מחקר 20_01_0080

דו"ח שנתי לשנת 2019 ומסכם

מחקר: **<u>טיפוח מכלואים עמידים לעקות אביוטיות בעזרת כלים גנומיים ופנומיים</u>**

1. תקציר

<u>הבעיה:</u> עקות אביוטיות של חום ויובש מהוות מחסום משמעותי לתפוקות חקלאיות ובעיקר עבור גידולי שדה. ישנו צורך למצוא חומר גנטי שישפר עמידות וסבילות גידולים כגון שעורה כנגד טמפרטורות גבוהות ויובש. יתרה מזאת, נדרש לפתח כלים לפרדיקציה של עמידות קווים שונים כבר בשלב המוקדם על מנת לשפר תהליכי טיפוח של זנים חדשים ועמידים יותר.

<u>שיטות העבודה:</u> פרויקט זה מחולק בין אפיון השפעות החום על שעורה והשוואה בין מדידות בשטח לבין קבלת נתוני מקצבי שעון צירקדי בפלטפורמות פנומיות חדשניות. השוואות אלו כוללות בחינת הקווים (עד קציר) תחת טמפרטורות אופטימליות (;Optimal or ambient temperature OT or HT) לעומת גבוהות (High temperature; HT) למאפייני צמיחה ופריון בשדה. כמו כן, בחינה באמצעות מערכת SensyPAM לתכונות של אמפליטודה ומחזור של שעון צירקדי תחת OT ו HT על פי נתוני פוטוסינתזה בשלבים מוקדמים של הצמח.

<u>תוצאות עיקריות:</u> התוצאות מצביעות על כך שצמחים תחת HT הפחיתו את ערכי היצרנות לכל התכונות שנמדדו כולל עיכוב של זמן הפריחה שהוביל לחנטה לא אופטימלית בחום. עם זאת, כאשר ניתחנו את התוצאות להשפעת [קו X טמפרטורה], מצאנו כמה קווים ייחודיים עם תשואה גבוהה יותר ב- H. הפרמטרים של קצב השעון הושפעו באופן משמעותי מהטמפרטורה בתנאי יום קצר, שם לצמחים ב- HT היה מחזור (period) ארוך יותר, משרעת (amplitude) גבוהה יותר. בניתוחים אלה, כמה קווים הראו תגובה ייחודית ל- HT. עבור מקטע בר ספציפי אחד, HsHeat3.1, נמצא כי האלל בר השפיע על תכונות שדה ופרמטרים של שעון היממה. שתי משפחות שהתפצל ב HsHeat3.1 נותחו על פי תכונות שדה ושעון. ניסויים אלה נערכו על צמחים בודדים ועל חלקות עם קוי BC2S1 הומוזיגוטיים. התוצאות הראו כי ההשפעות החיוביות של אלל הבר מובהקות רק בצמחים בודדים ולא בחלקות. לאחר מכן, רצינו לשאול על הקשר בין פנוטיפ שדה לבין פרמטרים של שעון בסביבות HT ו- AT. נמצא מתאם לינארי שלילי בין תגובת המשרעת ל- HT (משרעת הדלתא) ליציבות ה- GY, כלומר עם שינוי מקדם השונות (iCV_GY) בין HT ל- AT. תוצאות אלו נבדקו עוד על ידי ניתוח ביתר פירוט של הדוקרנים ומצאנו מתאם משמעותי בין היענות משרעת לפוריות בחום. לאחר מכן נבדקו ניסויים אלה במערך גדול יותר של גנוטיפים תוך שימוש ב -230 HEB-25 קווים, ובחלקות קטנות בשטח כדי לאמת קשרים אלו ולבדוק את האפשרות כי שימוש מוקדם ב- SensyPAM בשלב 3-4 העלים יכול לספק חיזוי ל ביצועים בשטח תחת חום. אכן, גם בניסוי גדול יותר זה היעדר תגובות משרעת תחת חום היה בקורלציה חיובית עם הפחתה פחותה בפריון הדוקרנים.

<u>מסקנות והמלצות לגבי ישום הדו״ח:</u> מחקר זה מדגים את חשיבות המצב הסביבתי במנגנונים הכרוכים בתכונה הנחשבת מהותית ויציבה, כלומר מקצב השעון היממה. זה גם מרמז על קשר עם ביצועי השדה בסביבה מסוימת, ובכך מצביע על כך שניתוח שעון היממה באמצעות פלטפורמה כמו SensyPAM בשלבים מוקדמים יכול לשמש מנבא לתכונות שדה. זה גם מדגיש את הפוטנציאל של אללים של מיני בר לווסת את הפלסטיות של הצמחים ולשפר את עמידותם במתח אביוטי, אם כי נדרשים מחקרים נוספים כדי להבין את תפקידו של אללים ספציפיים מהבר לטובת טיפוח קווים עמידים יותר.

דו״ח סופי
לתכנית מחקר מספר **20_01_0080**

מוגש לקרן המדען הראשי במשרד החקלאות

על ידי:

אייל פרידמן, מכון וולקני, חוקר ראשי

רחל גרין, מכון למדעי החיים, האוניברסיטה העברית

נועם שנטל, מחלקה למדעי המחשב

עזרא רבינס, מו״פ יטבתה

3. גוף הדו״ח

מטרות המחקר

1. פיתוח שיטה לאיתור מוטציות בגני מטרה
2. טיפוח חומר גנטי בעל אללים ממין בר המקנים עמידות לחום
3. פיתוח חומרה ותוכנה לאיפיון בתפוקה גבוהה של מאפייני שעון צירקדי באמצעים לא פולשניים
4. איתור עקרות זיכרית שתאפשר יצור יעיל של מכלואים בשעורה

(מטרה זו שונתה בשנה השניה של המחקר, והוחלט בהסכמה להתרכז במטרות האחרות)

1. Background

High temperature stress is one of the primary causes of crop loss worldwide, with predicted average yield loss of approximately 7% for every 1°C increase. One of the important sources of new genetic variation for improving resilience of modern varieties against stress are wild relatives of crops, which harbor most of the gene pool that existed before domestication of the crop species. Reshuffling of alleles back from the wild has been a tool for identifying and studying variation of yield traits, e.g. fruit size and sugar content. However, wild alleles can either positively or negatively correlated with yield increase and a majority of the QTL from wild species have negative effects on yield, either under optimal or stress conditions.

In plants, the circadian system regulates numerous physiological and molecular processes including chlorophyll biosynthesis, starch metabolism, growth, stomatal opening, photoperiodic flowering and the expression of around 30% of the genome (Hsu and Harmer, 2014). Although by definition circadian rhythms have ~24 hour periods (de Montaigu et al., 2010), exact period lengths can vary by a few hours between tissues within a single plant (Takahashi et al., 2015, Endo et al., 2014) and among different accessions within a species (Kusakina et al., 2014). Rhythm phase, the timing of the rhythm peak, and amplitude, the difference between peak and midpoint of the rhythm may also differ. Increasingly, the circadian clock is known to be intimately involved in regulating metabolism. For example, a tight interplay exists between sugar metabolism and signaling by the circadian clock genes (Webb and Satake, 2015). Recently, a shorter period circadian clock and natural variation in buffering capacity at high temperature was shown for Arabidopsis accessions (Kusakina et al., 2014); the period shortening was correlated with earlier expression of *TOC1* and *LHY* under 27°C, indicating this may be an escape mechanism against heat (Kusakina et al., 2014). This study showed that accessions with poor buffering against temperature, i.e. having greater

period difference between 17°C and 27°C, gained more weight, suggesting that having a more flexible circadian system may be an advantage, although reproductive output was not reported. Output of the circadian clock and their rhythmicity are often used as proximation to the core clock activities, e.g. leaf movement and carbon dioxide fixation (Tindall et al., 2015). Moreover, continued advancements in the throughput of measuring circadian rhythms in plants have paved the way to large scale comparisons between naturally adapted populations and interspecific populations. These advancements are utilized to find the genetic basis of the circadian clock variation (Müller et al., 2015; Müller et al., 2018), i.e. circadian clock research had become a significant phenotype in the phenomics arena. While the initially used qRT-PCR for clock genes is destructive and time-consuming, and LUC assays require expensive reagents and apparatus, other non-destructive methods developed to allow species-wide, non-transgenic and high temporal resolutions (Tindall et al., 2015). These remote methods are including prompt (F) and delayed fluorescence (DF) from Chl (Gould et al., 2009; Dakhiya et al., 2017; Bdolach et al., 2019), and more recently, measurement of circadian thermocycles of the plant organs (Dakhiya and Green, 2019). Other techniques to follow rhythmicity, each with its pros and cons (Tindall et al., 2015; Dakhiya and Green, 2019), include camera following of the leaf movement (Edwards and Millar, 2007), or other measurements of physiological traits such as photosynthetic rate using infra-Red Gas Exchange Analyzer (IRGA) (Bohn et al., 2001). In this proposal we wish to test the utilization of a new platform, SensyPAM, for measuring clock rhythms and test if it can predict plant performance in the field, under optimal and high temperatures. **We wish to explore the importance of clock flexibility and examine in depth the fitness values of temperature compensation. Fitness defined as reproductive success is linked to yield and output and one of the important questions that we will be asking is whether there is necessarily a positive correlation between greater vegetative growth under stress and agricultural output since growth may come on the expense of grain yield and reduce harvest index.** A number of studies have shown that the domestication and spread of important crops to more stable agriculture environments has been accompanied by changes in canonical circadian oscillator genes that cause altered period length and amplitude (Müller et al., 2015). These altered rhythms may affect the ability of plants to grow under stressful conditions and/or change photoperiod-sensitive development. Moreover, variation in flowering time regulation may help plants better adapt to harsh environments. Thus, for example, commercial barley varieties bred to adapt to the high-latitude short growing seasons typical of northern Europe carry the *early*

maturity8 (*eam8*) allele, which allows rapid day-length independent flowering. *eam8* affects a gene homologous to an Arabidopsis oscillator gene (Faure et al., 2012). In the future, farmers will face serious environmental challenges including significant warming with extended heat-wave and unpredictable weather patterns (Moore and Lobell, 2015). These challenges may, in part, be met by developing next-generation crop varieties with circadian clock variants that are tailored to optimize growth and yield under stressful conditions. **The general aim of this proposal is to gain better understanding of the circadian clock characteristics that could provide crop plants with resilience against heat, one of the most important abiotic stresses.**

One QTL that we recently identified (*Hordeum spontaneum* Heat 3.1; HsHeat3.1) to be related with heat resilience includes the barley ortholog of the Arabidopsis *GIGANTEA* (*GI*) gene. This circadian clock gene that also acts as an important component of several major pathways in plants including the diurnal expression of majority of the transcriptome under open field conditions in rice and salt-stress adaptation (Fowler et al., 1999; Izawa et al., 2011). In Arabidopsis, the rhythmic expression of the F-box protein ZEITLUPE (ZTL) is associated with its binding to AtGI, an interaction specifically enhanced by blue light, through the amino-terminal flavin-binding LIGHT, OXYGEN OR VOLTAGE (LOV) domain of ZTL (Kim et al., 2007). Finally, it was recently found that GIGANTEA forms a ternary complex with HSP90 and ZEITLUPE and its co-chaperone action synergistically enhances HSP90/HSP70 maturation of ZEITLUPE in vitro (Cha et al. 2017). **These studies suggest the central role of GIGANTEA in stress responses likely depends on stress-conditioned protein-protein interaction with key players in these pathways.** Nevertheless, relationship between GI expression and reproductive output has not been shown (Bendix et al., 2015), nor GI activity or alteration was shown to confer heat tolerance.

2. Materials and Methods

2.1 Plant material and heat experiments

For the GWAS, we used 338 barley lines from the interspecific multiparent barley population HEB-25 that includes the introduction of 25 wild barley accessions into a common cultivated (cv. Barke) genetic background (Maurer *et al.*, 2015). . In the Yotveta heat experiments we relied our heat treatment on covering one nethouse with polyethene, and comparing performance of a barley panel under two set-ups (covered and non-covered net houses).

2.2 Clock phenotype under optimal and high temperature

Plants were grown to emergence of the fourth leaf under 14 h: 10 h, light: dark at a constant temperature of 20-22 °C. Following this entrainment of the plants for 4 wk, we moved them to the high-throughput SensyPAM (SensyTIV, Aviel, Israel), custom-designed to allow F measurements in up to 240 plants for each experiment (see details in (Bdolach *et al.*, 2019)). For the clock measurement, F was measured every 2.5 h for 3 d, in continuous light. Plants are growing in a 6-pack pots with planting mixture "Green" (Aven Ari, Israel) with radiance of 200 μmol/(m^2 s) and irrigated daily. We measured the same plant once under OT and second under HT, with four to five plants per genotype. Between each run the day length was returned to 14 h: 10 h, light: dark for two nights. For the clock analysis the non-photochemical quenching values (NPQlss, (Fm-Fmlss)/Fmlss) was calculated and normalized to the mean of each experimental cycle as previously shown (Bdolach et al., 2019). The circadian clock's free-running period (period), amplitude (amplitude) and amplitude error were extracted from the BioDare2 website (https://biodare2.ed.ac.uk). The input data were set to "cubic dtr" and "MFourFit" was used as the analytical method (Zielinski *et al.*, 2014).

2.3 Statistical analysis and GWAS

We used the JMP version 14.0 statistical package (SAS Institute, Cary, NC, USA) for statistical analyses and to generate reaction norms for the means and SE of the different traits. Student's t-tests between treatments were conducted per panel using the 'Fit Y by X' function. A factorial model was employed for the ANOVA (Table S1), using the 'Fit model', with temperature treatment and panel as fixed effects. A GWAS was carried out to identify trait variations, per se, under OT and HT, and to assess digenic interactions (2D scan). We performed the genome scan using three different analyses: extended Bayesian LASSO (EBL), linear mixed model (LMM) and Tassel pipeline.

3. Results and Discussion

Comment: Previous two reports (Year 1 and Year 2) provided most of the results obtained, and current report summarize these, as well as adding the parts achieved recently. Moreover, the report is arranged and geared towards summarizing the conclusion drawn from the project, rather than showing just experimental output.

3.1 Field trials and responses under heat- first round

One of the uttermost challenges in testing plants under heat was experienced in the field of Yotvata. While the initial set-up (without allowing way-out to night humidity) did lead to

increased temperatures in the high-temperature (HT) compared to ambient, it also prevented us from achieving harvesting of the plants as they grew wildly under HT. Moreover, the relatively high humidity in the HT part also had adverse effect on the plants. Combination of these difficulties, with knowing that in separate experiments conducted in our ARO HT/OT site in which we managed to obtain much better growth of the plants (Bdolach et al. 2019), led us to continue experiments in ARO and improve Yotveta set-up in the last year. Results of the first Yotvata experiment appear in report of the second year, and summary of the responses for 18 lines is presented in Fig. 1. Additional results appear in the last part of the results, after we managed to improve field set-up and obtain mini-plot phenotype under both AT and HT in the last experiment of 2020.

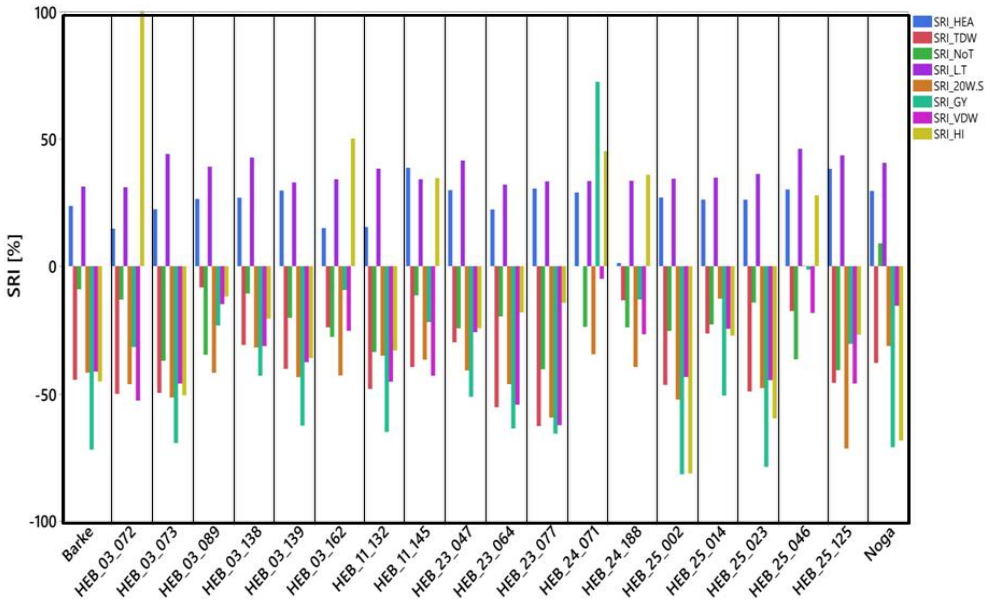


Fig. 1. Field traits stress resistance index for each line (trait by color). When the [Line X Environment] interaction effect was analyzed, only GY and HEA had a significant effect ($P<0.05$, ANOVA).

2.2 *The circadian clock rhythm phenotype: Temperature and genotype effects*

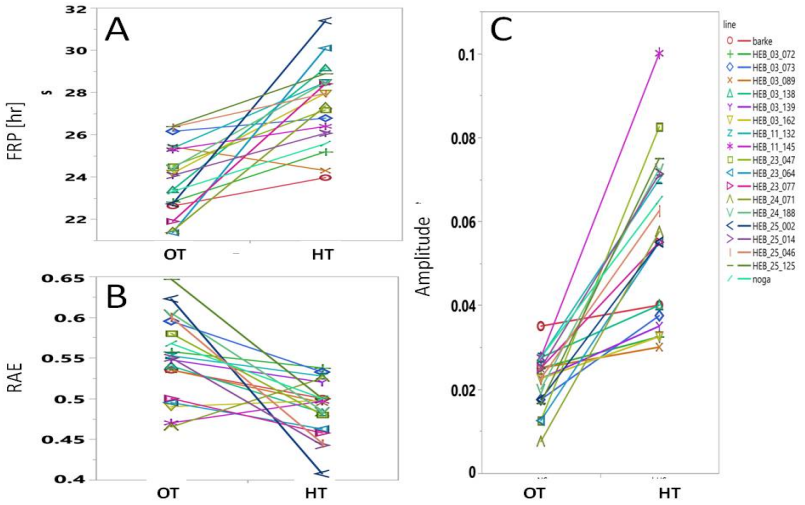


Fig. 2. Least square means for lines in AT and HT (reaction norm).(A) FRP (hr) , (B) RAE and (C) Amplitude in optimal temperature (OT) and in high temperature (HT).

Overall, across all HEB lines tested, regardless of the wild donor, the clock characteristics were similar. Except HEB-03-089, higher temperatures (32°C vs 22°C) were associated with deceleration of the clock (higher period), increased amplitude and noisy rhythms as realized by reduced relative amplitude errot (RAE; the closer values are to 1 the more rhythmic they are). Nevertheless, there seem to be a spectrum of responses between the lines, which prompted us later to extend the number of lines and perform genome scan using the iSELECT genotypic data (Prusty et al., 2020). This allowed us to identify few major QTL that affect these responses and in which the wild allele is associated with increased plasticity of both amplitude and period. These results were summarized to a manuscript that was submitted recently (Prusty et al., New Phytologist, under Revision).

3.2 Relationship between growth and circadian clock responses - can early clock phenotype predict field performance, and which attributes of it?

A major question of this study is, how circadian clock properties affect or interconnected with field traits under different thermal environments? The relationship between the change of the circadian clock and field traits examined for both means and coefficient of variation (iCV). The later used as a measurement for the stability in the field trait by line (Fridman 2015). The most significant finding was that lines with higher amplitude had lower CV in GY. Furthermore, the iCV for GY, an index corresponding to the change in CV between HT and OT (Fridman

2015), was negatively and significantly correlated with amplitude response (delta amplitude; Fig 3 B).

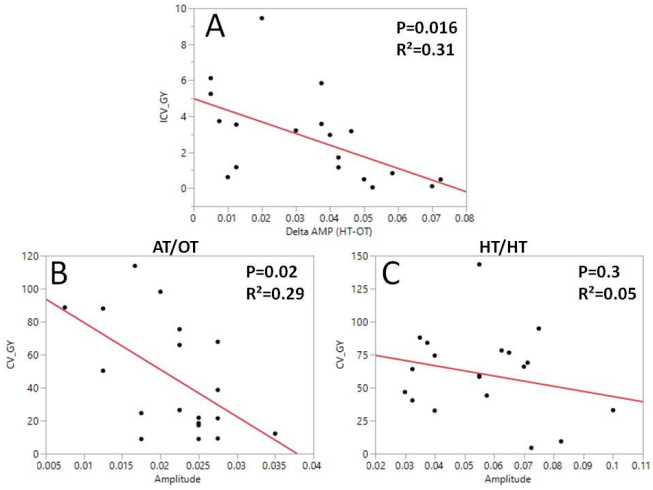


Fig. 3. Relationship between amplitude plasticity and yield stability under heat in single plants stand. (A) Linear correlation between delta of amplitude (HT-OT) and iCV of GY, (B) linear correlation between the amplitude in OT and CV of GY in AT, (C)) linear correlation between the amplitude in HT and CV of GY in HT .

Following on these results we wished to examine in more detail the relationship between the amplitude rather than period of the clock, since the former seemed correlated with reproductive yield. In the next set of experiments, conducted during 2019, several lines were examined in ARO under AT and HT. In this experiment the spikes were dissected to learn of possible effects on fertility. We defined fertility as the mean proportion of filled spikelet in a spike based on 10 spikes for a plant. A pair-wise correlation analysis compared between the means difference of period, amplitude and ERR to all means difference of field phenotype. Fig 4 depict the overall scatterplot and zooming-in on some of the more significant correlation found. Overall, this analysis revealed a positive relationship between **stability of the clock amplitude** (less change between the treatments) to **reproductive mass**, **fertility** and **spike uniformity** (less change in the CV of trait). This could be observed by the significant correlation ($r=-0.867$, $P<0.0053$) of delta amplitude (dAMP) and 10 spike weight (10SW), i.e. the more the AMP increased under heat (high dAMP) the more reduction observed in the reproductive spike output. Similar trend, albeit not significant ($P<0.15$), observed between the dAMP and that of fertility. Other significant correlations with the dAMP were found with change in uniformity or stability of the spikelet (dCV_SPK) and fertility (dCV_FER), i.e. the lesser responsive were the lines the more they kept stability under heat. In contrast to these significant correlation for the dAMP, those of the clock period were not significant for any of the reproductive traits or spikelet uniformity,

however there is still positive relationship between stability of the clock to reproductive mass, fertility and spike uniformity as depicted by the CV between spikes on the same plant (Fig. 5)

3.3 Testing the possible pleiotropic effects of hsHeat3.1 on circadian clock and growth plasticity

The possible correlation between variation at the Heat3.1 locus with differences in thermal plasticity of the circadian clock traits was conducted for the three clock parameters (FRP, AMP, ERR) by drawing a reaction norm and testing for QTL by environment interaction (QxE) using ANOVA. Overall, this analysis indicated higher responsiveness for the Heat3.1$^{Hs/Hs}$ vs Heat3.1$^{Hv/Hv}$ genotypes (homozygous for wild vs cultivated allele). Period reaction norm between genotype and treatment, revealed a very sharp decline of the Heat3.1$^{Hs/Hs}$ genotype, and at lesser level for Heat3.1$^{Hv/Hv}$ and Barke control (Fig. 6A) moreover, AMP reaction norm revealed larger difference between the means of Heat3.1$^{Hs/Hs}$ (0.017) in compare to Heat3.1$^{Hv/Hv}$ (0.009) (Fig. 6B). Overall, Heat3.1$^{Hv/Hv}$ represent more stability in the clock parameters than Heat3.1$^{Hs/Hs.}$.

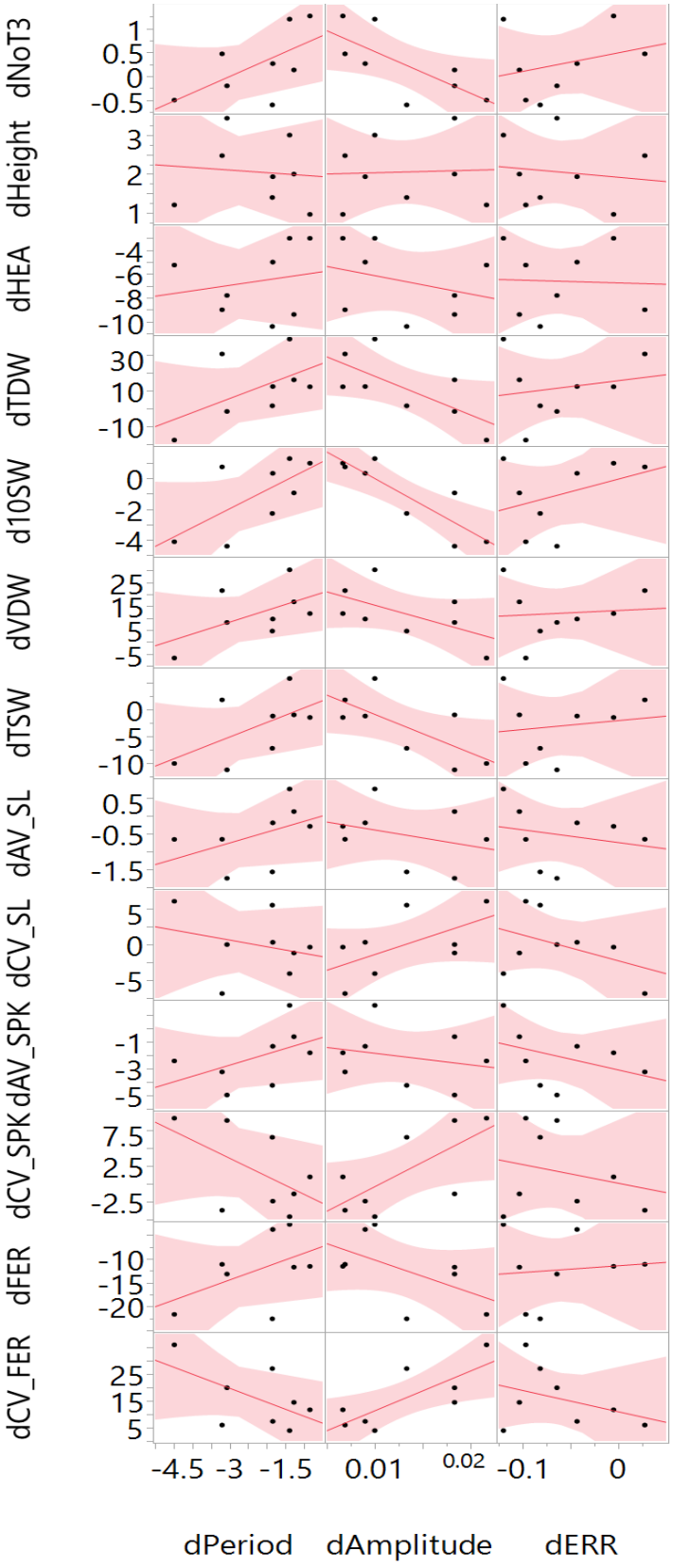
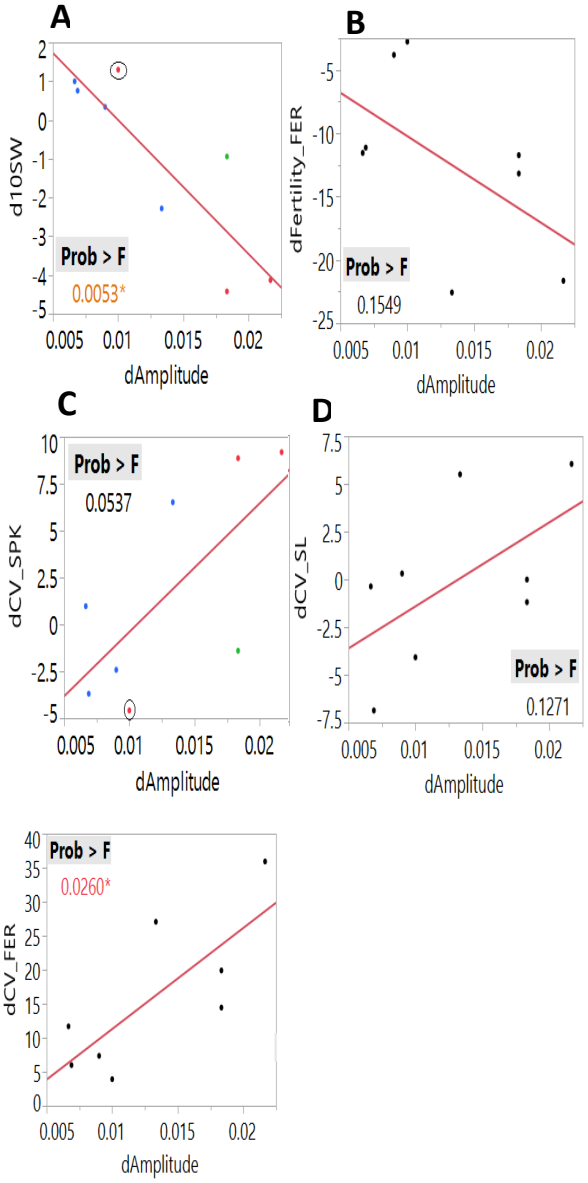


Figure 4. Pair-wise correlation between circadian clock and growth responses to heat. On the left are the whole correlation and at the right zoom-in on some the correlation in more detail. d correspond to the Delta of the trait, i.e. the value under HT minus that under AT.



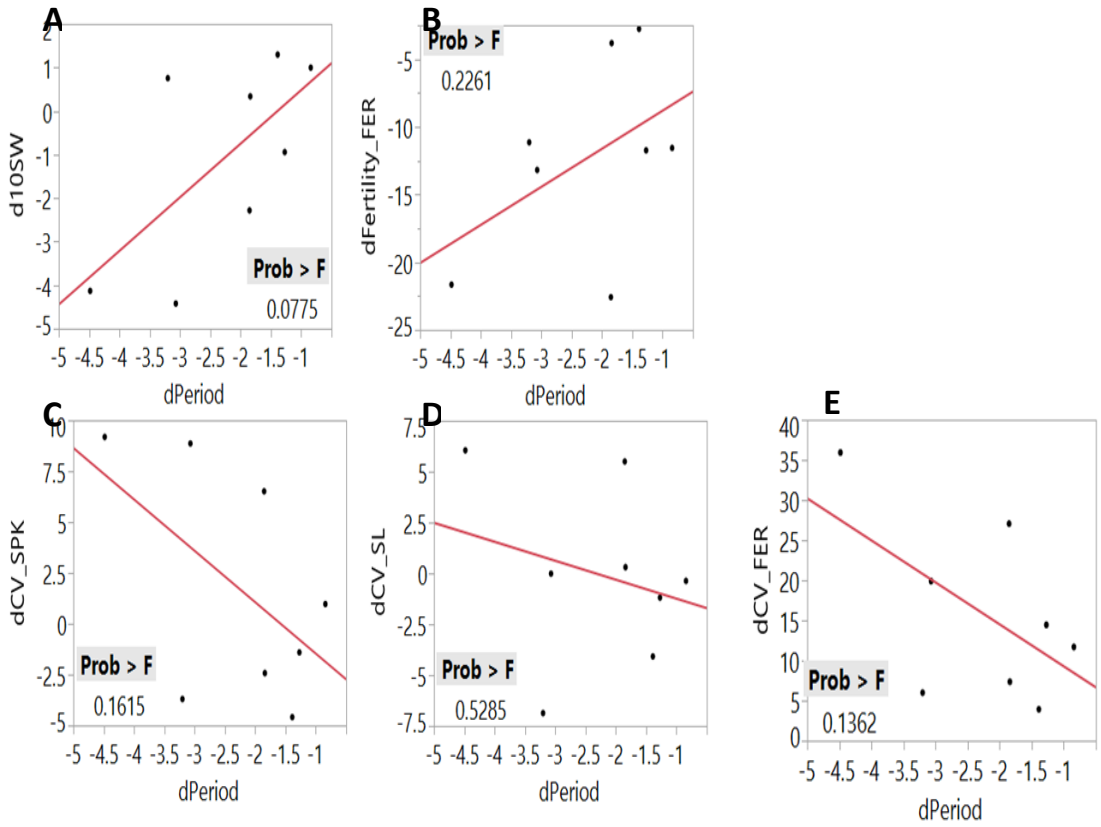
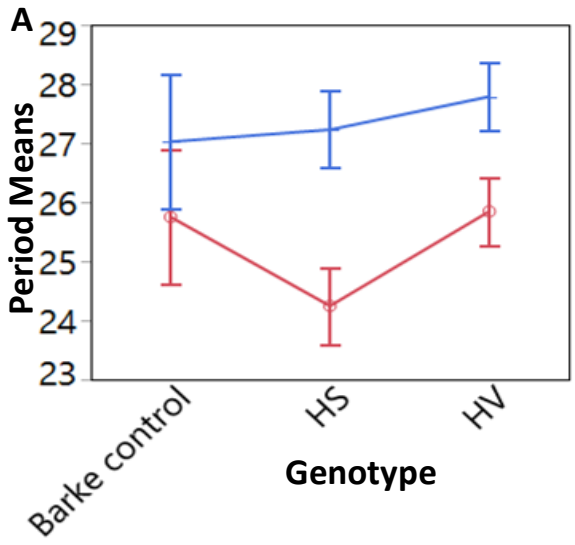


Figure 5. Relationship between the delta of Period, reproductive traits and their stability (CV).

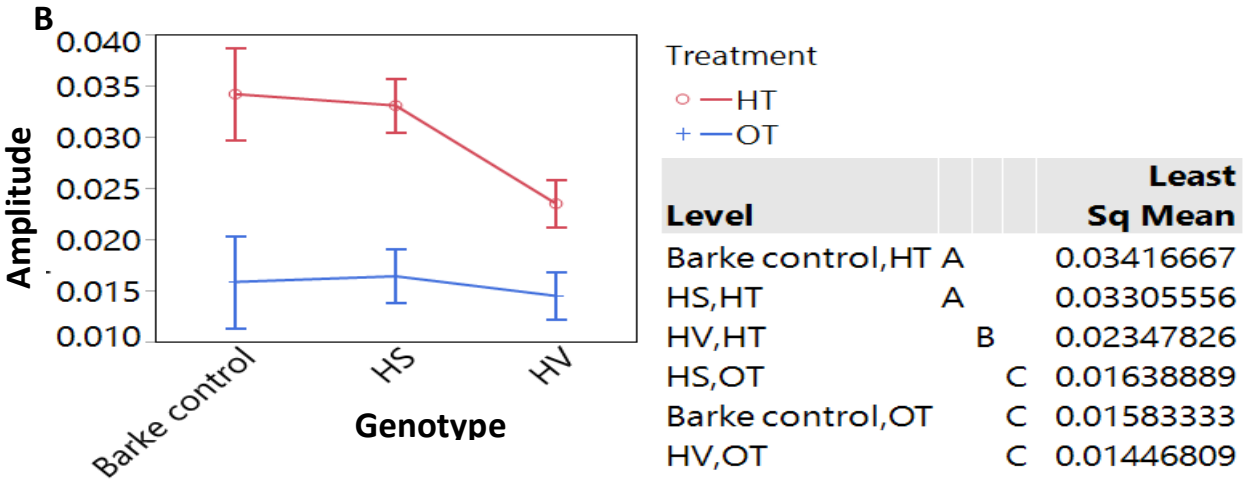


Treatment

HT

OT

Level		Least Sq Mean
HV,OT	A	27.786809
HS,OT	A B	27.226667
Barke control,OT	A B C	27.023333
HV,HT	C	25.845000
Barke control,HT	B C D	25.750833
HS,HT	D	24.245833



Level				Least Sq Mean
Barke control,HT	A			0.03416667
HS,HT	A			0.03305556
HV,HT		B		0.02347826
HS,OT			C	0.01638889
Barke control,OT			C	0.01583333
HV,OT			C	0.01446809

Figure 6 The effect of Heat3.1 genotype on clock period and amplitude under heat and ambient treatment. Reaction norm of genotype* treatment for three characters: (A) Period, (B) Amplitude and (C) ERR. Blue line represents OT treatment and red HT treatment. A Tukey Kramer tests significance of the differences between means of the three genotypes (Heat3.1$^{Hv/Hv}$; Heat3.1$^{Hs/Hs}$ and Barke) under HT and AT. HS and HV correspond to *Hordeum spontanem* (wild allele) and *H. vulgare* (cultivated allele).

3.4 Testing the relationship between clock robustness and fertility in a large field trial

Our last experiment in this project (still under analysis due to COVID-19 related delays) followed up on the earlier observation that found a correlation between amplitude thermal responses (in the 4th leaf) and fertility (Fig. 4). We improved our field set-up in Yotveta by providing humidity way-out during the night, which improved the overall growth of the 230 HEB lines that we measured under AT and HT. To allow larger scale analysis of fertility we have undertaken a combined image analysis using X-ray and thermal camera imaging, together with RGB imaging of the whole experiment (7 spikes/plot; Fig. 7A-C). This allowed us to calculate the average fertility of each genotype and to correlate it with the clock phenotype that we performed in the SensyPAM. Again, the significant and negative correlation between amplitude responses and spike fertility repeated itself (Fig. 7E &F).

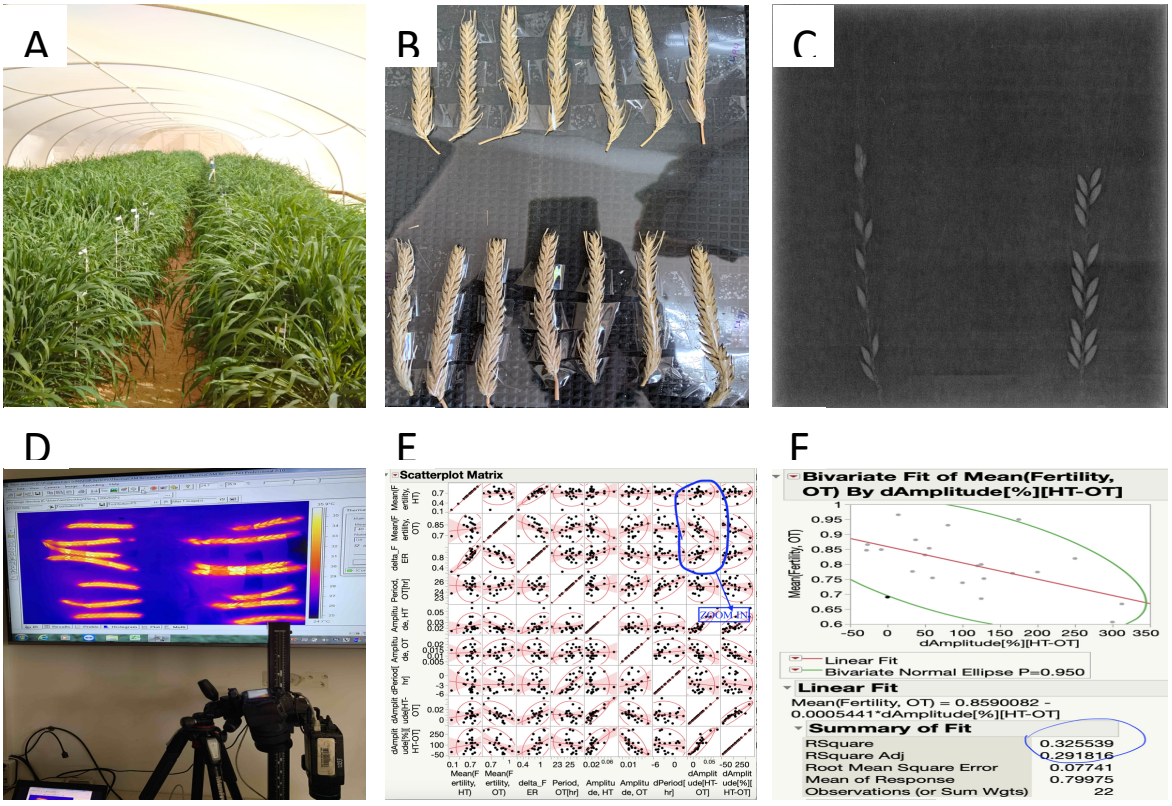


Figure 7. Relationship between amplitude plasticity and fertility under heat in mini-plots field experiment. **A)** This experiment in Yotveta including 230 HEB genotypes grown under HT and AT that were scored for spike phenotypes. Results are shown for selected 22 accessions under HT and AT. **B)** Seven spikes were sampled and analyzed by **C)** X-ray and **D)** FLIR thermal camera to calculate percentage of filled spikelets in a spike (=fertility). **E)** Pair-wise correlation between circadian clock period and amplitude and fertility, repeat the **F)** negative correlation between amplitude responses to heat (dAMP) and changes in fertility or fertility per se under OT.

3.5 Genome scan for clock and field data to examine pleiotropic QTL that effect both traits under stress

Our last set of experiments examined if indeed genomic loci that will be associated with clock traits, and therefore could be found on the SensyPAM at early stages of growth, will show effects on true field traits as biomass and grain yield. We already examined the whole HEB-25 population (N=1,420) in the open field in Gilat, under optimal and drought conditions few years back, and this year in Yotveta. Therefore, we could examine reproducibility of the results and the conditioning of two major abiotic stresses (heat and drought) on this genetic overlap between clock plasticity (change under heat at this point) and yield traits QTL. Also, the fact that the HEB

population was tested in field trails across the globe allowed us to further test these QTL effects on yield elsewhere. We compared genome scans to QTLs results that were published for the same population by other groups, i.e.at 'Kühnfeld Experimental Station' of Martin Luther UniversityHalle-Wittenberg (51°29′46.47″N; 11°59′41.81″E)(Maurer et al., 2015), a the International Center for Biosaline Agriculture (ICBA), Dubai, United Arab Emirates (N 25° 05.847; E 055° 23.464)(Saade et al., 2016) and finally at Dundee, Scotland(Herzig et al., 2018).

Genome scan with clock phenotypes: Genome scan for the loci affecting clock trait per-se (under OT or HT), or their plasticity (delta of trait), was initially conducted with 3013 markers using a linear mixed model (LMM), which took into consideration the population structure and HEB familial relationships. By using a significant threshold determined by Bonferroni correction or BH FDR 0.1 altogether, we identified four significant QTL, two for each period and amplitude plasticity. The QTL we identified named as Driver of Clock (DOC). One locus, *DOC3.1*, which resides on chromosome 3 (position 29,085,440 -36,987,723; PVE= 4.5 %) was associated with variation of the amplitude under HT and delta amplitude (Fig. 8a). We also identified *DOC3.2,* which is also associated with variation of the amplitude (position, 51,509,488, LOD = 4.63; PVE= 4.5 %), nearby to the *DOC3.1* (Fig. 8a). Notably, the increase in the amplitude under HT for both *DOC3.1* and *3.2* loci was attributed due to the positive effect of the wild allele for all the markers in these loci (Fig. 4b, 4c). It is interesting to note that based on LD pattern, the region of *DOC3.2* spans the barley domestication gene *BTR* and circadian clock gene *GI*. The third QTL loci named as *DOC5.1*, resides on chromosome 5 (position 605,805,151-609517829)and it was associated with variation of the period under OT and delta period (Fig. 8d). Similarly, *DOC1.1*on chromosome 1 (position, 548,354,572 – 556,777,555) was found to be associated for period under HT (Fig. 8d). In both *DOC1.1* and *DOC 5.1* the wild allele accelerated the period (HsHs vs HvHv; Fig 8 e,f).

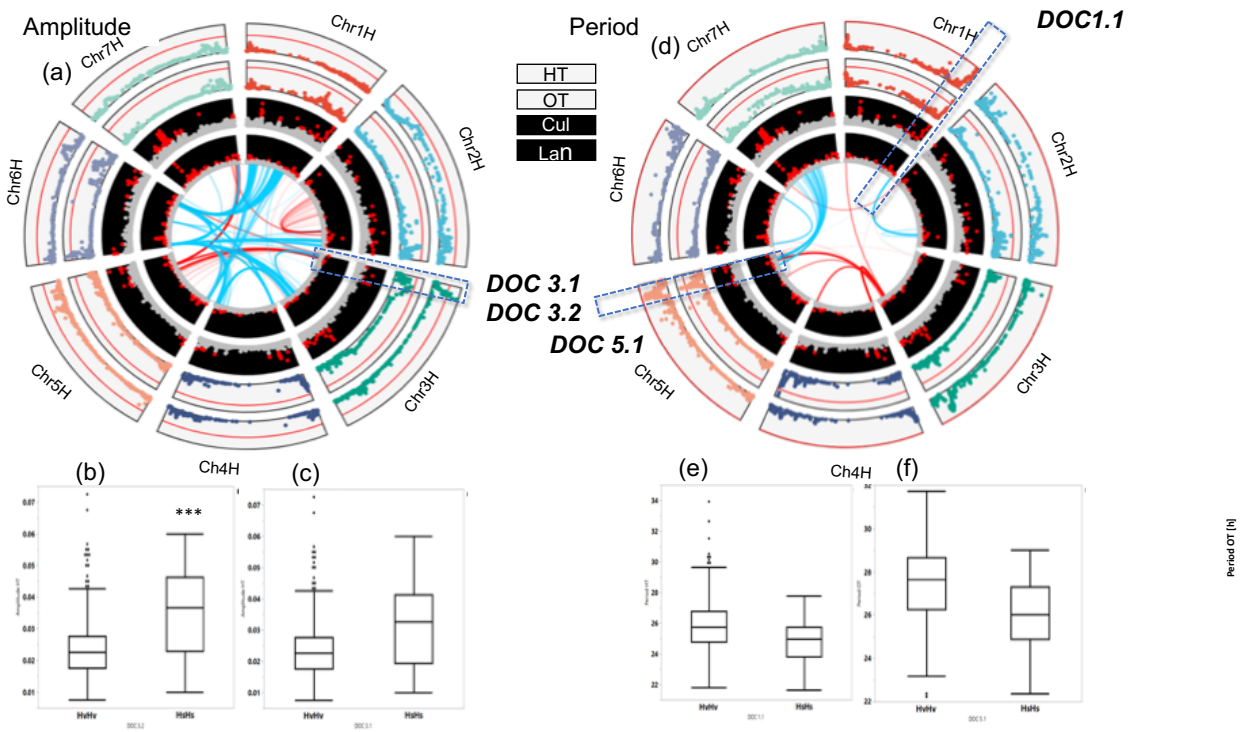


Fig. 8 Genome scan identifies drivers of the clock (DOC) loci. Circos plots depicting the genome-wide association study (GWAS) results in the HEB-25 multiparent population for (**a**) period and (**d**) amplitude and their plasticity. Barley chromosomes in the plot are depicted in different colors. The outermost layer and the one in from that represent Manhattan plots with $-\log_{10}(p)$ of 1D GWAS for high temperature (HT; 32 °C) and optimal temperature (OT; 22 °C), respectively. Blue and red lines within the center of the circles indicate significant digenic interactions detected by 2D two-locus GWAS under OT and HT, respectively ($P = 0.05$, established by 1,000 permutations). Box plot for DOC markers that show differences in the allele effect for wild and cultivated markers: (**b**) SCRI_RS_156009 of *DOC1.1*, (**c**) SCRI_RS_196175 of *DOC5.1*, (**e**) BOPA1_4844_1737 of *DOC3.1* and BOPA2_12_31475 of (**f**) *DOC3.2*. ***, $P < 0.001$.

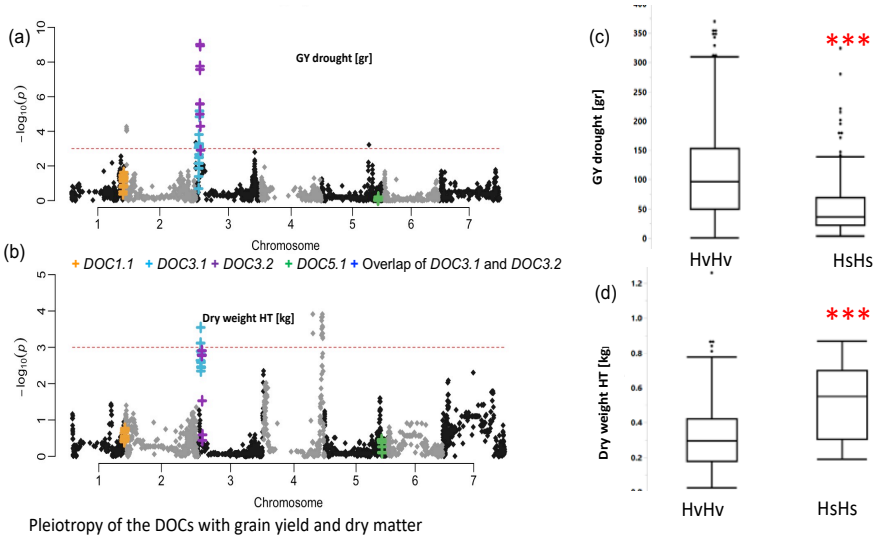


Fig. 9 Pleiotropy of the drivers of the clock (DOC) loci for plant growth and grain yield (GY) in field experiments at Gilat and Yotveta. (**a**) Manhattan plot with $-\log_{10}(p)$ of 1D genome-wide association study (GWAS) for GY under drought conditions in Gilat, and for (**b**) plant DW in Yotveta. The different DOCs are color-coded with crosses (*DOC1.1* – yellow, *DOC3.1* – blue, *DOC3.2* – violet and *DOC5.1* – green). Box plot for *DOC3.1* with the wild genotype (HsHs) showing (**c**) significant decreasing effect ($P < 0.0001$) on GY, and (**d**) significant increasing effect ($P < 0.0001$) on DW compared to carriers of the cultivated alleles (HvHv).

Genome scan with yield and growth phenotypes: In Gilat experiment, the wild allele at the *DOC3.1* and 3.2, which we linked with higher amplitude for the wild allele under HT (Fig. 8b,c), was associated with significant reduction of GY and GY per plant by 50% and 25% in drought and control environments (Fig. 9a,9c). The wild allele doubled total dry matter (TDW) and almost doubled vegetative dry weight (VDW) under high temperature in Yotveta (Fig. 9b,9d). In experiments conducted previously at Halle, this QTL reduced 1000-grain weight (TGW) and increased plant height (HEI) (Maurer et al., 2015). Apart from *DOC 3.1* and *3.2*, we also found association by GLM analysis of the wild allele of *DOC1.1*with shortening of the period under HT by the wild allele (Fig. 8e). Moreover, wild allele was found to be linked with 26% and 12% increased grain yield (GY) under drought and control (in Gilat), respectively (Fig 9a,9 b). The wild allele also increased GY per plant under the two treatments in Gilat. The same QTL is found for shortening plant development by 2-3 days in comparison between carriers of the wild vs the cultivated allele(Maurer et al., 2015; Herzig et al., 2018). In these studies theses effects on flowering time were associated to HvELF3(EARLY FLOWERING3), which is located nearby. Similarly, (Saade et al., 2016) showed that the wild allele in this QTL causes earlier flowering and maturity under both control and saline conditions and increases the harvest index.

To summarize, these genome scans for QTL carrying wild alleles that co-affect SensyPAM and field (yield) phenotypes show that we are able to identify at least three loci in

which the change of the clock is correlated significantly with the yield. This identification is done on very young seedling, at 3-4 leaf stage. Yet it correlates significantly with field performance, and the QTL identified will serve as a source to explore the mechanism underlying, as well as for pre-breeding. Interestingly, in one locus, DOC3.1, this relationship is with increase of the amplitude by the wild alleles that correlates with reduction of the grain yield (Fig. 8). In another genetic locus, DOC1.1, this pleiotropic QTL is affecting the period of the clock and the wild allele is significantly correlated with yield increase (Fig. 8). These two loci, together with the Heat3.1, are currently in the process of making nearly-isogenic lines that will serve for pre-breeding heat resilient lines (at least for HsHeat3.1 and DOC1.1 that has a significant positive effect).

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