דו״ח סופי לתכנית מחקר מספר **203-1170-20**

איתור זנים וטיפולים המאפשרים קבלת יבול שמן זית איכותי גבוה תחת תנאי חום

Identification of cultivars as well as agronomical treatments to increase olive oil yield and quality under high temperature conditions

מוגש לקרן המדען הראשי במשרד החקלאות

קוד זיהוי: 203-1170-20

שמות השותפים למחקר:

גיורא בן-ארי (giora@agri.gov.il) – ריכוז הפרויקט, תכנון ניסויים, עריכתם וניתוח התוצאות. עובד תקן.

רן חובב – אפיון ביטוי גנים במסלול סינתזת השמן. עובד תקן.

חניתה צמח – אפיון היסטולוגי. עובדת זמנית.

זוהר כרם – אפיון הרכב שמן. עובד תקן.

איריס ביטון – עריכת ניסויים וניתוח נתונים. עובדת תקן.

יאיר מני – עריכת ניסויים. עובד תקן.

הצהרת החוקר הראשי:

הממצאים בדו״ח זה הינם תוצאות ניסויים.

הניסויים מהווים המלצות לחקלאים: כן

חתימת החוקר: **תאריך: 31.12.2020**

תקציר

הצגת הבעיה – הזית התרבותי (*Olea europaea* L.) ידוע כגידול רב שנתי עמיד לעקות אביוטיות וככזה הוא נטוע גם באזורים שוליים שבהם מספר האפשרויות לגידול, בייחוד רב שנתי, נמוך. לפיכך, במקרים רבים, נאלץ הזית להתמודד עם עקות כגון מים מליחים או תנאי יובש וכן טמפרטורות גבוהות. על מנת למקסם את יבול שמן הזית באזורים המגיעים לטמפרטורות גבוהות בקיץ – מועד התפתחות הפרי וצבירת השמן, יש לאתר זנים מתאימים ו/או לפתח פרקטיקה אגרוטכנית שתסייע לעץ להתגבר על סטרס החום. כיום קיים פער בידע על צוואר הבקבוק הגורם לפחיתה ביבול ולפגיעה באיכות השמן, בעקבות טמפרטורות גבוהות בזמן התפתחות הפרי וצבירת השמן.

מטרת המחקר – לאתר זנים ולהגדיר טיפולים אגרוטכניים שיאפשרו קבלת יבול שמן איכותי גבוה גם בתנאי חום. לצורך כך הוגדרו מטרות המחקר המשניות הבאות: א. איתור צוואר הבקבוק הגורם ליבול שמן נמוך בתנאי חום. ב. איתור צוואר הבקבוק הגורם לאיכות שמן נמוכה בתנאי חום. ג. איתור זנים הנותנים יבול שמן גבוה ואיכותי גם בתנאי חום. ד. פיתוח פרקטיקה חקלאית שתאפשר קבלת יבול שמן גבוה ואיכותי בתנאי חום בזנים רגישים.

שיטות העבודה – הניסוי המבוקר כולל עציצי זיתים מניבים מחמישה זנים שהוצבו בטירת צבי (תנאי חום גבוה מאוד) ובצובה (אקלים ממוזג). נערך אפיון חודשי של התפתחות פרי וסינתזת השמן וכן אפיון טרנסקריפטום של שלבי התפתחות שונים. בסוף העונה נבחן הרכב השמן. על מנת לבחון את התקופה הקריטית להשפעת תנאי החום על הפחיתה ביבול ובאיכות השמן, נבחנו תקופות שונות במהלך העונה שבהם עצי הזית שהו בטירת צבי והושוו ההשפעות של כל מועד על יבול ואיכות השמן לאחר מסיק.

תוצאות עיקריות –נראה כי זן הברנע מתאים לגידול בתנאי החום של עמק בית שאן מתוך הזנים שנבחנו. זן זה מצליח גם בתנאי חום לייצר פרי באותו גודל ושמן באותה כמות כמו באזורים ממוזגים. הזנים פישולין וקורטינה מצליחים לייצר שמן באותם אחוזים בתנאי קיץ חם כמו בתנאים ממוזגים אבל גודל הפרי שלהם קטן באזור של קיץ חם ביחס לאזור של קיץ ממוזג. הקורונייקי והסורי מייצרים בתנאי חום פירות קטנים יותר המכילים פחות שמן ביחס לגידול בתנאי קיץ ממוזגים. איכות השמן נפגמה בתנאי קיץ חם בכל הזנים. יחד עם זאת נראה כי הסורי הינו הזן בעל העמידות הגבוהה ביותר לקיץ חם מבחינת איכות שמן ובזן זה קיבלנו שמן איכותי גם לאחר גידול בקיץ חם. התפתחות הפרי נפגעת כתוצאה משהות באזור עם טמפרטורות גבוהות במהלך כל העונה. לעומת זאת, אחוזי השמן ואיכות השמן נפגעים רק עקב טמפרטורות גבוהות בחודשים יולי ואוגוסט. ניתן לכוון את הפעולות האגרוטכניות למועד זה לשם פגיעה מינימאלית ביבול ואיכות השמן.

מסקנות והמלצות – מהזנים הנבחנים נראה כי הברנע מתאים לאזורים עם קיץ חם. יש לנסות לקרר את העצים בימים חמים במהלך יולי אוגוסט. יתכן שהשקיה בשעות החום בחודשים אלו תגרום לפחיתה בנזק. יש לבצע סריקה רחבה יותר שתכלול מגוון גדול יותר של זנים.

מבוא ותאור הבעיה

הזית התרבותי (*Olea europaea* L.) ידוע כגידול רב שנתי עמיד לעקות אביוטיות וככזה הוא נטוע גם באזורים שוליים שבהם מספר האפשרויות לגידול, בייחוד רב שנתי, נמוך. לפיכך, במקרים רבים, נאלץ הזית להתמודד עם עקות כגון מים מליחים או תנאי יובש וכן טמפרטורות גבוהות. כיום קיים פער בידע על צוואר הבקבוק הגורם לפחיתה ביבול ובאיכות בעקבות טמפרטורות גבוהות בזמן התפתחות הפרי וצבירת השמן. לא ידוע באיזה שלב של סינתזת השמן ישנה חשיבות לטמפרטורה ומתי בדיוק נוצר העיכוב בסינתזה והפגיעה באיכות. במדינת ישראל גדלים כיום זיתים בהיקף גידול של 330,000 דונם, יותר מכל מין פרי אחר. גידול הזית, בן השאר, מבוסס על שטחים שוליים בדרום ובעמק בית שאן, בהם טמפרטורות הקיץ נוסקות לגבהים בעייתיים. הרכב חומצות השומן בשמן הינו גורם קריטי בהיבטים רבים של איכות השמן ומחירו. הרכב חומצות השומן קובע את דרגתו והאוטנטיות של השמן כשמן זית, משפיע על הטעם, על חיי המדף ואפילו על תהליך ההפקה בבית הבד. כל אלו הם גורמים שבסיכום מתבטאים בתמורה שגובה המגדל על השמן, ומנגד זוהי בדיקה שמתבצעת תדיר בישראל על ידי כל הגורמים, הקניינים משרד הבריאות ומועצת הצמחים. בניגוד לזרעי חמנייה ששם התקבלה קורלציה חיובית בין הטמפרטורה לאחוז החומצה האוליאית, בזית שגדל בטמפרטורות גבוהות נמצא יחס הפוך בין הטמפרטורה לאחוז החומצה האוליאית עד לירידה מתחת ערך הסף של 55% בזנים עיקריים. הרכב השמן נמדד באחוזים ומכאן ברור שירידה בערך האוליאית גוררת מיד עליה באחרות: נמצא אכן שאחוז החומצות הפלמיטית, הלינוליאית (אומגה 6) והלינולינית (אומגה 3), שלהן ערך סף עליון בשמן זית, עולות עם העליה בטמפרטורה. שיחות עם מגדלים מאזור עמק בית שאן ועם חוקרי זיתים שעוסקים בתחום שנים רבות העלה טענה ברורה מצד כולם שיבול השמן שלהם נמוך משמעותית מיבול השמן אליו מגיעים חקלאים באזורים ממוזגים יותר. טענה זו נבדקה ומגובה בנתוני יבול של השנים האחרונות שנלקחו מבתי בד שונים.

מטרת המחקר

מטרת המחקר הנוכחי היא לאתר זנים ולהגדיר טיפולים אגרוטכניים שיאפשרו קבלת יבול שמן גבוה גם בתנאי חום.

לצורך כך הוגדרו מטרות המחקר המשניות הבאות:

א. איתור צוואר הבקבוק הגורם ליבול שמן נמוך בתנאי חום.

ב. איתור צוואר הבקבוק הגורם ליבול שמן שאינו איכותי בתנאי חום.

ג. איתור זנים הנותנים יבול שמן גבוה ואיכותי גם בתנאי חום.

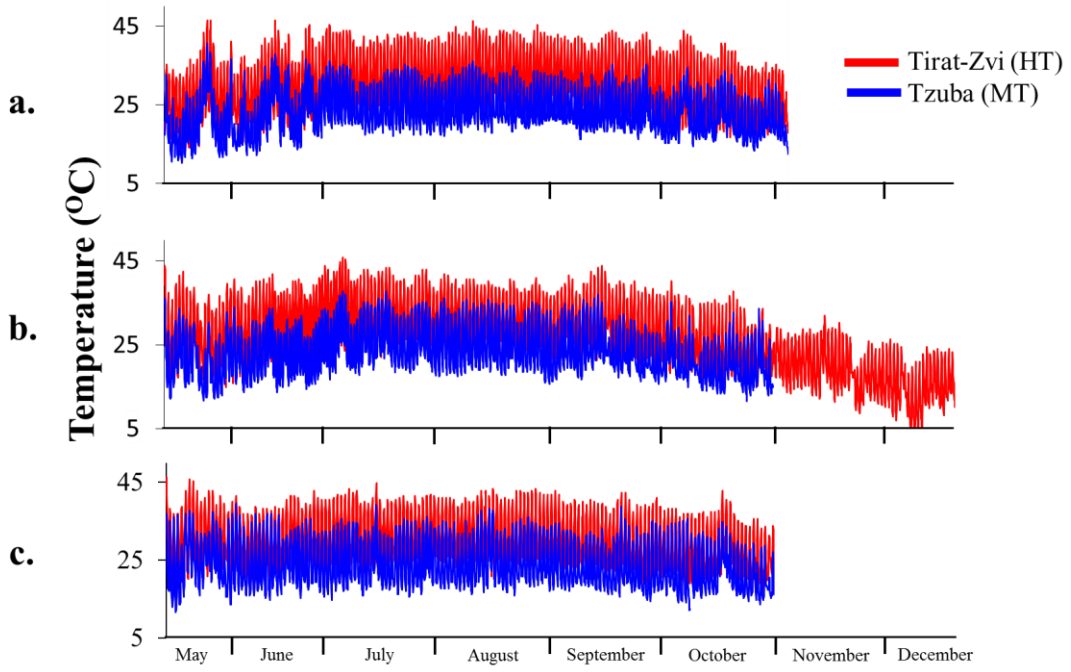
ד. פיתוח פרקטיקה חקלאית שתאפשר קבלת יבול שמן גבוה ואיכותי בתנאי חום בזנים רגישים.

<u>תוצאות</u>

בכל שלוש שנות הניסוי, עד לאחר חנטה, עציצי הזית נשמרו בבית דגן. לאחר חנטה, במאי, פוזרו העציצים (המכילים עץ זית מניב בן 7-5 שנים) לצובה וטירת צבי (איור 1). שם גדלו העצים במהלך כל העונה. כל העצים קיבלו 2 כפות של גרגירי אוסמוקוט בסוף החורף. בשנתיים הראשונות של המחקר, העציצים הכילו 5 עצים לכל זן מחמשת הזנים הבאים: ברנע, קורונייקי, קורטינה, פישולין וסורי בכל אתר (טירת צבי וצובה). מערכת ההשקיה שהורכבה עבדה במהלך כל העונה כל פעם 25 דקות למשך 3 פעמים ביום בצובה וארבע פעמים ביום בטירת צבי. כל עציץ קיבל 4 טפטפות של 2 ליטר לשעה. כך שכל זית קיבל השקיה של 10 ליטר ליום בצובה ו- 13.3 ליטר בטירת צבי. בחודשים יולי אוגוסט, תוגברה ההשקיה ברבע שעה נוספת ליום בשני האתרים (צובה וטירת צבי).

דגימות פרי נלקחו במהלך כל עונה עד המסיק. כל זן שהגיע לאינדקס הבשלה 3 (זיתים שחיצונית הם מעל 50% השחרה ופנימית לא התחילו להשחיר) סיים את הניסוי וכל הפירות נמסקו ושימשו להפקת שמן במערכת Abencor בבית דגן לשם אפיון הרכב ואיכות השמן.

הטמפרטורה בצל נמדדה בשני אתרי הניסוי לאורך כל העונה (אחת לשעתיים, איור 1). ניתן לראות שבכל שלושת שנות הניסוי, הטמפרטורה בטירת צבי ואילו בלילה הטמפרטורה היתה גבוהה משמעותית ביחס לצובה.



איור 1: הטמפרטורה בצל לאורך כל העונה בטירת צבי (אדום) ובצובה (כחול) בשנה הראשונה (a), בשנה השניה (b) ובשנת הניסוי השלישית (c). בשנה השניה הניסוי המשיך בטירת צבי יותר זמן מבצובה בגלל הבשלה מאוחרת.

בטבלה 1 ניתן לראות את הממוצעים החודשיים של הטמפרטורות הכי גבוהות בכל יום, את הטמפרטורות הכי גבוהות בכל חודש ואת הממוצעים החודשיים של הטמפרטורות הכי נמוכות

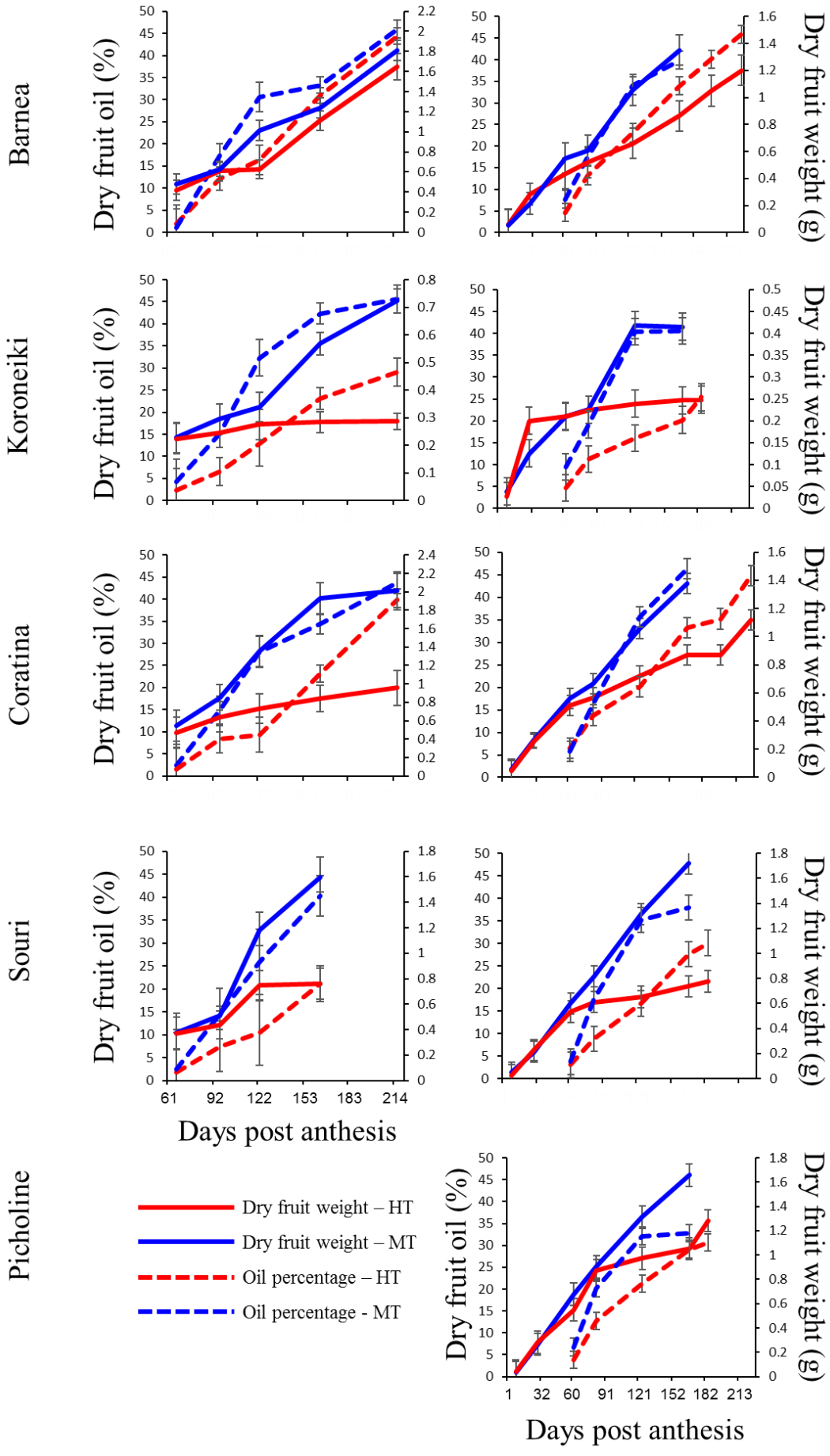
בכל יום. ההבדל ברב החודשים בין טירת צבי לצובה הוא כעשר מעלות ביום וכחמש מעלות בלילה.

Month	Mean (Max (Tzuba (°C)))	Mean (Max (Tirat Zvi (°C)))	Max (Max (Tzuba (°C)))	Max (Max (Tirat Zvi (°C)))	Mean (Min (Tzuba (°C)))	Mean (Min (Tirat Zvi (°C)))
1st year						
May	27.7	36.6	40.6	46.4	14.1	17.8
June	32.8	41.8	37.8	46.4	18.4	21.7
July	32.8	42.1	35.2	44.8	18.5	24.4
August	32.6	42.9	36.1	46.3	20.0	25.2
September	30.8	40.7	35.2	43.9	17.8	21.9
October	29.4	37.2	34.4	43.9	16.8	18.4
2nd year						
May	28.8	37.6	36.1	43.9	14.8	18.0
June	30.2	39.3	33.6	43.9	16.6	20.9
July	34.1	41.6	37.8	45.8	20.1	25.1
August	33.8	39.1	36.1	41.9	18.6	24.5
September	31.4	39.2	36.9	43.9	18.5	22.7
October	27.3	33.7	35.2	40.1	15.5	17.8
November		26.2		31.9		13.4
December		22.7		26.3		8.9
3rd year						
May	35.7	41.4	37.8	46.3	16.3	20.1
June	34.4	39.5	39.6	42.9	17.3	21.5
July	33.8	41.1	39.2	44.8	17.7	23.4
August	34.2	41.5	38.3	43.4	18.3	24.4
September	32.5	39.7	38.7	42.9	17.3	22.4
October	30.7	36.4	35.7	42.9	16.1	20.5

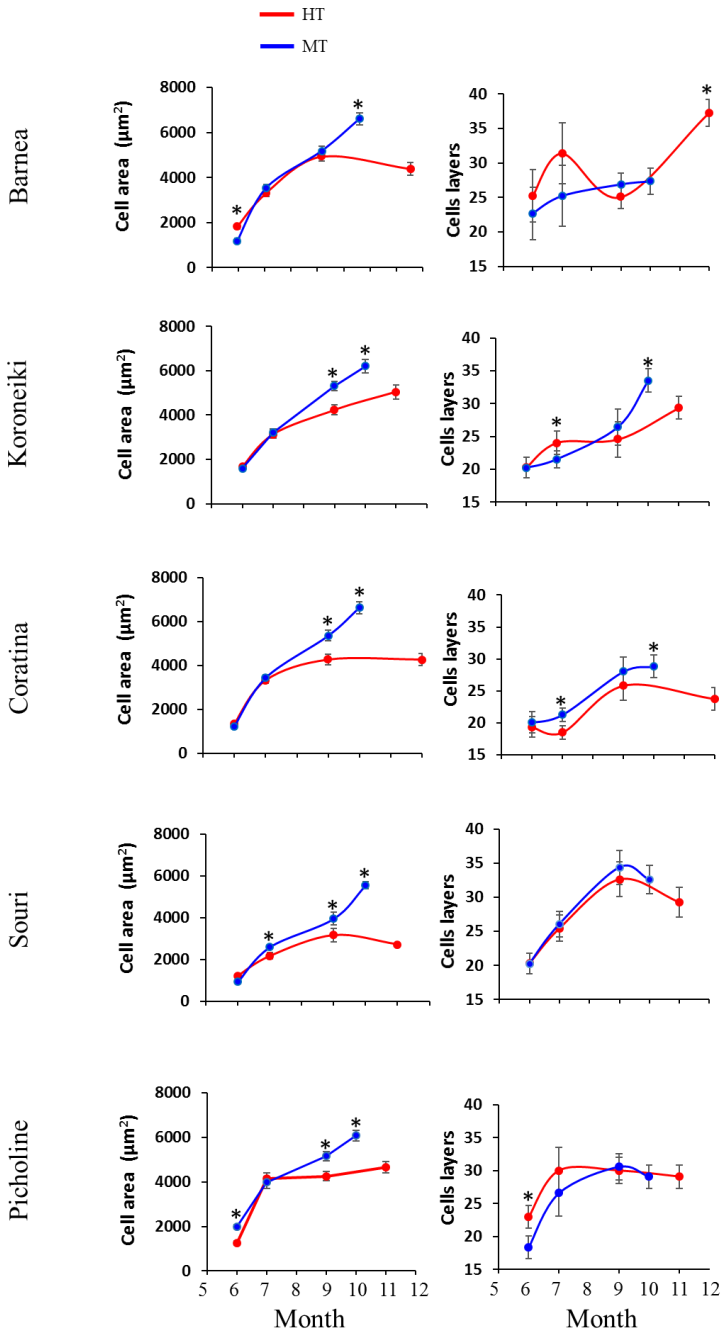
טבלה 1: הממוצעים החודשיים של הטמפרטורות הכי גבוהות בכל יום ((Mean (Max), הטמפרטורות הכי גבוהות בכל חודש ((Max(Max) והממוצעים החודשיים של הטמפרטורות הכי נמוכות בכל יום ((Mean (Min).

בשנתיים הראשונות של המחקר רצינו לבחון את השפעת טמפרטורות קיציות גבוהות על התפתחות הפרי, צבירת השמן ואיכותו. תמונת התפתחות הפרי וצבירת השמן בשנתיים הראשונות לניסוי מראה שהזנים השונים שנבחנו מתנהגים שונה זה מזה והשפעת טמפרטורות קיץ גבוהות משפיעות באופן שונה על כל זן וזן. נראה כי זן הברנע עמיד יחסית לטמפרטורות קיץ גבוהות ובמשך השנתיים של הניסוי הראה משקל פרי סופי ואחוז שמן סופי שהיו דומים בטירת צבי ובצובה (על אף שתוך כדי העונה היו הבדלים בין שני אתרי הניסוי ובצובה היו פירות יותר גדולים ואחוזי שמן גבוהים יותר במהלך הקיץ). הפישולין והקורטינה מראים שטמפרטורות הקיץ החמות לא משפיעות על אחוזי השמן הסופיים אך כן משפיעות על גודל הפרי. לכן ניתן לראות שאחוזי השמן הסופיים בשני הזנים דומים בטירת צבי ובצובה, אך הפירות שעברו את הקיץ בטירת צבי קטנים יותר מאלו שעברו אותו בצובה. הקורונייקי והסורי רגישים לטמפרטורות קיץ גבוהות ובהם ניתן לראות שמשקל הפרי הסופי ואחוזי השמן בפירות שעברו את הקיץ בטירת צבי נמוכים יותר באופן מובהק בהשוואה לפירות שעברו את הקיץ בצובה (איור 2).

איור 2: משקל פרי יבש (ציר ה- Y הימני) ואחוז שמן מחומר יבש (ציר ה- Y השמאלי) במהלך העונה. ציר ה- X הינו ימים מפריחה. משקל הפרי בקו רציף ואחוז השמן בקו מקווקו. העצים שבילו את הקיץ בטירת צבי מיוצגים על ידי קווים בצבע אדום (HT=High Temp.) והעצים של צובה על ידי קווים כחולים (MT=Mild Temp.). תוצאות שנת הניסוי הראשונה בטור השמאלי והשניה בטור הימני. שם הזן רשום משמאל.


Barnea
Koroneiki
Coratina
Souri
Picholine
Dry fruit oil (%)
Dry fruit weight (g)
Days post anthesis
Dry fruit weight – HT
Dry fruit weight – MT
Oil percentage – HT
Oil percentage - MT

בנוסף, בעזרת אנאליזות הסטולוגיות, מדדנו גם גודל תא ומספר שכבות תאים במהלך התפתחות הפרי, כדי להבין את מועדי חלוקת התאים וגדילת התאים (איור 3).



איור 3: גדילת תאים ומספר שכבות תאים (כמדד לחלוקת תאים) אשר נמדדו בעונת הניסוי השניה בכל הזנים בפירות שהתפתחו בצובה (קוים כחולים) או בטירת צבי (קוים אדומים). כוכבית מסמנת הבדל מובהק בין טירת צבי לצובה ($P<0.05$).

על פי הספרות במיני פרי אחרים שהתפתחותם דומה להתפתחות פרי הזית ונראית כסיגמואיד כפול, נמצא שתחילת התפתחות הפרי מתרחשת כתוצאה מחלוקת תאים בעיקר והמשך התפתחות הפרי נובע בעיקר מגדילת התאים ללא חלוקתם. ממצאינו מראים שבזית בכל הזנים ובשני אתרי הניסוי תחילת התפתחות הפרי נבעה מחלוקת תאים ברב המקרים אך בכל המקרים גם מגדילת התאים באופן משמעותי. בנוסף, ניתן לראות בבירור שבברנע שגדל בטירת צבי היתה חלוקת תאים מאוחרת שכנראה איפשרה לו להשוות את משקל הפרי לפירות ברנע שגדלו בצובה.

לשם בחינת הגורם המשפיע על תכונות הפרי המושפעות מהקיץ החם בטירת צבי, ערכנו אנאליזה שבה החנו את הקורלציות בין מדדי הטמפרטורה השונים לבין ההבדלים בתכונות השונות בין טירת צבי לצובה (טבלה 2). כשכללנו את כל הזנים באנאליזה קיבלנו קורלציה שלילית מובהקת בין משקל פרי לכל שלושת המדדים. כאשר כללנו רק את הזנים הרגישים באנאליזה, קיבלנו קורלציה שלילית מובהקת של כל מדדי הטמפרטורה עם משקל פרי ושל הטמפרטורה המקסימאלית על צבירת שמן. בכל האנאליזות, הקורלציה המקסימלית היתה בין הטמפרטורה המקסימלית לתכונות הפרי המושפעות. לפיכך, ניתן להסיק שהגורם העיקרי המשפיע של השינוי בהתפתחות הפרי וצבירת השמן הוא הטמפרטורה המקסימאלית. זה מנוגד לממצאים שפורסמו לאחרונה [1] וקבעו שמשקל פרי מושפע בעיקר מעליה בטמפרטורה יומית ממוצעת ואחוז שמן מושפע בעיקר מעליה באמפליטודה בין יום ללילה. שני המדדים האלו נבדקו גם אצלנו ולא הראו קורלציה גבוהה ומובהקת. המחקר המוזכר השתמש בטמפרטורה של 35 מעלות כטמפרטורת המקסימום ולכן לדעתינו לא הגיע לטמפרטורות הגבוהות שלהם השפעה ברורה ומובהקת על התכונות הללו.

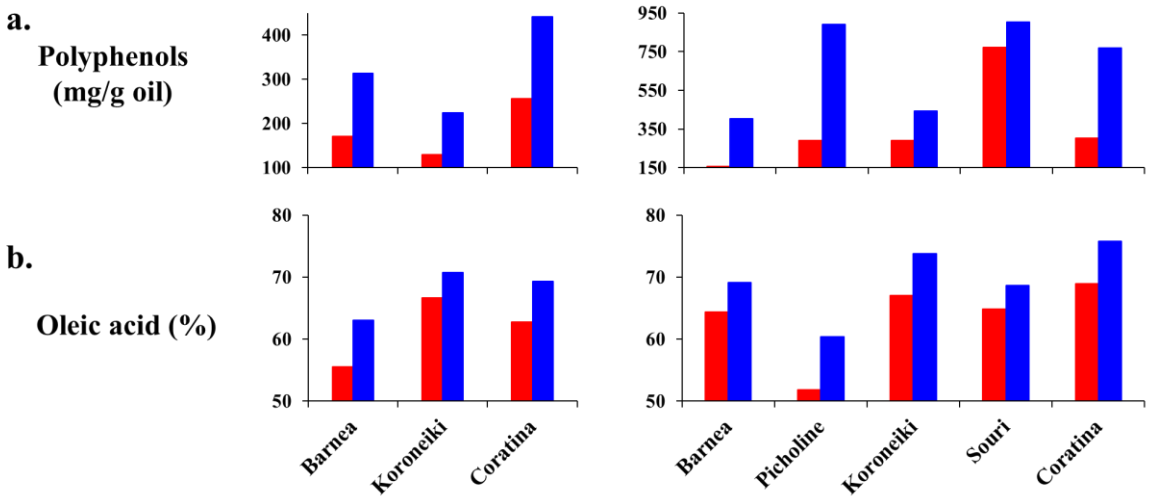
All cultivars

Variable	Variable	Correlation	P value
Dry fruit weight	Tmax	-0.346	0.0016
Dry fruit weight	Tmin	-0.2782	0.0119
Dry fruit weight	Tmean	-0.3366	0.0021
Dry fruit oil percentage	Tmax	-0.1827	0.1245
Dry fruit oil percentage	Tmin	-0.0897	0.4538
Dry fruit oil percentage	Tmean	-0.1222	0.3067
Only sensitive cultivars			
Variable	**Variable**	**Correlation**	**P value**
Dry fruit weight	Tmax	-0.4356	0.0004
Dry fruit weight	Tmin	-0.362	0.0038
Dry fruit weight	Tmean	-0.4195	0.0007
Dry fruit oil percentage	Tmax	-0.4015	0.0309
Dry fruit oil percentage	Tmin	-0.2414	0.2072
Dry fruit oil percentage	Tmean	-0.3218	0.0887

טבלה 2: מקדמי הקורלציה בין מדדי הטמפרטורה השונים שנלקחו לבין השינוי בתכונות משקל פרי יבש ואחוז שמן ממשקל פרי יבש.

גם איכות השמן היתה ירודה בזיתים שעברו את הקיץ בטירת צבי. נערכה אנליזה של ריכוז הפוליפנולים בשמן והרכב חומצות השומן בשמן (שנחשבים כמדדי איכות שמן זית). נמצא שכמות הפוליפנולים בשמנים בצובה גובהה משמעותית מהשמנים בטירת צבי. בנוסף, נמצא שבצובה השמן מכיל יותר חומצה אוליאית ופחות חומצה פלמיטית ולינוליאית (איור 3). ניתן לראות שהזן סורי מראה ריכוז פוליפנולים גבוה מאוד גם בטירת צבי וההבדל באחוז חומצה אוליאית בין פירות שעברו את הקיץ בטירת צבי לעומת כאלו שעברו את הקיץ בצובה, הינו הנמוך ביותר בין הזנים. בנוסף נמצא כי השמן שהופק מפירות פישולין שעברו את הקיץ בטירת צבי מכיל פחות מ-

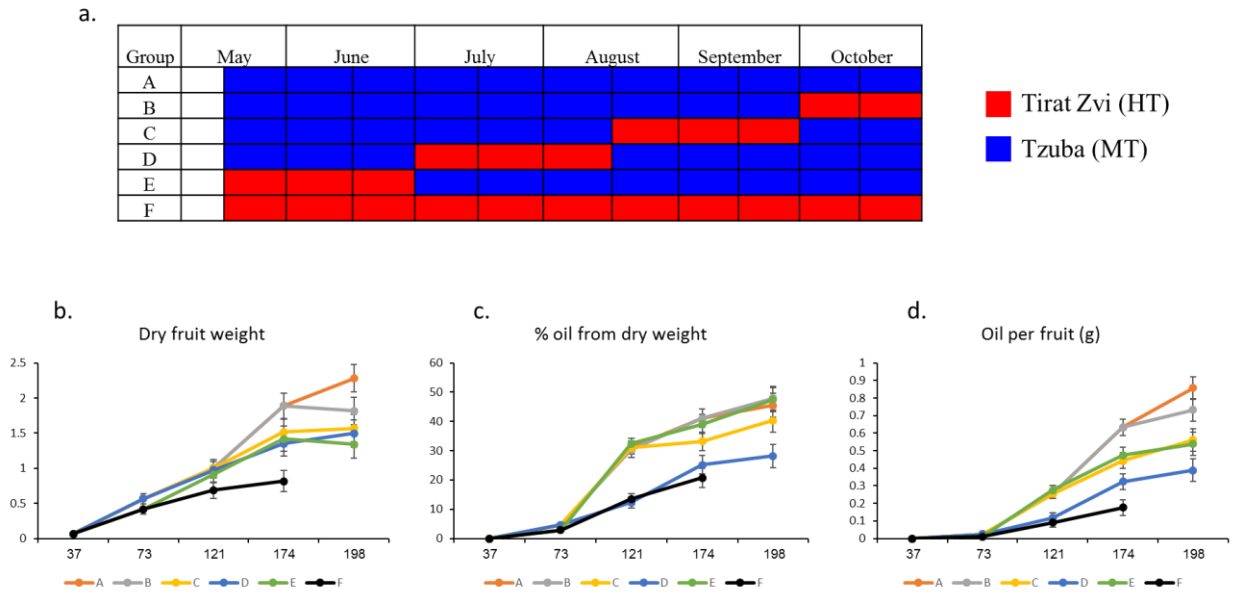
55% חומצה אוליאית שזה ערך הסף התחתון לשמן זית על פי ארגון השמן העולמי. כלומר שמן הפישולין שהופק מפירות שעברו את הקיץ בטירת צבי, לא יכול להיחשב לשמן זית.



איור 3: ריכוז פוליפנולים (גרפים עליונים) ואחוז חומצה אוליאית (גרפים תחתונים) בשמן זית מהזנים השונים שנבחנו בניסוי, שהופק מפירות שעברו את הקיץ בטירת צבי (עמודות אדומות) או בצובה (עמודות כחולות), בשנת הניסוי הראשונה (גרפים שמאליים) ובשניה (גרפים ימניים).

בשנת המחקר השלישית רצינו לבחון מהי התקופה הקריטית שבה טמפרטורה גבוהה משפיעה על התפתחות הפרי וצבירת ואיכות השמן. לשם כך נבחר הזן סורי שנצפה כרגיש לטמפרטורות קיץ גבוהות גם מבחינת התפתחות פרי וגם בצבירת שמן (אך פחות רגיש מבחינת איכות שמן). נבחרו 24 עצי סורי בני 7 בעציצים של 55 ליטר, אשר חולקו לשש קבוצות בנות ארבעה עצים כל אחת. קבוצה אחת שהתה כל תקופת התפתחות הפרי בטירת צבי, קבוצה אחרת שהתה כל תקופת התפתחות הפרי בצובה ושאר ארבעת הקבוצות שהו בטירת צבי לתקופה של כחודש וחצי וכל שאר התקופה שהו בצובה (איור a4). הפירות שגדלו על עצים ששהו במהלך כל העונה בטירת צבי, הבשילו מוקדם והגיעו לאינדקס הבשלה של 3 כבר בסוף ספטמבר ולפיכך נמסקו בשלב זה. פירות אלו נמצאו קטנים יותר ועם אחוזי שמן נמוכים יותר באופן מובהק מכל הקבוצות האחרות. נמצא שלגבי גודל פרי, כל הקבוצות ששהו בטירת צבי נפגעו מהטמפרטורה הגבוהה ופיתחו פרי קטן יותר באופן מובהק ביחס לפירות שהיו על עצים ששהו כל העונה בצובה. בעיקרון, ככל שהשהות בטירת צבי היתה בשלב מוקדם יותר בהתפתחות הפרי, כך הפגיעה בגודל הפרי. כלומר, גודל הפרי הקטן ביותר (מלבד הפירות שהיו בטירת צבי כל העונה) התקבל בפירות ששהו בטירת צבי מאמצע מאי ועד סוף יוני (איור b4). לעומת זאת, בצבירת השמן, נראה שרק פירות ששהו בטירת צבי מתחילת יולי עד אמצע אוגוסט נפגעו ולא הגיעו לאחוזי שמן מספקים. לעומתם, לא נמצא הבדל מובהק באחוזי השמן של פירות ששהו בכל התקופות האחרות בטירת צבי (איור c4). יבול השמן הינו תוצאה של גודל הפרי ואחוזי השמן. לפיכך, חישבנו את המדד של כמות שמן לפרי, הבא לייצג בקירוב את ערך יבול השמן. נמצא שמבין הקבוצות ששהו בטירת צבי לתקופה אחת מבין ארבעת התקופות, הקבוצה ששהתה בטירת צבי מתחילת יולי עד אמצע אוגוסט הראתה את משקל השמן לפרי הנמוך ביותר. לאחריה, הקבוצה ששהתה בטירת צבי בשלב מוקדם (אמצע מאי עד סוף יוני) והקבוצה ששהתה בטירת צבי מאמצע אוגוסט עד סוף ספטמבר. לבסוף, הפגיעה

המינימלית ביבול שמן לפרי נראתה בקבוצה ששהתה בטירת צבי במהלך חודש אוקטובר, אך גם בקבוצה זאת היה יבול נמוך באופן מובהק מפירות ששהו כל העונה בצובה (איור 4d).



איור 4: תיאור הניסוי בשנה שלישית (a) וזמן השהות בצובה ובטירת צבי של כל קבוצת עצים (A-F). תוצאות הניסוי מובאות בסעיפים הבאים: עקומת משקל הפירות הממוצע בכל אחת מהקבוצות, כאשר בציר ה Y מתואר משקל הפרי בגרם ובציר ה- X מתואר המועד כימים מפריחה (b). אחוז השמן מחומר יבש בכל אחת מהקבוצות כאשר בציר ה Y מתואר אחוז השמן ובציר ה- X מתואר המועד כימים מפריחה (c). יבול השמן לפרי בכל אחת מהקבוצות כאשר בציר ה Y מתואר משקל השמן בגרם ובציר ה- X מתואר המועד כימים מפריחה (d). קווי השגיאה בכל נקודה מתארים את רווח הסמך במבחן ANOVA ($p=0.05$) כך שכאשר אין חפיפה בין הקווים, קיים הבדל מובהק בין הנקודות. הקבוצות השונות מתוארות בקווים בצבעים שונים ע"פ המקרא.

איכות שמן בשנת המחקר השלישית:

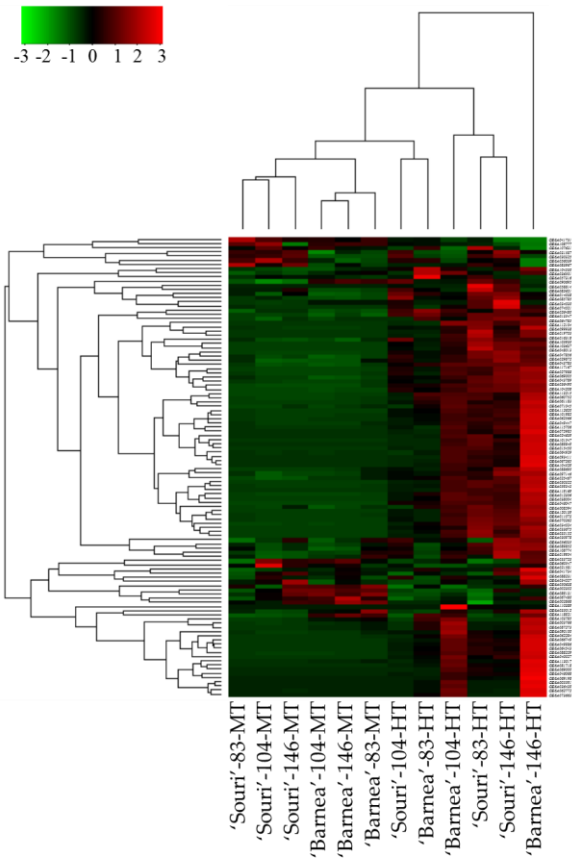
על מנת להבין את המועד הקריטי שבו ישנה השפעה של טמפרטורות גבוהות על איכות השמן אפיינו את הרכב חומצות השומן בקבוצות השונות, בהנחה שהטמפרטורות החמות משפיעות על ירידה באחוז החומצה האוליאית ביחס לחומצות השומן האחרות. כצפוי, מצאנו שהשמן שהופק מזיתי סורי ששהו במשך כל העונה בצובה הכיל את אחוז החומצה האוליאית הגבוה ביותר מבין כל הקבוצות (62.3). לעומתו השמן שהופק מזיתים ששהו במשך כל העונה בטירת צבי הכיל את אחוז החומצה האוליאית הנמוך ביותר מבין כל הקבוצות (56.78). בשאר הקבוצות היו ערכי ביניים, כאשר רב הקבוצות היו קרובות יותר לקבוצה ששהתה כל העונה בצובה, מבחינת תכולת חומצה אוליאית, מלבד קבוצה D שהראתה אחוז חומצה אוליאית דומה לשמן שהופק מקבוצת הזיתים ששהתה במהלך כל העונה בטירת צבי (טבלה 3). כלומר, נראה שהזמן שבו השפעת הטמפרטורות הגבוהות על איכות השמן הוא הקריטי ביותר הוא במהלך יולי ועד אמצע אוגוסט. יש לציין שערכי החומצה האוליאית בשנה זו היו נמוכים משמעותית מהערכים שהתקבלו בשנה השניה. אין לנו באמת הסבר לכך אך ניתן לומר שגם בזנים אחרים ובחלקות אחרות ראינו הבדל בין שנים.

	Palmitic	Palmitoleic	Margaric	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Eicosenoic	Docosanoic	Lignoceric	Squalene
A	13.98	0.60	0.14	3.14	62.30	15.01	2.71	0.59	0.49	0.12	0.00	0.92
B	14.33	0.67	0.15	3.27	61.58	14.74	2.83	0.65	0.53	0.14	0.00	1.11
C	15.59	0.69	0.18	3.24	60.02	14.96	2.78	0.70	0.56	0.15	0.09	1.04
D	15.41	0.99	0.16	3.95	57.67	15.81	3.29	0.93	0.62	0.16	0.10	0.90
E	14.77	0.79	0.14	3.71	60.17	14.97	3.02	0.65	0.53	0.13	0.00	1.12
F	15.65	1.53	0.22	4.21	56.78	15.24	3.30	1.21	0.70	0.19	0.13	0.82

טבלה 3: הרכב חומצות השומן (באחוזים) בשמנים שהופקו לאחר המסיק של סוף השנה השלישית מהקבוצות השונות.

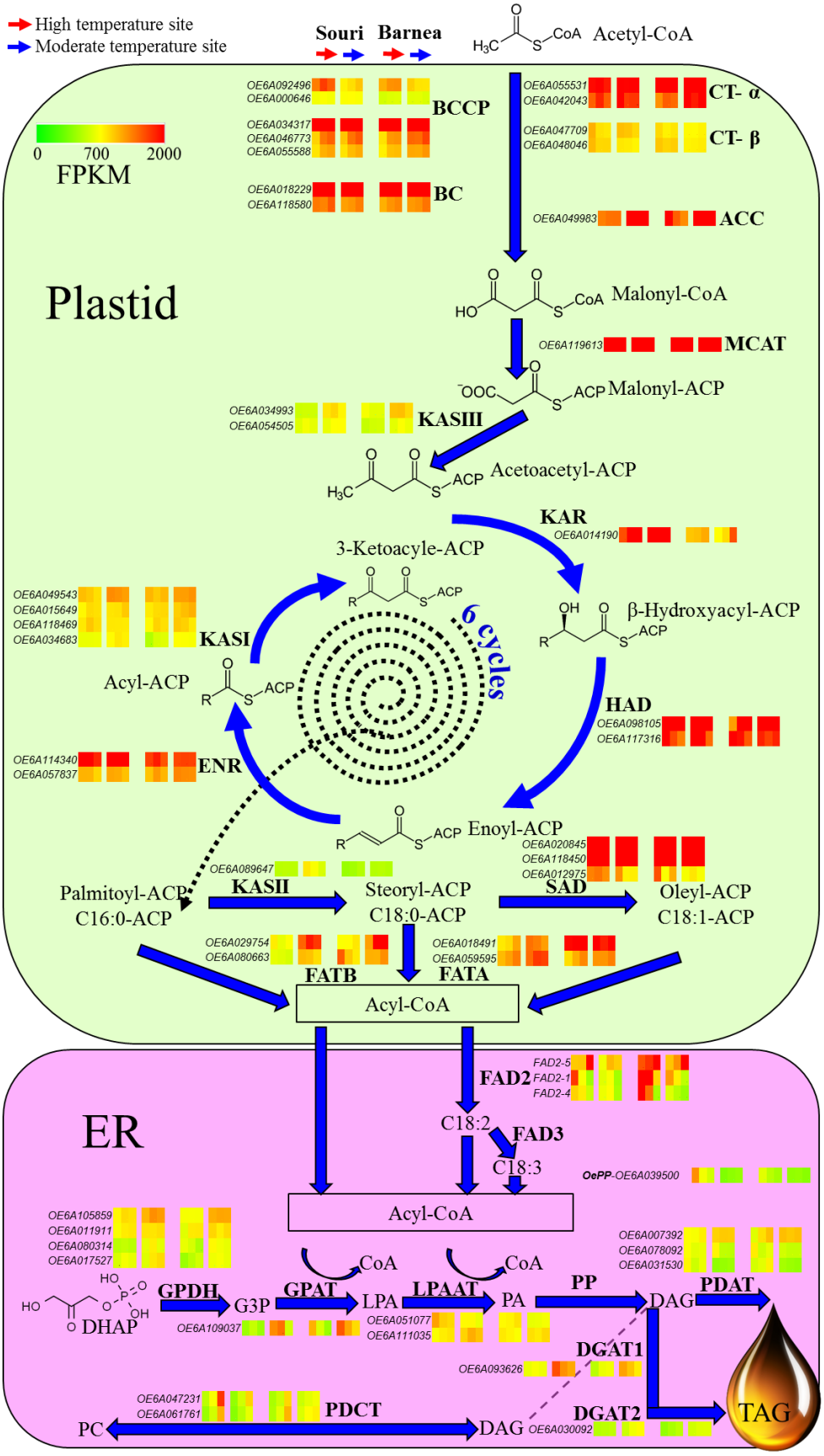
אנליזת טרנסקריפטום:

נבחרו 2 זנים, 'סורי' שהראה רגישות גדולה לחום בטירת צבי והזן 'ברנע' שהראה רגישות נמוכה והראה ביצועים דומים בטירת צבי ובצובה. נבחרו 3 מועדים, תחילת יולי, סוף יולי ותחילת ספטמבר (בהם מתרחשת צבירת השמן העיקרית). הופק רנ״א משתי חזרות ביולוגיות מכל מועד, מכל זן, מכל אתר (טירת צבי וצובה) ונשלח לריצוף עמוק במכון וויצמן. תחילה, על מנת לבחון האם העצים ששהו בטירת צבי אכן היו בסטרס חום, בחנו את תבנית הביטוי של הגנים השייכים למשפחת חלבוני סטרס החום (heat shock proteins) (איור 5). ניתן לראות שבעיקר בברנע בימים 146 (תחילת ספטמבר) ו- 104 (סוף יולי) ובסורי בימים 146 ו- 83 (תחילת יולי) הזיתים בהחלט היו בסטרס חום ורמת הביטוי של משפחת הגנים המעורבים בסטרס חום היתה גבוהה מאוד.



איור 5: Hierarchical clustering של תבנית ביטוי הגנים המעורבים בסטרס חום (Heat shock protein) בברנע וסורי במועדי הדגימה השונים (83,104 ו- 146 ימים מפריחה). כל טור מייצג דגימה וכל שורה מייצגת את אחד הגנים ממשפחת ה heat shock protein בזית. כל גן בכל דוגמא צבוע על פי רמת הביטוי שלו מגבוה (אדום) לנמוך (ירוק). ניתן לראות ששת הטורים השמאליים המייצגים את הפירות שהיו בצובה (Moderate Temperatures – MT) במהלך הקיץ מכילים בעיקר תאים ירוקים המצביעים על ביטוי נמוך של הגנים השייכים למשפחת Heat shock protein. לעומתם, במיוחד ארבעת הטורים הימניים המייצגים עצי ברנע וסורי ששהו בטירת צבי (High Temperatures – HT) במהלך הקיץ מכילים הרבה גנים שהתבטאו ביתר (אדומים). דבר שמצביע על כך שהעצים ששהו בטירת צבי היו סטרס חום.

בהמשך בחנו את תבנית הביטוי של הגנים המעורבים בביוסינתזה של שמן הזית (איור 6)



איור 6: תבנית הביטוי של הגנים המעורבים בביוסינתזה של שמן הזית מטירת צבי (HT) בהשוואה לצובה (MT). רמות הביטוי לכל גן מופיעות בסקאלה של ירוק לאדום מעל או בצד שמות האנזימים. לכל אנזים, רק גנים שרמת הביטוי שלהם היתה מעל 200 FPKM בלפחות מועד אחד ואתר אחד, נכנסו לאנליזה ומוצגים. לכל גן, ששת הריבועים משמעל מייצגים את הביטוי בזן הסורי וששת הריבועים מימין מייצגים את רמות הביטוי בזן הברנע. בתוך כל זן, שלושת הריבועים השמאליים מייצגים את רמת הביטוי ב 83, 104 ו- 146 ימים מפריחה בטירת צבי ושלושת הריבועים הימניים מייצגים את רמות הביטוי באותם מועדים בצובה. אברי התא השונים מופיעים בצבעים שונים. הביוסינתזה מתחילה בציטוזול (רקע לבן), ממשיכה בפלסטידה (רקע ירוק) ומסתיימת ברשתית האנדופלזמתית (רקע ורוד). הקיצורים בתמונה הם:

BCCP—biotin carboxyl carrier protein, *BC*—biotin carboxylase, *CT*—carboxyl transferase, *ACC*—acetyl-CoA carboxylase, *MCAT*— malonyl-CoA: ACP transacylase, *ACP*—acyl carrier protein, *KAS*—β-ketoacyl-ACP synthase, *KAR*— β-ketoacyl-ACP reductase, *HAD*—β-hydroxyacyl-ACP dehydrase, *ENR*—enoyl-ACP reductase, *SAD*— stearoyl-ACP desaturase, *FAT*—fatty acyl-ACP thioesterases, *FAD*—fatty acid desaturases, *GPDH*—glycerol 3-phosphate dehydrogenase, *GPAT*—glycerol 3-phosphate acyltransferase, *LPAAT*—lysophosphatidate acyltransferase, *PP*—phosphatidate phosphohydrolase, *PDCT*— phosphatidylcholine diacylglycerol cholinephosphotransferase, *DGAT*— diacylglycerol acyltransferase, *PDAT*—phospholipid; diacylglycerol acyltransferase, *DHAP*—dihydroxyacetone phosphate, *G3P*—glycerol 3-phosphate, *LPA*—lysophosphatidate, *PA*—phosphatidate, *DAG*—diacylglycerol, *PC*—phosphatidylcholine, *TAG*—triacylglycerol.

הגנים שנמצאו כרגישים לטמפרטורות גבוהות וביטויים ירד בטירת צבי לעומת צובה הם: *OeACC1*, *OeKASI, II, III*, *OeFATA*, *OeFATB*, *OeGPDH*, *OeGPAT*, *OeDGATs*, *OePDAT*.

שלושה גנים הראו רגישות דיפרנציאלית לחום בהתאם לרגישות הזנים ברנע וסורי לחום.

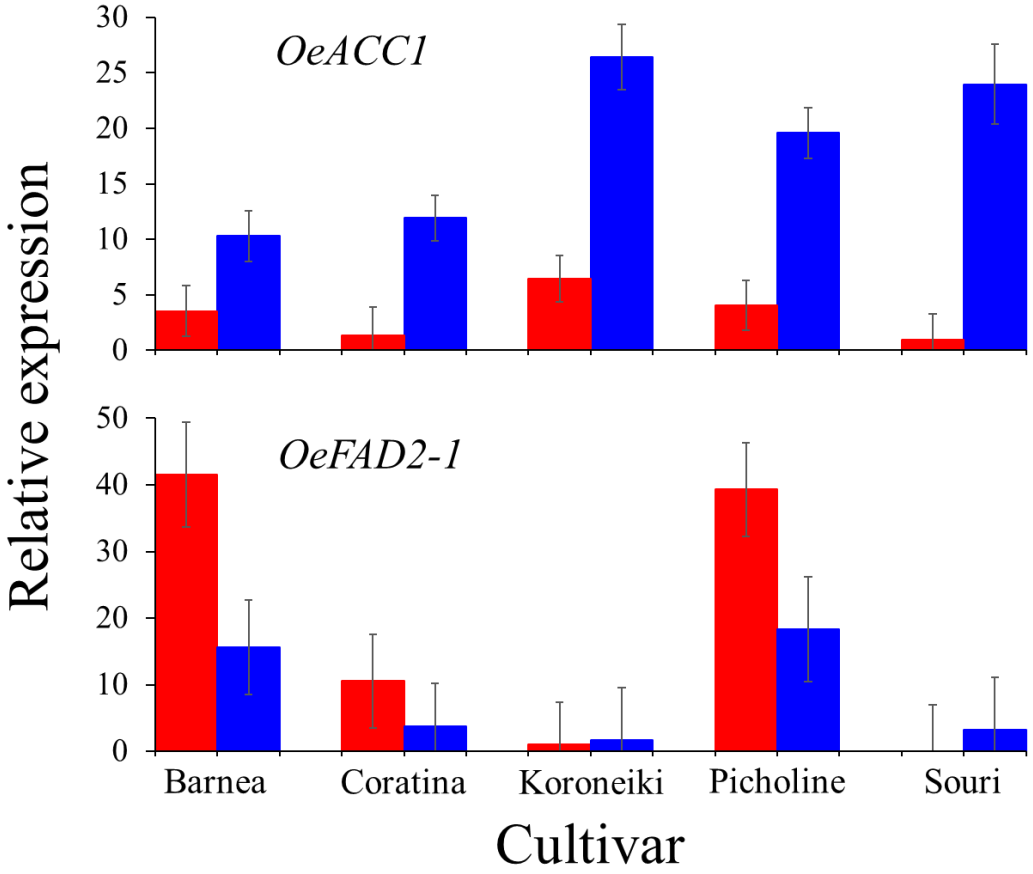
הגן *OeACC1* שהוא הגן העיקרי שפעיל בתחילת מסלול סינתזת השמן ומעודד סינתזת שמן, הראה הבדל בין רמת הביטוי בצובה לעומת רמת הביטוי בטירת צבי שהיה גבוה אצל הזן הרגיש יותר מבחינת סינתזת שמן, הסורי ונמוך יחסית אצל הזן העמיד יותר לחום, הברנע.

הגן *OePDCT* שנמצא בסוף מסלול סינתזת השמן ומעכב סינתזת שמן, הראה הבדל בין רמת הביטוי בטירת צבי לעומת רמת הביטוי בצובה שהיה נמוך אצל הזן הרגיש יותר מבחינת סינתזת שמן, הסורי וגבוה יחסית אצל הזן העמיד יותר לחום, הברנע.

הגן *OeFAD2* שמעביר חומצה אוליאית לחומצה לינולאית ומעודד ירידה בחומצה האוליאית וכך ירידה באיכות השמן, הראה הבדל בין רמת הביטוי בטירת צבי לעומת רמת הביטוי בצובה שהיה נמוך אצל הזן העמיד יותר מבחינת איכות שמן, הסורי וגבוה יחסית אצל הזן הרגיש יותר לחום, הברנע.

מכיוון ששלושת הגנים האלו נתפסו כקנדידטים להיות מרקרים לעמידות לחום, בחרנו את השניים העיקריים בהם *OeACC1* כמרקר לעמידות לחום בהיבט של סינתזת שמן ו- *OeFAD2* כמרקר לעמידות לחום בהיבט של איכות שמן. שני גנים אלו המשיכו לאנליזה מדוקדקת יותר

שבה נבחנה רמת הביטוי של הגנים בעזרת RT-PCR על חמשת הזנים הנבחנים במועד 146 יום מפריחה, בטירת צבי ובצובה (איור 7).



איור 7: רמות הביטוי של הגנים OeACC1 ו- OeFAD2-1 בציפת הפרי ביום 146 מפריחה בחמשת הזנים שהשתתפו במחקר הנוכחי, ברנע, קורטינה, קורונייקי, פישולין וסורי, בטירת צבי (עמודות אדומות) ובצובה (עמודות כחולות). עמודות השגיאה מייצגות רווח סמך ($p<0.05$).

נמצא שרמת הביטוי של OeACC1, שמתחיל את סינתזת השמן, בחודש ספטמבר תאמה בקירוב את רגישות הזנים לטמפרטורות קיץ גבוהות ביחס לסנתזת שמן, במיוחד בזני הקיצון, כאשר ההבדל ברמת הביטוי של הגן בין צובה לטירת צבי היה גדול ביותר בזנים סורי וקורונייקי שנמצאו כרגישים לחום ואילו הקטן ביותר בברנע שנמצא כעמיד לחום. תמונה דומה מתקבלת מתבנית הביטוי של הגן OeFAD2-1 שתפקידו להפוך חומצה אוליאית (חומצת שומן חד בלתי רוויה שמקנה לשמן הזית את מרכיב האיכות העיקרי שלו) לחומצה לינוליאית (חומצת שומן רב בלתי רוויה שמורידה את איכות השמן). גן זה מתבטא ביתר בטירת צבי לעומת צובה בעיקר בזנים הרגישים ביותר לטמפרטורות קיץ גבוהות בהקשר של איכות שמן, הברנע והפישולין. לעומת זאת, בזן סורי שנמצא כזן העמיד ביותר לטמפרטורות גבוהות מבחינת איכות שמן, הסן מתבטא ברמה נמוכה בצובה וברמה זניחה בטירת צבי.

דיון

על מנת להבין את השפעת החום על יבול שמן זית ומנגנוני התגוננות שפתחו זנים שונים, בניסוי זה נבחנת רגישות זנים שונים לחום בתקופת התפתחות הפרי וצבירת השמן. טמפרטורות הקיץ בטירת צבי, בעמק בית שאן מגיעות לטמפרטורות מאוד גבוהות וככל הנראה פוגמות ביבול השמן, לפחות בחלק מהזנים. הממצאים משתי העונות הראשונות של הניסוי מצביעים על זן הברנע כבעל עמידות יחסית לתנאי החום הסוררים בטירת צבי במהלך הקיץ וזן זה הראה התפתחות פרי וצבירת שמן יחסית דומות בין טירת צבי לצובה. זני הקורטינה והפישולין מראים עמידות לתנאי קיץ חם בתכונת צבירת השמן אך רגישים בהתפתחות פרי. הקורוניקי והסורי הראו רגישות יחסית ובהם התפתחות הפרי וצבירת השמן בטירת צבי מעוכבים ביחס לצובה שבה יש תנאי אקלים נוחים.

איכות שמן הזית נקבעת על פי ריכוז הפוליפנולים וריכוז חומצת השומן העיקרית החד בלתי רוויה, החומצה האוליאית. בזנים הנבחנים, שני מדדים אלו היו נמוכים באופן משמעותי בשמן שהופק מזיתים שגדלו בטירת צבי לעומת זיתים שגדלו בצובה. על פי ארגון שמן הזית העולמי, שמן הזית חייב להכיל לפחות 55% חומצה אוליאית. בטירת צבי, אחוז החומצה האוליאית בפישולין היה 52%, לכן על פי ארגון השמן העולמי, שמן זה פסול מלהיקרא שמן זית. זו בעיה אמיתית שיש לתת עליה את הדעת. שמני הזנים האחרים, למרות ירידה באיכות השמן בטירת צבי ביחס לצובה, גם בטירת צבי עברו את ערכי הסף ויכולים להיחשב כשמן זית. העמידויות של חמשת הזנים הנבחנים מסוכמת בטבלה 1.

High temperatures summer resistant			
Cultivar	Fruit weight	% oil	Oil quality
Barnea	✓	✓	X
Picholine	X	✓	X
Coratina	X	✓	X
Koroneiki	X	X	X
Souri	X	X	✓

טבלה 1: העמידויות שהראו הזנים השונים לטמפרטורות קיץ גבוהות בבחינה של התכונות הבאות: התפתחות פרי, צבירת שמן ואיכות שמן.

על מנת להבין מהו הפרמטר המשפיע ביותר על עיכוב התפתחות הפרי וצבירת השמן נערכו אנאליזות קורלציות עם המדדים השונים ונמצא כי טמפרטורת היום המקסימלית הינה הפרמטר הקריטי בהקשר של הפגיעה ביבול השמן. במחקר אחר [1] נמצא כי טמפרטורת היום הממוצעת

היא הפרמטר המשפיע ביותר על התפתחות פרי וההבדל בין טמפרטורת יום ללילה הוא הפרמטר המשפיע ביותר על צבירת שמן. פרמטרים אלו נבחנו גם אצלנו באנליזה ולא נצפו כמשפיעים ביותר. במחקר המוזכר טמפרטורות המקסימום היו נמוכות בהרבה מטמפרטורות המקסימום שהיו במחקר שלנו ולכן ניתן לומר שטמפרטורת המקסימום היא הפרמטר החשוב ביותר בקביעת עיכוב התפתחות הפרי וצבירת השמן.

עד כה היה ידוע שבזית, כמו בשאר הגלעיניים, עקומת התפתחות הפרי היא סגמואיד כפול, כאשר בעליה הראשונה, ההתפתחות היא בעיקר עקב חלוקת תאים ובעליה השניה, ההתפתחות נובעת מגדילת התאים [2]. במחקר אחר [3] החוקרים מצאו שברב זני הזית הנבחנים, בשלב השני התפתחות הפרי נבעה מגדילת תאים, אולם היו זנים בהם נצפתה גם חלוקת תאים בשלב מאוחר יחסית. במחקר הנוכחי, הכולל זנים שלא נבחנו במחקרים המוזכרים, ניתן לראות בבירור שבחלק מהזנים התפתחות הפרי המאוחרת נבעה מחלוקת תאים מוגברת (איור 3). זו יכולה להיות תופעה הקשורה לזן או תופעה הקושרה להשפעת הטמפרטורות הגבוהות בקיץ. התופעה בולטת במיוחד בברנע שהיה בטירת צבי. לפיכך, יכול להיות שאפשר לקשר את זה לעמידות לטמפרטורות גבוהות בהקשר של התפתחות פרי. במחקר הנוכחי רק ברנע נצפה כעמיד לטמפרטורות גבוהות מבחינת גודל פרי (איור 2).

באנליזה הנוכחית, ראינו ששלושה מתוך חמשת הזנים היו עמידים לטמפרטורות קיץ גבוהות בהקשר של אחוזי שמן (איור 2). ברנע, קורטינה ופישולין. מחקרים קודמים [4-7] לא הצביעו על הבדלים בין זנים. החידוש במחקר שלנו הוא שניתן למצוא זנים עמידים ולגדל אותם באזורים בעלי טמפרטורות קיץ גבוהות. טמפרטורות גבוהות משפיעות גם על איכות השמן. איכות השמן של כל זן נקבעת בעיקר על סמך אחוז החומצה החד בלתי רוויה בו, החומצה האוליאית – ככל שיש יותר חומצה אוליאית שטובה מאוד מבחינה בריאותית, כך עולה איכות השמן. פרמטר נוסף שמאפיין איכות שמן של כל זן וזן הוא רמת הפוליפנולים, אנטיאוקסידנט שנחשב כבריא מאוד לצריכה. שני הפרמטרים האלו נפגעו בקיץ עם טמפרטורות גבוהות כגון זה בטירת צבי, אולם רמת הפגיעה שונה בין הזנים. הזן הסורי הראה את הפגיעה המינימלית ומבין הזנים הנבחנים, מהווה את הזן העמיד ביותר לטמפרטורות גבוהות מבחינת איכות השמן. כאשר בחנו את המועד הקריטי לפגיעה (בשנה השלישית) מצאנו שהפגיעה של טמפרטורות גבוהות בהתפתחות הפרי מתרחשת במהלך כל עונת הגידול ושהות לזמן קצוב באזור עם טמפרטורות גבוהות פגע בגודל הפרי הסופי בכל המועדים שבהם העצים שהו בטירת צבי. מצד שני, פגיעה של טמפרטורות גבוהות באחוזי השמן ובאיכות השמן היתה בעיקר כאשר העצים שהו בטמפרטורות חמות מתחילת חודש יולי ועד אמצע חודש אוגוסט. כלומר, זהו המועד הקריטי (לאחר התקשות גלעין) שבו טמפרטורות חמות ישפיעו על צבירת השמן ואיכותו. כלומר, פרקטיקה חקלאית למניעת פגיעה, צריכה להתמקד בתקופה הזו.

לשם העמקה והבנת המנגנון של העיכוב, נערכה אנליזת טרנסקריפטום בזנים ברנע וסורי שמראים מנגנונים שונים ברגישותם לחום. בהתפתחות פרי וצבירת שמן הסורי רגיש והברנע עמיד ובאיכות שמן התמונה הפוכה. לגבי הרגישות לטמפרטורות גבוהות בהתפתחות פרי, לא הצלחנו לשים את האצבע על המנגנון הגורם לעיכוב ההתפתחות. לעומת זאת, לגבי הרגישות בצבירת שמן ואיכות, מצאנו את האנזימים המשפיעים על העיכוב. הגנים המקודדים לאנזימים המשתתפים בביוסינתזה של שמן הזית, שנצפו כרגישים לחום וביטויים נמוך יותר בטמפרטורות גבוהות היו

OeACC1, OeKASI, II, III, OeFATA, OeFATB, OeGPDH, OeGPAT, OeDGATs, OePDAT. ואילו הגן המקודד ל *OeFAD2-1* נמצא כגן המשפיע על ירידה באיכות השמן בטמפרטורות גבוהות (איור 6). איור 7 מדגים כי הגנים *OeACC1* ו- *OeFAD2-1* יכולים לשמש כמרקרים לבחינת זנים עמידים לטמפרטורות גבוהות מבחינת צבירת שמן ואיכות השמן בהתאמה.

מסקנות:

ניתן לראות כי תגובת הזית לתנאי טמפרטורות קיץ גבוהות מתחלקת לשלוש תגובות בלתי תלויות. טמפרטורות קיץ גבוהות גורמות לעיכוב בהתפתחות הפרי, עיכוב בצבירת השמן ופגיעה באיכות השמן. על פי אנליזת חמשת הזנים שהשתתפו במחקר זה, ניתן ללמוד שהתגובות אינן תלויות אחת בשניה. למדנו במחקר שיש זנים עמידים ויש זנים רגישים אך לא מצאנו, מבין חמשת הזנים הנבחנים, זן העמיד לטמפרטורות גבוהות בכל שלושת הפרמטרים. לשם כך יש להרחיב את הסריקה. איתרנו מרקרים שיוכלו לשמש לסריקה רחבה יותר אולם סריקה כזו מחייבת שהייה של הזנים הנסרקים בטמפרטורות קיץ גבוהות. מנגנון העמידות לתנאי טמפרטורות קיץ גבוהות: מהאנליזה שנערכה עד כה, ניתן לראות שבחודשים החמים של אמצע הקיץ, משקל הפרי ואחוזי השמן היו נמוכים בטירת צבי ביחס לצובה בכל הזנים. יחד עם זאת, לקראת סוף העונה, הזנים שהראו עמידות, עלו בצורה דרמטית במשקל הפרי ובצבירת השמן וכך השתוו למשקל פרי הסופי או לאחוזי השמן הסופיים של חבריהם שגדלו בצובה. נראה שהמנגנון שמאפשר משקל פרי סופי ואחוז סופי של שמן גבוהים גם באזורים של טמפרטורות קיץ גבוהות הוא היכולת של הזן להשהות את התפתחות הפרי וצבירת השמן במהלך החודשים החמים, אך לאחר מכן להשלים את החסר על ידי התפתחות פרי מאוחרת וצבירת שמן מסיבית בסוף העונה בחודשים שבהם הטמפרטורות מתמתנות גם באזורים כמו טירת צבי. יתכן שהיכולת הזו טמונה ביכולת להמשיך בחלוקת תאים גם בשלב התפתחותי מאוחר יחסית, כמו שראינו אצל הזן העמיד, הברנע. מצאנו שהמועד הקריטי לפגיעה בצבירת השמן ואיכותו הוא במהלך החודשים יולי אוגוסט.

מטרת המחקר בהצעת המחקר היתה:
לאתר זנים ולהגדיר טיפולים אגרוטכניים שיאפשרו קבלת יבול שמן גבוה גם בתנאי חום.
לצורך זה הוגדרו מטרות המשנה הבאות:

א) איתור צוואר הבקבוק הגורם ליבול שמן נמוך בתנאי חום.
ב) איתור צוואר הבקבוק הגורם ליבול שמן שאינו איכותי בתנאי חום.
ג) איתור זנים הנותנים יבול שמן גבוה ואיכותי גם בתנאי חום.
ד) פיתוח פרקטיקה חקלאית שתאפשר קבלת יבול שמן גבוה ואיכותי בתנאי חום בזנים רגישים.

בהתאמה מצאנו:

א. צוואר הבקבוק הגורם ליבול שמן נמוך בתנאי חום הינו מספר גנים, אך העיקרי בניהם הוא הגן *OeACC1* המתחיל את סינתזת השמן.
ב. הגורם העיקרי ליבול שמן שאינו איכותי בתנאי חום הוא הגן *OeFAD2-1*.

ג. הברנע נותן יבול שמן ראוי גם באזורים חמים. הסורי והקורונייקי הם הזנים הרגישים ביותר לחום מבין הזנים הנבחנים בהקשר של יבול שמן. הסורי נותן שמן איכותי גם בתנאי חום. לעומתו, כל ארבעת הזנים האחרים ובעיקר הברנע והפישולין, נותנים שמן בעל איכות נמוכה יחסית בתנאי חום. יש לבצע סריקת זנים רחבה יותר.

ד. מכיוון שהתקופה הקריטית היא במהלך יולי ואוגוסט, בזנים הרגישים, בתקופה זו, יש לצנן את העצים בימים חמים בשעות הצהריים, לדוגמא על ידי השקיה במהלך הצהריים. המלצה זו צריכה עדיין להיבדק בניסויי שדה.

יישום תוצאות המחקר, הבעיות שנותרו לפתרון והמשך המחקר

את תוצאות המחקר יש בהחלט ליישם כבר בנטיעות קרובות (בבחירת הזנים). על מנת לאתר זנים עמידים נוספים וכן לאשש את הפרקטיקה החקלאית המוצעת במחקר זה, יש לבצע מחקר המשך. לשם כך, לפני שנתיים ניטע אוסף של 100 זנים בעמק בית שאן וברמת הגולן לשם בחינת השפעת תנאי חום על מגוון זנים רחב. ניתן להשתמש בתשתית זו למחקר המשך.

פרסום הדו"ח: אני ממליץ לפרסם את הדו"ח ללא הגבלה.

מצורפים לדו"ח זה 2 מאמרים שהתפרסמו על מחקר זה:

1. Nissim Y, Shloberg M, Biton I, Many Y, Doron-Faigenboim A, Zemach H, Hovav R, Kerem Z, Avidan B, Ben-Ari G. (2020). High temperature environment reduces olive oil yield and quality. *PLoS One* 15(4):e0231956.

2. Nissim Y, Shloberg M, Biton I, Many Y, Doron-Faigenboim A, Hovav R, Kerem Z, Avidan B, Ben-Ari G. (2020). A High Temperature Environment Regulates the Olive Oil Biosynthesis Network. *Plants* 9, 1135; doi:10.3390.

References:

.1 García-Inza, G.P.; Hall, A.J.; Rousseaux, M.C. Proportion of oleic acid in olive oil as influenced by the dimensions of the daily temperature oscillation. *Scientia Horticulturae* **2018**, *227*, 305-312, doi:https://doi.org/10.1016/j.scienta.2017.09.030.

.2 Rallo, P.; Rapoport, H. Early growth and development of the olive fruit mesocarp. *The Journal of Horticultural Science and Biotechnology* **2001**, *76*, 408-412, doi:10.1080/14620316.2001.11511385.

.3 Hammami, S.B.M.; Manrique, T.; Rapoport, H.F. Cultivar-based fruit size in olive depends on different tissue and cellular processes throughout growth. *Scientia Horticulturae* **2011**, *130*, 445-451, doi:https://doi.org/10.1016/j.scienta.2011.07.018.

.4 García-Inza, G.P.; Castro, D.N.; Hall, A.J.; Rousseaux, M.C. Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid

composition in olive (Olea europaea L. var. ‘Arauco’). *European Journal of Agronomy* **2014**, *54*, 107-115, doi:https://doi.org/10.1016/j.eja.2013.12.005.

.5 Miserere, A.; Searles, P ;.García-Inza, G.P.; Rousseaux, M. Elevated temperature affects vegetative growth and fruit oil concentration in olive trees (Olea europaea). *Acta Horticulturae* **2018**, 10.17660/ActaHortic.2018.1199.83, 523-528, doi:10.17660/ActaHortic.2018.1199.83.

.6 Rondanini, D.P.; Castro, D.N.; Searles, P.S.; Rousseaux, M.C. Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. *European Journal of Agronomy* **2014** ,237-246 ,52 , doi:https://doi.org/10.1016/j.eja.2013.09.002.

.7 Trentacoste, E.R.; Puertas, C.M.; Sadras, V.O. Modelling the intraspecific variation in the dynamics of fruit growth, oil and water concentration in olive (Olea europaea L.). *European Journal of Agronomy* **2012**, *38*, 83-93, doi:https://doi.org/10.1016/j.eja.2012.01.001.

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RESEARCH ARTICLE

High temperature environment reduces olive oil yield and quality

Yael Nissim[1], Maya Shloberg[1,2], Iris Biton[1], Yair Many[1], Adi Doron-Faigenboim[1], Hanita Zemach[1], Ran Hovav[1], Zohar Kerem[2], Benjamin Avidan[1], Giora Ben-Ari[1]*

1 Institute of Plant Sciences, ARO, The Volcani Center, Rishon LeZion, Israel, **2** Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

* giora@agri.gov.il

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Abstract

Global warming is predicted to have a negative effect on plant growth due to the damaging effect of high temperatures. In order to address the effect of high temperature environments on olive oil yield and quality, we compared its effect on the fruit development of five olive cultivars placed in a region noted for its high summer temperatures, with trees of the same cultivars placed in a region of relatively mild summers. We found that the effects of a high temperature environment are genotype dependent and in general, high temperatures during fruit development affected three important traits: fruit weight, oil concentration and oil quality. None of the tested cultivars exhibited complete heat stress tolerance. Final dry fruit weight at harvest of the 'Barnea' cultivar was not affected by the high temperature environment, whereas the 'Koroneiki', 'Coratina', 'Souri' and 'Picholine' cultivars exhibited decreased dry fruit weight at harvest in response to higher temperatures by 0.2, 1, 0.4 and 0.2 g respectively. The pattern of final oil concentration was also cultivar dependent, 'Barnea', 'Coratina' and 'Picholine' not being affected by the high temperature environment, whereas the 'Koroneiki' and 'Souri' cultivars showed a decreased dry fruit oil concentration at harvest under the same conditions by 15 and 8% respectively. Regarding the quality of oil produced, the 'Souri' cultivar proved more tolerant to a high temperature environment than any other of the cultivars analyzed in this study. These results suggest that different olive cultivars have developed a variety of mechanisms in dealing with high temperatures. Elucidation of the mechanism of each of these responses may open the way to development of a variety of olives broadly adapted to conditions of high temperatures.

Introduction

Fluctuations in temperature occur naturally during plant growth and reproduction. However, extreme hot summers can damage the intermolecular interactions needed for proper growth, thus impairing plant development and fruit set. The increasing threat of climate change is already having a substantial impact on agricultural production [1]. High temperatures may cause visual symptoms of sunburn, leaf abscission and growth inhibition of plants [2, 3]. High

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temperature shocks during the reproductive phase of cereal crops can cause substantial reduction in yield. Oilseed crops are also negatively affected by heat stress, which has been shown to reduce starch, protein and oil content [4, 5]. Significant reduction of yield due to heat stress has been reported in peanut and tomato [6, 7] and due to high temperatures has been reported in wheat, rice and bean [8–10]. Plants exposed to temperatures above their optimal growing temperatures, exhibit cellular and metabolic responses which enable the plants to survive [11–15]. The level of damage to crops caused by high temperatures depends on the growth stage at the time of exposure, and the severity of the stress. The reproductive phase is more sensitive to high temperatures, causing a reduction in yield [16].

As in other drupes, olive fruit development is characterized by a double sigmoid growth curve for fruit size and weight [17, 18]. Rallo and Rapoport [19] examined the development of 'Manzanilla' olive fruit mesocarp. They found that as in other drupes, both cell division and expansion contribute to initial mesocarp growth. From six weeks post anthesis, mesocarp growth is determined solely by cell expansion. Hence, the early difference in fruit size between cultivars is mostly a result of the rate of cell division. Later fruit growth is a function of increased cell size [20].

Oil accumulation in olive fruits is known to begin in the second half of the summer. It lasts for about 8 weeks during summer and fall and then slows down during fruit ripening. Oil accumulation is strongly influenced by the cultivar type and the climactic conditions prevalent during fruit development. During ripening, the olive fruit undergoes various modifications [21–24].

The period of drupe development and maturation lasts 18–20 weeks after flowering (WAF). During this period, the drupe passed through four developmental stages. The first stage begins soon after fertilization, lasts for 2 weeks and is characterized by ovary growth through cell division. The second stage lasts up to 6 WAF, and is characterized by drupe growth by cell expansion. The third stage extends for 7–10 WAF. During this stage, the fruit continues to grow by cell expansion. Lipids are stored in oil bodies in the mesocarp cells. The first oil bodies (2–4 per cell, on average) appear at about seven WAF. As the fruit matures, oil bodies increase in number and begin to coalesce into one main oil droplet per cell, occupying $\sim$40% of the cell volume, and to several minor oil bodies. By the completion of pit hardening at about 10 WAF, the oil bodies have fused into a large oil droplet comprising $\sim$60% of the cell volume. The fourth stage at 10–20 WAF is characterized by continued oil accumulation and at about 16–17 WAF the color of the epicarp begin to change to purple due to intense anthocyanin deposition in the vacuole. At that time, the oil droplets occupy about 80% of the cell volume [25–28].

Virgin olive oil is characterized by its sensorial and nutritional properties, which are different from those of other plant oils. Its health benefits are due to both its fatty acid composition and minor compounds such as polyphenols, tocopherols, pigments and vitamins. Olive oil contains mainly monounsaturated fats. The main fatty acid is oleic acid (C18:1), which represents between 55 and 83% of the total oil. It also contains a moderate amount of linoleic acid (C18:2; 3.5–21%) and palmitic acid (C16:0; 7.5–20%) and a small amount of stearic acid (C18:0) and linolenic acid (C18:3). Fatty acid composition of olive oil is strongly affected by several agronomical factors such as cultivar type, fruit ripeness, crop yield, and growing medium [29]. Several studies have examined the effect of high temperatures over a long period of time on olive fruit weight [30, 31], olive oil accumulation [30–33] and olive oil fatty acid composition [30, 33–37]. Garcia-Inza et al. [30] studied the effect of temperature during the oil accumulation phase on these parameters, using transparent plastic chambers with individualized temperature control to manipulate temperatures. One experiment was carried out for four months with four treatments of different temperatures ranging from16.7 to 30.7˚C. Another experiment was carried out for four successive one month long treatment periods

with two treatments at differing temperatures. They found that fruit dry weight was not affected by average temperatures within a range of 16–25˚C, but was reduced with further increases in temperature. Oil concentration decreased linearly at 1.1%˚C^{-1} across the entire range (16–32˚C) of average seasonal temperatures explored, while oleic acid concentration decreased 0.7%˚C^{-1} over the same range. In the one month long experiment, an additional 7˚C above the control had a permanent negative effect on oil concentration at final harvest, particularly when the exposure to high temperature was at the beginning of oil accumulation. Oleic acid concentration was also negatively affected by the high temperature treatment. However, oleic acid concentration recovered upon removal of the chamber, with the exception being that just as with total oil accumulation, oleic acid accumulation was retarded when the heat treatment was applied at the beginning of fruit development. In general, they concluded that high temperatures during oil accumulation negatively affected olive oil yield and quality in warm regions, particularly if the high-temperature event occurs early. Garcia-Inza et al. [30] used chambers which may distort the effects of a natural environment. Other studies tested the effect of high temperatures comparing fruits of various cultivars in different locations during different years. When comparing the yields of trees from different orchards, which were subjected to different methods of agriculture, many factors can influence the parameters being tested. In order to avoid these problems, we compared adult plants in pots, all of the same age, and whose fertilization, pruning and other treatments were equal, the only difference between the two groups being their location during the period of fruit development.

Olives are a major crop in Israel and are planted all over the country including semi-desert regions such as the area surrounding Tirat Zvi, where summer temperatures often rise above 40˚C. The objective of this study was to characterize the effect of a high temperature environment during olive oil accumulation, on oil yield and quality. In order to address this we used five year old potted olive trees of five selected cultivars and placed one group in Tirat Zvi (high temperatures during the summer) during the period of fruit development, and a second group in Tzuba, a village with relatively mild summers, for two consecutive years. Characterizing fruit development, oil accumulation and oil quality in both groups revealed that unsurprisingly, high temperatures during oil accumulation can decrease fruit size, oil yield and oil quality. We demonstrated however, that sensitivity to high summer temperatures is genotype dependent.

Materials and methods

Experimental design

The olive cultivars 'Barnea', 'Coratina', 'Koroneiki', 'Souri' and 'Picholine' were used in this study. Twelve olive trees from each of the five cultivars brought from the Boxer nursery (Bnei Darom, Israel) and planted in pots in 2010 at the Volcani Center, Israel (31˚59'N 34˚49'E; 42 m above sea level). During the summer of 2015, the trees were replanted in 50 liter pots. Right after fruit set, at the beginning of May 2016, six saplings of each cultivar were placed in an extremely hot climate zone at Tirat Zvi village (32˚25'N 35˚31'E; 225 m below sea level). The remaining six trees of each cultivar were placed in a more temperate environment at Tzuba village (31˚47'06.0"N 35˚06'33.5"E; 705 m above sea level). Both locations are private land and the owners of the land gave permission to conduct the study on these sites. Temperature measurements were determined using a HOBO USB Micro battery-powered station, which is a weatherproof data logger for monitoring temperature. The HOBO devices were stationed in the shade at both locations and measured the temperature every two hours throughout the entire period of the experiment. Data regarding humidity, rainfall and wind speed was taken from the Israel Meteorological Service database (https://ims.data.gov.il), measured at meteorological stations located 5 km from Tirat Zvi and 4.5 km from Tzuba.

The plants were watered using a drip irrigation system which delivered 2L of water per hour spread over three 25 minute cycles per day (07.00, 12.00 and 17.00 h) at Tzuba, the moderate temperature (MT) site, and four such cycles (06.00, 10.00, 14.00 and 18.00 h) at Tirat Zvi, the high temperature (HT) site. Each plant was irrigated with four drippers. An additional fifteen minutes of irrigation was added at each location in mid-summer to ensure an adequate supply of water. Irrigation was delivered in excess, in order to prevent dry soil. The pots had perforated bottoms in order to allow the escape of excess water. Taking into account the differences in evaporation between the two locations, the trees in the HT site were watered one third more than the trees at the MT site. In total, each tree in the HT site was watered with 13.3 liters per day and the trees in the MT site with 10 liters per day per tree. During July and August 2 liters per day per tree were added in both locations. Weeds were controlled by manual removal. Olive fruit fly control was done only in Tzuba site using Vertimec EC, Vertigo EC and Score EC as necessary, as well as Rogor L-40 EC which was applied once a month throughout the experiment. Plants were fertilized at the beginning of May with Osmocote, smart-release plant food; one application contains 11 essential nutrients and is effective for 6 months.

In order to address the effect of a high temperature environment on olive fruit development as well as oil accumulation and quality, we characterized various physiological parameters of the five olive cultivars 'Barnea', 'Picholine Languedoc', 'Koroneiki', 'Souri' and 'Coratina' during the fruit development period of two consecutive years. Trees of each cultivar were placed at two different locations, two weeks after full bloom: The HT site (Tirat Zvi), was characterized by very warm summers and the MT site, Tzuba, with a mild summer. Temperatures were measured at both locations every two hours during the entire season by portable data loggers. Olives from each of the five cultivars on both locations were sampled every month throughout the experiment for physiological and histological analysis as well as for evaluation of olive oil content. At the end of the season, all fruits remaining on the trees were harvested, the oil extracted and analyzed for quality. Trees were returned to the Volcani center for the winter and spring, and in May 2017, two weeks after full bloom, the trees were transported once more to Tirat Zvi (HT site) and Tzuba (MT site). Transportation in both years was carried out very carefully and no loss of fruit was experienced as a result of the move. In total, the experiment lasted from the beginning of May till the end of October 2016 and from the beginning of May till Mid December 2017. At the end of the 2016 season, we harvested only a limited number of 'Souri' and 'Picholine' fruits. Therefore, the 'Souri' fruits were only partially analyzed and the 'Picholine' fruits were not analyzed at all. In 2017, all five cultivars were fully analyzed. Fruit load was not measured, even though it may have had an effect on fruit weight and oil concentration. However, plants were randomly chosen to be placed in either of the two locations and therefore we believe that the average fruit load was similar at the HT and the MT sites.

Evaluating olive oil concentration

Whole fruit fresh weight was recorded, and the fruit was then dissected to mesocarp and seed for further analysis. The fresh weight of each dissected section was recorded. Olive mesocarp and endocarp was oven dried at 90˚C for 48 h and the dry weight was recorded. Oil content (dry weight basis) was determined using chemical oil extraction with petroleum ether as a solvent in quintuple (approximately 5 gr each).

Cell size—histological analysis

A section of the fruit was analyzed for the number of cell layers (S1 Fig) and cell area using differential staining. Fresh fruit sections from each sampling date were preserved throughout the experiment using FAA (Formaldehyde 10%, Ethanol 50%, Acetic Acid 5% and water 35%) as a

fixative. At the end of the experiment, we analyzed only samples representing different developmental stages. The beginning of June, 50 days post anthesis (DPA), represented the developmental stage preceding pit hardening. The beginning of July, 83 DPA, represented the developmental stage just after pit hardening, and September, 146 DPA, represented the period before ripening and the end of fruit growth. The last time-point for this analysis was at harvest. Analysis was done as described [38] with safranin/fast green staining.

Oil drops—histological analysis

A fresh section of the fruits was analyzed for oil drop size and density using Sudan IV staining protocol. The specimens were stained for 6 min with Sudan IV (0.5% w/v in 90% ethanol) and then transferred to a slide, differentiated rapidly in ethanol 50% to remove excess stain and the images observed and photographed under a light microscope DMLB (Leica, Germany) with a DS-Fi1 camera attached (Nikon, USA). Measurement of oil drop size and density was done using NIS elements software (Nikon, USA).

Cold-press—olive oil extraction

At the end of the experiment, olives of each cultivar and location were harvested after developing a semi-black skin color, and a maturity index (MI (of approximately three, which occurred around November. The MI was calculated from three repeats of 100 fruits, as a subjective evaluation of the skin color and flesh as developed in the Research Station of Venta del Llano (Jaen, Spain) and proposed by Uceda and Frias [39]. Oil was extracted from healthy fruits using a laboratory-scale Abencor system (Comercial Abengoa, S.A., Seville, Spain) equipped with a hammer crusher, malaxer and centrifuge that simulates the industrial process of EVOO production.

Determination of phenolic compounds

In 2016, Phenolic compounds were isolated from a solution of oil in hexane by double-extraction with methanol/water (60:40, v/v). Total phenols, expressed as tyrosol equivalents (ppm), were determined with a UV–visible spectrophotometer (Beckman Coulter, Fullerton, CA, USA) at 735 nm using Folin–Ciocalteu reagent.

In 2017, the phenolic compounds were isolated from olive oil to evaluate Ortho-diphenols by solid-phase extraction as developed by Mateos et al., [40]. Determination of biophenols by HPLC was done according to International Olive Council (COI/T.20/Doc No 29/Rev.1). The calculation of Biophenol content, expressed in mg/kg, was calculated by measuring the sum of the areas of the related chromatographic peaks.

Determination of oil fatty acid composition

Fatty acids were transformed into fatty acid methyl esters (FAMEs (using trans-esterification with cold methanolic solution of potassium hydroxide, according to International Olive Council (COI/T.20/Doc. 24) and European Union (EU Regulation- EN 1991R2568) protocols. Data analyzed by Chemstation software.

The calculation of the percentage of each fatty acid identified by gas chromatograph was done according to the formula: %fatty acid = (area of fatty acid x 100) / (total area)

Statistical analysis

The parameters tested were fresh and dry fruit weight, dry fruit oil concentration, wet oil concentration, amount of oil per fruit, cell size and number of cell layers and oil droplet size and

density. These were subjected to three-way analysis of variance (ANOVA) including full factorial analysis for each year, for their dependence on the three independent variables of sampling date, tree location and cultivar type, including the various interactions between them.

Fruit was sampled during the entire period from fruit set to ripening. Obviously, fruit growth and oil accumulation continued throughout this period. Therefore, we chose two sampling days and performed a full factorial two-way ANOVA analysis for the independent variables of tree location and cultivar type for each. The first sampling date chosen (146 days post-anthesis), was that on which the differences between the two locations were most evident. Harvesting time was chosen as the obvious summation date of the experiment. When we encountered significant interaction between factors, a Tukey-Kramer test was performed in order to rank the various levels of interaction. All statistical analyses were performed using JMP software [41].

Results

Different locations typify different climate conditions

Summer temperatures in Tirat Zvi (the HT site) were higher than in Tzuba (the MT site) by almost 10˚C in daytime and 5˚C at night. For example, in 2016 the average daily maximum temperature at the HT site was above 40˚C during June, July August and September, whereas at the MT site it was 32.8, 32.8, 32.6 and 30.8˚C respectively during these months. During this period, there was only one day with a temperature above 40˚C in the MT site (40.6˚C). The average daily minimum temperature at the HT site was above 21˚C from June to September, whereas in the MT site it never exceeded 20˚C. During 2017, the maximum monthly temperature was above 40˚C in the HT site from May till October. During November and December, temperatures decreased dramatically at the HT site. During July, the maximum temperature at the HT site was 45.8˚C whereas at the MT site it was 37.8˚C. In 2017, the average daily minimum temperature in the HT site was above 20.5˚C from June to September, and below 20.5˚C in the MT site. The average difference in maximum daily temperature between the HT site and the MT site was 9.2˚C and 7.5˚C during 2016 and 2017 respectively and a difference of 4˚C and 4.15˚C in the average minimum daily temperatures during 2016 and 2017 respectively (Fig 1 and S1 Table). During both seasons, there were only few rainy days. The humidity at the MT site was slightly higher compared to the HT site and wind speed in both locations differed in an average speed of 0.47 m/s (S2 Table). Since all trees were kept together at the volcani center until fruit set, differences in flowering time of the various cultivars were not recorded. In both years, fruits were harvested at a maturity index of approximately 3. In 2016, the time of ripening of the fruits was uniform for all cultivars at both locations and the harvest was carried out at the beginning of November with no significant differences between maturity index at the time of harvest for all cultivars at both locations. In 2017, fruits at the MT site ripened earlier than at the HT site. In contrast to the MT site, differences in ripening time between cultivars were observed at the HT site. Therefore, in the MT site, all cultivars were harvested at 168 days post anthesis (October 23rd). In the HT site, the cultivars 'Koroneiki', 'Picholine' and 'Souri' were harvested 198 days post anthesis (November 22nd) and 'Barnea' and 'Coratina' were harvested 226 days post anthesis (December 20th). The maturity index for the various cultivars is presented in S3 Table.

A high temperature environment affects fruit weight and oil accumulation

The difference between summer temperatures at the HT site and the MT site was greater in 2016 compared to 2017. Therefore, differences between the two locations in all parameters measured were greater in 2016. Interaction between sampling date, cultivar type and tree

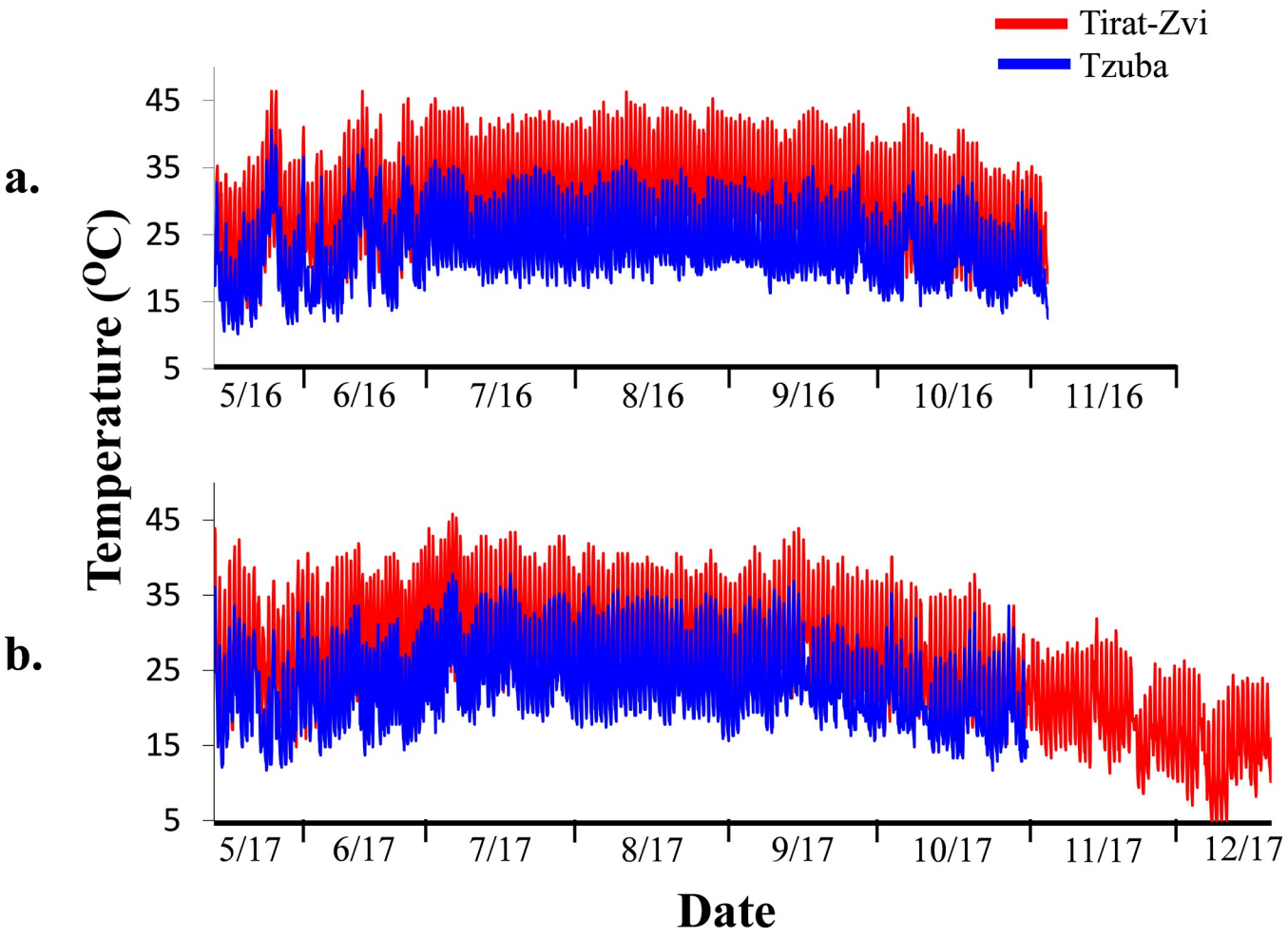

Fig 1. Temperatures measured at Tirat Zvi, the HT site (red line), indicate a very warm summer and Tzuba, the MT site, (blue line), represents a mild summer, during 2016 (a) and 2017 (b). Temperature was measured by a data logger every 2 hours during the entire fruit development period from fruit set till harvest.

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location was found to be significant for dry fruit weight as well as dry fruit oil concentration (Three-ways ANOVA—$P<0.001$ and $P<0.005$ in 2016 and $P<0.001$ and $P<0.001$ in 2017 respectively). We tested the effects of cultivar type and tree location and the interaction between them on dry fruit weight and dry fruit oil concentration, by two-ways ANOVA at 164 DPA and at harvest in 2016 and at 146 DPA and harvest time in 2017 (S4A and S4F Table). These independent variables and the interaction between them were found to have significant effects on both parameters at both sampling dates for both years.

'Barnea' dry fruit weight in August 2016 was significantly lower in the HT site trees than that measured at the MT site. In 2017, dry fruit weight of 'Barnea' in the HT site was significantly lower from August till the end of October (harvest time at the MT site). However, when temperatures decreased in November-December, 'Barnea' dry fruit weight at the HT site rose to the same level as at the MT site.

'Koroneiki' dry fruit weight during 2016 was significantly higher at the MT site compared to the HT site, since its dry fruit weight at the HT remained constant from July till harvest. In 2017, dry fruit weight was equal at both locations from fruit set till the end of July. However, during August and September 'Koroneiki' fruit weight increased dramatically at the MT site, while at the HT site fruit weight was roughly constant during this period. At harvest,

'Koroneiki' fruit weight at the MT site was significantly higher than at the HT site (405.3 and 254 mg respectively).

'Coratina' dry fruit weight was equal at the HT and MT sites until July, but after July of both years, there was a significant rise in dry weight at the MT compare to the HT site. At the 2016 harvest, dry fruit weight was double at the MT site compared to the HT site. In 2017, at the MT harvest time, 'Coratina' dry fruit weight at the MT site was 1.38 g, and at the HT site, 0.871. However, when temperatures decreased in November-December, fruit weight at the HT site rose and at HT harvest time reached 1.12 g, still significantly lower compared to the MT 'Coratina' dry fruit weight at harvest. 'Souri' cultivar dry fruit weight was significantly higher at the MT site from the end of July till harvest during both years.

'Picholine' dry fruit weight was significantly higher at the MT site than at the HT site, from August until harvest. During harvest, 'Picholine' dry fruit weight was 1.66 g at the MT site, significantly higher than 1.28 g at the HT site (Fig 2). Fresh fruit weight, commercial oil percentage and oil per fruit data are presented in S5 Table.

The 'Barnea' dry fruit oil content was not significantly different between the two locations in 2016 except in August when dry fruit oil percentage was 30.6 at the MT site and only 16.3 at the HT site. In 2017, beginning in September, dry fruit oil content was higher in the MT trees compared to the HT site (34% and 23.1% respectively). However, since the HT 'Barnea' was harvested much later than in the MT, the final dry fruit oil content at harvest was similar at both locations. The dry fruit oil content in the 'Koroneiki' cultivar was significantly higher at the MT compared to the HT site during both years from August until harvest. The oil percentage of dry fruit of the 'Coratina' cultivar was significantly higher at the MT site compared to the HT site during most of the season. However, in both years, at harvest time, the dry fruit oil percentage was similar at the MT and HT sites (43.6 and 40% in 2016, 46.3 and 44.8% in 2017 respectively). From August till harvest time, the dry fruit oil percentage of the 'Souri' cultivar was significantly higher at MT compared to the HT site during both years. The dry fruit oil percentage of the 'Picholine' cultivar was significantly higher at the MT compared to the HT site during August and September. However, at harvest, the dry fruit oil percentage in the MT and HT sites were similar (32.7% and 30.6% respectively) (Fig 2).

We analyzed the variation in the gain of dry fruit weight and dry fruit oil concentration in all the cultivars tested, during each month from June to September at the two locations in 2016 and 2017. We found that the main disparity in gain of fruit weight between the HT and MT sites occurred during August of both years. Similarly, the main difference in oil concentration between the HT and MT sites was measured during July and August of 2016 and in August of 2017. Since the interaction between cultivar type and tree location was significant for both traits, during August (164 DPA in 2016 and 146 DPA in 2017) and at harvest time, we ranked the cultivars by their performance at the MT site compared to that at the HT site (S4B–S4E and S4G–S4J Table). In 2016, at harvest, the, 'Koroneiki' and 'Coratina' cultivars showed the most significant difference in dry fruit weight, whereas the 'Barnea' cultivar exhibited the least difference between the MT and HT sites. A similar analysis for oil content of dry fruit demonstrated that the 'Koroneiki' cultivar showed a highly significant difference between the two locations, whereas variation for this trait in the 'Coratina' and 'Barnea' cultivars was significantly lower. In 2017, at harvest, the 'Souri' cultivar showed the most significant difference in dry fruit weight between the MT and HT sites. The cultivars 'Koroneiki', 'Picholine' and 'Coratina' exhibited lesser differences between sites, whereas the 'Barnea' cultivar exhibited the least difference between the MT and HT sites. A similar analysis for oil content of dry fruit demonstrated that the 'Koroneiki' and 'Souri' cultivars showed a highly significant difference between the two locations, while the cultivars 'Picholine', 'Coratina' and 'Barnea' had significantly lower variation between locations. We also analyzed the association between the gain of dry

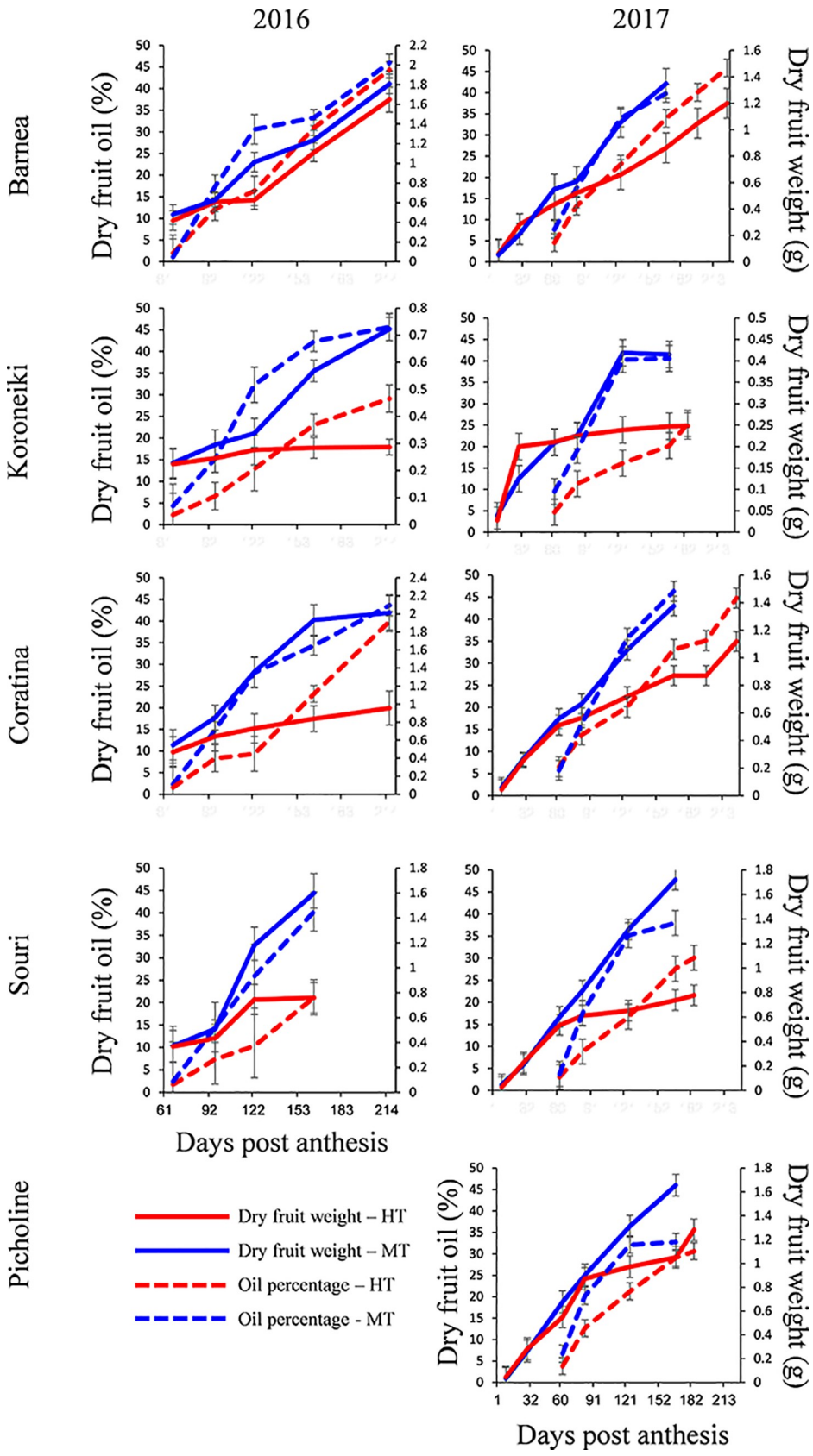
2016
2017
Barnea
Koroneiki
Coratina
Souri
Picholine
Dry fruit oil (%)
Dry fruit weight (g)
Days post anthesis
Dry fruit weight – HT
Dry fruit weight – MT
Oil percentage – HT
Oil percentage - MT

Fig 2. Dry fruit weight and oil accumulation of all analyzed cultivars during 2016 and 2017. Dry fruit weight (continuous line) and dry fruit oil percentage (dashed line), of fruits grown at the HT site, Tirat Zvi (red line) and the MT site, Tzuba (blue line), during the entire season are presented. The right Y axis is the dry fruit weight, while the left Y axis is the dry fruit oil percentage. Error bars represent confidence limits ($\alpha = 0.05$). Error bars that do not overlap represent significant differences.

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fruit weight and dry fruit oil concentration in all analyzed cultivars during each month in relation to the monthly average maximum daily temperature (Tmax), minimum daily temperature (Tmin) and average daily temperature (Tmean). We found that dry fruit weight in all cultivars was correlated (negatively and significantly) to all the above temperature variables. In contrast however, dry fruit oil concentration was not correlated to any of those temperature variables monitored. When we applied these analyses to those cultivars in which dry fruit weight was sensitive to high temperatures ('Koroneiki', 'Coratina', 'Souri' and 'Picholine'), we found that gain of dry fruit weight exhibited a significantly negative correlation with all temperature variables. Similarly, when we applied our analysis to those cultivars in which oil concentration had proved itself to be sensitive to high temperatures ('Koroneiki' and 'Souri'), we found a significant negative correlation between dry fruit oil concentration and Tmax (S6 Table).

High temperature environment affects mesocarp growth

In order to address the effect of a high temperature environment on fruit growth, we measured mesocarp cell size and the number of cell layers in the mesocarp at various developmental stages (Fig 3). The effect of cultivar type and tree location on mesocarp cell size and the number of cell layers in the mesocarp was found to be significant at both 146 DPA and harvest time. The interaction between cultivar type and tree location was found to have a significant effect on cell size at both sampling dates, but affected the number of cell layers at harvest time alone (S4F Table). The 'Barnea' fruit weight curve at the MT site exhibits the expected double sigmoid pattern, in which fruit weight increases linearly after fruit set, stays steady during pit hardening and increases again after pit hardening until September, just before ripening. At the HT site, the 'Barnea' fruit weight curve increases in approximately linear fashion from fruit set to harvest (Fig 2). The number of cell layers in the 'Barnea' fruits at the MT site did not change statistically during fruit growth from the beginning of June until harvest-time at the end of October. However, cell size increased dramatically from 1181 μm^2 at the beginning of June to 6604 μm^2 at the end of October. The number of cell layers in 'Barnea' fruits at the HT site was constant from June till September. However, it increased from 27 to 37 layers from September until harvest time in December. The cell size curve of the 'Barnea' fruits at the HT site showed the opposite tendency and increased from June till September but remained steady from September until December (Fig 3 and S2A Fig).

The 'Koroneiki' fruit weight curve at the MT and HT sites is similar to that of 'Barnea'. At the MT site it exhibits double sigmoid growth and at the HT site growth is linear. However, the slope of the 'Koroneiki' curve at the HT site is flatter than in the 'Barnea'. The number of mesocarp cell layers as well as cell size in the 'Koroneiki' fruits in the MT site increased during the entire period of fruit development. In June, the 'Koroneiki' mesocarp in the MT site consisted of 20 layers of 1596 μm^2 cells, whereas in October (at harvest) it consisted of 34 layers of 6210 μm^2 cells. At the HT site, mesocarp cell size and the number of cell layers increased dramatically from June to July, before pit hardening, and then increased at a slower pace till harvest. During this period cell size as well as the number of cell layers was significantly lower in the mesocarp of 'Koroneiki' fruits from the HT site compared to those which grew at the MT site (Fig 3 and S2B Fig). The 'Coratina' mesocarp showed a tendency similar to that of 'Koroneiki', however the number of cell layers, unlike in the 'Koroneiki' mesocarp, was higher at the

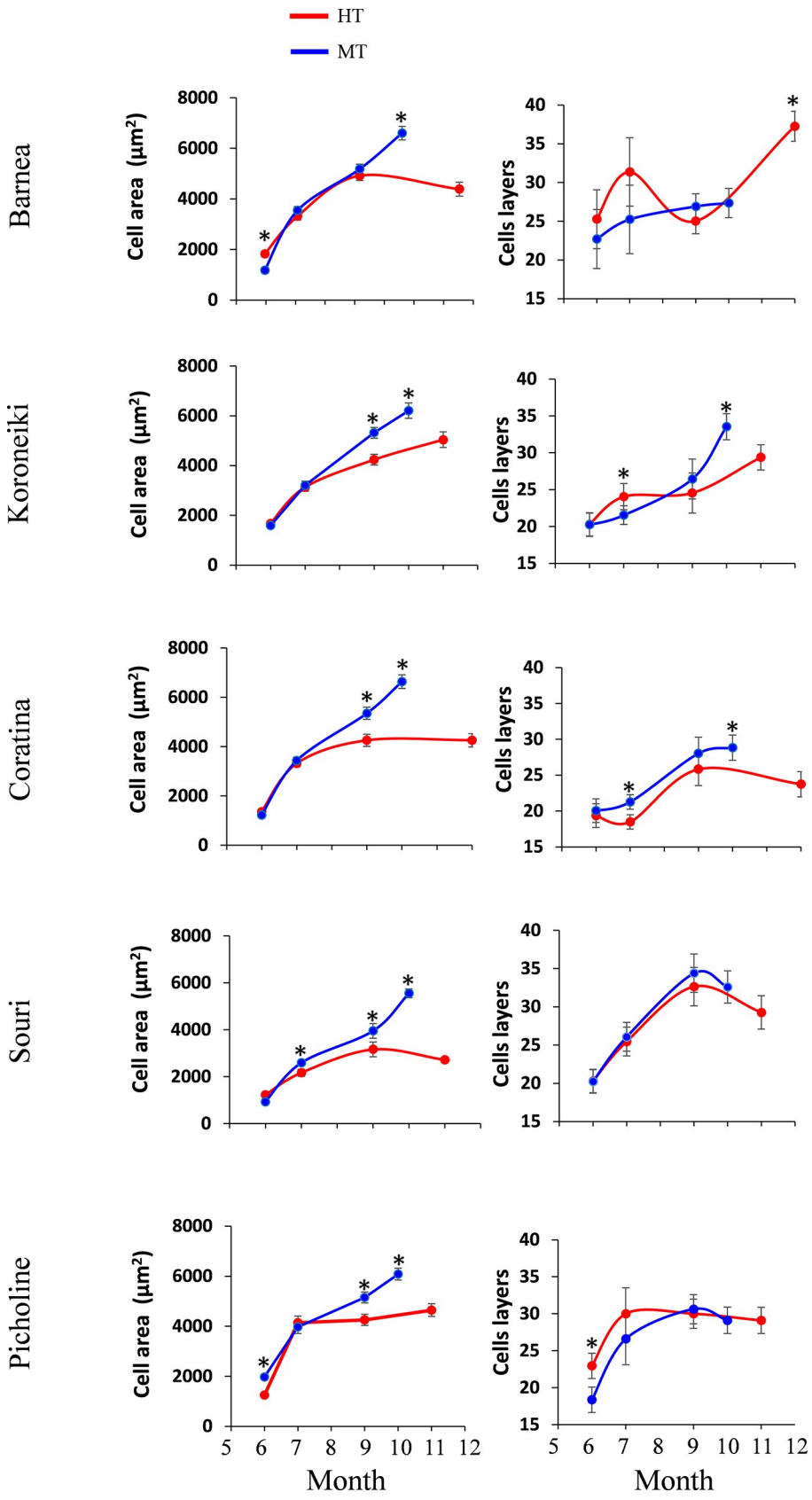
HT
MT
Barnea
Koroneiki
Coratina
Souri
Picholine
Cell area (µm²)
Cells layers
Month
0
2000
4000
6000
8000
15
20
25
30
35
40
5
6
7
8
9
10
11
12

Fig 3. Mesocarp cell characterization in all 5 cultivars during 2017. Quantification of the average cell area and the number of cell layers during the season (June, July, September and at harvest) are presented. Error bars represent confidence limits ($\alpha = 0.05$). Asterisks represent significant difference ($\alpha = 0.05$).

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MT site all season. The number of cell layers in the 'Souri' and 'Picholine' mesocarp was similar at both sites, whereas cell size in both cultivars was significantly higher at the MT site compared to the HT site in three of the four dates sampled (Fig 3).

September fruit weight of all five cultivars at the MT site was significantly higher than at the HT site. However, at that time, the number of mesocarp cell layers in the fruit of all cultivars at the MT site was not significantly higher than at HT. In contrast to 'Barnea', the mesocarp of the other four cultivars consisted of significantly larger cells in the MT site trees compared to the same cultivars grown at the HT site (S4 Fig).

A high temperature environment affects oil drop size and density

In order to address the effect of a high temperature environment on oil accumulation, we measured oil drop size and density at various stages of development: 50, 83 and 146 DPA (Fig 4). At 146 DPA, the effects of cultivar type and tree location and their interaction, on oil drop size, was found to be significant. In contrast, the effect of tree location on oil drop density was not found to be significant. However, the effects of cultivar type and the interaction between cultivar type and tree location were significant (S4F Table). 'Barnea' fruit at the HT site, at 50 DPA, showed significantly larger, denser oil drops compared to those at the MT site. Average oil drop size was 75 and 54 μm^2 and average oil drop densities were 476 and 352 drops per cm^2, in the HT and the MT sites respectively. However, later in the season, at 83 as well as 146 DPA, the oil content and oil drop size were higher at the MT compared to the HT site, whereas oil drop density was similar at both locations. Oil drop sizes at 83 DPA were 286 and 220 μm^2 at the MT and the HT sites respectively and at 146 DPA, 860 and 658 μm^2. The differences between the two locations on both dates are significant. Oil drop densities at 83 DPA were 747 and 650 drops per cm^2 and at 146 DPA, 677 and 645 drops per cm^2 at the MT and the HT sites respectively. These differences on both dates are not statistically significant (Figs 4 and S4F). 'Koroneiki' olives showed the same trend as the 'Barnea' with bigger oil drops at the HT site at 50 DPA and bigger oil drops at the MT site at 83 DPA and at 146 DPA. However, at 146 DPA, in 'Barnea', the ratio of oil drop size between the MT and the HT sites is 1.3 and in 'Koroneiki', the ratio reaches 2.6. In addition, at 146 DPA, oil drop density of 'Koroneiki' olives is higher in trees at the HT compared to the MT site (Fig 4 and S4B Fig). 'Coratina' oil drop density was higher at the MT site compared to the HT site. However, the differences between the two sites was not significant at all three sample dates. Surprisingly, 'Coratina' oil drops were significantly larger at the HT site compared to the MT site at 83 DPA. However, later in the season, at 146 DPA, oil drops were significantly larger at the MT site compared to the HT site. The oil drop density in 'Souri' and 'Picholine' was similar at both sites at all sampling dates. However, oil drops were significantly larger at the MT sites compared to the HT sites in both cultivars (Fig 4). By September, the oil content of all five cultivars was significantly higher in olives at the MT site than in those grown in the HT site. At that time, oil drop size was also significantly greater in the MT site olives than in those from the HT site. However, oil drop density in 'Koroneiki' olives was higher at the HT than at the MT site (S5 Fig).

High temperature environment affects oil composition

In order to characterize the effect of high temperature environments on oil composition and quality, we measured the polyphenol content in the oil extracted from the various cultivars at

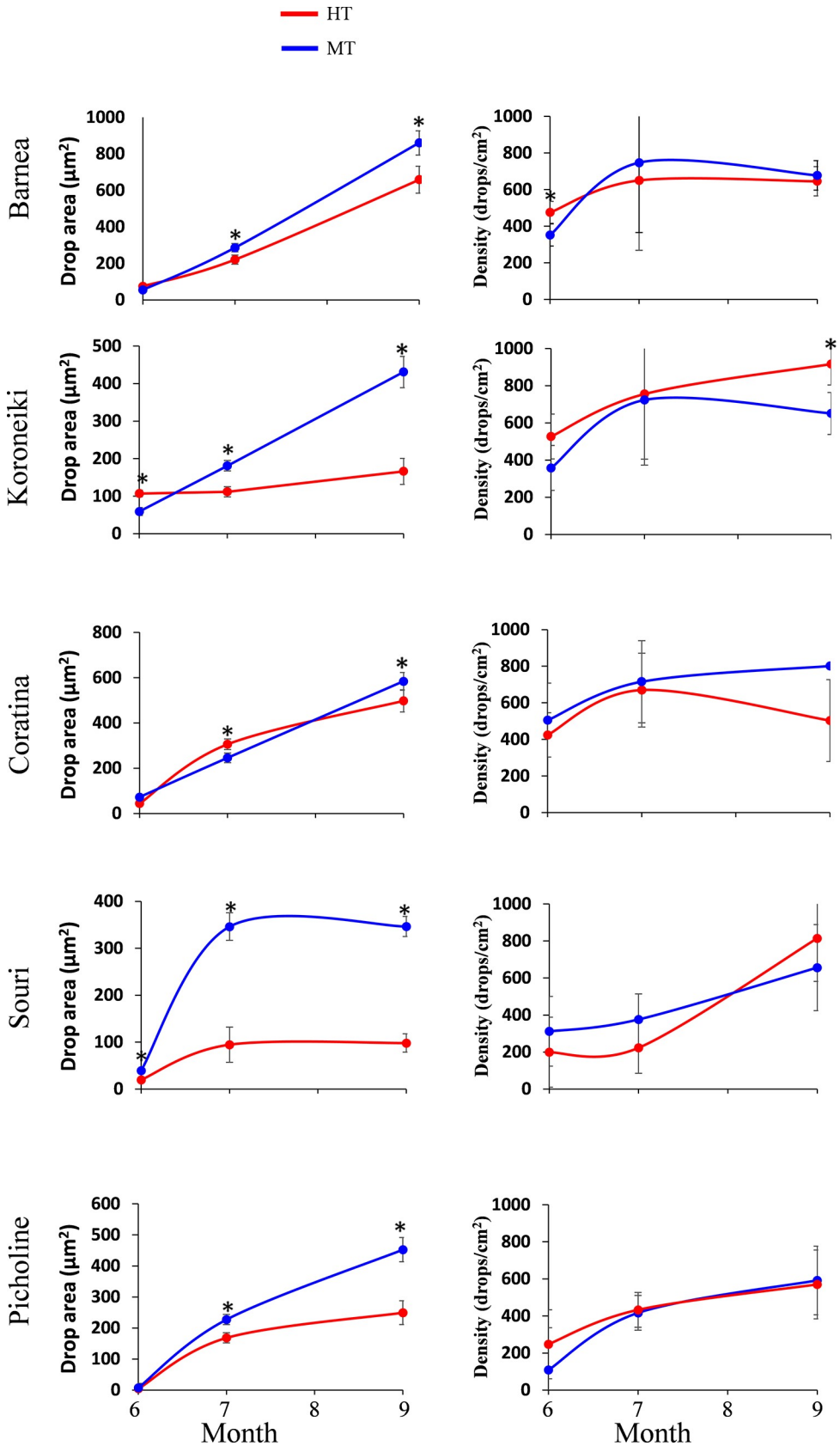
HT
MT
Barnea
Koroneiki
Coratina
Souri
Picholine
Drop area (µm²)
Density (drops/cm²)
Month
6
7
8
9

Fig 4. Oil accumulation in all cultivars during 2017. Quantification of the average oil drop area, the density of oil drops as well as the oil content during the season (June, July and September) are presented. Error bars represent confidence limits (α = 0.05). Asterisks represent significant difference (α = 0.05).

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both locations (Fig 5A). During the 2016 season, polyphenol levels varied between cultivars. In general, levels were almost double in oil extracted from fruits from the MT site compared to those grown at the HT site. In 2017, polyphenol levels in all cultivars at the HT site were lower than at the MT site. The lowest polyphenol level, 156 mg/g oil, was measured in the oil extracted from 'Barnea' from the HT site. The polyphenol level in oil extracted from 'Barnea' grown at the MT site was more than double that from the HT fruits—404 mg/g oil. Oil extracted from 'Coratina' showed the same pattern as that of 'Barnea'. Polyphenol levels in oil extracted from 'Picholine' grown at the MT site were more than three times higher than in oil extracted from 'Picholine' from the HT site (893 and 291 mg/g oil respectively). Polyphenol levels in oil extracted from 'Koroneiki' at the MT site were 150% higher than the levels at the HT site. The smallest difference between polyphenol levels in oil extracted from the two locations was found in oil extracted from the 'Souri' cultivar. This was mainly due to the unusually high level of polyphenols found in oil extracted from 'Souri' grown at the HT site, 772 mg/g oil (Fig 5A).

The main fatty acid found in olive oil is oleic acid. We found that oil extracted from all five cultivars grown at the HT site contained lower levels of oleic acid than oil extracted from olives grown in a milder environment (Fig 5B). During 2016, the differences between sites in the percentage of oleic acid in oil extracted from olives grown at the MT site compared to olives from the HT site was 8, 4 and 7% in 'Barnea', 'Koroneiki' and 'Coratina' respectively. During 2017, oleic acid levels were higher than in 2016 for all cultivars in both climate regions. Oleic acid levels in oil extracted from olives grown at the MT site reached 74% in 'Koroneiki' and 76% in 'Coratina', whereas the levels in oil extracted from olives grown at the HT site was 67 and 69% respectively. Oleic acid level in oil extracted from 'Picholine' olives grown at the HT site was 51.8%, whereas at the MT site it was 60.4% (Fig 5B). We also characterized all other fatty acids in the oil of the various cultivars in both climate regions (S7 Table). The decrease in oleic acid content in the oil extracted from olives grown at the HT site coincides with an increase in the level of palmitic acid (C16:0) and linoleic acid (C18:2) in oil extracted from olives grown there. The palmitic acid content was about 2% more in oil from the HT site compared to the MT olives in all cultivars during both years. Excessively high levels of palmitic and linoleic acid were detected in 'Barnea' oil extracted in 2016, and in 'Picholine' oil extracted in 2017. 'Picholine' oil extracted from olives grown in 2017 in the HT site contained 21.6% palmitic acid, and 'Barnea' oil extracted from olives grown in 2016 at the HT site contained 23.3% linoleic acid.

Discussion

The most critical stage of development of olive fruit, when fruit growth as well as oil accumulation rate are maximized, is between 60 and 120 days after flowering, between pit hardening and fruit ripening [42]. In the northern hemisphere, this period falls in July and August. In both 2016 and 2017, this period was particularly hot at the HT site. Temperatures reached 46˚C in 2016 and 45˚C in 2017, whereas the mean daily maximum temperatures during July and August were 42.5˚C in 2016 and 40.4˚C in 2017. In general, the summer of 2016 at the HT site was hotter than the summer of 2017. This may be the reason for the early ripening in 2016 at the HT site compared to 2017. However, it was shown that several parameters such as irrigation regime or fruit size can affect ripening time [43].

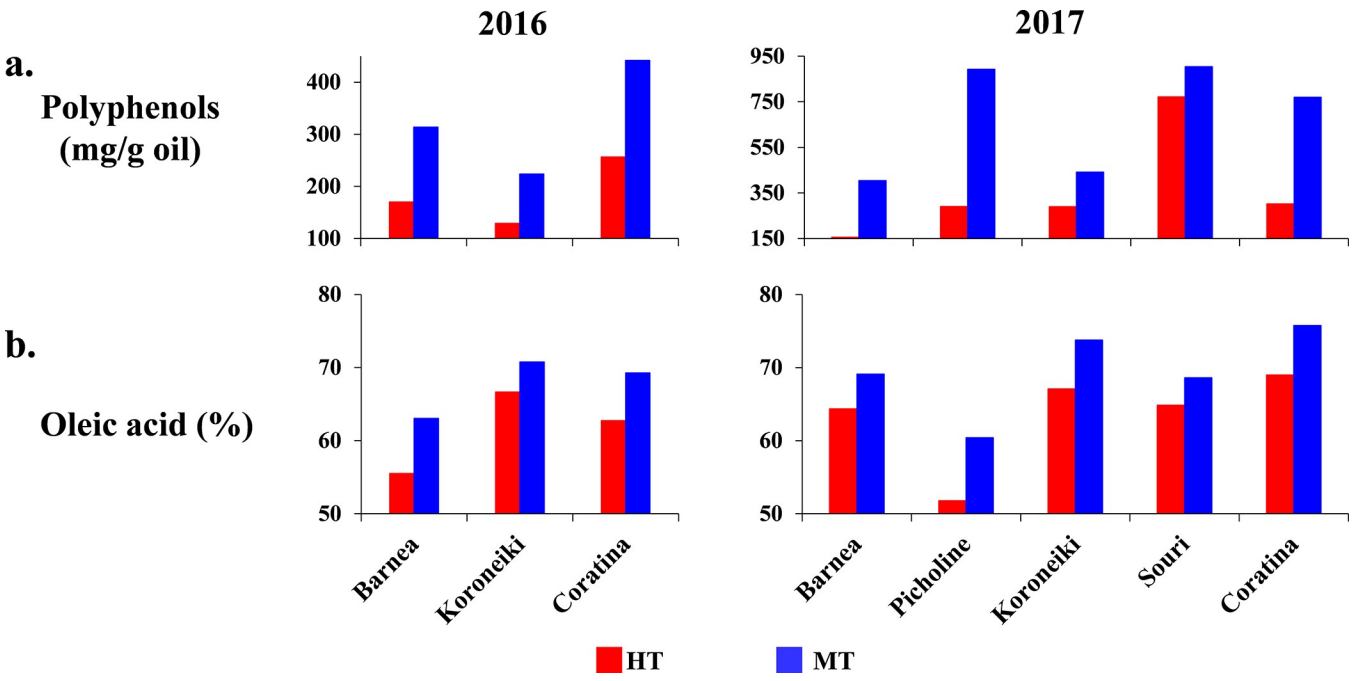

Fig 5. Oil quality, represented by polyphenol (a) and oleic acid (b) levels in the oil extracted from the various cultivars in the two climatic regions, during both years.

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Olive oil yield is the function of the number of fruits, average fruit weight and oil concentration at maturity. In this study, we addressed the effects of an environment of consistently high temperatures on fruit development, oil accumulation and oil composition in five olive cultivars. We found that different olive cultivars respond to high temperatures of the environment in a genotypic specific manner. A high temperature environment repressed fruit development and oil accumulation and also affected oil composition. We found that the 'Koroneiki' and 'Souri' cultivars are highly temperatures sensitive, and were affected to an extreme degree by the high environmental temperatures. We demonstrated that fruit weight and oil accumulation in 'Koroneiki' and 'Souri' olives harvested in the HT site were much lower compared to these parameters at the MT site. In contrast to 'Koroneiki' and 'Souri', the 'Barnea' cultivar exhibited greater tolerance to high temperatures, and although fruit growth as well as oil accumulation were at some stages significantly lower at the HT site, the final fruit weight as well as oil percentage at harvest was virtually the same at both the HT and MT sites (S4 Table). These results were repeated in 2016 and 2017 (Fig 2). Oil quality, defined by oleic acid content as well as by polyphenol levels in the oil after harvest, was found to be affected by high temperatures in all five cultivars.

Whether the effects of high temperatures can be attributed mainly to average, maximum or minimum daily temperatures or to other temperature parameters, is still not clear. We found significant association between dry fruit weight and Tmax, Tmin and Tmean. However, when we analyzed sensitive cultivars only, we found a significant association between Tmax and dry fruit oil concentration (S6 Table). Daily maximum temperature differences between the HT and the MT site were higher in 2016 compared to 2017. On the other hand, differences between the HT and MT site in the daily minimum temperatures were higher in 2017. Differences in dry fruit weight and dry fruit oil concentration were also higher in 2016. It would seem therefore, that the most relevant parameter for predicting the effects of high

temperatures, is the daily maximum temperature. A recent study [35] found that olive fruit dry weight showed a tendency to decrease with increasing mean temperature, while the proportion of oil in the fruit exhibited a significant correlation with mean daily thermal amplitude, and weaker correlation with mean daily maximum and minimum temperatures. The proportion of oleic acid in the oil showed a negative correlation with mean daily minimum temperature and with mean daily temperature, and a linear relationship with mean daily thermal amplitude [35]. This study used lower maximum temperatures to cause heat stress compared to the ambient temperatures at our HT site (S1 Table). We can therefore surmise that olive plants suffered from high temperatures as a result of the maximum daily temperatures encountered at the HT site.

A high temperature environment affects fruit weight

We found that final dry fruit weight at harvest of 'Barnea' cultivar was not affected by the location of the trees nor by the resulting difference in temperatures. In comparison to 'Barnea', the 'Koroneiki', 'Coratina', 'Souri' and 'Picholine' cultivars showed a decreased dry fruit weight at harvest in response to the higher temperatures at the HT site. The influence of temperature during early stages of fruit development has been identified in several fruit tree species [44–47]. Dry fruit weight in the olive cultivar 'Arauco' was not affected by temperatures in the range of 16–25˚C. However, at higher temperatures it showed a decrease of 0.08 grams in dry fruit weight for each additional 1˚C [30]. We found a cultivar dependent decrease of between 0.026 to 0.044 grams in 2016, and 0.015 to 0.078 grams in 2017, in dry fruit weight per 1˚C increase, measured in September, before harvest.

It has been suggested that temperature during fruit development may have a greater effect on cell division than its effect on cell expansion in tomato fruits [48]. Another study found that continuous heating of tomato fruit reduced cell expansion [49]. We found that in 'Barnea' olives grown at the MT site, cell expansion alone contributed to mesocarp growth beginning 7 weeks after full bloom. At the HT site, 'Barnea' fruits followed this pattern from 50 to 146 days (7 to 21 weeks) after full bloom. However, during the period of 146 to 247 days after full bloom, cell expansion ceased and cell division alone contributed to mesocarp growth. In the 'Koroneiki' cultivar we found a similar pattern to 'Barnea', the exception being, that at the MT site, cell division also contributed to mesocarp growth in the late stages of fruit development. 'Coratina' showed the same trend as 'Koroneiki' whereas in 'Souri' and 'Picholine', as in 'Barnea', it was cell expansion alone that was responsible for the differences between the two sites (Fig 3). Rallo and Rapoport [19] found that in olives, as in other drupes, both cell division and cell expansion contribute to mesocarp growth at early stages of fruit development. Six weeks after full bloom, cell division ceased, and cell expansion alone contributed to further mesocarp growth. Hammami et al. [20] found that fruit size differences among six olive cultivars are due to cell division throughout fruit growth, which occurs mainly in the first six weeks after bloom. However, they were surprised to find that a substantial number of cells formed after these six weeks, and continued during the following 6 months. The six cultivars they tested did not include the five cultivars tested in this study. In our study, it is possible that in reaction to the unusually high temperatures at the HT site during the early stages of fruit development, the "Barnea" cultivar delayed mesocarp cell division until the arrival of milder weather (October-December). The 'Koroneiki' cultivar, which has been shown to be relatively sensitive to high temperatures, may have been stressed by the temperatures at the MT site during the spring and as a result, exhibited delayed cell division. In September 2017, when fruit weight differences between olives grown at the HT site and those of the MT site were at their greatest, the number of cell layers was identical, but cell area was significantly greater at the MT site in

all cultivars but the 'Barnea'. This suggests that unlike satsuma mandarins [47], the main effect of high temperatures on olive fruit weight is through repression of cell expansion and not cell division (S3 Fig).

High temperature environment affects oil accumulation

We found that the effect of a high temperatures environment on oil concentration is genotype dependent. In 2016, the average difference in maximum daily temperature between the HT and the MT site was 9.2˚C, and that of minimum daily temperature was 4˚C. In 2016 these temperature differences did not affect 'Barnea' and 'Coratina's' final oil concentration. However, the 'Koroneiki' cultivar of the same year had a final dry fruit oil concentration at harvest of 45.6% at the MT site and 29.1% at the HT site, which is an average of about a 1.79% decrease per degree of increased maximum daily temperature and 4.13% decrease per degree of increased minimum daily temperature. The 'Souri' cultivar had a 2.08% decrease per degree of increased maximum daily temperature and a 4.78% decrease per degree of increased minimum daily temperature. In 2017, decrease in oil concentration as a function of the severity of high temperatures, was more moderate than in 2016. However, just as in 2016, dry fruit oil concentration of 'Barnea' and 'Coratina' cultivars was not affected by the location of the trees and the resulting difference in temperatures, whereas the 'Koroneiki' and 'Souri' cultivars showed a decreased oil concentration at harvest in response to the higher temperatures at the HT location. 'Picholine' dry fruit oil concentration, like that of 'Barnea' and 'Coratina', was similar at both locations. Other studies have found that heat stress reduced oil concentration in sunflower hybrids by 6% [50]. However, corn which had undergone heat stress during grain fill, was found to have the same oil content as control plants [51]. Olive oil concentration as a percentage of dry weight in 'Arauco' cultivars was found to decrease linearly at 1.1% per degree of increased temperature. [30]. Our finding is also consistent with the results of Rondanini et al. [33] which measured the oil accumulation of six olive cultivars, at three locations over two years and found a negative association between oil concentration and average temperature. The response to heat stress treatment of potted 'Coratina' and 'Arbequina' trees was found to reduce dry fruit weight by 0.34 and 0.22 g, respectively. In this study, fruit oil concentration (%) was 4.6 and 6.2% less on a dry-weight basis in fruit exposed to elevated temperatures. Higher temperature was found to promote vegetative growth but negatively affected oil concentration [32]. Trentacoste et al. [31] found that fruit oil concentration in 10 olive varieties decreased with increasing maximum daily temperature. It is accepted that oil accumulation begins only after pit hardening. However, while pit hardening occurs about 10 weeks after flowering, Matteucci et al. [28] has shown that oil bodies appear in the mesocarp cells 7 weeks after flowering, and large oil droplets, derived from their fusion, are present in each cell 10 weeks after flowering. In accord with Matteucci et al. [28], we also observed very clear oil bodies 50 days after flowering. In September, 146 days after flowering, oil drops were significantly larger at the MT site compared to the HT site in all five cultivars. At this stage, oil drops comprise between 3% to 17% of the cell volume. However, these values do not represent the true oil concentration in the mesocarp, because each cell may include many small oil drops that had not yet fused at the time the picture was taken.

Differences between years of the experiment

Although development patterns of the olive fruits were similar during both years of the study, the differences between those grown at the MT site and those at the HT site, were significant. Humidity, wind speed and rainfall were similar in 2016 and 2017 (S2 Table). However, the yearly average temperature difference between the HT and the MT site was higher in 2016

compared to 2017. Another result of our study indicates that during the course of the growing season, August is the month in which temperature has the greatest effect on fruit weight and oil accumulation, since during this month we found the greatest divergence between the two locations in 2016 and 2017 (S6 Table).

High temperatures environment affects oil quality

Among the effects of high temperatures on chemical parameters characterized in the current study, were total polyphenols and the fatty acid profile of olive oil. Polyphenol levels are known to decrease during fruit development [52, 53]. These levels are often positively correlated with water stress but in some cases show a divergent trend [54, 55]. Our analysis shows that a high temperature environment caused a decrease in the total polyphenol content of all analyzed cultivars. In 2016, total polyphenol level of oil from the HT site was approximately 55% of the level in oil from the MT site in all 3 analyzed cultivars. In 2017, total polyphenol level oil from the HT site was approximately 35% the level found in oil from the MT site in 'Barnea', 'Picholine' and 'Coratina'. However, in the 'Koroneiki' cultivar, polyphenol level at the HT site was 65% the level in the MT site, and in 'Souri', polyphenol level was very high in the HT site olives, reaching 772 mg/kg oil, compared to 905 mg/kg oil in the MT site. The decrease in total polyphenols in the HT site may also be explained by the differences in the irrigation regime. It is recognized that excess irrigation during fruit development results in lower phenolic concentrations in the olive oil due to changes in the biosynthetic and catabolic polyphenol pathways in the olive fruit [56–60].

The olive trees in the HT site got one third more water than those in the MT site; In our opinion, the difference between polyphenol levels at the two locations are too dramatic to be explained only by the level of irrigation [55]. The total polyphenol levels in 2017 were higher than in 2016 and were very high compared to levels found in previous studies [55]. This can be explained as an effect of climate change. Total polyphenol levels were dramatically lower in oil from the HT compared to the MT site in all cultivars except for the 'Souri', which as mentioned above, had a very high total polyphenol level at the MT site as well as at the HT. This might suggest that, at least in terms of oil quality, the 'Souri' cultivar is relatively tolerant to high temperatures.

One of the major criteria of oil quality is its fatty acid composition. In accord with other studies on olive oil, in our study all cultivars showed a reduction in their oleic acid content in both years in response to the high temperature environment at the HT site. In sunflower oil, heat stress caused an increase in oleic acid content and a reduction of linoleic acid [50]. In contrast, it has been demonstrated that in the olive, high temperatures caused a decreased level of oleic acid and an increase of linoleic and palmitic acids [30]. This may be partly explained by the gene expression level of genes involved in the oil biosynthesis pathway [61]. The cultivars 'Arbequina', 'Barnea', 'Koroneiki', 'Manzanillo' and 'Picual', showed lower level of oleic acid and higher level of linoleic acid in regions with high temperatures compared to the levels found in areas of mild temperatures [37]. This study compared olive oil composition in Northern New South Wales and Southern Queensland, which is a warm area to Tasmania, a milder region. The 'Koroneiki' fatty acid composition was not analysed in Tasmania. However, the 'Barnea' and 'Coratina' showed a decrease in oleic acid content of 16.6 and 7.8% respectively in the warm region compared to the mild region. Our results showed smaller differences between oil extracted from the HT compare to the MT sites in oleic acid content. The temperatures during the 2 season analysed by Mailer et al. [37] were not mentioned. However, the larger decrease in oleic acid content compared to our findings can be explained by larger differences in temperatures or in other agronomic parameters, since the sites in their experiment differed

in many variables: they were cultivated by different farmers, possibly with different soil, water quality and many other parameters. The influence of temperatures during the growing season on the fatty acids composition of 188 Italian cultivars was studied between 2001 and 2005. Significantly lower oleic acid and higher palmitic and linoleic acids levels were found in the warmest year of the study (2003) compared to the coolest year (2005) [36].

The cultivars used in our study all exhibited, in both years of the trial, elevated levels of palmitic as well as linoleic acids as a result of exposure to the high temperatures of the HT site. According to the International Olive Council (IOC; http://www.internationaloliveoil.org), olive oil must contain 55–83% oleic acid and 3.5–21% linoleic acid. The oil of the 'Barnea' grown at the HT site in 2016 contained 23.34% linoleic acid and 'Picholine' oil from the HT site in 2017 contained 51.8% oleic acid and 21.62% linoleic acid. Thus, neither meets the standards of the IOC. Analysis of the oils extracted from the five cultivars in 2017, showed 'Souri' to have the smallest disparity in its oil composition between olives grown at the MT site compared to those from the HT site. Oleic acid in 'Souri' decreased by less than 4% and linoleic acid increased by only 1.5% at the HT compared to the MT site. These results, taken with those of total polyphenol levels, suggest that in regard to oil quality, the 'Souri' cultivar is more tolerant to high temperature environments than any of the other cultivars analyzed in this study.

Conclusions

Our study demonstrates the negative effect of a high temperature environment on several key characteristics critical to the quality of olive oil. High temperature environments are shown to negatively influence fruit development as well as oil accumulation and thereby reduce yield. These high temperatures diminish oil quality by modifying fatty acid composition and causing a reduction of polyphenols and oleic acid, the most important components of olive oil. However, high temperature effects are genotype dependent and each cultivar responds differently to this stress. We found that each of the tested cultivars responded differently to high temperatures environment; none were completely heat tolerant. The 'Koroneiki' cultivar was negatively affected by high temperatures in all analyzed parameters. In the 'Picholine' and 'Coratina' cultivars, fruit development and oil quality were negatively affected by the high temperatures of the HT site, but oil concentration remained unaffected. The 'Souri' cultivar responded negatively to the high temperature environment with regard to fruit development and oil concentration, but was relatively tolerant to high temperatures in terms of oil quality. In contrast, in the 'Barnea' cultivar, exposure to high temperatures reduced oil quality, but did not impair final fruit weight or oil concentration. Although our results should be treated cautiously since the experiment was carried out for only two years, we found that the tested cultivars were tolerant regarding the effect of a high temperature environment on some of the traits examined, while exhibiting sensitivity to others. This suggests that the response to a high temperature environment is different for each of these traits and indicates induction of three different signal transduction mechanisms, resulting in a reduction of fruit development, oil accumulation and oil quality. Different olive cultivars have developed a variety of mechanisms to deal with different aspects of high temperature damage. Elucidation of the mechanism of each of these three responses is a vital step in the process of developing a variety of olives tolerant to high temperature damage.

Supporting information

S1 Fig. Microscope image of 'Barnea' fruit in June 2017. The region of the mesocarp cell layers counted is marked.
(PPTX)

S2 Fig. Microscope images of the mesocarp cells of 'Barnea' (a) and 'Koroneiki' (b) sampled in June, July, September and at harvest-time of the 2017 season from Tirat Zvi (HT site) and Tzuba (MT site).
(PPTX)

S3 Fig. Mesocarp cell characterization in all five cultivars in September 2017. Microscope images of the mesocarp cells sampled in September are presented at the left with quantification of the average cell area and the number of cell layer as well as fruit weight at the time, are presented at the right. Error bars represent confidence limits ($\alpha = 0.05$). Asterisks represent significant difference ($\alpha = 0.05$).
(PPTX)

S4 Fig. Oil accumulation in 'Barnea' (a) and 'Koroneiki' (b) cultivars during 2017. Microscope images of the mesocarp cells sampled in June, July and September of 2017 season from Tirat Zvi (HT site) and Tzuba (MT site).
(PPTX)

S5 Fig. Oil content in the mesocarp in all five cultivars in September 2017. Microscope images of oil drops in fruits sampled at September are presented at the left and quantification of the average oil drop area, the density of oil drops as well as the oil content in September 2017 are presented at the right. Error bars represent confidence limits ($\alpha = 0.05$). Asterisks represent significant difference ($\alpha = 0.05$).
(PPTX)

S1 Table. Maximum and average of the daily maximum and minimum temperature at both locations for each month, in years 2016 and 2017
(DOCX)

S2 Table. Climate data on both seasons at both locations.
(DOCX)

S3 Table. Maturity index at harvest of all cultivars at both locations and years.
(DOCX)

S4 Table. Two-way ANOVA test. a., f. The probability of the effects of cultivar type and tree location and their interaction, on the various parameters measured in this study, at two sampling dates in each year, 164 DPA and at harvest in 2016 and 146 DPA and at harvest time in 2017. Significant effects ($P<0.05$) are in bold font. **b-e, g-j.** Tukey-Kramer test, ranking the differences in dry fruit weight and dry fruit oil content between locations in the tested cultivars in 2016 at 164 DPA (b-c) and at harvest (d-e) and in 2017, at 146 DPA (g-h) and at harvest (i-j). The values appearing in the tables b-e and g-j are the added value in performance at the MT site compared to the HT site, calculated as a proportion of the HT results.
(DOCX)

S5 Table. Fruit weight, oil percentage and oil per fruit in all cultivars during 2016 and 2017. Errors are confidence limits ($\alpha = 0.05$).
(DOCX)

S6 Table. Correlation between Tmax, Tmean and Tmin to dry fruit weight and dry fruit oil percentage. The monthly gain of dry fruit weight and dry fruit oil percentatge for each cultivar, at each site for both years and was correlated to the temperatures data for each month (average daily maximum temp.–Tmax; average daily temp.–Tmean; average daily minimum

temp.–Tmin). Correlation coefficient was calculated for all cultivars or for heat sensitive cultivars only. Significant correlation coefficients are highlighted in red font.
(DOCX)

S7 Table. Olive oil composition in both climate regions during 2016 and 2017.
(DOCX)

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Author Contributions

Data curation: Yael Nissim, Maya Shloberg, Iris Biton, Yair Many, Adi Doron-Faigenboim, Hanita Zemach, Benjamin Avidan, Giora Ben-Ari.

Formal analysis: Giora Ben-Ari.

Methodology: Giora Ben-Ari.

Project administration: Yael Nissim, Giora Ben-Ari.

Resources: Ran Hovav, Giora Ben-Ari.

Supervision: Ran Hovav, Zohar Kerem, Giora Ben-Ari.

Validation: Yael Nissim, Giora Ben-Ari.

Visualization: Giora Ben-Ari.

Writing – original draft: Giora Ben-Ari.

Writing – review & editing: Giora Ben-Ari.

References

1. Gray SB, Brady SM. Plant developmental responses to climate change. Developmental Biology. 2016; 419(1):64–77. https://doi.org/10.1016/j.ydbio.2016.07.023 PMID: 27521050
2. Ismail AM, Hall AE. Reproductive-Stage Heat Tolerance, Leaf Membrane Thermostability and Plant Morphology in Cowpea Research partially supported by USDA NRICGP Award No. 98-35100-6129 and USAID Grant No. DAN-G-SS-86-00008-00 to AEH. The opinions and recommendations are those of the authors and not necessarily those of USAID. Crop Science. 1999; 39(6):1762–8. https://doi.org/10.2135/cropsci1999.3961762x
3. Vollenweider P, Günthardt-Goerg MS. Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environmental Pollution. 2005; 137(3):455–65. https://doi.org/10.1016/j.envpol.2005.01.032 PMID: 16005758
4. Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N. Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology. 2002; 48(5):667–81. https://doi.org/10.1023/a:1014826730024
5. P. Wilhelm E, Mullen R, Keeling P, Singletary G. Heat stress during grain filling in maize: effects on kernel growth and metabolism. Crop Science. 1999; 39:1733–41. https://doi.org/10.2135/cropsci1999.3961733x
6. Camejo D, Rodríguez P, Angeles Morales M, Miguel Dell'Amico J, Torrecillas A, Alarcón JJ. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. Journal of Plant Physiology. 2005; 162(3):281–9. https://doi.org/10.1016/j.jplph.2004.07.014 PMID: 15832680
7. Vara Prasad PV, Craufurd PQ, Summerfield RJ. Fruit Number in Relation to Pollen Production and Viability in Groundnut Exposed to Short Episodes of Heat Stress. Annals of Botany. 1999; 84(3):381–6. https://doi.org/10.1006/anbo.1999.0926
8. Fahad S, Hussain S, Saud S, Hassan S, Tanveer M, Ihsan MZ, et al. A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes

of rice. Plant Physiology and Biochemistry. 2016; 103:191–8. https://doi.org/10.1016/j.plaphy.2016.03.001 PMID: 26995314

9. Ferris R, Ellis RH, Wheeler TR, Hadley P. Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Annals of Botany. 1998; 82(5):631–9. https://doi.org/10.1006/anbo.1998.0740.
10. Rainey KM, Griffiths PD. Evaluation of Phaseolus acutifolius A. Gray plant introductions under high temperatures in a controlled environment. Genetic Resources and Crop Evolution. 2005; 52(2):117–20. https://doi.org/10.1007/s10722-004-1811-2
11. Barnabas B, Jager K, Feher A. The effect of drought and heat stress on reproductive processes in cereals. Plant, cell & environment. 2008; 31(1):11–38. Epub 2007/11/01. https://doi.org/10.1111/j.1365-3040.2007.01727.x PMID: 17971069.
12. Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in plant science. 2013; 4:273–. https://doi.org/10.3389/fpls.2013.00273 PMID: 23914193.
13. Bray EA, Bailey-Serres J, Weretilnyk E. Biochemistry and molecular biology of plants, responses to abiotic stresses. American Society of Plant Biologists. 2000:1158–249.
14. Guy C. Molecular responses of plants to cold shock and cold acclimation. Journal of molecular microbiology and biotechnology. 1999; 1:231–42. PMID: 10943554
15. Weis E, Berry JA. Plants and high temperature stress. Symposia of the Society for Experimental Biology. 1988; 42:329–46. Epub 1988/01/01. PMID: 3077863.
16. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, et al. Crop production under drought and heat stress: plant responses and management options. Frontiers in Plant Science. 2017; 8(1147). https://doi.org/10.3389/fpls.2017.01147 PMID: 28706531
17. Hartmann H, editor Growth of the olive fruit. Proceedings of the American Society for Horticultural Science; 1949: American society horticultural science 701 north Saint Asaph Street, Alexandria, VA 22314–1998.
18. Lavee S. Olive. In: Monselise SP, editor. Handbook of fruit set and development. Boca Raton, Florida USA: CRC Press Inc.; 1986. p. 261–76.
19. Rallo P, Rapoport H. Early growth and development of the olive fruit mesocarp. The Journal of Horticultural Science and Biotechnology. 2001; 76(4):408–12. https://doi.org/10.1080/14620316.2001.11511385
20. Hammami SBM, Manrique T, Rapoport HF. Cultivar-based fruit size in olive depends on different tissue and cellular processes throughout growth. Scientia Horticulturae. 2011; 130(2):445–51. https://doi.org/10.1016/j.scienta.2011.07.018.
21. Andrea B, Giovanni C, Fabio M, Riccardo G. Oil accumulation in intact olive fruits measured by near infrared spectroscopy–acousto-optically tunable filter. Journal of the Science of Food and Agriculture. 2013; 93(6):1259–65. https://doi.org/10.1002/jsfa.5899 PMID: 23023831
22. Conde C, Delrot S, Gerós H. Physiological, biochemical and molecular changes occurring during olive development and ripening. Journal of Plant Physiology. 2008; 165(15):1545–62. https://doi.org/10.1016/j.jplph.2008.04.018 PMID: 18571766
23. Gucci R, Lodolini EM, Rapoport HF. Water deficit-induced changes in mesocarp cellular processes and the relationship between mesocarp and endocarp during olive fruit development. Tree Physiology. 2009; 29(12):1575–85. https://doi.org/10.1093/treephys/tpp086 PMID: 19825868
24. Tombesi A, editor Olive fruit growth and metabolism1994: International Society for Horticultural Science (ISHS), Leuven, Belgium.
25. Breton C, Souyris I, Villemur P, Berville A. Oil accumulation kinetic along ripening in four olive cultivars varying for fruit size. OCL. 2009; 16(1):58–64.
26. Hartmann HT. Growth of the olive fruit. Proceedings of the American Society for Horticultural Science. 1949; 54:86–94.
27. Lavee S, Wodner M. Factors affecting the nature of oil accumulation in fruit of olive (Olea europaea L.) cultivars. Journal of Horticultural Science. 1991; 66(5):583–91. https://doi.org/10.1080/00221589.1991.11516187
28. Matteucci M, D'Angeli S, Errico S, Lamanna R, Perrotta G, Altamura MM. Cold affects the transcription of fatty acid desaturases and oil quality in the fruit of Olea europaea L. genotypes with different cold hardiness. J Exp Bot. 2011; 62(10):3403–20. Epub 02/28. https://doi.org/10.1093/jxb/err013 PMID: 21357772.
29. Servili M. Olive oil processing technologies and investments. In: Caballero J, D'Andria R, Fernández M, Garrido A, Rallo L, Arroyo López FN, et al., editors. Present and future of the Mediterranean olive

sector. Options Méditerranéennes: Série A. Séminaires Méditerranéens. 106: Zaragoza: CIHEAM / IOC; 2013. p. 55–66.

30. García-Inza GP, Castro DN, Hall AJ, Rousseaux MC. Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid composition in olive (Olea europaea L. var. 'Arauco'). European Journal of Agronomy. 2014; 54:107–15. https://doi.org/10.1016/j.eja.2013.12.005.

31. Trentacoste ER, Puertas CM, Sadras VO. Modelling the intraspecific variation in the dynamics of fruit growth, oil and water concentration in olive (Olea europaea L.). European Journal of Agronomy. 2012; 38:83–93. https://doi.org/10.1016/j.eja.2012.01.001.

32. Miserere A, Searles PS, García-Inza GP, Rousseaux MC. Elevated temperature affects vegetative growth and fruit oil concentration in olive trees (Olea europaea). ActaHortic2018. p. 523–8.

33. Rondanini DP, Castro DN, Searles PS, Rousseaux MC. Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. European Journal of Agronomy. 2014; 52:237–46. https://doi.org/10.1016/j.eja.2013.09.002.

34. García-Inza GP, Castro DN, Hall AJ, Rousseaux MC. Opposite oleic acid responses to temperature in oils from the seed and mesocarp of the olive fruit. European Journal of Agronomy. 2016; 76:138–47. https://doi.org/10.1016/j.eja.2016.03.003.

35. García-Inza GP, Hall AJ, Rousseaux MC. Proportion of oleic acid in olive oil as influenced by the dimensions of the daily temperature oscillation. Scientia Horticulturae. 2018; 227:305–12. https://doi.org/10.1016/j.scienta.2017.09.030.

36. Lombardo N, Marone E, Alessandrino M, Godino G, Madeo A, Fiorino P. Influence of growing season temperatures in the fatty acids (FAs) of triacilglycerols (TAGs) composition in Italian cultivars of Olea europaea. Advances in Horticultural Science. 2008; 22(1):49–53.

37. Mailer RJ, Ayton J, Graham K. The Influence of Growing Region, Cultivar and Harvest Timing on the Diversity of Australian Olive Oil. Journal of the American Oil Chemists' Society. 2010; 87(8):877–84. https://doi.org/10.1007/s11746-010-1608-8

38. Goldental-Cohen S, Biton I, Many Y, Tavrizov K, Dourou AM, Zemach H, et al. Removal of flowers or inflorescences affects 'Barnea' olive fruitlet post-anthesis abscission. The Journal of Horticultural Science and Biotechnology. 2019; 94(4):488–98. https://doi.org/10.1080/14620316.2018.1562313

39. Uceda M, Frías L. Épocas de recolección. Evolución del contenido graso del fruto y de la composición y calidad del aceite. Proceedings of II Seminario Oleícola Internationa. Córdoba, Spain: IOOC; 1975.

40. Mateos R, Espartero JL, Trujillo M, Ríos JJ, León-Camacho M, Alcudia F, et al. Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. Journal of Agricultural and Food Chemistry. 2001; 49(5):2185–92. https://doi.org/10.1021/jf0013205 PMID: 11368575

41. SAS Institute. JMP® version 7 User's Guide. SAS Institute Inc. Cary, NC. 2007.

42. Galla G, Barcaccia G, Ramina A, Collani S, Alagna F, Baldoni L, et al. Computational annotation of genes differentially expressed along olive fruit development. BMC Plant Biology. 2009; 9(1):128. https://doi.org/10.1186/1471-2229-9-128 PMID: 19852839

43. Inglese P, Barone E, Gullo G. The effect of complementary irrigation on fruit growth, ripening pattern and oil characteristics of olive (Olea europaea L.) cv. Carolea. Journal of Horticultural Science. 1996; 71 (2):257–63. https://doi.org/10.1080/14620316.1996.11515404

44. Atkinson CJ, Taylor L, Taylor JM, Lucas AS. Temperature and irrigation effects on the cropping, development and quality of 'Cox's Orange Pippin' and 'Queen Cox' apples. Scientia Horticulturae. 1998; 75 (1):59–81. https://doi.org/10.1016/S0304-4238(98)00117-4.

45. Austin PT, Hall AJ, Gandar PW, Warrington IJ, Fulton TA, Halligan EA. A Compartment Model of the Effect of Early-season Temperatures on Potential Size and Growth of 'Delicious' Apple Fruits. Annals of Botany. 1999; 83(2):129–43. https://doi.org/10.1006/anbo.1998.0804

46. Léchaudel M, Génard M, Lescourret F, Urban L, Jannoyer M. Modeling effects of weather and source–sink relationships on mango fruit growth. Tree Physiology. 2005; 25(5):583–97. https://doi.org/10.1093/treephys/25.5.583 PMID: 15741151

47. Marsh KB, Richardson AC, Macrae EA. Early- and mid-season temperature effects on the growth and composition of satsuma mandarins. The Journal of Horticultural Science and Biotechnology. 1999; 74 (4):443–51. https://doi.org/10.1080/14620316.1999.11511135

48. Bertin N. Analysis of the tomato fruit growth response to temperature and plant fruit load in relation to cell division, cell expansion and DNA endoreduplication. Annals of botany. 2005; 95(3):439–47. Epub 12/06. https://doi.org/10.1093/aob/mci042 PMID: 15582899.

49. Fanwoua J, de Visser P, Heuvelink E, Angenent G, Yin X, Marcelis L, et al. Response of Cell Division and Cell Expansion to Local Fruit Heating in Tomato Fruit. Journal of the American Society for Horticultural Science. 2012; 137(5):294–301.

50. van der Merwe R, Labuschagne MT, Herselman L, Hugo A. Effect of heat stress on seed yield components and oil composition in high- and mid-oleic sunflower hybrids. South African Journal of Plant and Soil. 2015; 32(3):121–8. https://doi.org/10.1080/02571862.2015.1018354

51. Goldman SD. The Effects of Heat Stress on High Oil Corn: Western Kentucky University; 2005.

52. Alowaiesh B, Singh Z, Fang Z, Kailis SG. Harvest time impacts the fatty acid compositions, phenolic compounds and sensory attributes of Frantoio and Manzanilla olive oil. Scientia Horticulturae. 2018; 234:74–80. https://doi.org/10.1016/j.scienta.2018.02.017.

53. Mougiou N, Trikka F, Trantas E, Ververidis F, Makris A, Argiriou A, et al. Expression of hydroxytyrosol and oleuropein biosynthetic genes are correlated with metabolite accumulation during fruit development in olive, Olea europaea, cv. Koroneiki. Plant Physiology and Biochemistry. 2018; 128:41–9. https://doi.org/10.1016/j.plaphy.2018.05.004 PMID: 29753981

54. Arnon D, Amos N, Alon B-G, Guy H, Isaac Z, Doron S, et al. The effect of water stress on super-high-density 'Koroneiki' olive oil quality. Journal of the Science of Food and Agriculture. 2015; 95(10):2016–20. https://doi.org/10.1002/jsfa.6913 PMID: 25214192

55. Dag A, Ben-Gal A, Yermiyahu U, Basheer L, Nir Y, Kerem Z. The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed 'Souri' olive orchard converted to irrigation. Journal of the Science of Food and Agriculture. 2008; 88(9):1524–8. https://doi.org/10.1002/jsfa.3243 WOS:000257176500005.

56. Alagna F, Mariotti R, Panara F, Caporali S, Urbani S, Veneziani G, et al. Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC plant biology. 2012; 12:162–. https://doi.org/10.1186/1471-2229-12-162 PMID: 22963618.

57. Cirilli M, Caruso G, Gennai C, Urbani S, Frioni E, Ruzzi M, et al. The Role of Polyphenoloxidase, Peroxidase, and β-Glucosidase in Phenolics Accumulation in Olea europaea L. Fruits under Different Water Regimes. Frontiers in plant science. 2017; 8:717–. https://doi.org/10.3389/fpls.2017.00717 PMID: 28536589.

58. Gómez-Rico A, Salvador MD, La Greca M, Fregapane G. Phenolic and Volatile Compounds of Extra Virgin Olive Oil (Olea europaea L. Cv. Cornicabra) with Regard to Fruit Ripening and Irrigation Management. Journal of Agricultural and Food Chemistry. 2006; 54(19):7130–6. https://doi.org/10.1021/jf060798r PMID: 16968073

59. Servili M, Esposto S, Lodolini E, Selvaggini R, Taticchi A, Urbani S, et al. Irrigation Effects on Quality, Phenolic Composition, and Selected Volatiles of Virgin Olive Oils Cv. Leccino. Journal of Agricultural and Food Chemistry. 2007; 55(16):6609–18. https://doi.org/10.1021/jf070599n PMID: 17636938

60. Tovar MJ, Romero MP, Girona J, Motilva MJ. L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (Olea europaea L cv Arbequina) fruit grown under different irrigation regimes. Journal of the Science of Food and Agriculture. 2002; 82(8):892–8. https://doi.org/10.1002/jsfa.1122

61. Hernández ML, Padilla MN, Sicardo MD, Mancha M, Martínez-Rivas JM. Effect of different environmental stresses on the expression of oleate desaturase genes and fatty acid composition in olive fruit. Phytochemistry. 2011; 72(2):178–87. https://doi.org/10.1016/j.phytochem.2010.11.026.

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A High Temperature Environment Regulates the Olive Oil Biosynthesis Network

Yael Nissim [1], Maya Shlosberg [1,2], Iris Biton [1], Yair Many [1], Adi Doron-Faigenboim [1], Ran Hovav [1], Zohar Kerem [2], Benjamin Avidan [1] and Giora Ben-Ari [1,*]

[1] Institute of Plant Sciences, ARO, The Volcani Center, Rishon LeZion 7528809, Israel; yaelns@volcani.agri.gov.il (Y.N.); Maya.shlosberg@mail.huji.ac.il (M.S.); ivrb28@volcani.agri.gov.il (I.B.); yairm@volcani.agri.gov.il (Y.M.); adif@volcani.agri.gov.il (A.D.-F.); ranh@volcani.agri.gov.il (R.H.); vhavidan@volcani.agri.gov.il (B.A.)

[2] Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; zohar.kerem@mail.huji.ac.il

* Correspondence: giora@agri.gov.il

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Abstract: Climate change has been shown to have a substantial impact on agriculture and high temperatures and heat stress are known to have many negative effects on the vegetative and reproductive phases of plants. In a previous study, we addressed the effects of high temperature environments on olive oil yield and quality, by comparing the fruit development and oil accumulation and quality of five olive cultivars placed in high temperature and moderate temperature environments. The aim of the current study was to explore the molecular mechanism resulting in the negative effect of a high temperature environment on oil quantity and quality. We analyzed the transcriptome of two extreme cultivars, 'Barnea', which is tolerant to high temperatures in regard to quantity of oil production, but sensitive regarding its quality, and 'Souri', which is heat sensitive regarding quantity of oil produced, but relatively tolerant regarding its quality. Transcriptome analyses have been carried out at three different time points during fruit development, focusing on the genes involved in the oil biosynthesis pathway. We found that heat-shock protein expression was induced by the high temperature environment, but the degree of induction was cultivar dependent. The 'Barnea' cultivar, whose oil production showed greater tolerance to high temperatures, exhibited a larger degree of induction than the heat sensitive 'Souri'. On the other hand, many genes involved in olive oil biosynthesis were found to be repressed as a response to high temperatures. *OePDCT* as well as *OeFAD2* genes showed cultivar dependent expression patterns according to their heat tolerance characteristics. The transcription factors *OeDof4.3*, *OeWRI1.1*, *OeDof4.4* and *OeWRI1.2* were identified as key factors in regulating the oil biosynthesis pathway in response to heat stress, based on their co-expression characteristics with other genes involved in this pathway. Our results may contribute to identifying or developing a more heat tolerant cultivar, which will be able to produce high yield and quality oil in a future characterized by global warming.

Keywords: high temperature; olive; oil; gene expression; biosynthesis

1. Introduction

Abiotic stresses, including high temperatures have a substantial negative impact on the reproductive phase of the plant. Oilseed crops are also negatively affected by heat stress, which has been shown to reduce starch, protein and oil content [1,2]. Plants exposed to temperatures above their

optimal growing temperatures, exhibit cellular and metabolic responses which enable the plants to survive [3–7]. The level of damage to crops caused by high temperatures depends on many parameters. The reproductive phase of plant development is more sensitive to high temperatures than the vegetative phase, causing a reduction in yield [8]. Yield reduction in response to heat stress or high temperatures has been reported in wheat, peanut, rice bean and tomato [9–13]. Olive oil accumulation in the mesocarp cells of the fruit is influenced by cultivar type and climactic conditions. It occurs mainly during the summer and decreases during fruit ripening in the fall [14,15].

Olive oil quality is characterized by its sensorial and nutritional properties. Its health benefits are due to both its fatty acid composition, especially oleic acid concentration and minor compounds such as polyphenols. These are strongly affected by factors such as cultivar type, degree of ripeness, fruit load, and soil quality [16]. Several studies have examined the effect of high temperatures on olive oil accumulation [17–20] and olive oil fatty acid composition [17,19,21–24]. Oleic acid concentration in olive oil was found to be negatively affected by a high temperature environment [17].

1.1. Heat-Shock Proteins

Plants interact with climatic factors such as heat stress by triggering certain mechanisms of defense such as a specific gene expression program. A response to heat stress on the molecular level is found in all living organisms and results in an increase in the induction and synthesis of a group of proteins called heat-shock proteins [25]. Heat-shock proteins positively regulate antioxidant enzymes which detoxify reactive oxygen species. They also enhance plant immunity by the accumulation and stability of pathogenesis-related proteins produced under biotic stresses [26]. During the onset of stress, plants reduce normal protein production, and transcribe and translate heat-shock proteins. Heat-shock proteins have been reported in a wide range of organisms and are highly conserved [26]. Based on molecular weight, HSPs are generally classified into the following sub-families: HSP100, HSP90, HSP70, HSP60, and small HSPs. Several studies have indicated that many high molecular weight HSPs exhibited a response to high-temperature stress [26]. Among the many heat-shock proteins, HSP70s and HSP60s whose response to heat stress has been most intensively studied, have been shown to maintain proper folding under conditions of heat stress with the aid of ATPs [26]. Hsp70s are a class of highly conserved proteins which act as molecular chaperones and play a crucial role in protecting the plant cells from the harmful effects of heat stress. Hsp70 is known to accumulate in heat-stressed tissue and overexpression of Hsp70 was shown to enhance tolerance to heat stress in several plant species including brassica, tobacco and rice [27–30].

1.2. Olive Oil Biosynthesis

Fatty acid biosynthesis begins with Acetyl-CoA carboxylase (ACCase) catalyzing the formation of malonyl-CoA from acetyl-CoA by the ACCase enzyme, which is a complex consisting of three separate proteins or domains: BC, CT and BCCP. ACCase has two isoforms, the prokaryotic enzyme is a heteromeric multisubunit complex (htACC). In eukaryotic cells, the enzyme takes the form of a homomeric multidomain polypeptide (hmACC). The hmACC is a single multifunctional polypeptide, with all three domains forming part of a single polypeptide (ACC1). htACC consists of four separate proteins, BC, BCCP, and CT, which is a heterodimer with α and β subunits. The majority of plants require both types of ACCase, localizing htACC in plastids and hmACC in the cytosol [31]. The MCAT enzyme catalyzes formation of malonyl-ACP from malonyl-CoA. Fatty acids are subsequently produced by a complex of six enzymes referred to as fatty acid synthase (FAS). This complex includes KAS I, KASII and KASIII, KAR, HAD and ENR enzymes. The first step is the condensation of malonyl-ACP with acetyl- CoA by the action of KAS III to produce acetoacetyl-ACP in the plastid. This subsequently forms a β-hydroxyacyl derivative by KAR, which is then dehydrated by HAD and finally reduced by ENR, resulting in a four-carbon acyl-ACP derivative. This forms 3-Katoacyle-ACP by the action of KAS I which can undergo five more chain elongation cycles. The final product of these cycles is palmitoyl-ACP with 16 carbons (C16:0-ACP). Finally, this compound can be elongated to form stearoyl-ACP (C18:0) by KAS II. The most abundant fatty acid in olive oil, oleic acid, is formed by SAD enzyme. The release of the acyl segment from the ACP derivatives is

performed by FATA and FATB. These thioesterases show different substrate specificities. FATA preferentially hydrolyzes the 18:1 acyl-ACP, whereas the FATB type is most active with 16:0 and 18:0 acyl-ACPs. The process is localized on the endoplasmic reticulum (ER) by a reaction catalyzed by an oleate desaturase (FAD2) to form linoleic acid (18:2) from oleic acid 18:1. Further desaturation may occur, catalyzed by FAD3 to produce α-linolenic acid (18:3). The assembly of triacylglycerol (TAG) from glycerol-3-phosphate and fatty acids take place on the ER, by the Kornberg–Pricer acylation reactions followed by the Kennedy pathway mediated by the enzymes GPDH, GPAT, LPAAT and PP to form diacylglycerol (DAG). Finally, DAG is an important precursor for synthesis of phospholipids such as PC or storage lipids such as TAG. In order to form TAG, DAG is acylated at the third position by a diacylglycerol acyltransferases (DGATs) or by a phospholipid; diacylglycerol acyltransferase (PDAT) [32]. Another pathway for the DAG is through reactions of phosphatidylcholine diacylglycerol cholinephosphotransferase (PDCT), in which the acyl groups enter PC and can then return to DAG after they are desaturated or otherwise modified on PC [33].

1.3. Transcription Factors Regulate Olive Oil Biosynthesis

Regulation of oil biosynthesis by transcription factors (TFs) has been widely investigated and many transcription factors have been reported to affect TAG production [34]. *Leafy cotyledon 1* (*LEC1*) and *LEC1-LIKE* (*L1L*) TFs have been reported to regulate fatty acid synthesis through ACCase in *Arabidopsis*, maize and bean. *DOF4* and *DOF11* TFs were found to regulate fatty acid synthesis through ACCase, *ENR* and *KASII* in *Arabidopsis* and soybean. *MYB73* was reported to regulate fatty acid synthesis through *FATB* and KAS genes in *Arabidopsis* and *Camelina sativa* [34]. However, *WRINKLED1* (*WRI1*) transcription factor is vital to synthesis, and is the "master regulator" of the transcriptional control of the plant oil biosynthesis pathway [35]. Several other TFs were reported to control fatty acid biosynthesis not directly through the biosynthesis genes, but through other genes or transcription factors. These TFs include *LEC2*, *FUS3*, *ABI3*, several bZIP TFs, *MYB96*, *MYB30*, *PICKLE*, *VAL1*, *VAL2*, *ASIL1* and *AP2* [34].

We have lately addressed the effect of high temperature environments on olive oil yield and quality, by comparing the fruit development and oil accumulation and quality of five olive cultivars placed in high temperature (HT) and low temperature (MT) environments [36]. We found that the effects of a high temperature environment are genotype dependent and in general, high temperatures during fruit development decreased oil accumulation and quality. 'Barnea' cultivar final oil content was not affected by the high temperature environment, but 'Souri' cultivar exhibited a dramatic decrease of 8% in oil concentration calculated as a percentage of dry fruit weight at harvest in response to high ambient temperatures. Regarding the quality of oil produced, the 'Souri' cultivar proved more tolerant to a high temperature environment than any other of the tested cultivars. The 'Barnea' oil had a polyphenol level of 404 mg/g oil in the MT environment and only 156 mg/g oil in the HT environment. In addition, the 'Barnea' oil showed a decrease of 4.8% in oleic acid concentration of oil extracted from fruit grown in HT conditions compared to the MT environment. The 'Souri' cultivar oil contained high polyphenol levels of 905 and 772 mg/g oil in the MT and HT environments respectively. In addition, the 'Souri' oil showed a decrease of only 3.7% in oleic acid concentration of oil extracted from fruit grown under HT conditions compared to oil from MT fruit. The aim of the current study was to explore the molecular mechanism underlying the negative effect of the high temperature environment on oil quantity and quality. In order to identify the molecular mechanism and the possible blocks, in which HT negatively affects oil accumulation and quality, we analyzed the transcriptome of the two extreme cultivars, 'Barnea' and 'Souri'. Transcriptome analyses were carried out at three different time points during fruit development, focusing on the genes involved in the oil biosynthesis pathway.

2. Materials and Methods

2.1. Experimental Design and Plant Material

The olive cultivars 'Barnea', 'Coratina', 'Koroneiki', 'Souri' and 'Picholine' were used in this study. Of these, 'Barnea', which is tolerant to HT environment, regarding fruit development and oil accumulation and 'Souri', tolerant to HT environment, regarding oil quality, were used for transcriptome analysis. Twelve olive trees in 50 L pots of each of the five cultivars were placed in an extremely hot climate zone at Tirat Zvi village (HT environment) and in a more temperate environment (MT environment) during 2016 and 2017. Experiment design and all measurements were described previously in detail [36]. During 2017, the mesocarp of five fruits from each of the six trees was collected and three replicates of mesocarp from a mix of ten fruits from two trees each were used for transcriptome analysis. Mesocarp was sampled at three time points during the season, at 83, 104 and 146 days post anthesis (DPA).

2.2. Transcriptome Analysis

Fruits were removed from the tree, the mesocarp of a pool of 10 fruits from two trees was excised, mixed in a tube and immediately frozen in liquid nitrogen. Total RNA was extracted from olive mesocarp tissue using Sigma (Sigma Aldrich, Darmstadt, Germany) plant total RNA extraction kit according to the manufacturer's instructions. DNA was removed by treatment with RNAase-free DNAase at 37 °C for 30 min. Total RNA samples were used to prepare 24 cDNA libraries of the two cultivars ('Barnea' and 'Souri') at three time points during fruit development (83, 104 and 146 DPA) in two locations (HT and MT environments) and two biological replicates, using Illumina's TruSeq RNA library preparation kit according to the manufacturer's instructions. cDNA libraries were sequenced on NextSeq high output 75 cycles. Sequencing was performed by the Sequencing unit of the Crown Institute for Genomics in the Weizmann Institute of Science, Israel. Raw reads were subjected to a filtering and cleaning procedure using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/, version 0.0.13.2) for: (1) trimming read-end nucleotides with quality scores <30 using fastq_quality_trimmer; (2) removing reads with less than 70% base pairs with quality score ≤30 using fastq_quality_filter. Cleaned reads, obtained after processing, were assembled de novo using Trinity software (GitHub Inc., San Francisco, CA, USA) [37] with trimmomaticSE option to remove adaptors [38]. Only transcripts with a minimum length of 200 bp were analyzed against OE6 transcriptome reference. The transcript quantification (the number of reads per gene) from RNA-Seq data was performed using bowtie2 aligner [39] and the Expectation-Maximization method (RSEM) [40]

2.3. RT-PCR

Specific primers were designed using Primer3 software (Elixir, Narva maantee, Estonia) [41]. Primers for the various genes were designed to amplify a region containing more than one exon, so that genomic DNA would not be amplified. A total of 2.5 μg RNA from each sample was treated with RQ DNase (Promega, Madison, WI, USA) and reverse-transcribed using random hexamer primers (Promega). Real-time PCR was carried out using the SYBR green amplification kit (ABgene, Blenheim Road, Epsom, UK) according to manufacturer's instructions. Each reaction contained 1 μL cDNA and 1 μM of each primer from the relevant primer pair in a final volume of 10 μL. Quantification of real-time PCR products was carried out by detection of SYBR green fluorescence on a StepOnePlus™ system (Applied Biosystems, Foster City, CA, USA). Dilution series of cDNA were created for each set of and a standard curve was established for each gene. Triplicate of cDNA were used and each reaction was subjected to melting-point analysis to confirm single amplified products. At least two biological repeats were carried out for each gene. Transcript levels were estimated using a standard curve for each gene, and these levels were normalized against the amount of *OeACTIN* transcript level in each sample. The sequences of the primers used are listed in the Table S1.

2.4. Data Analysis

Differential expression analysis was done with edgeR package (Bioconductor, Buffalo, NY, USA) [42], transcripts that were more than two fold differentially expressed with false discovery corrected statistical significance of at most 0.01 were considered differentially expressed [43]. Hierarchical clustering of gene expression and visualization of heat map were performed using R Bioconductor (Bioconductor, Buffalo, NY, USA) [44], using the normalized log-FPKM values (Z score) of each gene. Venn diagrams were constructed using the online Venny 2.0 software (http://bioinfogp.cnb.csic.es/tools/venny/). From each gene family participating in the oil biosynthesis pathway, we presented only genes that showed a normalized expression level of at least 200 FPKM in at least one time-point and environment, assuming that genes with low expression levels are not involved in the biosynthesis pathway. Co-expression network analysis inferred by using Conet plugin of cytoscape software (Cytoscape.js, Toronto, ON, Canada) that was based on a Pearson correlation (cut-off of $r > 0.75$).

2.5. Statistical Analysis

RT-PCR statistical analyses were conducted using JMP software (SAS, Cary, NC, USA) [45]. The RT-PCR results were subjected to two-way analysis of variance (ANOVA) including full factorial analysis, for their dependence on the two independent variables of tree location and cultivar type, including the interaction between them. Since we encountered significant interaction between the two factors in all gene expression analysis, a Tukey-Kramer test, based on multiple comparison correction was performed, in order to compare the various levels of interaction.

3. Results

3.1. Gene Expression Regulation

In order to understand the mechanism in which high temperature summers affect olive oil biosynthesis we used transcriptome analysis. Two out of five analyzed [36] olive cultivars were chosen, 'Barnea' and 'Souri'. 'Barnea' exhibited tolerance to HT environment in term of oil concentration at harvest, but its oil quality dramatically decreased in an HT environment. 'Souri' exhibited sensitivity to HT environment in term of oil concentration at harvest, but proved more tolerant to HT in oil quality, compared to all the tested cultivars. Mesocarp transcriptomic of the two cultivars was analyzed at three time points during fruit development in HT and MT environments, 83, 104 and 146 days post anthesis (DPA).

The transcriptome sequencing resulted in an average of 21,748,879 reads for each sequenced sample. Among them, 96.54% were clean reads and 83.47% of the reads were significantly mapped to the olive genome [46] (Table S2). The sequencing data were deposited in the NCBI Sequence Read Archive (SRA) database as bioproject PRJNA638790.

Comparing expression levels of each gene in the HT and MT environments for each cultivar and each time point, identified the regulated genes in response to different environments. The 'Barnea' cultivar had 2437, 2795 and 3265 genes that were significantly upregulated in the HT environment at 83, 104 and 146 DPA respectively. The 'Souri' cultivar had 3009, 2625 and 3129 genes that were significantly upregulated in the HT environment at 83, 104 and 146 DPA respectively. The 'Barnea' cultivar had 2500, 2634 and 3473 genes that were significantly upregulated in the MT environment at 83, 104 and 146 DPA respectively. The 'Souri' cultivar had 2953, 3053 and 3174 genes that were significantly upregulated in the MT environment at 83, 104 and 146 DPA respectively. Among the differentially expressed genes, more genes were up-regulated at all three time points (1193, 1226, 1291 and 1362 for 'Barnea'-MT-Up, 'Barnea'-HT-Up, 'Souri'-MT-Up and 'Souri'-HT-Up respectively) compared to those that were unique for specific time point or common to only two time points (Figure S1a). Comparing the identity of the regulated genes between the three selected time points showed that the highest number of regulated genes is common at all three time points. However, when comparing the regulated genes between the two cultivars, we found that the number of regulated

genes that were cultivar specific in each of the two cultivars was similar to the number of regulated genes common to both cultivars, in all three time points (Figure S1b).

3.2. Heat-Shock Protein Genes are Upregulated in the HT Environment

Hierarchical clustering of all 110 heat-shock protein genes revealed that the gene expression profile of the heat-shock protein genes in 'Barnea' and 'Souri' in all three time points in the MT environment is similar (Figure 1). Most of these genes were expressed at low rates in both cultivars and at all three time points. In addition, The MT samples were separated by cultivar into two cluster groups. For the 'Souri', three time points in MT were clustered in one group whereas for 'Barnea', three time points in MT were clustered in another group. In the HT samples, 'Souri' at 104 DPA and 'Barnea' at 83 DPA clustered together and most of the heat-shock protein genes were expressed at low rates in these samples. 'Souri' at 83 and 146 DPA and 'Barnea' at 104 DPA were clustered in one group and most of the heat-shock protein genes were highly expressed. Above all, the heat-shock protein genes of 'Barnea' at 146 DPA in the HT environment were expressed at extremely high rates. Validation of the heat-shock protein gene expression pattern in HT and MT environments was carried out by RT-PCR analysis of the expression pattern of *OeHSP70* known to be induced by heat shock.

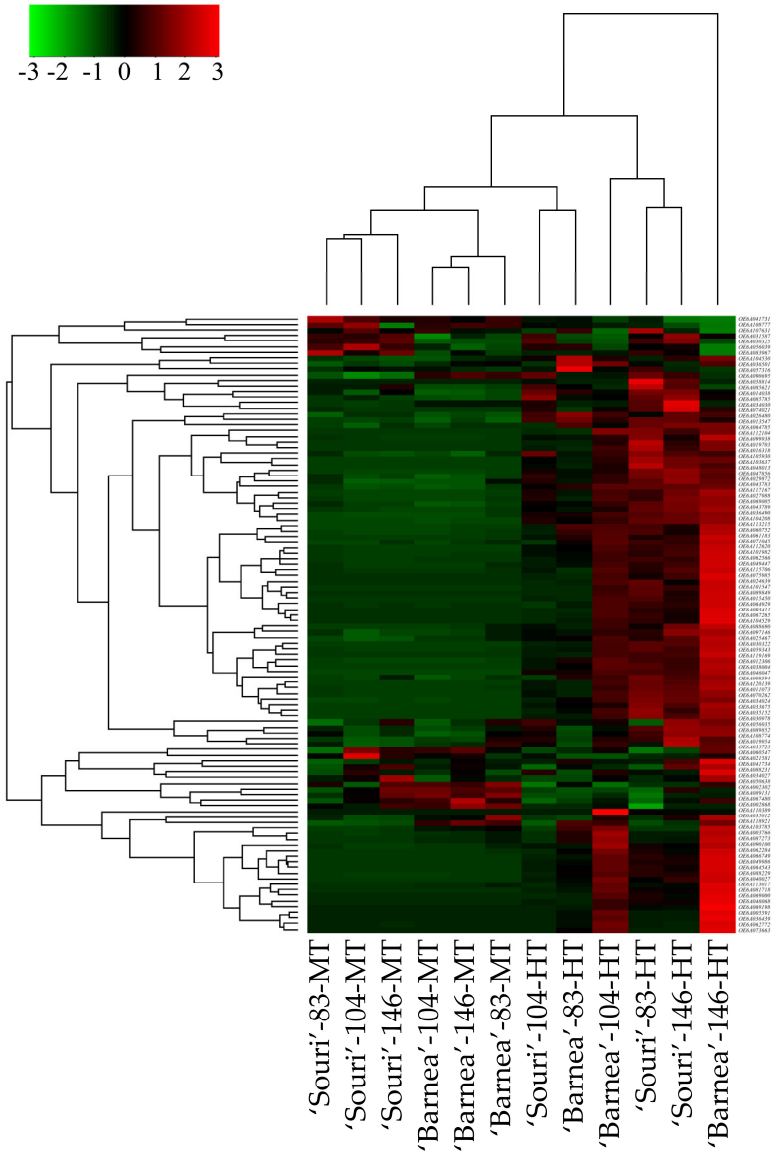

Figure 1. Hierarchical clustering of all heat-shock protein genes. Genes (rows) and samples (columns) were clustered according to their expression pattern. Sample names below include the cultivar-sample DPA-environment. Expression levels are indicated on an abundance scale of green to red.

Expression level of *OeHSP70* (*OE6A062772*) at 146 DPA, in all five cultivars [36] was analyzed (Figure S2). *OeHSP70* expression level in the MT environment in all five cultivars was similar and relatively low compared to its expression in the HT environment. The expression level of *OeHSP70* in the HT environment varied between the cultivars. The 'Souri' cultivar showed the lowest expression, 'Picholine' and 'Coratina' exhibited moderate expression, and 'Barnea' and 'Koroneiki' showed the highest expression, in which, 'Barnea' *OeHSP70* expression was found to be 8.7 times greater than *OeHSP70* expression in the 'Souri' cultivar in the HT environment.

3.3. The Effect of High Summer Temperatures on the Olive Oil Biosynthesis Pathway

The biochemical pathways leading to oil (triacylglycerol) synthesis in the olive fruit mesocarp involve many subcellular organelles and multiple enzymes, beginning with the precursor, acetyl-CoA and concluding with triacylglycerol (TAG). Based on the transcriptome analysis of 'Barnea' and 'Souri' at the two contrasting environments at three time points during fruit development, we focused on the gene expression pattern of all genes involved in olive oil biosynthesis (Figure 2). Figure 2 presents the expression pattern of the 'Souri' and the 'Barnea' cultivars. The following stages describe in detail oil synthesis in the 'Souri', a cultivar we have shown to be sensitive to a high temperature environment as reflected by oil biosynthesis [36]. The first step of the fatty acids biosynthesis is catalyzing the formation of malonyl- CoA from acetyl-CoA by Acetyl-CoA carboxylase (ACCase). Gene expression pattern of the genes involved in the multienzyme complex was similar in the HT and MT environments beside *OeBCCP1* (*OE6A092496*), which was induced in the HT environment. On the other hand, *OeACC1* (*OE6A049983*) was expressed three to six fold (depending on the sample date) higher in the MT environment compared to the HT. *OeMCAT*, which catalyzed malonyl-CoA was expressed similarly in both environments. Fatty acid synthase (FAS) is an easily dissociated multisubunit complex consisting of the monofunctional enzymes KASI, KASII, KASIII, KAR, HAD and ENR. *OeHAD*, *OeENR* and *OeKAR* were expressed similarly in the HT and MT environments at the first sample date (83 DPA), at which *OeKAR* expression was higher in the MT compared to the HT environment. However, *OeKASI*, *OeKASII* and *OeKASIII* expression was higher in the MT compared to the HT environment in all three sample dates. Both stearoyl-ACP Δ9-desaturase (SAD) family genes (*OE6A020845* and *OE6A118450*) were very highly expressed in both environments. The last stage of de novo fatty acid synthesis is regulated by the acyl-ACP thioesterases, FATA and FATB. The two FAT genes, *OeFATA* and *OeFATB* were induced in the MT environment in comparison to their expression in the HT. This is especially true for the *OeFATB* gene (*OE6A029754*), which is expressed at a threefold level in the MT environment compared to its expression in the HT at all three time points. The modification of oleic acid (18:1) into linoleic acid (18:2) and linolenic acid (18:3) is catalyzed by the fatty acid desaturases FAD2 and FAD3 respectively in the Endoplasmic Reticulum (ER). OeFAD3 genes were not expressed in our samples. However, OeFAD2 genes showed higher expression in the HT compared to the MT environment, especially regarding *OeFAD2-5* (*OE6A098403*) at the last sample date (146 DPA) and *OeFAD2-1* (*OE6A069627*) at the first sample date (83 DPA). The expression level of *OeFAD2-5* in the 'Souri' cultivar at 146 DPA was found to be 11,700 FPKM in the HT environment, significantly higher than the 1976 FPKM level found in the MT environment. Assembly of fatty acids onto the glycerol backbone to create triacylglycerol (TAG) begins with a series of reactions with dihydroxyacetone phosphate (DHAP) as a precursor and diacylglycerol (DAG) as the product with four enzymes involved (GPDH, GPAT, LPAAT and PP). The expression level of the main genes involved in this reaction, *OeGPDH* (*OE6A105859*) and *OeGPAT* (*OE6A109037*) was significantly higher in the MT environment compared to that in the HT. However, *OeLPAAT* gene family consists of one gene (*OE6A051077*) that was induced in the HT environment and another single gene (*OE6A111035*) significantly induced in the MT environment. *OePP* genes were marginally expressed in both environments at all three time points.

DAG induction can proceed along three different pathways, two of them with TAG as the final product, and one, regulated by PDCT, to return to the phosphatidylcholine pool. DAG can create TAG by the reaction of PDAT or by the reaction of the two DAG proteins, DGAT1 and DGAT2.

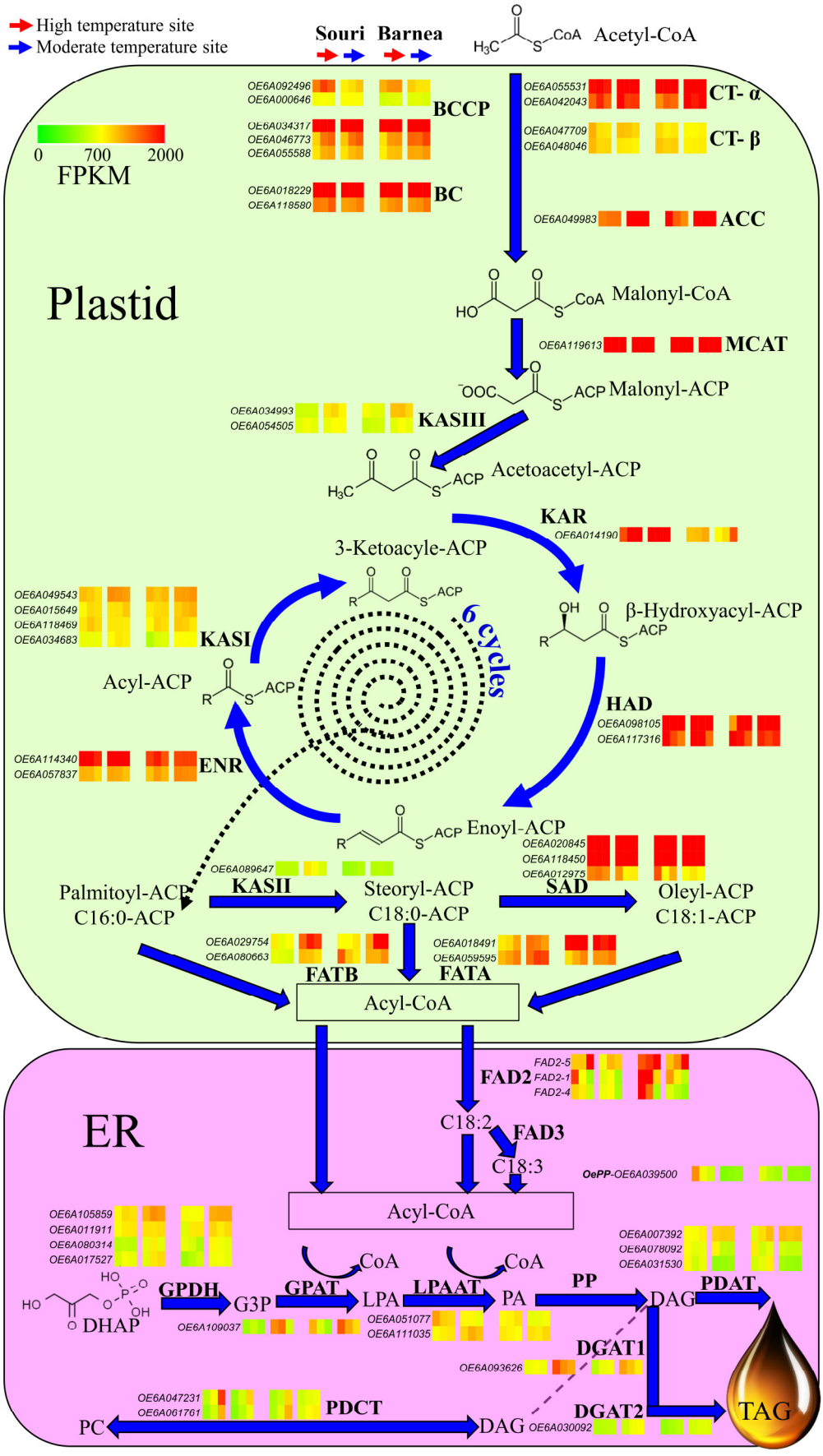

Figure 2. Expression pattern of the genes involved in the olive oil biosynthesis pathway in fruit from the HT site compared to that of fruit grown in the MT environment. Expression levels for each gene are presented in a green to red scale above or beside the name of the enzyme. For each enzyme, only

genes with a FPKM of above 200 in at least one time point and environment appears. For each gene, expression level at the three time points sampled at the two environments is presented. In each gene, the six left squares represent the expression pattern in the 'Souri' cultivar, whereas the six right squares represent the expression pattern in the 'Barnea' cultivar. Within each cultivar, the three left squares represent the expression level at 83, 104 and 146 DPA in the HT environment, whereas the three right squares represent the expression level at the same sample dates in the MT environment. The various cell components appear in different colors. The reaction begins with cytosol (white background), then advances to the plastid (light green background) and terminates at the endoplasmic reticulum (ER; purple background). Abbreviations: *BCCP*—biotin carboxyl carrier protein, *BC*—biotin carboxylase, *CT*—carboxyl transferase, *ACC*—acetyl-CoA carboxylase, *MCAT*—malonyl-CoA:ACP transacylase, *ACP*—acyl carrier protein, *KAS*—β-ketoacyl-ACP synthase, *KAR*—β-ketoacyl-ACP reductase, *HAD*—β-hydroxyacyl-ACP dehydrase, *ENR*—enoyl-ACP reductase, *SAD*—stearoyl-ACP desaturase, *FAT*—fatty acyl-ACP thioesterases, *FAD*—fatty acid desaturases, *GPDH*—glycerol 3-phosphate dehydrogenase, *GPAT*—glycerol 3-phosphate acyltransferase, *LPAAT*—lysophosphatidate acyltransferase, *PP*—phosphatidate phosphohydrolase, *PDCT*—phosphatidylcholine diacylglycerol cholinephosphotransferase, *DGAT*—diacylglycerol acyltransferase, *PDAT*—phospholipid; diacylglycerol acyltransferase, *DHAP*—dihydroxyacetone phosphate, *G3P*—glycerol 3-phosphate, *LPA*—lysophosphatidate, *PA*—phosphatidate, *DAG*—diacylglycerol, *PC*—phosphatidylcholine, *TAG*—triacylglycerol.

The *OePDAT* gene family consists of three genes that were highly expressed in our study. *OE6A007392* had a significantly higher expression level in the MT compared to the HT environment at all three time points, whereas *OE6A078092* and *OE6A031530* had a significantly higher expression level in the HT compare to the MT. *OeDGAT1* (*OE6A093626*) was significantly expressed at a higher level in the MT compare to the HT environment, especially at 83 DPA, whereas *OeDGAT2* (*OE6A030092*) expression was higher in the MT only at the latter two time points at 104 and 146 DPA. The two expressed *OePDCT* genes (*OE6A047231* and *OE6A061761*) showed higher expression levels in the HT compare to the MT environment at the time points 104 and 146 DPA.

The 'Barnea' gene expression pattern was similar to that of the 'Souri' with the same trends between the two environments (Figure 2). The exceptional genes were FATA, in which one, *OeFATA* (*OE6A018491*), was highly expressed in both environments at all three time points. The expression pattern of two *FAD2* genes, *OeFAD2-5* and *Oe-FAD2-1* was also different in the 'Barnea' cultivar compared to the 'Souri'. The 'Barnea' cultivar showed extremely high expression of *OeFAD2-5* in the HT environment at all three time points and relatively high expression in the MT environment, especially at 146 DPA. *Oe-FAD2-1* was very highly expressed in the first two sample dates (83 and 104 DPA) in the HT environment and moderately expressed in the MT environment at all time-points. The 'Barnea' cultivar showed the same trend in the expression of *OeDGAT1* and *OeDGAT2* in both environments as the 'Souri' cultivar, only in the 'Barnea' cultivar, the differences between the expression levels in the MT and HT environments were smaller. Unlike the 'Souri' cultivar, the 'Barnea' *OePDCT* genes were expressed similarly in both environments.

Validation of the oil biosynthesis gene expression pattern in HT and MT environment was carried out by RT-PCR analysis of the expression pattern of *OeACC1* and *OeFAD2-1* at 146 DPA, in all five cultivars [36] (Figure 3).

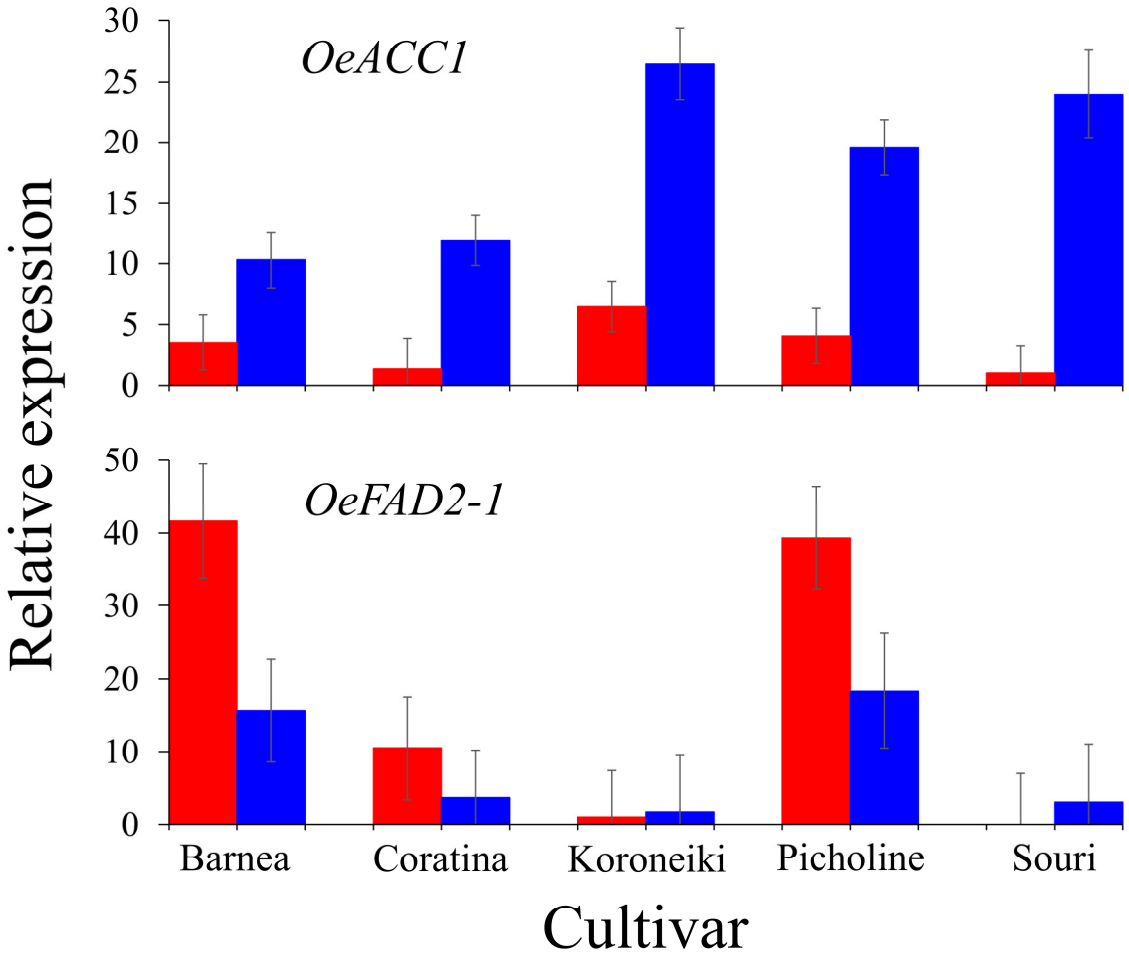

Figure 3. *OeACC1* and *OeFAD2-1* expression analyzed by RT-PCR at 146 DPA. Relative expression levels of *OeACC1* (above) and *OeFAD2-1* (below) in the five cultivars 'Barnea', 'Coratina', 'Koroneiki', 'Picholine' and 'Souri', in HT (red columns) and MT (blue columns) environments. Error bars represent confidence intervals ($p < 0.05$).

OeACC1 expression was significantly higher in the MT compared to the HT environment in all five cultivars, with the greatest differences between the two environments in the s 'Koroneiki' and 'Souri' cultivars. *OeFAD2-1* expression level was relatively high in the MT environment in 'Barnea' and 'Picholine' cultivars and low in the other three. In the HT environment, *OeFAD2-1* expression level in the 'Koroneiki' cultivar was low, and in the 'Souri' cultivar it was no expressed at all. In 'Coratina', it was expressed at a relatively high level and in the 'Barnea' and 'Picholine' cultivars was induced at extremely high levels.

3.4. Transcription Factors Regulating Olive Oil Biosynthesis Pathway

We next explored the regulatory roles of transcription factors in the oil biosynthesis pathway. In order to identify putative targets of the transcription factors known to directly regulate the oil biosynthesis pathway genes, we used co-expression analysis to identify significant and high ($r > 0.75$) expression pattern correlation between pairs of transcription factors and genes involved in the oil biosynthesis pathway (Figure 4). We identified eight WRI1 genes in the olive genome (*OE6A039756*, *OE6A079258*, *OE6A099997*, *OE6A092113*, *OE6A061030*, *OE6A094680*, *OE6A054009* and *OE6A025576*), four Dof4 genes (*OE6A082367*, *OE6A085395*, *OE6A104771* and *OE6A039076*), four LEC1 genes (*OE6A094722*, *OE6A016910*, *OE6A001317* and *OE6A033717*), one Dof11 gene (*OE6A032821*), one L1L gene (*OE6A077959*) and one MYB73 gene (*OE6A056119*). The transcription factors with the highest number of putative target genes, based on co-expression, were *OeDof4*.3, *OeWRI1.1*, *OeDof4.4* and *OeWRI1.2* with 15, 12, 11 and 10 positive co-expressed oil biosynthesis genes. The oil biosynthesis genes with the highest number of putative transcription factors control, based on co-expression, were

OeACC1, *OeGPDH.3*, *OsDGAT1*, *OeFATB.2* and *OePDAT.3* with 9, 7, 6, 6 and 6 co-expressed transcription factors.

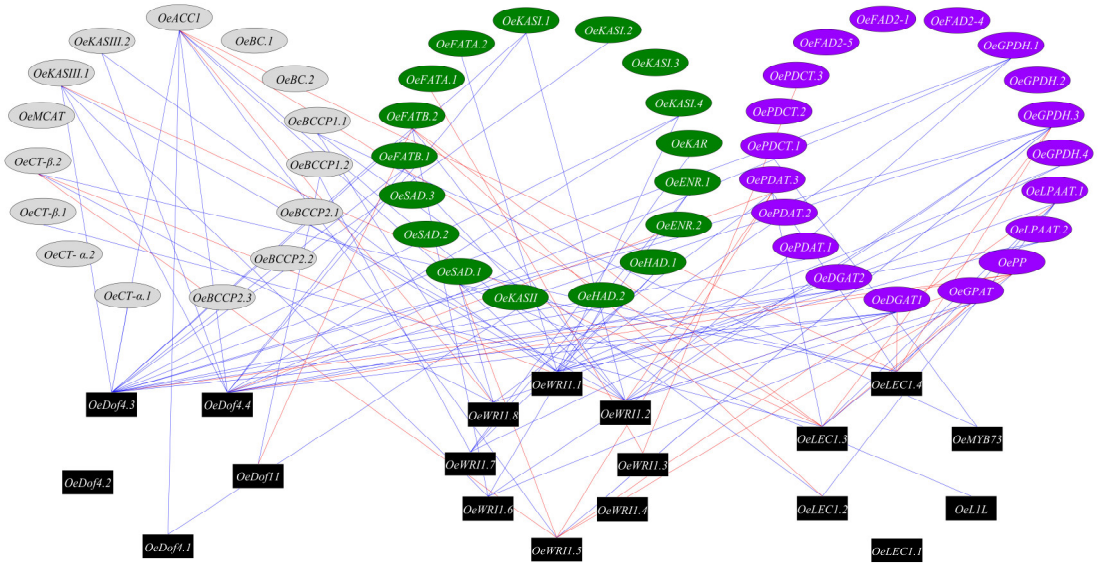

Figure 4. TF-oil biosynthesis network. Co-expressed network of the correlation among Transcription factors (colored in black) and Oil biosynthesis genes. The blue and red lines indicate positive and negative correlations between two genes, respectively. The oil biosynthesis genes are colored in green, gray and purple for plastid, cytosol and ER, respectively.

4. Discussion

In this study, we addressed the molecular effects of an environment of consistently high temperatures on oil accumulation and composition in different cultivars. We used two cultivars that differ in their response to HT environment. The 'Barnea' cultivar, which had been found to be relatively resistant to HT environment in term of oil accumulation, but not in its oil quality and the 'Souri' cultivar, found to be sensitive to HT environment in term of oil accumulation, but relatively resistant to HT environment in term of oil quality [36]. We found that during fruit development and during the most critical period of oil accumulation, both cultivars, the heat tolerant 'Barnea' cultivar as well as the heat sensitive, 'Souri', induced a heat stress protein response in the HT environment. We characterized the gene expression pattern of the genes involved in the olive oil biosynthesis and found that many genes are repressed in response to a high temperature environment. Finally, we characterized the TF-oil biosynthesis network by its response to an MT vs. HT environment in the two analyzed cultivars, 'Barnea' and 'Souri'.

4.1. HT Environment Induced A Heat Stress Response in Both Cultivars

Heat stress or high temperature affects the metabolism of plants, especially cell membranes and physiological processes such as photosynthesis, respiration, and water regulation. This defense at the molecular level is very important for survival and growth of plants. Plants show a series of molecular responses to these stresses, among them is the synthesis of heat-shock proteins. Heat-shock proteins act as molecular chaperones regulating the folding, accumulation, localization and degradation, of proteins in plants [25]. Our study focused on heat stress only. We treated the plant with enough water and fertilization to attempt to isolate heat stress from other abiotic stresses [36]. Characterization of the expression pattern of heat-shock proteins in our study, revealed that most of the heat-shock proteins induced dramatically in an HT environment were HSP20-like chaperones, although some genes belonging to the HSP70 family had the same expression pattern. Most heat-shock proteins in our study underwent high expression in the HT environment and low expression in the MT

environment at all three time-points (Figure 1). The 'Souri' cultivar presents a unique expression pattern of many HSP genes that were induced in 83 DPA, then repressed in 104 DPA, and up-regulated again in 146 DPA. We could not find any reasonable explanation for this gene expression pattern. At 104 and 146 DPA, the ratio between the expression of the heat-shock protein genes in the HT and the MT environment was higher in the 'Barnea' compared to the 'Souri' cultivar. Assuming that the 'Barnea' cultivar is more tolerant to heat stress compared to the 'Souri' cultivar in regard to fruit development and oil accumulation [36], suggests that induction of heat-shock proteins may contribute to the olive plant's tolerance to heat stress. This assumption is in agreement with other studies which found that heat-shock proteins are involved in heat tolerance [26,47,48]. Analysis of *OeHSP70* expression also showed the same pattern, in which the differences between the expression levels in the HT and the MT environments were much higher in the 'Barnea' cultivar than in the 'Souri'. However, contrary to that, the 'Koroneiki' cultivar, which is as sensitive to high temperatures as the 'Souri' [36], showed a similar pattern of *OeHSP70* expression as the 'Barnea' and not, as we might have predicted, to the 'Souri' (Figure S2). The *OeHSP70* expression pattern in the 'Souri' and 'Barnea' cultivars as revealed in the RT-PCR experiment showed the same trend as was seen in the RNA-seq results. However, the ratio between the expression level in HT and MT environments was slightly different. This ratio was 1308:1 and 12:1 in the 'Barnea' and 'Souri' cultivars respectively according to the RNA-seq results and 630:1 and 101:1 respectively according to the RT-PCR results.

4.2. High Summer Temperatures Repress Genes Involved in the Olive Oil Biosynthesis Pathway

Formation of malonyl-CoA from acetyl-CoA is catalyze by ACCase. In our study, both the genes composing the multienzyme complex (htACC) as well as hmACC (*OeACC1*) were expressed in the olive mesocarp (Figures 2 and 3). It has been suggested that in monocotyledons both forms are active, whereas in dicotyledons, such as olives, hmACC alone is active [32]. However, according to the expression levels of both forms, our results suggest that in olives both forms may be active and contribute to the malonyl-CoA, a precursor of fatty acids. Many genes involved in olive oil biosynthesis are not sensitive to warm temperatures during fruit development and show similar expression in both HT and MT environments. These genes include the genes responsible for forming the htACC complex, MCAT, KAR, HAD, ENR, SAD and LPAAT. However, many genes involved in oil biosynthesis are sensitive to a high temperature environment and showed significant lower expression in the HT compare to the MT environment. The high temperature sensitive genes were ACC1, KASI, II and III, FATA and FATB, GPDH, GPAT, DGATs and PDAT. FAD2 and PDCT were also high temperature sensitive genes, but showed increased expression in HT compared to the MT environment. Oil accumulation was shown to be temperature sensitive and high temperatures resulted in decreased olive oil content in the mesocarp [17,36]. Conversion of acetyl-CoA to malonyl-CoA is catalyzed by ACC1 which is a key regulator of fatty acid synthesis. Overexpression of *ACC1* in yeast significantly increased the total fatty acid content [49–51]. We found that *OeACC1* (*OE6A049983*) is repressed in an HT environment. At 146 DPA, the ratio of the *OeACC1* expression level between MT and HT environment was 2.9:1 in the heat tolerant 'Barnea' cultivar and 24:1 in the heat sensitive cultivar 'Souri'. However, in the sensitive cultivar 'Koroneiki' the ratio was 4.1:1 and in the tolerant cultivars 'Coratina' and 'Picholine' the ratio was 9:1 and 4.8:1, respectively (Figure 3). Based on these results, we can conclude that *OeACC1* is repressed at high temperatures. However, this gene cannot serve as a marker to discriminate between tolerant and sensitive cultivars in regard to heat stress. All three KAS genes were found to be sensitive to high temperatures and their expression was repressed in the HT environment. Characterizing only the gene with the highest expression from each gene family, we found that KASI (*OE6A049543*) was significantly overexpressed in the MT environment at all three time-points in the 'Barnea' cultivar, but only at 146 DPA in the 'Souri' cultivar. KASII (*OE6A089647*) was significantly overexpressed in the MT environment at all three time-points in the 'Souri' cultivar, but only at 83 and 104 DPA in the 'Barnea' cultivar. KASIII (*OE6A034993*) was significantly overexpressed in the MT environment at all three time-points in both cultivars. KAS genes, like *OeACC1*, are repressed in high temperatures. However, they also cannot serve as markers to discriminate between tolerant and sensitive cultivars to heat

stress. Most of the genes involved in the fatty acid synthesis occurring in the ER were found to be HT regulated. GPDH, GPAT, DGAT1, DGAT2 and PDAT expression was repressed in HT environment. DGAT plays a key role in determining the carbon flux into TAG [52], and repression of its expression may lead to decreased oil accumulation as we found earlier [36]. However, FAD2 and PDCT were induced in under these conditions (Figure 2). Fatty acid modification from oleic acid to linoleic acid (C18:2) is catalyzed by FAD2 and FAD6 and from oleic acid to linolenic acid (C18:3) by FAD3 [32]. FAD3 genes were not expressed and the OeFAD6 gene was minimally expressed in both cultivars and in both environments. Among the FAD2 genes, *OeFAD2-2* and *OeFAD2-3* were also expressed at low levels in both cultivars and in both environments. *OeFAD2-5*, *OeFAD2-1* and *OeFAD2-4* were highly expressed in some samples. In the 'Souri' cultivar, *OeFAD2-4* was expressed similarly in both environments, *OeFAD2-1* expressed similarly in both environments at 104 and 146 DPA but induced in the HT environment at 83 DPA, *OeFAD2-5* induced in the HT environment at 83 and 146 DPA. In the 'Barnea' cultivar, with the exception of *OeFAD2-4*, which was expressed similarly in both environments at 146 DPA, all three expressed FAD2 genes were induced in the HT environment at all sampled time-points. This is in agreement with other studies which found that FAD2 genes expression was induced by high temperatures [53]. However, other studies found that low temperatures also induced FAD2 genes in olives [54]. PDCT genes were induced in the HT environment in the 'Souri' cultivar, but not in the 'Barnea' cultivar. The PDCT enzyme uses the DAG as precursor and returns it to the PC pool, thus reducing the amount of DAG that continues to synthesis of TAG and repressing oil production. The main genes whose expression was cultivar specific regarding 'Barnea' and the 'Souri', are PDCT and FAD2. PDCT was induced in the HT environment only in the heat sensitive cultivar, 'Souri', and it's activity repressed oil production in that environment. However, in the 'Barnea' cultivar, its expression was constant in both environments. FAD2 was highly induced in the HT environment in the heat sensitive cultivar, 'Barnea' and its' effect was evident in the quality of the oil produced under high temperature conditions. It was only slightly induced under similar environmental conditions in the heat resistant cultivar, 'Souri'. *OeFAD2-1* expression level in all five cultivars in both environments (Figure 3), was found to be in relatively good agreement with the oil quality sensitivity of these cultivars [36]. The cultivars 'Barnea' and 'Picholine' were found to be the most heat sensitive in terms of oil quality and these cultivars showed the highest expression level of *OeFAD2-1* in the HT environment. The 'Souri' cultivar was characterized as the most heat resistant of the five in terms of oil quality and showed no expression of *OeFAD2-1* under HT conditions. However, the cultivars 'Koroneiki' and 'Coratina' showed oil quality sensitivity under high temperature conditions, with relatively low expression of *OeFAD2-1*. The 'Koroneiki' cultivar, although found to be heat sensitive in term of oil quality, showed similar expression levels of *OeFAD2-1* in both environments. *OeACC1* and *OeFAD2-1* expression patterns in the 'Souri' and 'Barnea' cultivars as demonstrated in the RT-PCR experiment showed the same trend as in the RNA-seq results. However, the ratio between the expression level in HT and MT environments was slightly different. This ratio in *OeACC1* was 1:2.5 and 1:2 in the 'Barnea' and 'Souri' cultivars respectively according to the RNA-seq results and 1:2.9 and 1:25 respectively as appeared in the RT-PCR results. For *OeFAD2-1*, this ratio was 2.7:1 and 1:1.5 in the 'Barnea' and 'Souri' cultivars respectively according to the RNA-seq results and 2.7:1 and 1:10 respectively according to the RT-PCR results.

4.3. WRI1 and Dof4 Are the Main Transcription Factors Regulating Olive Oil Biosynthesis Pathway

Network analysis of the transcription factors and oil biosynthesis genes reveals transcription factors hubs, which hypothetically could regulate the genes involved in fatty acid biosynthesis in response to high temperatures. We found that among the transcription factors known to regulate oil biosynthesis, the transcription factors *OeDof4*.3, *OeWRI1.1*, *OeDof4.4* and *OeWRI1.2* were co-expressed with a high number of oil biosynthesis genes. The LEC1, L1L, MYB73 and Dof11 transcription factors were co-expressed with a relatively low number of genes involved in oil biosynthesis, suggesting that these TFs are not the main factors affecting the response of oil production to high temperatures. WRI1 was already suggested as the main regulator in

transcriptional control of plant oil biosynthesis [35]. However, in order to validate the role of the suggested hub, more studies need to be carried out, including identifying the binding motifs of the transcription factors in the target gene promoters as well as chromatin immunoprecipitation experiments to show that indeed the suggested transcription factors interact with the promotor genes involved in oil biosynthesis.

5. Conclusions

Our study demonstrates the negative effect of a high temperature environment on several key genes critical to the production and quality of olive oil. Different olive cultivars have developed a variety of mechanisms to deal with different aspects of high temperature damage. In order to elucidate the mechanism of high temperature damage to olive oil, we characterized the expression pattern of genes involved in the olive oil biosynthesis pathway in a high temperature (HT) environment compared to expression patterns under moderate conditions (MT). We found that most of the genes regulated by high temperatures are common to different stages of fruit development. However, many of them are cultivar dependent. We hypothesize that a strong induction of heat-shock proteins, especially during the late stage of fruit development, can indicate heat tolerance. Many genes involved in the olive oil biosynthesis are down-regulated as a response to high temperatures. *OePDCT* as well as *OeFAD2* genes showed cultivar dependent expression patterns strongly related to their heat tolerance. Hence, these genes are recommended as markers for screening of various cultivar to test their tolerance level for high summer temperatures. We also found that the transcription factors *OeDof4.3*, *OeWRI1.1*, *OeDof4.4* and *OeWRI1.2* were co-expressed with a high number of olive oil biosynthesis genes and therefore seem to be key factors in regulating the oil biosynthesis pathway in response to heat stress. Due to climate changes in recent years and the forecast for the future, the mechanisms of the various olive cultivars response to heat stress should be characterized in detail in order to identify existing cultivars or to develop new ones tolerant to high summer temperatures. These will hopefully produce high yields as well as quality oil in a changing environment.

Supplementary Materials: The following are available online at www.mdpi.com/2223-7747/9/9/1135/s1, Figure S1: Venn diagrams of the significantly regulated genes. Comparison of the regulated gene identities between the different sampled time points in each cultivar and environment (**a**) and between the two cultivars in each time point and environment (**b**), Figure S2: *OeHSP70* expression analyzed by RT-PCR at 146 DPA, Table S1: Primers used in the real-time quantitative PCR, Table S2: Sequencing results. The left column describe the number of Days Post Anthesis (DPA). #raw-reads are the average number of reads for sequencing lane. #clean-reads are the average number of reads after filtering and % mapping is the percentage of reads mapped to the olive genome.

Author Contributions: The original design of the study was set up by G.B.-A., Z.K. and B.A.; Y.N. and M.S. performed all the molecular analyses; Y.N., M.S., I.B., Y.M. and G.B.-A. performed the field experiment; A.D.-F., R.H. and G.B.-A. performed the data analysis; G.B.-A. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maestri, E.; Klueva, N.; Perrotta, C.; Gulli, M.; Nguyen, H.T.; Marmiroli, N. Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Mol. Biol.* **2002**, *48*, 667–681, doi:10.1023/a:1014826730024.
2. Wilhelm, E.P.; Mullen, R.; Keeling, P.; Singletary, G. Heat stress during grain filling in maize: Effects on kernel growth and metabolism. *Crop Sci.* **1999**, *39*, 1733–1741, doi:10.2135/cropsci1999.3961733x.
3. Barnabas, B.; Jager, K.; Feher, A. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* **2008**, *31*, 11–38, doi:10.1111/j.1365-3040.2007.01727.x.

4. Bita, C.E.; Gerats, T. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* **2013**, *4*, 273–273, doi:10.3389/fpls.2013.00273.
5. Bray, E.A.; Bailey-Serres, J.; Weretilnyk, E. *Biochemistry and Molecular Biology of Plants, Responses to Abiotic Stresses*; Gruissem, W., Jones, R., Eds.; American Society of Plant Biologists: Rockville, MD, USA, 2000; pp. 1158–1203.
6. Guy, C. Molecular responses of plants to cold shock and cold acclimation. *J. Mol. Microbiol. Biotechnol.* **1999**, *1*, 231–242.
7. Weis, E.; Berry, J.A. Plants and high temperature stress. *Symp. Soc. Exp. Biol.* **1988**, *42*, 329–346.
8. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; et al. Crop production under drought and heat stress: Plant responses and management options. *Front. Plant Sci.* **2017**, *8*, doi:10.3389/fpls.2017.01147.
9. Camejo, D.; Rodríguez, P.; Angeles Morales, M.; Miguel Dell'Amico, J.; Torrecillas, A.; Alarcón, J.J. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **2005**, *162*, 281–289, doi:10.1016/j.jplph.2004.07.014.
10. Fahad, S.; Hussain, S.; Saud, S.; Hassan, S.; Tanveer, M.; Ihsan, M.Z.; Shah, A.N.; Ullah, A.; Nasrullah; Khan, F.; et al. A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. *Plant Physiol. Biochem.* **2016**, *103*, 191–198, doi:10.1016/j.plaphy.2016.03.001.
11. Ferris, R.; Ellis, R.H.; Wheeler, T.R.; Hadley, P. Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. *Ann. Bot.* **1998**, *82*, 631–639, doi:10.1006/anbo.1998.0740.
12. Rainey, K.M.; Griffiths, P.D. Evaluation of Phaseolus acutifolius A. Gray plant introductions under high temperatures in a controlled environment. *Genet. Resour. Crop Evol.* **2005**, *52*, 117–120, doi:10.1007/s10722-004-1811-2.
13. Vara Prasad, P.V.; Craufurd, P.Q.; Summerfield, R.J. Fruit Number in Relation to Pollen Production and Viability in Groundnut Exposed to Short Episodes of Heat Stress. *Ann. Bot.* **1999**, *84*, 381–386, doi:10.1006/anbo.1999.0926.
14. Bellincontro, A.; Caruso, G.; Mencarelli, F.; Gucci, R. Oil accumulation in intact olive fruits measured by near infrared spectroscopy-acousto-optically tunable filter. *J. Sci. Food Agric.* **2013**, *93*, 1259–1265, doi:10.1002/jsfa.5899.
15. Conde, C.; Delrot, S.; Gerós, H. Physiological, biochemical and molecular changes occurring during olive development and ripening. *J. Plant Physiol.* **2008**, *165*, 1545–1562, doi:10.1016/j.jplph.2008.04.018.
16. Servili, M. Olive oil processing technologies and investments. In *Present and Future of the Mediterranean Olive Sector*; Caballero, J., D'Andria, R., Fernández, M., Garrido, A., Rallo, L., Arroyo López, F.N., Arcas, N., Fernandez Escobar, R., López-Miranda, J., Msallem, M., et al., Eds.; CIHEAM IOC: Zaragoza, Spain, 2013; Volume 106, pp. 55–66.
17. García-Inza, G.P.; Castro, D.N.; Hall, A.J.; Rousseaux, M.C. Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid composition in olive (Olea europaea L. var. 'Arauco'). *Eur. J. Agron.* **2014**, *54*, 107–115, doi:10.1016/j.eja.2013.12.005.
18. Miserere, A.; Searles, P.S.; García-Inza, G.P.; Rousseaux, M.C. Elevated temperature affects vegetative growth and fruit oil concentration in olive trees (Olea europaea). *Acta Hortic.* **2018**, *1199*, 523–528.
19. Rondanini, D.P.; Castro, D.N.; Searles, P.S.; Rousseaux, M.C. Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. *Eur. J. Agron.* **2014**, *52*, 237–246, doi:10.1016/j.eja.2013.09.002.
20. Trentacoste, E.R.; Puertas, C.M.; Sadras, V.O. Modelling the intraspecific variation in the dynamics of fruit growth, oil and water concentration in olive (Olea europaea L.). *Eur. J. Agron.* **2012**, *38*, 83–93, doi:10.1016/j.eja.2012.01.001.
21. García-Inza, G.P.; Castro, D.N.; Hall, A.J.; Rousseaux, M.C. Opposite oleic acid responses to temperature in oils from the seed and mesocarp of the olive fruit. *Eur. J. Agron.* **2016**, *76*, 138–147, doi:10.1016/j.eja.2016.03.003.
22. García-Inza, G.P.; Hall, A.J.; Rousseaux, M.C. Proportion of oleic acid in olive oil as influenced by the dimensions of the daily temperature oscillation. *Sci. Hortic.* **2018**, *227*, 305–312, doi:10.1016/j.scienta.2017.09.030.

23. Lombardo, N.; Marone, E.; Alessandrino, M.; Godino, G.; Madeo, A.; Fiorino, P. Influence of growing season temperatures in the fatty acids (FAs) of triacilglycerols (TAGs) composition in Italian cultivars of Olea europaea. *Adv. Hortic. Sci.* **2008**, *22*, 49–53.
24. Mailer, R.J.; Ayton, J.; Graham, K. The Influence of Growing Region, Cultivar and Harvest Timing on the Diversity of Australian Olive Oil. *J. Am. Oil Chem. Soc.* **2010**, *87*, 877–884, doi:doi:10.1007/s11746-010-1608-8.
25. Al-Whaibi, M.H. Plant heat-shock proteins: A mini review. *J. King Saud Univ. Sci.* **2011**, *23*, 139–150, doi:10.1016/j.jksus.2010.06.022.
26. Ul Haq, S.; Khan, A.; Ali, M.; Khattak, A.M.; Gai, W.-X.; Zhang, H.-X.; Wei, A.-M.; Gong, Z.-H. Heat shock proteins: Dynamic biomolecules to counter plant biotic and abiotic stresses. *Int. J. Mol. Sci.* **2019**, *20*, 5321, doi:10.3390/ijms20215321.
27. Janni, M.; Gullì, M.; Maestri, E.; Marmiroli, M.; Valliyodan, B.; Nguyen, H.T.; Marmiroli, N. Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. *J. Exp. Bot.* **2020**, *71*, 3780–3802, doi:10.1093/jxb/eraa034.
28. Montero-Barrientos, M.; Hermosa, R.; Nicolás, C.; Cardoza, R.E.; Gutiérrez, S.; Monte, E. Overexpression of a Trichoderma HSP70 gene increases fungal resistance to heat and other abiotic stresses. *Fungal Genet. Biol.* **2008**, *45*, 1506–1513, doi:10.1016/j.fgb.2008.09.003.
29. Qi, Y.; Wang, H.; Zou, Y.; Liu, C.; Liu, Y.; Wang, Y.; Zhang, W. Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice. *FEBS Lett.* **2011**, *585*, 231–239, doi:10.1016/j.febslet.2010.11.051.
30. Wang, X.; Yan, B.; Shi, M.; Zhou, W.; Zekria, D.; Wang, H.; Kai, G. Overexpression of a Brassica campestris HSP70 in tobacco confers enhanced tolerance to heat stress. *Protoplasma* **2016**, *253*, 637–645, doi:10.1007/s00709-015-0867-5.
31. He, M.; Qin, C.-X.; Wang, X.; Ding, N.-Z. Plant unsaturated fatty acids: Biosynthesis and regulation. *Front. Plant Sci.* **2020**, *11*, doi:10.3389/fpls.2020.00390.
32. Salas, J.; Harwood, J.; Martínez Force, E. Lipid metabolism in olive: Biosynthesis of triacylglycerols and aroma components. In *Handbook of Olive Oil*, 2nd ed.; Aparicio, R., Harwood, J., Eds.; Springer: New York, NY, USA, 2013, doi:10.1007/978-1-4614-7777-8_4pp. 97-127.
33. Hu, Z.; Ren, Z.; Lu, C. The phosphatidylcholine diacylglycerol cholinephosphotransferase is required for efficient hydroxy fatty acid accumulation in transgenic Arabidopsis. *Plant Physiol.* **2012**, *158*, 1944–1954, doi:10.1104/pp.111.192153.
34. Manan, S.; Chen, B.; She, G.; Wan, X.; Zhao, J. Transport and transcriptional regulation of oil production in plants. *Crit. Rev. Biotechnol.* **2017**, *37*, 641–655, doi:10.1080/07388551.2016.1212185.
35. Kong, Q.; Yuan, L.; Ma, W. WRINKLED1, a "Master Regulator" in transcriptional control of plant oil biosynthesis. *Plants* **2019**, *8*, 238, doi:10.3390/plants8070238.
36. Nissim, Y.; Shloberg, M.; Biton, I.; Many, Y.; Doron-Faigenboim, A.; Zemach, H.; Hovav, R.; Kerem, Z.; Avidan, B.; Ben-Ari, G. High temperature environment reduces olive oil yield and quality. *PLoS ONE* **2020**, *15*, e0231956, doi:10.1371/journal.pone.0231956.
37. Grabherr, M.G.; Haas, B.J.; Yassour, M.; Levin, J.Z.; Thompson, D.A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R.; Zeng, Q.; et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol*. **2011**, *29*, 644–652.
38. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, doi:10.1093/bioinformatics/btu170.
39. Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **2009**, *10*, R25, doi:10.1186/gb-2009-10-3-r25.
40. Li, B.; Dewey, C.N. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* **2011**, *12*, 323, doi:10.1186/1471-2105-12-323.
41. Rozen, S.; Skaletsky, H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **2000**, *132*, 365–386.
42. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **2010**, *26*, 139–140, doi:10.1093/bioinformatics/btp616.
43. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc.* **1995**, *57*, 289–300.

44. Gentleman, R.C.; Carey, V.J.; Bates, D.M.; Bolstad, B.; Dettling, M.; Dudoit, S.; Ellis, B.; Gautier, L.; Ge, Y.; Gentry, J.; et al. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol.* **2004**, *5*, R80, doi:10.1186/gb-2004-5-10-r80.
45. *SAS Institute JMP® Version 7 User's Guide*; SAS Institute Inc.: Cary, NC, USA, 2007.
46. Cruz, F.; Julca, I.; Gómez-Garrido, J.; Loska, D.; Marcet-Houben, M.; Cano, E.; Galán, B.; Frias, L.; Ribeca, P.; Derdak, S.; et al. Genome sequence of the olive tree, Olea europaea. *GigaScience* **2016**, *5*, 29, doi:10.1186/s13742-016-0134-5.
47. Huang, L.-J.; Cheng, G.-X.; Khan, A.; Wei, A.-M.; Yu, Q.-H.; Yang, S.-B.; Luo, D.-X.; Gong, Z.-H. CaHSP16.4, a small heat shock protein gene in pepper, is involved in heat and drought tolerance. *Protoplasma* **2019**, *256*, 39–51, doi:10.1007/s00709-018-1280-7.
48. Xu, Y.; Zhan, C.; Huang, B. Heat shock proteins in association with heat tolerance in grasses. *Int. J. Proteom.* **2011**, *2011*, 529648, doi:10.1155/2011/529648.
49. Fernandez-Moya, R.; Da Silva, N.A. Engineering Saccharomyces cerevisiae for high-level synthesis of fatty acids and derived products. *FEMS Yeast Res.* **2017**, *17*, doi:10.1093/femsyr/fox071.
50. Gomma, A.E.; Lee, S.-K.; Sun, S.M.; Yang, S.H.; Chung, G. Improvement in oil production by increasing Malonyl-CoA and Glycerol-3-Phosphate pools in scenedesmus quadricauda. *Indian J. Microbiol.* **2015**, *55*, 447–455, doi:10.1007/s12088-015-0546-4.
51. Shin, G.-H.; Veen, M.; Stahl, U.; Lang, C. Overexpression of genes of the fatty acid biosynthetic pathway leads to accumulation of sterols in Saccharomyces cerevisiae. *Yeast* **2012**, *29*, 371–383, doi:10.1002/yea.2916.
52. Liu, Q.; Siloto, R.M.P.; Lehner, R.; Stone, S.J.; Weselake, R.J. Acyl-CoA:diacylglycerol acyltransferase: Molecular biology, biochemistry and biotechnology. *Prog. Lipid Res.* **2012**, *51*, 350–377, doi:10.1016/j.plipres.2012.06.001.
53. Li, Q.; Zheng, Q.; Shen, W.; Cram, D.; Fowler, D.B.; Wei, Y.; Zou, J. Understanding the biochemical basis of temperature-Induced lipid pathway adjustments in plants. *Plant Cell* **2015**, *27*, 86–103, doi:10.1105/tpc.114.134338.
54. Luisa Hernández, M.; Dolores Sicardo, M.; Arjona, P.M.; Martínez-Rivas, J.M. Specialized functions of olive FAD2 gene family members related to fruit development and the abiotic stress response. *Plant Cell Physiol.* **2019**, *61*, 427–441, doi:10.1093/pcp/pcz208.