 0.003	0.07 ± 0.013	0.58 ± 0.02
MM	0.014 ± 0.0035	0.58 ± 0.005	0.05 ± 0.011*	0.49 ± 0.02

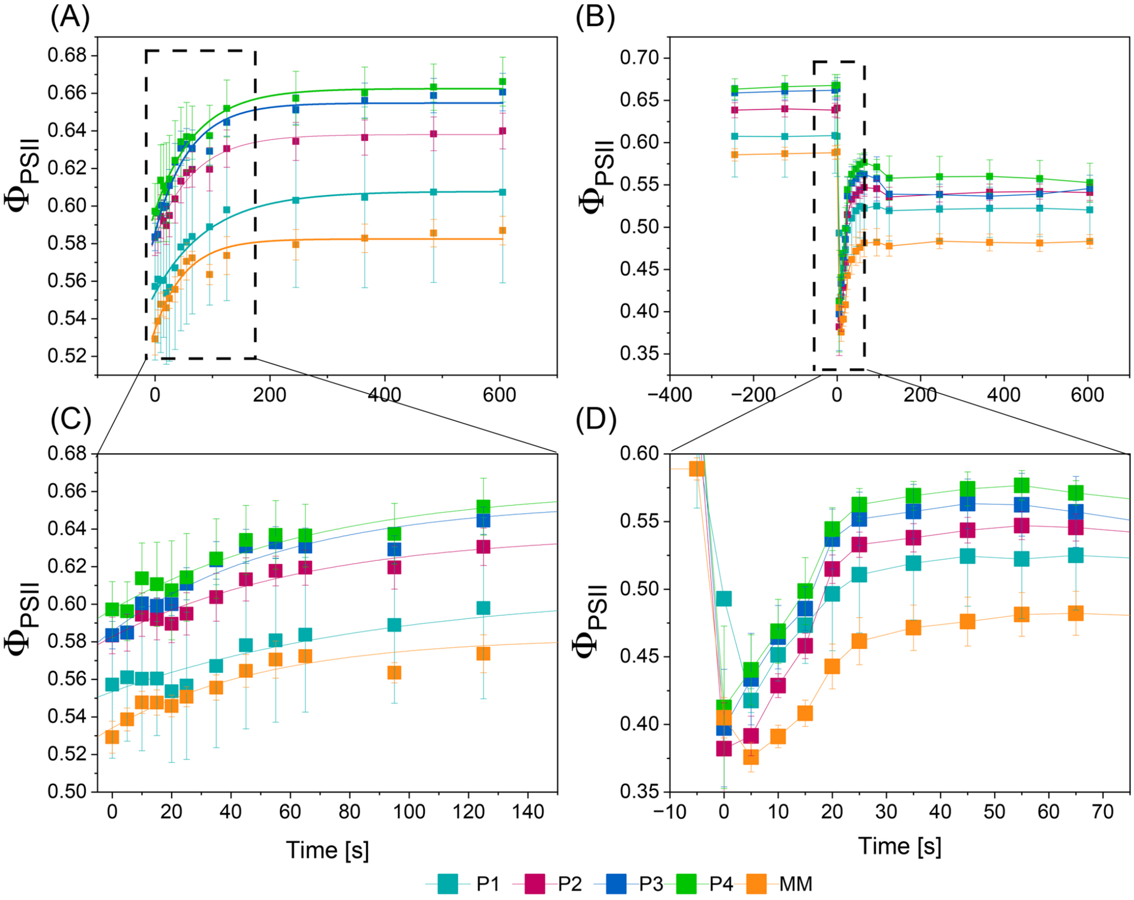

FIGURE 4 Time course plot illustrating kinetics of Φ_{PSII} for all genotypes (19 DAS) following a step change in irradiance at 14°C. (A) The kinetics for a step decrease in irradiance from 400 µmol m^{-2} s^{-1} to 200 µmol m^{-2} s^{-1}. Solid lines indicate the fitted curves. (B) The kinetics for a step increase in irradiance from 200 µmol m^{-2} s^{-1} to 600 µmol m^{-2} s^{-1}. Solid lines are the linear interpolations between each point. (C) Enlarged plot of initial change to step decrease in irradiance (indicated with dashed lines in A). (D) Enlarged plot of initial change to step increase in irradiance (indicated with dashed lines in B). Each point shows a mean value ($n = 3$) and error bars indicate standard error. Nagcarlan (P1), North Carolina Heatset-1 (NCHS-1; P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM).

significantly under chilling conditions (Figure 4 D, Table 2). The rate constant of MM was the lowest (Table 2). The steady-state Φ_{PSII} after a step decrease to 200 µmol m^{-2} s^{-1} and step increase in irradiance to 600 µmol m^{-2} s^{-1} decreased compared to those under control conditions; the Φ_{PSII} of MM (0.49) decreased the most (Figure 4C, D, Table 2). MM seemed to be the most sensitive to chilling, with both the speed of response and quantum efficiencies decreasing significantly. On the other hand, the steady-state Φ_{PSII} of P2, P3 and P4 attained following a step increase or step decrease in irradiance were less sensitive to chilling, indicating some resilience in this response.

3.5 | Change in kinetics of short-term responses of photosynthesis with chilling and recovery

With chilling, the rate constants of responses to both step increase and step decrease of all genotypes decreased. However, the observed decreases were different for different genotypes. For example, the genotype that responded the fastest to a step decrease in irradiance under control conditions did not respond the fastest when subjected to chilling. Similarly, the degree to which rate constants decreased with chilling were also different between different genotypes. The variation in the effect of chilling on the rate constants suggest that each genotype might respond differently to prolonged exposure to chilling and subsequent restoration of temperature. Therefore, the rate constants of the time series change in Φ_{PSII} with step increase and step decrease in irradiance towards the end of the chilling treatment (24 DAS) and during the recovery phase (25 DAS and 30DAS) was monitored.

The changes in rate constant for the response of Φ_{PSII} to a step increase or decrease in irradiance with temperature change is shown in Figure 5. Notably, the rate constants obtained from a response to a step decrease in irradiance of most genotypes remain relatively unchanged with a decrease in temperature and during recovery (Figure 5; left). P1 showed only a slight decrease in the rate constants (Figure 5B), while the effect of chilling was much more prominent for P3 and MM (Figure 5F, J). For genotypes P2 (Figure 5C; cold) and P4 (Figure 5G; cold), where the rate constants decrease with chilling, the rate constants recovered to pre-chill values during the recovery period. On the other hand, for the response to step increase in irradiance, all genotypes except P2 and P4 (Figure 5D, H), showed a marked decrease in the rate constant under chilling conditions (Figure 5; right; cold). The rate constants during the step increase in irradiance for all genotypes remained the same during prolonged exposure to chilling (Figure 5B, D, F, H, J; cold_acc). Surprisingly, the rate constants, during the 'recovery' phase for P1 (Figure 5B, Rec) and MM (Figure 5J; Rec) were higher than during the control conditions. The rate constant of P3 on the other hand increased gradually to pre-chilling levels (Figure 5F; Rec and Rec_acc).

3.6 | Changes in steady-state Φ_{PSII} attained following step changes in irradiance under chilling and recovery

While the kinetics (rate constants) are crucial in understanding how quickly each genotype responds to each condition, the changes in the steady-state values of Φ_{PSII}, after step increase or step decrease in irradiance, are also crucial as they indicate the scale of the adaptation to the irradiance step and parallel photosynthetic rates at the irradiance reached by the step. Moreover, the influence of chilling on steady-state Φ_{PSII} reached after step change, for both increase and decrease in irradiance, was genotype-specific. This indicates that, much like rate constants, the effect of prolonged chilling and recovery on the attained steady state Φ_{PSII} might also be different for different genotypes.

After the initial exposure to chilling (19 DAS), the steady-state Φ_{PSII} after step decreased in irradiance for all genotypes except the P1 decrease (Figure 6 A). At the end of the prolonged exposure to chilling (Figure 6 left; Cold_acc), the Φ_{PSII} of P2 and MM (Figure 6C, I) remained unchanged while the Φ_{PSII} of P1 and P4 increased (Figure 6A, G). P3 was the only genotype where the Φ_{PSII} decreased after prolonged chilling exposure (Figure 6E; Cold_acc). The Φ_{PSII} of all genotypes increased when the temperature was restored to 21°C (Figure 6 left; Rec). The Φ_{PSII} during the 'recovery' phase of all genotypes except P4, were higher than the Φ_{PSII} observed before exposure to chilling. This pattern of increased Φ_{PSII} in the recovery phase compared to the control conditions was also observed for the steady state Φ_{PSII} at 400 µmol m^{-2} s^{-1} (Figure 2).

The effect of chilling on the steady-state Φ_{PSII} after the step increase in irradiance was different from that observed during the step decrease in irradiance. Only MM (Figure 6J) had lower Φ_{PSII} than that observed during the control conditions. At the end of the chilling treatment (24 DAS) the Φ_{PSII} of P1 dropped significantly (Figure 6B; Cold_acc) while the Φ_{PSII} of all the other genotypes had the same Φ_{PSII} as the start of the chilling. Much like steady state Φ_{PSII} after step decrease and Φ_{PSII} at growth irradiance, during the 'recovery' phase the Φ_{PSII} was higher than the control conditions. P4 was the only exception, wherein the Φ_{PSII} remains constant throughout the changes in temperature (Figure 6H).

4 | DISCUSSION

4.1 | Effect of chilling on Fv/Fm and steady-state Φ_{PSII} was reversible

A decrease in Fv/Fm has been commonly used as a measure of the plants' responses to stress. While the reduction of Fv/Fm might not correspond to actual stress in certain conditions (Murchie and Lawson 2013), in cold environments the reduction in Fv/Fm has shown to be correlated to stress (Krause and Somersalo 1989; Rizza et al., 2001; Ogaya et al., 2011). We observed a decrease in Fv/Fm when the temperature was lowered to 14°C, however, the Fv/Fm recovered when temperatures were restored to 21°C (growth temperature), indicating that the effect of chilling in the light was reversible. The decrease in Fv/Fm at 19 DAS could be due to the slow repair of PSII (D1 proteins), owing to the decrease in flexibility of thylakoid membranes under low temperature conditions (Moon et al., 1995; Murata et al., 2007), or the slow reversible downregulation of PSII (Groom and Baker 1992). The decrease in Fv/Fm from 20 DAS to

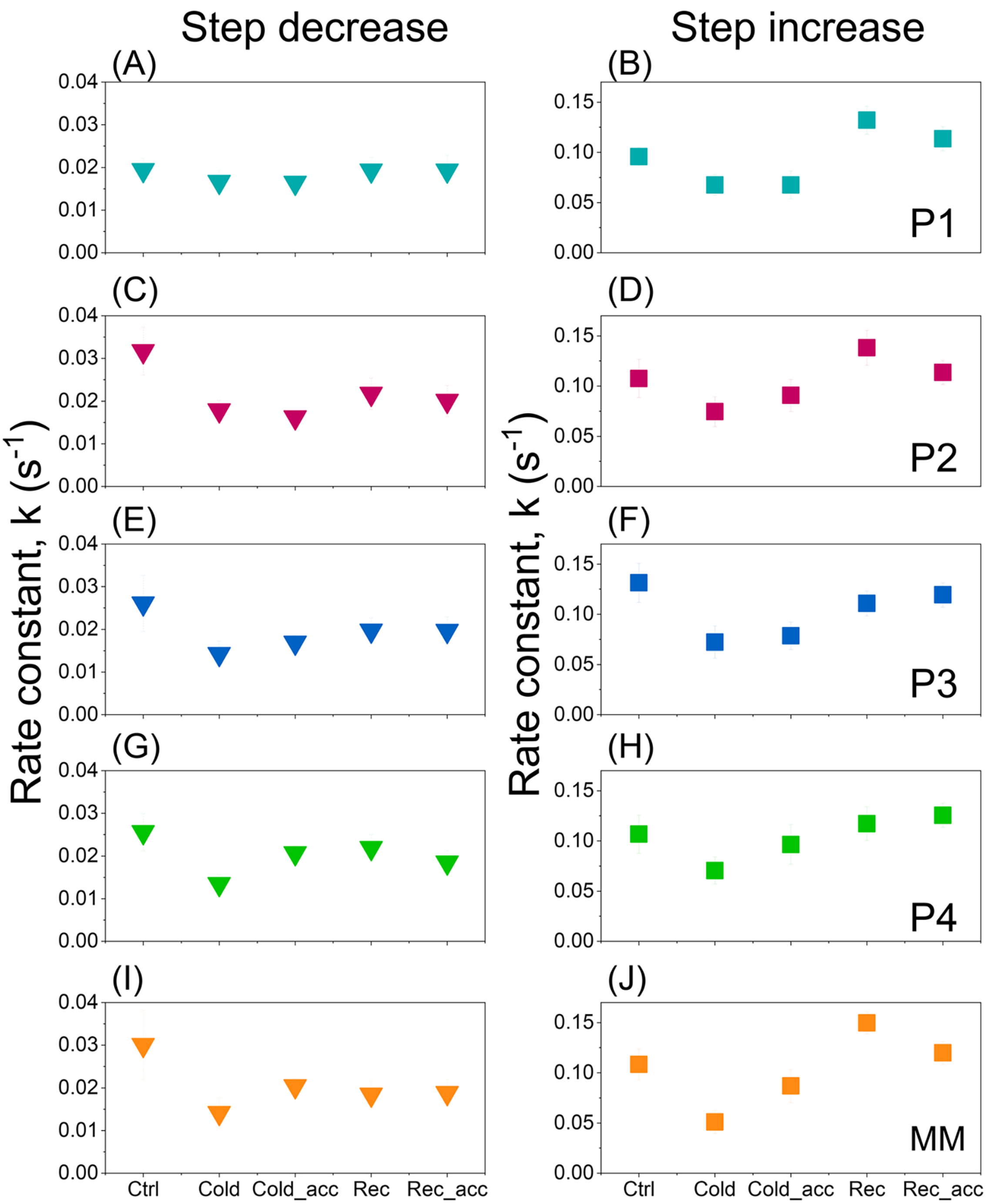

FIGURE 5 Plot indicating the trend for kinetics to step change in irradiance during different conditions. Each point represents kinetics under different conditions as follows: Control (Ctrl; 18 DAS), Chilling (Cold;19 DAS), Chilling acclimation (Cold_acc; 24DAS), Recovery (Rec; 25DAS), acclimation to recovery conditions (Rec_acc; 30DAS). (A, C, E, G, and I) illustrate changes in kinetics to a step increase in irradiance from 200 μmol m^{-2} s^{-1} to 600 μmol m^{-2} s^{-1} for P1, P2, P3, P4 and MM respectively. (B, D, F, H, and J) illustrate changes in kinetics to a step decrease in irradiance from 400 μmol m^{-2} s^{-1} to 200 μmol m^{-2} s^{-1} for P1, P2, P3, P4 and MM respectively. Each point shows a mean value ($n = 3$) and error bars indicate standard error (±SE). Each time point is statistically different from the point to the left of it ($p < 0.05$; Repeated Measures ANOVA, Bonferroni post-hoc test; S.4.1). Nagcarlan (P1), North Carolina heatset-1 (NCHS-1) (P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker, MM).

24 DAS agrees with previous studies that report a decrease in Fv/Fm due to low temperatures and high light (Martin and Ort 1985; Krause and Somersalo 1989; Rizza et al., 2001; Ogaya et al., 2011; Powles 1984).

Much like Fv/Fm, the steady-state Φ_{PSII} (dawn and dusk) decreased when the temperature was lowered to 14°C and recovered when temperatures were restored to 21°C. The dusk Φ_{PSII} were slightly lower than the dawn Φ_{PSII} (Figure 2), potentially due to the

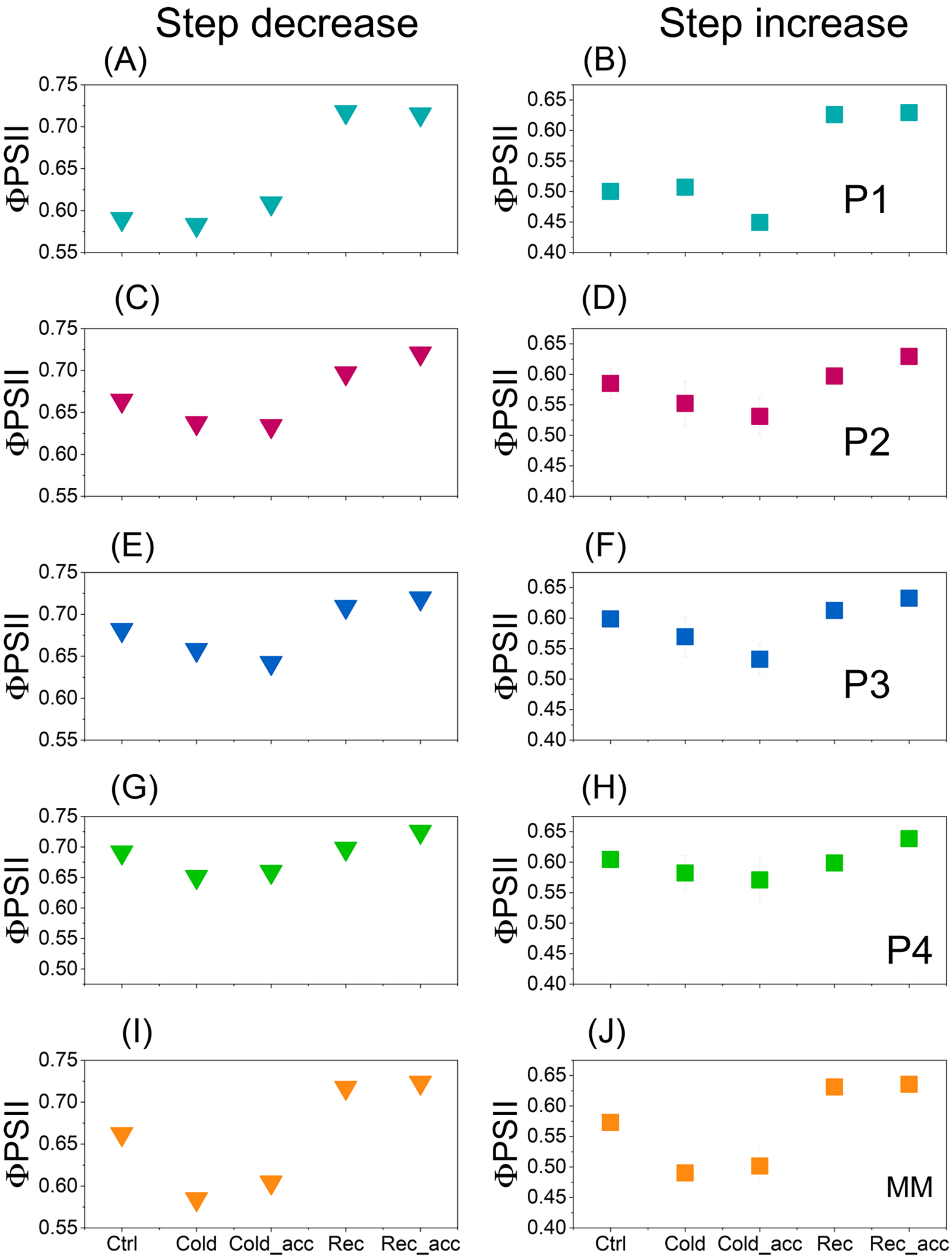

FIGURE 6 Plot indicating the trend for steady-state Φ_{PSII} reached after a step change in irradiance during different conditions. Each point represents kinetics under different conditions as follows: Control (Ctrl; 18 DAS), Chilling (Cold; 19 DAS), Chilling acclimation (Cold_acc; 24DAS), Recovery (Rec; 25DAS), acclimation to recovery conditions (Rec_acc; 30DAS). (A, C, E, G, and I) illustrate changes in kinetics to a step decrease in irradiance from 400 μmol m^{-2} s^{-1} to 200 μmol m^{-2} s^{-1} for P1, P2, P3, P4 and MM respectively. (B, D, F, H, and J) illustrate changes in kinetics to a step increase in irradiance from 200 μmol m^{-2} s^{-1} to 600 μmol m^{-2} s^{-1} for P1, P2, P3, P4 and MM respectively. Each point shows a mean value ($n = 3$) and error bars indicate standard error (± SE). Each time point is statistically different from the point to the left of it ($p < 0.05$; Repeated Measures ANOVA, Bonferroni post-hoc tes; S.4.2). Nagcarlan (P1), North Carolina heatset-1 (NCHS-1, P2), Delfo parent 1(P3) and Delfo parent 2 (P4) and a control; *S. lycopersicum* (cv. Moneymaker; MM).

effect of the light treatment, circadian rhythm or other diurnal processes (Lanoue et al., 2018; Matthews et al., 2018) or due to the accumulation of PSII damage. The decrease in Φ_{PSII} has been suggested to be due to the downregulation of electron transport because of decreased electron sinks (largely the Calvin-Benson-Bassham cycle) during cold, the slowly reversible down regulation of PSII and to the transfer of electrons to alternative electron sinks (e.g. Mehler-Ascorbate-peroxidase pathway and cyclic electrons transport; Allen and Ort 2001).

4.2 | Steady-state Φ_{PSII} reached following a step change in irradiance and rate constant can be considered as separate photosynthetic traits

The genotype MM that responded the slowest during a step increase, under control conditions, did not have the slowest response to a step decrease in irradiance (Figure 3B, D; Table 1). This agrees with the suggestion that processes that limit photosynthesis during an increase in irradiance are different from those limiting photosynthesis during a decrease in irradiance (Kaiser et al., 2015; Kaiser et al., 2018a). Studies on photosynthetic induction and relaxation have provided insights into the activation and deactivation of numerous processes that regulate photosynthesis (Sassenrath-Cole and Pearcy 1992, 1994; Sassenrath-Cole et al., 1994; Kaiser et al., 2018b; Kaiser et al., 2020; Kaiser et al., 2015, 2018a). However, the implication that the factors affecting transient change might be entirely different from the factors affecting the steady state reached following a change in irradiance, has not been explored extensively. In our investigation, the genotypes that responded quickly or slowly to changes in irradiances did not necessarily have the highest or lowest Φ_{PSII} attained following those changes in irradiances. During a step decrease in irradiance, P2 had the highest rate constant amongst all genotypes but P2 did not have the highest Φ_{PSII} after step decrease in irradiance, P3 did. Similarly, during step increase, MM had the lowest rate constant but did not have the lowest Φ_{PSII} after step increase in irradiance, P1 did. No correlation between rate constants and Φ_{PSII} was also observed under chilling conditions. There is thus no apparent link between having a higher or lower rate constant with higher or lower Φ_{PSII} or vice versa. Thus, factors affecting steady-state attained Φ_{PSII} following a step increase or step decrease in irradiance might be different to those regulating the transient response of photosynthesis to a changing irradiance. Therefore, the rate constants of the rate of response to irradiance and steady-state Φ_{PSII} reached following a change in irradiance, can be considered largely independent photosynthetic traits.

4.3 | The effect of chilling on the steady-state Φ_{PSII} reached following a step-change in irradiance and rate constants are different

Martin and Ort (1982) reported a decrease in the quantum yield of PSII electron transport in vitro, measured via the photo-reduction of hexacyanoferrate (III), in response to chilling (1°C for 16 h). They also observed a reduction in photosynthetic capacity, measured via the CO_2 saturated light response curve. This is similar to what we observed at 14°C, where the steady-state Φ_{PSII} after a step increase and step decrease in irradiance decreased when compared to the steady-state Φ_{PSII} after a step increase and step decrease in irradiance during control conditions. The decrease in steady-state Φ_{PSII} following an increase or decrease in irradiance observed in our study, is similar to the reductions in CO_2 assimilation observed by Powles et al., (1983) in *Phaseolus vulgaris* and Martin and Ort (1985) in *Solanum lycopersicum*, where they report a large drop in CO_2 assimilation following the combined effect of temperature and high light.

The rate constants of the changes in Φ_{PSII} to step increase and step decrease in irradiance were also affected by chilling exposure. Although, further studies are required to identify the mechanistic basis of this reduction, the changes in the processes that regulate the transient response of photosynthesis to irradiance could be responsible. Powles et al., (1983) observed a reduction in CO_2 assimilation and stomatal conductance with chilling temperatures (6°C) and Sassenrath et al., (1990) observed the loss of FBPase activity and reduction in RuBP concentrations under chilling conditions (8°C). RuBP regeneration has been suggested to limit fast photosynthetic response in light flecks (Sassenrath-Cole and Pearcy 1994; Sassenrath-Cole et al., 1994). Additionally, changes in stomatal conductance also limit photosynthesis during early stages of photosynthetic induction (Taylor et al., 2020; Sakoda et al., 2021). Thus, reduced FBPase activity, impaired RuBP regeneration and decreased stomatal conductance due to chilling could explain the observed decrease in rate constants. However, Martin et al., (1981) observed a reduction in photosynthesis in chilled leaves (1°C) acclimated to CO_2-saturated conditions (1500 ppm). Assuming that photosynthesis is not limited by chloroplast CO_2 (C_c) concentrations at 1500 ppm CO_2, this suggests that metabolic limitations, and not stomatal limitations, limit photosynthetic responses to short-term fluctuations in irradiance during chilling exposure. However, the aforementioned studies use different temperature and light treatments than those in our study. Therefore, the factors influencing the reduction of rate constants in our study could be entirely different.

4.4 | Considerable phenotypic variation exists between genotypes for short-term fluctuating light in response to chilling

The steady-state Φ_{PSII} of all genotypes, with the exception of P1, were similar within each temperature treatment, indicating only some phenotypic variation between the genotypes. However, when comparing the effect of chilling on steady-state Φ_{PSII}, the steady state Φ_{PSII} (dusk and dawn) of P1 and MM were cold sensitive. The Φ_{PSII} of P1 decreased the most during chilling and also recovered the most during the 'recovery' phase, compared to steady-state Φ_{PSII} at the control temperature. Similarly, MM experienced a large decrease in Φ_{PSII} after the onset of chilling temperatures. In contrast the other genotypes (P2, P3 and P4) had a relatively lower decrease in Φ_{PSII}, indicating some resilience to chilling. The effect of chilling on steady-state Φ_{PSII} thus shows phenotypic variation, and notably, the variation mostly lies in the effect of low temperature on photosynthesis (Figure 2A, B).

The effect of chilling on the rate constants and the Φ_{PSII} following step changes in irradiance are however different for the different genotypes. The rate constants observed during the step decrease in irradiance were relatively unaffected by chilling compared to the rate constants during the step increase in irradiance (Figure 5). Notably, the rate constants during the step decrease of irradiance of P1 changed the least, seeming to be insensitive to chilling. On the other hand, the effect of chilling on the rate constant for the response to an increase in irradiance varied considerably between genotypes, with the rate constant of MM decreasing the most and the other genotypes showing smaller decreases in rate constants. These rate constants recovered back to those observed under control conditions following the restoration of temperature to control values, much like the Fv/Fm and steady-state Φ_{PSII} at 400 µmol m^{-2} s^{-1}. This suggests that the effects of chilling on the processes that restrict the kinetics of the response of Φ_{PSII} are also reversible.

The response to chilling of the steady-state Φ_{PSII} reached after a step increase and decrease in irradiance, and their subsequent recovery after restoring the temperature to control values, were similar across genotypes. The steady-state Φ_{PSII}, of P2, P3 and P4 that was reached following a step increase or decrease in irradiance, decreased to a lesser extent, in chilling conditions, than did that of P1 and MM. Thus, there is phenotypic variation between genotypes for both the rate constants of change in Φ_{PSII}, and steady-state Φ_{PSII} reached after step change in irradiance. Moreover, P1 and P2 are known to be heat-tolerant lines for pollen viability. Although the heat tolerance for photosynthesis or vegetative growth has not been investigated (Villareal et al., 1977; Xu et al., 2017b; Xu et al., 2017a). The key genetic factors that impart heat tolerance characteristics along with the genetic factors underpinning the phenotypes discussed in this study could prove to be important targets for breeding climate-resilient crops. These targeted approaches can also be beneficial for tomato growers who can grow tomatoes at lower temperatures, cutting the cost of greenhouse heating. Brüggemann et al., (1996) have demonstrated that chilling-tolerant tomato lines can be obtained by crossing the cultivated tomato with a cold-tolerant species, *Solanum peruvianum*.

We observed phenotypic variation in different photosynthetic traits under steady state and also under short-term changes in irradiance. Especially the response of these traits to chilling. We also identified certain responses from specific genotypes that could be beneficial for developing climate-resilient crops with optimized photosynthesis. These genetic factors underpinning these phenotypes could be identified from the MAGIC population that these parental lines make up. The variation described was observed between tomato lines of the same species. However, by using wild-relatives of tomato, the phenotypic variation observed might be larger, owing to geographical and evolutionary differences (Yoshiyama et al., 2024). The current study only focuses on the variation of photosynthetic responses, measured using one parameter Φ_{PSII}, to short-term fluctuations in irradiance and the effect of chilling on these responses. Considering the tightly regulated nature of photosynthesis, extensive exploration of different traits using different techniques will be required to identify genetic factors that are necessary to generate climate-resilient crops. Hence, generating populations focused on photosynthetic traits would be necessary to carry out such investigations.

AUTHOR CONTRIBUTIONS

L.R. and K.J. designed the experiments, performed the research. K.J. wrote the manuscript. J.H. and M.G.M.A. supervised the study and revised the manuscript. All authors have read and approved of its content.

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CONFLICT OF INTEREST STATEMENT

The authors are not aware of any conflict of interest arising from drafting this manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new created data is already contained within this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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