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High non-photochemical quenching of VPZ transgenic potato plants limits CO_2 assimilation under high light conditions and reduces tuber yield under fluctuating light

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Abstract

Under natural conditions, photosynthesis has to be adjusted to fluctuating light intensities. Leaves exposed to high light dissipate excess light energy in form of heat at photosystem II (PSII) by a process called non-photochemical quenching (NPQ). Upon fast transition from light to shade, plants lose light energy by a relatively slow relaxation from photoprotection. Combined overexpression of violaxanthin de-epoxidase (VDE), photosystem II subunit S (PsbS) and zeaxanthin epoxidase (ZEP) in tobacco accelerates relaxation from photoprotection, and increases photosynthetic productivity. In *Arabidopsis*, expression of the same three genes (VPZ) resulted in a more rapid photoprotection but growth of the transgenic plants was impaired. Here we report on VPZ expressing potato plants grown under various light regimes. Similar to tobacco and *Arabidopsis*, induction and relaxation of NPQ was accelerated under all growth conditions tested, but, did not cause an overall increased photosynthetic rate or growth of transgenic plants. Tuber yield of VPZ expressing plants was unaltered as compared to control plants under constant light conditions and even decreased under fluctuating light conditions. Under control conditions, levels of the

phytohormone ABA were found to be elevated, indicating an increased violaxanthin availability in VPZ plants. The increased basal ABA levels, however, did not improve drought tolerance of VPZ tansgenic potato plants under greenhouse conditions. The failure to benefit from improved photoprotection is most likely caused by a reduced radiation use efficiency under high light conditions resulting from a too strong NPQ induction. Mitigating this negative effect in the future might help to improve photosynthetic performance in VPZ expressing potato plants.

Key-words: drought, photosynthesis, potato, non-photochemical quenching, Xanthophyll cycle

INTRODUCTION

Plant growth and development largely depend on the efficiency of photosynthesis to convert light into chemical energy and the proper allocation and utilization of derived photoassimilates (Sonnewald and Fernie, 2018). Photosynthesis can be divided into two processes, light-dependent reactions and the photosynthetic carbon reduction (PCR) or Calvin–Benson–

Bassham (CBB) cycle. During the light-dependent reactions, photons are absorbed by membrane-bound light harvesting complexes and the excitation energy is used to drive charge separation at the reaction centers of two photosystems and the electron transfer from water to $NADP^+$. In addition, protons are pumped into the thylakoid lumen fueling ATP synthesis via ATP-synthase. Produced chemical energy (ATP) and redox potential (NADPH) are subsequently used for carbon fixation via the CBB-cycle. Light is the primary energy source of photosynthesis and its absorption can significantly limit photosynthesis. To allow optimal light harvesting, plants have evolved several strategies to adapt to low and high light conditions (Shi et al., 2022). While low light might limit carbon fixation and plant growth, excessive light can damage plant cells by inducing the production of reactive oxygen species (ROS). ROS, generated through the Mehler reaction (Asada and Takahashi, 1987), can lead to membrane lipid peroxidation, base mutations, DNA strand breaks and protein damage but also programmed cell death (Asada, 2006). These processes can further be enhanced by abiotic stress such as heat or drought, leading to massive yield losses of major crop plants. Even under low light conditions, additional abiotic stress can lead to photoinhibition (Nishiyama and Murata, 2014; Wang et al., 2017). In this context, drought can play a major role, by inhibiting gas exchange due to stomatal closure (George et al., 2017; Anwar and Kim, 2020). It has been calculated that 20% yield losses are due to biotic stress, however, 80% can be attributed to abiotic stress (Valliyodan and Nguyen, 2006). In light of global climate change causing frequent

heat waves and drought periods, adaptation of the photosynthetic machinery to avoid photoinhibition and to allow high energy capture at the same time, becomes an important issue for crop improvement (Lohani et al., 2020).

To avoid light-induced damage of the photosynthetic machinery, plants have evolved several different photoprotection mechanisms, such as non-photochemical quenching (NPQ) (reviewed in Papageorgiou and Govindjee, 2014). Here excess light energy is dissipated as heat. The rate of NPQ positively correlates with the photosystem II subunit S (PsbS) protein and the interconversion of violaxanthin (V) and zeaxanthin (Z). Interconversion of V and Z is catalyzed by two enzymes, violaxanthin de-epoxidase (VDE) and zeaxanthin epoxidase (ZEP). NPQ induction works through VDE, which is induced at low pH (Hager and Holocher, 1994; Bugos and Yamamoto, 1996). VDE converts violaxanthin (V) via antheraxanthin (A) to zeaxanthin (Z) inducing NPQ under high light conditions, when protons accumulate in the lumen (Eskling et al., 1997). PsbS induces fast conformational changes of LHCⅡ further contributing to NPQ (Johnson and Ruban, 2010). The role of PsbS was validated in transgenic plants. In rice, PsbS overexpression resulted in higher NPQ and an increased radiation use efficiency under fluctuating light conditions (Hubbart et al., 2018). In tobacco, higher NPQ values could be confirmed but in addition, PsbS transgenic plants showed an improved water use efficiency due to a reduced stomata opening under high light conditions (Głowacka et al., 2018). Improved drought tolerance could also be observed in *Arabidopsis* overexpressing ZEP (Park et al., 2008). These plants showed increased abscisic acid (ABA) levels (Park et al., 2008) probably caused by improved provision of violaxanthin, a precursor for ABA biosynthesis.

Relaxation of NPQ under low light conditions requires zeaxanthin epoxidase (ZEP), transforming Z to V, which operates on the stromal side of the thylakoid membrane. Since both induction and relaxation of NPQ are enzyme dependent processes, they occur comparatively slow. Relaxation of NPQ occurs much slower than the induction (Eskling et al., 1997; Pérez-Bueno et al., 2008; Wang et al., 2008), which might lead to an unnecessarily reduced CO_2 fixation rate under fluctuating light conditions (Long et al., 1994; Werner et al., 2001; Zhu et al., 2004). Increased NPQ relaxation can thus probably improve crop yield (Murchie and Niyogi, 2011)

In previous experiments, faster induction and relaxation of NPQ was attempted by overexpression of the three genes coding for VDE, PsbS and ZEP (VPZ). In tobacco, VPZ transgenic plants showed improved NPQ and biomass production under field conditions (Kromdijk et al., 2016). In *Arabidopsis*, overexpression of the same genes

resulted in improved NPQ but reduced growth of the transgenic plants under fluctuating light (Garcia-Molina and Leister, 2020). A similar concept was followed in creeping bentgrass, but instead of using the *Arabidopsis* genes, VPZ genes from rice were used for overexpression. Similar to *Arabidopsis*, VPZ transgenic bentgrass showed growth penalties but improved drought and salt tolerance (Zhu, 2021). These obviously contradictory results could be explained by several mechanisms. Assuming that tobacco, *Arabidopsis* and bentgrass differ significantly in their source-sink relation, sink limitation could be one reason for the different outcomes.

Assuming that photosynthetic performance of source leaves is tightly linked to sink demand, we aimed to test the hypothesis of sink limitation by transferring the VPZ genes from *Arabidopsis* to potato plants. Potato form strong sink organs, the tubers, which could benefit from an improved photosynthetic performance, if source capacity is limiting.

Here we report that transgenic potato plants show accelerated induction and relaxation of NPQ. Transgenic plants did not suffer from VPZ overexpression under any growth condition tested, but they also did not benefit. Despite a slightly increased basal ABA content under ambient conditions, drought tolerance of transgenic potato plants was not significantly improved under greenhouse conditions. Under fluctuating light conditions, tuber yield of transgenic potato plants was reduced. This reduction might be caused by constitutively high levels of NPQ, reducing light perception of PS II and lowering the amount of absorbed irradiance under high light conditions. This might lead to a reduced electron transport and consequently limits the efficiency of the strongest electron sink, the Calvin-Benson-Bassham cycle. In accordance with this concept, leaves from VPZ transgenic potato plants show a reduced CO_2 assimilation rate under high light conditions.

RESULTS

Overexpression of the VPZ genes accelerates induction and relaxation of NPQ

To test the idea that optimized NPQ could improve potato growth and tuber yield, we followed the concept published by (Kromdijk et al., 2016) and created transgenic potato plants expressing *VDE*, *PsbS* and *ZEP* under control of the RbCoS1, GAPA and FBA promoter, respectively (Figure 1A). All cDNAs and promoter sequences, derived from *Arabidopsis* were cloned using the Golden Gate modular cloning system as described in Materials and Methods and Table S1. The resulting multi-gene construct was transformed into *Solanum tuberosum,* cultivar Solara, using *Agrobacterium tumefaciens*. From the primary transformants we selected three lines showing high (#14, #29) or medium (#24) expression levels (Figure 1B). Under greenhouse conditions, growth of transgenic plants was indistinguishable from untransformed control plants (Figure 1C).

According to previous studies, overexpression of the three selected genes was expected to improve qE, the reversible component of NPQ, via PsbS and qZ, the zeazanthin-dependent component of NPQ, by VDE and ZEP. To validate the functional expression of the VPZ construct, recovery from photoprotection was measured. As observed for transgenic tobacco and *Arabidopsis* plants, NPQ values were higher for all transgenic lines, especially for lines #14 and #24 (Figure 1D). However, whilst NPQ was higher in VPZ overexpressing lines, (Figure S1A) at the same time ϕ PSII, quantum yield of the PSII reaction center, was lower in the transgenics (Figure S1B). In the dark, NPQ levels of VPZ transgenic plants rapidly declined and reached wildtype levels six minutes after the shift to darkness (Figure 1D). This indicates a faster relaxation from photoprotection and provides evidence for the notion that the VPZ construct fullfils its physiological function in transgenic potato plants (Figure 1E).

Overexpression of the VPZ genes does not influence growth performance under constant light conditions

The results so far have shown that the VPZ construct is functional in transgenic potato plants. Therefore, the impact of the accelerated relaxation of NPQ on the overall growth performance of the transgenic plants was tested. Tissue culture plantlets were clonally propagated and ten clones of each line were transferred to the greenhouse together with untransformed control plants. Plants were grown under long day conditions (16 hours light / 8 hours darkness) and harvested 70 days after planting (dap). At harvest, VPZ transgenic plants were visually indistinguishable from untransformed controls. Under this light condition, plant height (Figure 2A), green

biomass (Figure 2B), tuber yield (Figure 2C) and harvest index (Figure 2D) were unaltered in VPZ expressing plants compared to the wild-type controls. This result was expected since the benefit of the VPZ construct should be more pronounced under fluctuating light conditions.

Reduced tuber yield of VPZ transgenic potato plants under fluctuating light conditions

Next, the impact of VPZ overexpression on plant growth and performance under fluctuating light conditions was evalutated. According to the results discussed above, VPZ transgenic plants should recover faster from NPQ after transfer to low light conditions. This faster relaxation should result in a more efficient photosynthesis under fluctuating light conditions and hence higher plant productivity, if photosynthesis is limiting. To test this hypothesis potato plants were grown under constant light conditions for 16 days. Thereafter the light regime was changed to a slow fluctuating light (5 minutes at 50 µmol*m^{-2} *s^{-1} followed by 1 minute at 500 µmol*m^{-2} *s^{-1}) cycling for 20 days followed by a fast fluctuating light cycle (1 minute at 50 µmol*m^{-2} *s^{-1} followed by 1 minute at 500 µmol*m^{-2} *s^{-1}) until harvest (Figure 3A). NPQ and CO_2 assimilation rates at steady state (500 µmol*m^{-2} *s^{-1} light intensity) were measured 15-16 days after the shift to the slow cycling regime (Figure 3B, D, F) and 20-23 days after plants have been shifted to the fast cycling conditions (Figure 3C, E, G). Again similar to the results obtained under constant light conditions (Figures 1, S1) we observed higher NPQ values accompanied with lower ϕ PSII values (Figure S2). Moreover, accelerated recovery from NPQ could be measured under all light conditions (Figure 3D, E). The CO_2 assimilation rates were found to be unaltered (Figure 3 F, G). Furthermore, we could not detect any differences in stomatal conductance (gsw), leaf internal CO_2 concentration (Ci), transpiration or water use efficiency (A/E) (Figures S3, S4). As a proxy for maximum efficiency of PSII we also measured chlorophyll fluorescence (F_v/F_m) but could detect neither a benefit nor a disadvantage in VPZ plants (Figure S5). Shoot growth of VPZ lines was indistinguishable from untransformed control plants (Figures 4A, S6). Interestingly, the tuber yield of VPZ transgenic potato plants grown under fluctuating light conditions was reduced, significantly in lines #14 and #29 (Figure 4B) leading to a slight reduction in harvest index (Figure 4C). This result indicates that VPZ expressing plants do not benefit from accelerated NPQ relaxation but instead may suffer from too high NPQ.

High NPQ limits CO_2 assimilation under high light conditions

The decreased tuber yield of VPZ expressing plants grown under fluctuating light conditions could be due to restrictions in CO_2 assimilation. We wondered whether a high NPQ of VPZ plants could limit CO_2 assimilation during the adaptation to the high light conditions and hence be responsible for the reduced tuber yield. To test this idea, the CO_2 assimilation rate was measured under conditions of decreasing light intensities interrupted by four minutes of high light (2,000 µmol*m^{-2} *s^{-1}) pulses. As shown in Figure 5A, CO_2 assimilation rates of transgenic lines #14 and #29 were indistinguishable from untransformed controls under low light conditions. Under high light conditions, however, transgenic lines showed a decreased CO_2 assimilation rate. This was accompanied by lower values for ϕPSII (Figure 5B) whilst values for gsw and Ci remained unaffected (Figure S7), supporting the assumption that photosynthetic performance of the trangenics is impaired during the high light phase.

Moderately increased abscisic acid (ABA) levels of VPZ transgenic potato plants under ambient conditions do not significantly alter drought tolerance

Violaxanthin not only serves as component of the xanthophyll cycle but also represents a precursor for ABA biosynthesis (Ivanov et al., 1995; Audran et al., 1998; Thompson et al., 2000). The phytohormone ABA plays multiple roles in plants including the regulation of stomatal closure (Cornish and Zeevaart, 1984; Zeevaart and Boyer, 1984; Zhang et al., 1987; Steuer et al., 1988) and stimulation of tuberization (Wareing and Jennings, 1980; Xu et al., 1998), both potentially influencing potato yield and stress tolerance (George et al., 2017). In previous studies it was shown that upregulation of the xanthophyll cycle can result in increased ABA levels and improved drought tolerance (Guan et al., 2015; Wang et al., 2017; Sun et al., 2019; Zhu, 2021). Therefore, a drought stress experiment was conducted (Figure 6A). To validate whether overexpression of VDE and ZEP would lead to altered ABA levels, accumulation of ABA was measured in leaves from well-watered and drought stressed potato plants. As shown in Figure 6B, ABA levels of VPZ expressing plants were found to be significantly elevated under well-watered conditions. During drought stress, ABA levels increased both in control and VPZ plants, however, differences between the genotypes observed under well-watered conditions were not reflected in drought stressed plants. Despite the differences in ABA levels, control and transgenic plants did not differ visually in their drought response. Upon drought stress, wilting of the leaves occurred simultaneously in control and VPZ expressing plants (Figure 6A). Additionally, we determined the expression of the ABA responsive gene P5CS1, an enzyme catalyzing the rate limiting step in proline biosynthesis (Yoshiba et al., 1999). As expected, expression of P5CS1 increased during drought stress, however no

significant difference was observed between VPZ expressing plants and the wild type (Figure 6C).

DISCUSSION

Overexpression of VPZ does improve NPQ induction and relaxation but not potato growth

Photoinhibition, i.e the high light-induced damage of PSII, is a common phenomenon occurring in nature and causing a decrease in photosynthetic rate (Murata et al., 2007). As one of the photoprotective mechanisms, a high level of NPQ naturally leads to stronger photoprotection, which is desired under excessive light conditions. In contrast, low light requires fast relaxation of NPQ to avoid inhibition of CO_2 assimilation due to a reduced radiation use efficiency. The proteins most relevant for induction and relaxation of NPQ are VDE, PsbS and ZEP (VPZ).

With overexpressed *VPZ*, the xanthophyll cycle is accelerated and should allow a more rapid response to changes in light intensity (Horton et al., 2007). Within this process, the enzymes fulfill different tasks. Most important and well-studied are VDE and ZEP. An overexpression of VDE would generate more zeaxanthin in a short time, inducing high NPQ (Zhao et al., 2016). Overexpression of ZEP accelerates NPQ relaxation, but can also lead to an increase in ABA levels, thereby influencing plant development and stress responses (Zhu, 2021).

In a previous work (Kromdijk et al., 2016) it was demonstrated that photosynthetic efficiency can be increased by accelerating the NPQ induction and relaxation processes. Using the model plant species *N. tabacum* cv. Petite Havana, the authors could show around 20% dry matter increase under field conditions. Based on the results in tobacco, the authors concluded that expression of the VPZ genes should enhance crop productivity in general. However, up to now this concept has not been confirmed in other crop species. In the model species *Arabidopsis*, growth rate and biomass accumulation appeared to be negatively affected under FL conditions (Garcia-Molina and Leister, 2020).

To validate whether the VPZ genes would be functional in potato and to test whether improved photosynthesis would result in increased tuber yield, transgenic potato lines overexpressing the VPZ genes were created. Potato was choosen since its tuber number and size is adapted to environmental conditions and thus provides an ideal model to study the impact of elevated source capacity (photosynthesis) on sink (tuber) development.

We verified that the transgenes were functionally expressed (Figure 1), however neither in control nor in fluctuating light growth regimes did VPZ transgenic potato plants produce more leaf or tuber biomass. Despite accelerated recovery from NPQ, transgenic plants did not show improved CO_2 fixation rates under fluctuating growth conditions (Figures 3F, G), or in low light that followed after high light (Figure 5A). The reason for this phenomenon remains elusive and might be explained by species-specific effects. Support for this assumption comes from studies in bentgrass (Zhu, 2021) and *Arabidopsis* (Garcia-Molina and Leister, 2020). In both species, expression of the VPZ genes resulted in reduced plant growth. Since the physiological function of the VPZ genes could be verified in both plant species, it was concluded that by manipulating NPQ additional regulatory processes might be altered which may cause perturbations in intra-organellar (energy transfer), intra-cellular (plastid-signalling) or inter-organ (source-sink) communication.

Alternatively, an imbalance between induction and relaxation caused by unapproriate expression of VDE and ZEP could result in extremely high NPQ levels hindering radiation use efficiency under fluctuating light conditions. Hence it might be possible that the differences reported in this study are the consequence of different expression levels/patterns of the transgenes rather than differences in central cellular processes between different plant species.

High NPQ limits CO_2 assimilation and tuber yield under fluctuating light conditions

We exposed the VPZ expressing plants to fluctuating light conditions, in order to increase the pressure on NPQ induction and relaxation processes. According to previous studies in tomato overexpressing a *LeVDE* gene (Han et al., 2010), we expected that VPZ transgenic potato plants would suffer less from photodamage under fluctuating light conditions. Under those growth conditions, improved stress tolerance should result in increased tuber yield, if the source activity would limit sink development. Whether potato is source or sink limited is still under debate. While (Zrenner et al., 1995) could show that an approx. 50% reduction in photosynthetic sucrose biosynthesis did not result in decreased tuber yields under greenhouse conditions, (Nölke et al., 2014) reported on 2.3-fold higher tuber yields of transgenic potato plants carrying a photorespiratory bypass, indicating a significant source limitation. In our experiments we could not observe improved performance of VPZ transgenic potato plants with respect to biomass production. Under long day conditions, growth performance of VPZ transgenic plants was indistinguishable from control plants. Under conditions of fluctuating light, tuber yield was not improved but instead decreased. To potentially explain this counter intuitive result, CO_2

assimilation rates were determined in a light response manner (Figure 5A). This analysis revealed reduced CO_2 assimilation rates if low light adapted leaves are shifted to high light (2,000 µmol*m^{-2} *s^{-1}) but not vice versa as discussed above. Thus, the reduced CO_2 assimilation rate coincides with decreased tuber yields of VPZ plants grown under fluctuating light conditions. Plants overexpressing the VPZ genes, especially when endogenous VDE activity is rather high, might accumulate more zeaxanthin under high light conditions. This high NPQ level most likely causes a reduced radiation use efficiency and overall decreased photosynthetic rate. Based on this observation we speculate that the ideal plant should have only very mildly augmented NPQ under high light but still relax very fast in low light. Whether transgenic potato plants carrying the desired effect on photosynthetic productivity (i.e. source capacity) would show increased tuber filling (i.e. sink strength) and thus tuber yield, remains to be shown.

Elevated basal ABA levels do not significantly improve drought tolerance of VPZ plants under greenhouse conditions

Contrary to our results and the previously published studies in *Arabidopsis* (Garcia-Molina and Leister, 2020) and bentgrass (Zhu, 2021), (Kromdijk et al., 2016) have a reported biomass gain in VPZ expressing tobacco plants grown under field conditions. These contradictory results might be explained by the different growth conditions. While under controlled greenhouse conditions and controlled fluctuating light conditions plants usually do not suffer from water limitations, this situation cannot be ruled out under open field conditions. In this context it is interesting to note that previous studies have shown that ectopic expression of VDE and/or ZEP can influence abiotic stress tolerance through ABA synthesis or photoprotection as outlined in the next section,.

Overexpression of AtZEP in transgenic *Arabidopsis* plants, for instance, resulted in enhanced tolerance to osmotic stress and increased ABA synthesis (Park et al., 2008), whereas ZEP overexpression in tomato enhanced the sensitivity of PSII to photoinhibition by high light and chilling stress (Wang et al., 2008). Overexpression of VDE led to improved drought tolerance in *Arabidopsis* (Guan et al., 2015) and improved cold and light stress tolerance in tobacco (Gao et al., 2010). The improved drought tolerance of VDE overexpressing plants is associated with increased ABA levels. This finding, on the first glance, is unexpected since VDE activity should reduce violaxanthin availability and limit ABA synthesis. This view is supported by rice plants in which VDE expression was downregulated by CRISPR/Cas9 (Wang et al., 2022). VDE mutant rice showed improved salt tolerance and higher ABA content, paralleled by increased stomatal closure, while overexpression of VDE had the

opposite effect (Wang et al., 2022). Why overexpression of ZEP or VDE as well as down-regulation of VDE can result in similar phenotypes depending on the species analyzed remains elusive. Possible explanations are an overall increased violaxanthin + antheraxanthin + zeaxanthin, (V+A+Z) pool due to a stimulation of the xanthophyll pathway. This is supported by studies in *Lycium chinense* which showed that an upregulation of VDE activity and enhanced ABA synthesis can occur concurrently under drought because of the upregulation of the entire xanthophyll cycle (Guan et al., 2015). The fact that both, ZEP and VDE overexpression, can enhance drought tolerance could be explained by the regulation of both enzymes by light-dependend pH changes. Depending on the light condition, either VDE (high light) or ZEP (low light) activity is favoured. This could lead to a shift in the V to Z ratio as discussed by (Jahns and Holzwarth, 2012) and explain the observed discrepancies.

The partially contradictory reports prompted us to investigate the drought tolerance and ABA content of VPZ transgenic potato plants. While indeed elevated ABA levels could be detected in well-watered transgenic potato plants, these plants did not show signs of improved drought tolerance (Figure 6). In our experiment, plants were placed under severe stress which was accompanied by induction of ABA accumulation and increased levels of P5CS1 expression. The outcome does not rule out that VPZ transgenic potato plants might show some drought tolerance under milder stress and/or high light conditions in an open field experiment.

In conclusion, we could demonstrate that overexpression of VDE, Psbs and ZEP in potato plants results in higher NPQ and a faster relaxation from photoprotection as described for other plant species previously. This intervention did not cause improved CO_2 assimilation rates. Growth of transgenic potato plants was unaltered, however, tuber yield was reduced under fluctuating light conditions. Reduced tuber yield coincides with the reduced CO_2 assimilation rate in response to high light pulses interrupting periods of low light. The decreased CO_2 asimilation rate can be explained by inhibition of the radiation use efficiency due to high NPQ. ABA content of well-watered VPZ transgenic potato plants was found to be elevated, but did not result in improved drought tolerance under greenhouse conditions.

MATERIALS AND METHODS

Plant material and growth conditions

Wild-type potato plants (*Solanum tuberosum* L. ‚Solara') plants were obtained from Bioplant, Germany. Transformation of potato plants was conducted as described previously (Rocha-Sosa et al., 1989). Plants were maintained and amplified in tissue

culture on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 2% sucrose. If not otherwise stated, plants were grown under greenhouse conditions with 16 h supplemental light (approx. 250-400 µE). Plant height was measured from soil surface to apical meristem. Harvest index was calculated as ratio of tuber fresh weight per plant divided by the sum of green fresh weight and tuber fresh weight per plant. Leaf samples were taken from fully developed source leaves (5th to 8th from top). Fluctuating light experiments were carried out in walk-in growth chambers with light intensities between 50 and 500 µE in 12h light/dark regime as described in (Garcia-Molina and Leister, 2020). Drought stress was applied by setting the relative water content (RWC) of all plants to approx. 70%, then, for drought conditions, watering was stopped, whereas watering was continued for control plants. Three days after the end of watering, samples for ABA and RNA measurement were taken. At this time point RWC under water-stress conditions was approx. 15%. RWC was determined by measurement of soil conductivity using the EM50 soil moisture sensor (Decagon, United States) and calibration of pot weight.

Generation of transgenic constructs

For cloning purposes *Escherichia coli* XL1 blue (Bullock et al., 1987) were used. Stable transformation of plants was conducted with *Agrobacterium tumefaciens* strain C58C1 (Van Larebeke et al., 1974) as described in (Rocha-Sosa et al., 1989). The plasmid expressing VDE, Psbs and ZEP was generated using L0 constructs as described in (Kromdijk et al., 2016) and further assembled as depicted in Figure 1A using the Golden Gate cloning system (Engler et al., 2008; Weber et al., 2011; Werner et al., 2012). This final L2 plasmid was based on the AGM4723 vector with a Kanamycin resistance marker for selection of transformed plants. A detailed depiction of the construct and the sequences are shown in Table S1.

Photosynthesis measurements

Photosynthesis and transpiration rates were measured on fully developed source leaves on the upper middle stem (5th–8th from top) under the respective greenhouse or phytochamber conditions as described previously in (Lehretz et al., 2021) using a LI-COR 6800 device. NPQ and light response curves were measured similar as described previously in (Kromdijk et al., 2016) with the same device. Chlorophyll fluorescence for F_v/F_m was measured with a PAR-FluorPen FP 110 portable PAM Fluorometer (PSI Photon Systems Instruments, Drásov, Czech Republic) on fully developed source leaves which were dark adapted for 30 min before measurement.

ABA measurements

Phytohormones were extracted as described by (Pan et al., 2010). Leaf samples were grinded in liquid nitrogen using acidic buffer (66 % Isopropanol, 0.0002 % HCl). Samples were shaken for 30 min at 4 °C. Subsequently the twofold volume of chloroform was added and the samples were shaken for another 30 min at 4 °C. After centrifugation for 10 min at 4000 rpm at 4 °C the lower phase was transferred to a new reaction tube and vacuum dried at room temperature for 40 min. The pellet was resolubilized in 80 µl methanol. For chromatography 10 µl were injected into a luna C18 RP-column (250x4.6mm, Phenomenex) with precolumn installed in an ICS 3000 HPLC system (Dionex). The ABA content was measured with a QTrap 3200 mass spectrometer (ABI, Sciex).

RNA extraction and quantitative qRT-PCR

Samples of source leaves were taken during the first half of the light period, 4 h after dawn. Total RNA was isolated from ca. 100 mg of frozen leaf material by grinding in 1 ml 8 M Guanidiumchloride with 0.7% β-ME and further processed as described in Logemann et al., 1987. RNA quantity was measured with ND-1000 spectrophotometer (NanoDrop Technologies). Complementary DNA synthesis and quantitative RT-PCR (qPCR) analysis were performed as described in Ferreira et al., 2010. Primers used for expression analysis are listed in Table S2.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

GGL performed all experiments and analyzed the data. GGL, AS, DL and US designed the experiments. GGL and US wrote the manuscript with input from all

authors. US was responsible for project planning. All authors res and approve of the manuscript.

REFERENCES

Anwar, A., and Kim, J.K. (2020). Transgenic breeding approaches for improving abiotic stress tolerance: Recent progress and future perspectives. Int. J. Mol. Sci. **21:** 1–29.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. **141:** 391–396.

Asada, K., and Takahashi, M. (1987). Production and Scavenging of Active Oxygen in Chloroplasts. in: Kyle, D.J., Osmond, C.B. Arntzen, C.J., Eds. *Photoinhibition*. Elsevier, pp: 227–287.

Audran, C., Borel, C., Frey, A., Sotta, B., Meyer, C., Simonneau, T., and Marion-Poll, A. (1998). Expression studies of the zeaxanthin epoxidase gene in Nicotiana plumbaginifolia. Plant Physiol. **118:** 1021–1028.

Bugos, R.C., and Yamamoto, H.Y. (1996). Molecular cloning of violaxanthin de-epoxidase from romaine lettuce and expression in Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. **93:** 6320–6325.

Bullock, W.O., Fernandez, J.M., and Short, J.M. (1987). XL1-blue - a high efficiency plasmid transforming recA Escherichia coli strian with ß-Galactosidase selection. Biotechniques **5:** 376–379.

Cornish, K., and Zeevaart, J. (1984). Abscisic acid metabolism in relation to water stress and leaf age in Xanthium strumarium. Plant Physiol. **76:** 1029–1035.

Engler, C., Kandzia, R., and Marillonnet, S. (2008). A one pot, one step, precision cloning method with high throughput capability. PLoS One 3.

Eskling, M., Arvidsson, P.O., and Åkerlund, H.E. (1997). The xanthophyll cycle, its regulation and components. Physiol. Plant. **100:** 806–816.

Ferreira, S.J., Senning, M., Sonnewald, S., Keßling, P.-M., Goldstein, R., and Sonnewald, U. (2010). Comparative transcriptome analysis coupled to X-ray CT reveals sucrose supply and growth velocity as major determinants of potato tuber starch biosynthesis. BMC Genomics **11:** 93.

Gao, S., Han, H., Feng, H.L., Zhao, S.J., and Meng, Q.W. (2010). Overexpression and suppression of violaxanthin de-epoxidase affects the sensitivity of photosystem ii photoinhibition to high light and chilling stress in transgenic tobacco. J. Integr. Plant Biol. **52:** 332–339.

Garcia-Molina, A., and Leister, D. (2020). Accelerated relaxation of photoprotection impairs biomass accumulation in *Arabidopsis*. Nat. Plants **6:** 9–12.

George, T.S., Taylor, M.A., Dodd, I.C., and White, P.J. (2017). Climate change and consequences for potato production: A review of tolerance to emerging abiotic stress. Potato Res. **60:** 239–268.

Głowacka, K., Kromdijk, J., Kucera, K., Xie, J., Cavanagh, A.P., Leonelli, L., Leakey, A.D.B., Ort, D.R., Niyogi, K.K., and Long, S.P. (2018). Photosystem II subunit s overexpression increases the efficiency of water use in a field-grown crop. Nat. Commun. **9:**

Guan, C., Ji, J., Zhang, X., Li, X., Jin, C., Guan, W., and Wang, G. (2015). Positive feedback regulation of a Lycium chinense-derived *VDE* gene by drought-induced endogenous ABA, and over-expression of this *VDE* gene improve drought-induced photo-damage in *Arabidopsis*. J. Plant Physiol. **175:** 26–36.

Hager, A., and Holocher, K. (1994). Localization of the xanthophyll-cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light-dependent) pH decrease. Planta **192:** 581–589.

Han, H., Gao, S., Li, B., Dong, X.C., Feng, H.L., and Meng, Q.W. (2010). Overexpression of violaxanthin de-epoxidase gene alleviates photoinhibition of PSII and PSI in tomato during high light and chilling stress. J. Plant Physiol. **167:** 176–183.

Horton, P., Ruban, A. V., and Young, A.J. (2007). Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll Cycle. Adv. Photosynth. Respir. 271–291.

Hubbart, S., Smillie, I.R.A., Heatley, M., Swarup, R., Foo, C.C., Zhao, L., and Murchie, E.H. (2018). Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. Commun. Biol. **1:** 1–12.

Ivanov, A.G., Krol, M., Maxwell, D., and Huner, N.P.A. (1995). Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle. FEBS Lett. **371:** 61–64.

Jahns, P., and Holzwarth, A.R. (2012). The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta - Bioenerg. **1817:** 182–193.

Johnson, M.P., and Ruban, A. V. (2010). *Arabidopsis* plants lacking PsbS protein possess photoprotective energy dissipation. Plant J. **61:** 283–289.

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K., and Long, S.P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science **354:** 857–862.

Lehretz, G.G., Sonnewald, S., Lugassi, N., Granot, D., and Sonnewald, U. (2021). Future-proofing potato for drought and heat tolerance by overexpression of hexokinase and SP6A. Front. Plant Sci. **11:** 1–9.

Logemann, J., Schell, J., and Willmitzer, L. (1987). Improved method for the isolation of RNA from plant tissues. Anal. Biochem. **163:** 16–20.

Lohani, N., Jain, D., Singh, M.B., and Bhalla, P.L. (2020). Engineering multiple abiotic stresstolerance in canola, Brassica napus. Front. Plant Sci. 11.

Long, S.P., Humphries, S., and Falkowski, G.P. (1994). Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Biol. **45:** 633–662.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15:** 473–497.

Murata, N., Takahashi, S., Nishiyama, Y., and Allakhverdiev, S.I. (2007). Photoinhibition of photosystem II under environmental stress. Biochim. Biophys. Acta - Bioenerg. **1767:** 414–421.

Murchie, E.H., and Niyogi, K.K. (2011). Manipulation of photoprotection to improve plant photosynthesis. Plant Physiol. **155:** 86–92.

Nishiyama, Y., and Murata, N. (2014). Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. Appl. Microbiol. Biotechnol. **98:** 8777–8796.

Nölke, G., Houdelet, M., Kreuzaler, F., Peterhänsel, C., and Schillberg, S. (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (Solanum tuberosum) plastids strongly enhances photosynthesis and tuber yield. Plant Biotechnol. J. **12:** 734–742.

Pan, X., Welti, R., and Wang, X. (2010). Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. Nat. Protoc. **5:** 986–992.

Papageorgiou, G.C., and Govindjee (2014). The Non-photochemical quenching of the electronically excited state of chlorophyll a in plants: Definitions, Timelines, Viewpoints, Open Questions, Non-Photochemical in Plants, Algae and Energy Dissipation Quenching and Cyanobacteria, Chapter **1:** 1-44.

Park, H.Y., Seok, H.Y., Park, B.K., Kim, S.H., Goh, C.H., Lee, B.H., and Moon,

Y.H. (2008). Overexpression of *Arabidopsis* ZEP enhances tolerance to osmotic stress. Biochem. Biophys. Res. Commun. **375:** 80–85.

Pérez-Bueno, M.L., Johnson, M.P., Zia, A., Ruban, A. V., and Horton, P. (2008). The Lhcb protein and xanthophyll composition of the light harvesting antenna controls the ΔpH-dependency of non-photochemical quenching in *Arabidopsis* thaliana. FEBS Lett. **582:** 1477–1482.

Rocha-Sosa, M., Sonnewald, U., Frommer, W., Stratmann, M., Schell, J., and Willmitzer, L. (1989). Both developmental and metabolic signals activate the promoter of a class I patatin gene. EMBO J. **8:** 23–29.

Shi, Y., Ke, X., Yang, X., Liu, Y., and Hou, X. (2022). Plants response to light stress. J. Genet. Genomics.

Sonnewald, U., and Fernie, A.R. (2018). Next-generation strategies for understanding and influencing source-sink relations in crop plants. Curr. Opin. Plant Biol. **43:** 63–70.

Steuer, B., Stuhlfauth, T., and Fock, H.P. (1988). The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure. Photosynth. Res. **18:** 327–336.

Sun, L.N., Wang, F., Wang, J.W., Sun, L.J., Gao, W.R., and Song, X.S. (2019). Overexpression of the *ChVDE* gene, encoding a violaxanthin de-epoxidase, improves tolerance to drought and salt stress in transgenic *Arabidopsis*. Biotech **9:** 1–10.

Thompson, A.J., Jackson, A.C., Parker, R.A., Morpeth, D.R., Burbidge, A., and Taylor, I.B. (2000). Abscisic acid biosynthesis in tomato: Regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid. Plant Mol. Biol. **42:** 833–845.

Valliyodan, B., and Nguyen, H.T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr. Opin. Plant Biol. **9:** 189–195.

Van Larebeke, N., Engler, G., Holsters, M., Van Den Elsacker, S., Zaenen, I., Schilperoort, R.A., and Schell, J. (1974). Large plasmid in agrobacterium tumefaciens essential for crown gall-inducing ability. Nature **252:** 169–170.

Wang, N., Fang, W., Han, H., Sui, N., Li, B., and Meng, Q.W. (2008). Overexpression of zeaxanthin epoxidase gene enhances the sensitivity of tomato PSII photoinhibition to high light and chilling stress. Physiol. Plant. **132:**

384–396.

Wang, Q.Z., Liu, Q., Gao, Y.N., and Liu, X. (2017). Review on the mechanisms of the response to salinity-alkalinity stress in plants. Acta Ecol. Sin. **37:** 5565–5577.

Wang, X., Ren, P., Ji, L., Zhu, B., and Xie, G. (2022). OsVDE, a xanthophyll cycle key enzyme, mediates abscisic acid biosynthesis and negatively regulates salinity tolerance in rice. Planta **255:** 1–15.

Wareing, P.F., and Jennings, A.M. V. (1980). The Hormonal Control of Tuberisation in Potato. Plant Growth Subst. 293–300.

Weber, E., Engler, C., Gruetzner, R., Werner, S., and Marillonnet, S. (2011). A modular cloning system for standardized assembly of multigene constructs. PLoS One **6:** 1–11.

Werner, C., Ryel, R.J., Correia, O., and Beyschlag, W. (2001). Effects of photoinhibition on whole-plant carbon gain assessed with a photosynthesis model. Plant, Cell Environ. **24:** 27–40.

Werner, S., Engler, C., Weber, E., Gruetzner, R., Werner, S., Engler, C., Weber, E., Gruetzner, R., Werner, S., Engler, C., Weber, E., Gruetzner, R., and Marillonnet, S. (2012). Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system and the MoClo system: 5979.

Xu, X., Van Lammeren, A.A.M., Vermeer, E., and Vreugdenhil, D. (1998). The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation *in vitro*. Plant Physiol. **117:** 575–584.

Yoshiba, Y., Nanjo, T., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K. (1999). Stress-responsive and developmental regulation of Δ1-Pyrroline-5-carboxylate synthetase 1 (P5CS1) gene expression in *Arabidopsis thaliana*. Biochem. Biophys. Res. Commun. **261:** 766–772.

Zeevaart, J., and Boyer, G. (1984). Accumulation and transport of abscisic acid and its metabolites in Ricinus and Xanthium. Plant Physiol. **74:** 934–939.

Zhang, J., Schurr, U., and Davies, W. (1987). Control of stomatal behaviour by abscisic acid which apparently originates in the roots. J. Exp. Bot. **38:** 1174–1181.

Zhao, X., Yang, H., Liu, R., Chen, T., Feng, B., Zhang, C., and Tao, L. (2016). Responses of heat dissipation in rice to stress. Chinese J. Rice Sci. **30:** 431–440.

Zhu, X.G., Ort, D.R., Whitmarsh, J., and Long, S.P. (2004). The slow reversibility of photosystem II thermal energy dissipation on transfer from high to low light may cause large losses in carbon gain by crop canopies: A theoretical analysis. J. Exp. Bot. **55:** 1167–1175.

Zhu, Z. (2021). Overexpression of non-photochemical quenching (NPQ) related genes alters plant development and enhances plant osmotic stress resistance in creeping bentgrass. PhD thesis: 1–56.

Zrenner, R., Salanoubat, M., Willmitzer, L., and Sonnewald, U. (1995). Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). Plant J. **7:** 79–107.

Figure legends

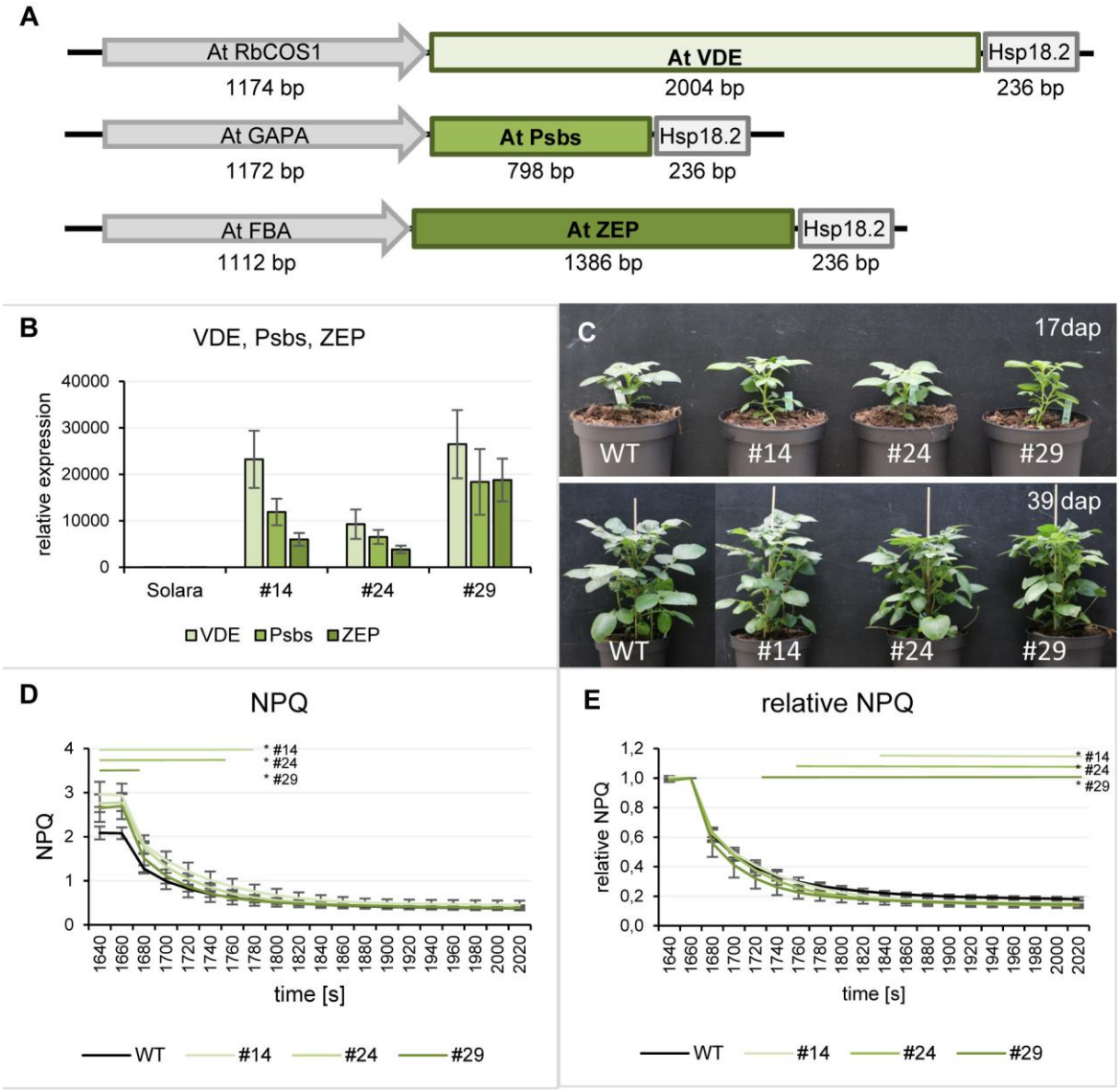

Figure 1. Characterization of VPZ expressing potato plants

(A) construct design **(B)** transgene expression in leaves, bars show mean of four biological replicates ± *SD*. **(C)** plant phenotype 17 dap and 39 dap **(D)** NPQ and **(E)** relative NPQ with relaxation in the dark (time to 50% relaxation $t_{50\%}$=16,4±2,0 (WT), $t_{50\%}$=15,7±2,0 (#14), $t_{50\%}$=15,2±2,6 (#24*), $t_{50\%}$=11,5± 4,4 (#29*), data points show mean of five plants ± SD, * p-value <0.05 compared to WT as indicated by colored lines.

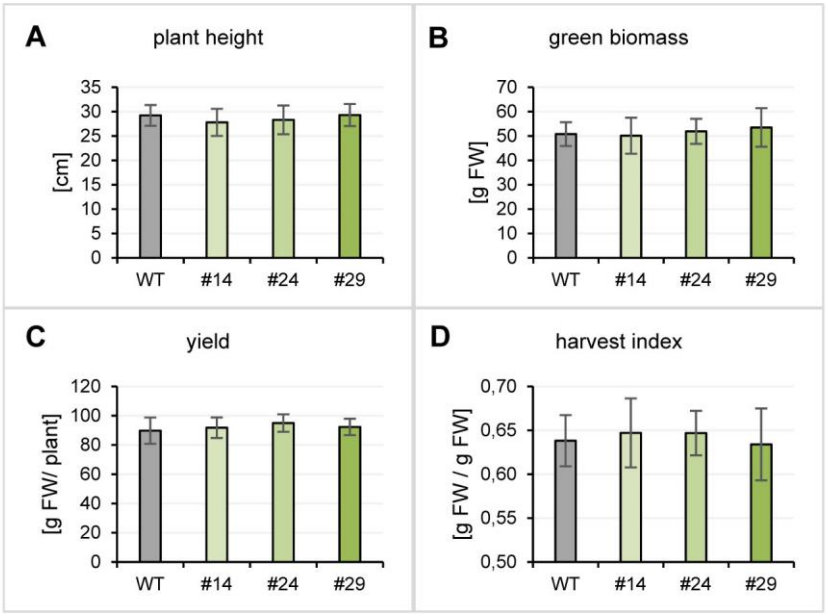

Figure 2. Harvest data of VPZ expressing potato plants

(A) plant height, **(B)** green biomass, **(C)** tuber yield, **(D)** harvest index, all bars show mean of ten plants ± *SD*, plants were grown under constant light greenhouse conditions and harvested at the age of ten weeks.

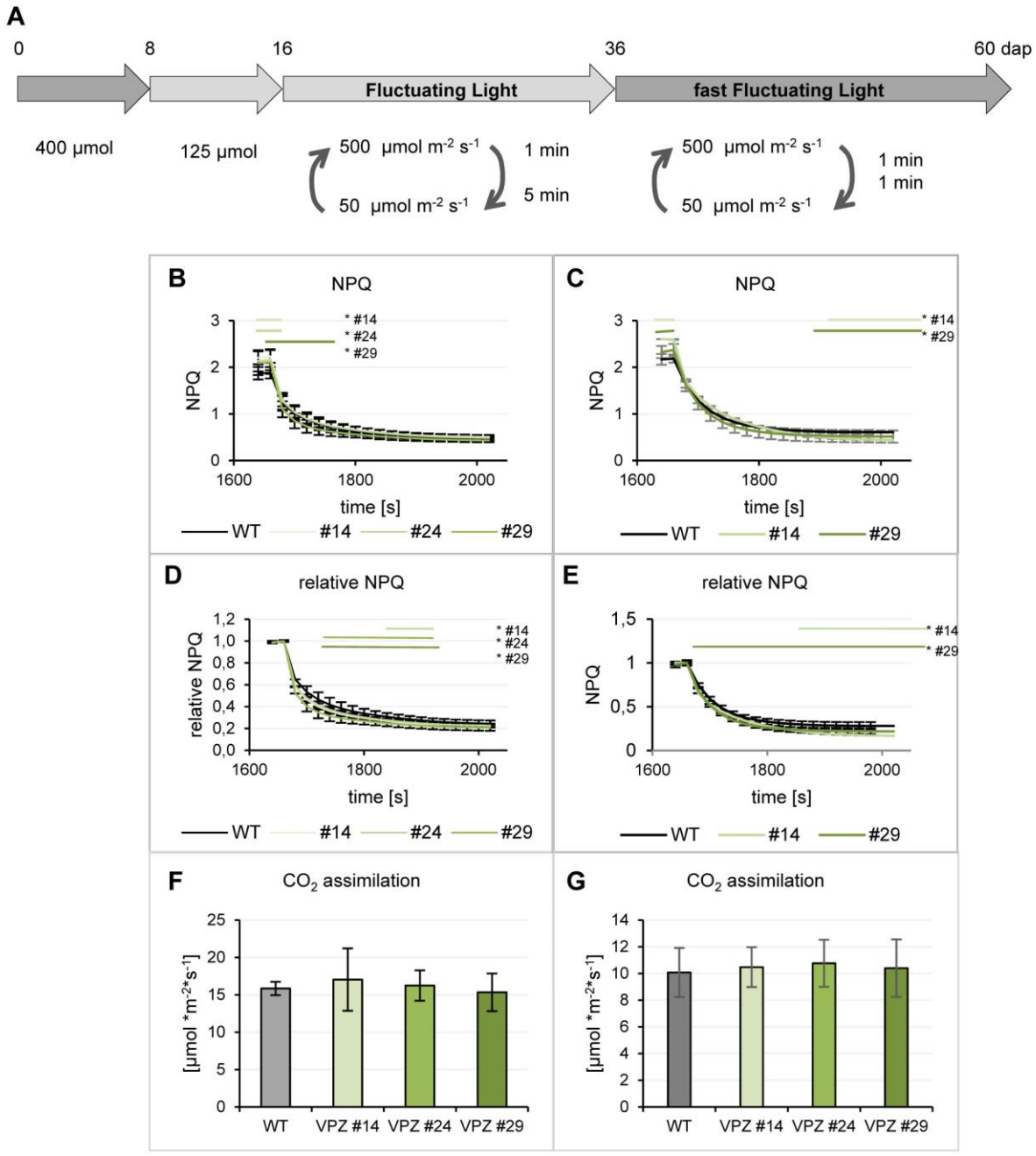

Figure 3. Non-photochemical quenching and CO_2 assimilation of VPZ potato plants

(A) experimental setup **(B)** NPQ in fluctuating light (FL) **(C)** NPQ in fast fluctuating light (fFL), **(D)** relative NPQ in FL, (time to 50% relaxation $t_{50\%}$=25,6±6,5 (WT), $t_{50\%}$=16,4±4,0 (#14*), $t_{50\%}$=20,0±4,6 (#24*), $t_{50\%}$=8,7± 2,5 (#29*), **(E)** relative NPQ in fFL, ($t_{50\%}$=26,6±6,1 (WT), $t_{50\%}$=21,7±7,0 (#14), $t_{50\%}$=21,4±5,6 (#29*) **(F)** CO_2 assimilation in FL, **(G)** CO_2 assimilation in fFL, bars show mean of five plants ± *SD*, * p-value <0.05 compared to WT as indicated by colored lines.

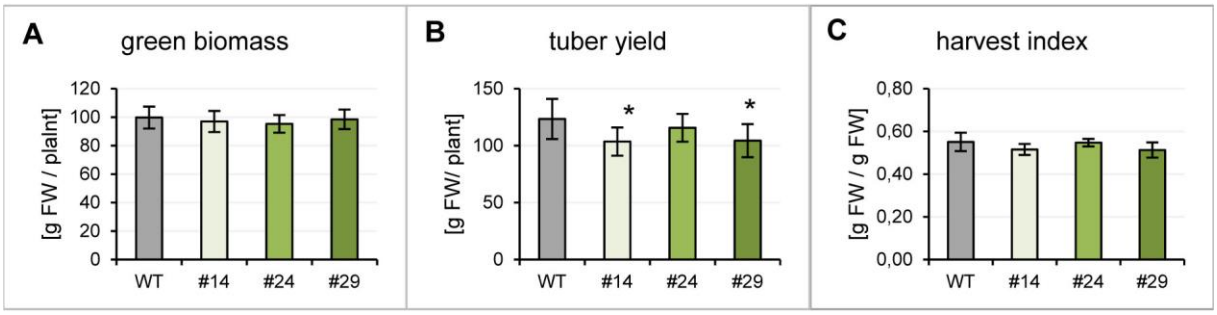

Figure 4. Harvest data of VPZ expressing potato plants under fluctuating light conditions

(A) green biomass, **(B)** tuber yield, **(C)** harvest index, bars show mean of ten plants ± *SD*, harvested at the age of nine weeks, * p-value <0.05 compared to WT.

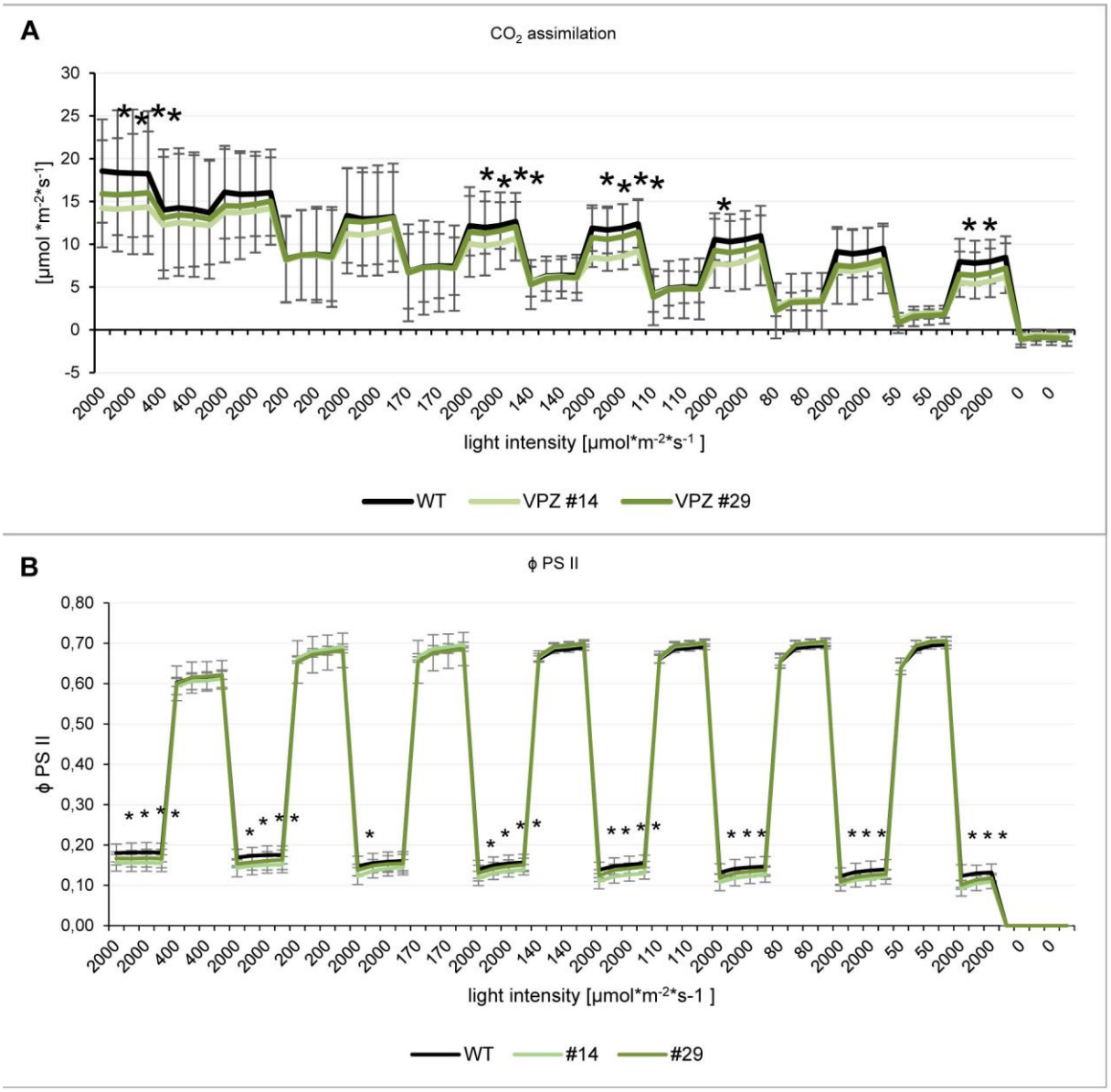

Figure 5. Light response of VPZ potato plants

(A) CO_2 assimilation, **(B)** ϕ PSII, plotted against light intensity, decreasing over time, low light was gradually decreased in 4 min timeframes interspaced by 4 min at 2000 µE high light, measuring points were taken every 1 min, bars show mean of nine biological replicates ± *SD*, * p-value <0.05 of line #14 compared to WT, line #29 showed no significant differences to WT.

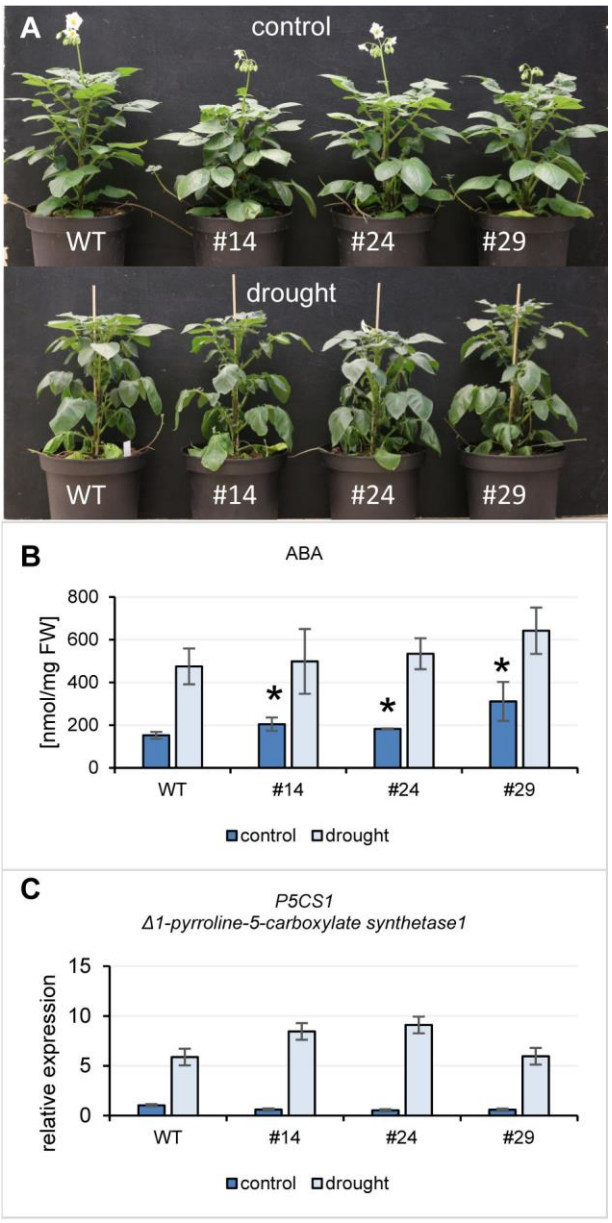

Figure 6. Drought experiments with VPZ expressing potato plants

(A) phenotype at 46 dap after three days of drought, **(B)** ABA contents in leaves, **(C)** P5CS1 expression in leaves, all samples taken after three days of drought, bars show mean of four biological replicates ± *SD*, * p-value <0.05 compared to respective WT.