Comparative studies of lipid metabolism in drought-tolerant and drought-sensitive plants and deep understanding of the resurrection grass *Oropetium thomaeum*

Dissertation

zur
Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der
Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Xiaomin Song

aus

Shaanxi, Volksrepublik China

Bonn, 2023

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

Gutachter / Betreuer: Prof. Dr. Dorothea Bartels

Gutachter: Prof. Dr. Peter Dörmann

Tag der Promotion: 14.06.2024

Erscheinungsjahr: 2024

DECLARATION

I hereby declare that this PhD dissertation is my own work, except where explicitly stated otherwise in the text or in the bibliography.

Xiaomin Song

Contents

ABBREVIATIONS

v/v: volume/volume

w/v: weight/volume

ABA: abscisic acid

ANOVA: analysis of variance

BLAST: blast local alignment search tool

bp: base pair

cDNA: complementary DNA

CDS: Coding sequence

CK: control

Col-0: Columbia-0

d: day

DW: dry weight

DNA: deoxyribonucleic acid

DNase: deoxyribonuclease

EDTA: ethylenediaminetetraacetic acid

et al.: lat. *et alii* = and others

DMSO: dimethyl sulfoxide

FW: fresh weight

g: gram

GC-MS: gas chromatograph with mass spectrometer

HPLC: high pressure liquid chromatography

h: hour

kb: kilobase

mm: Millimeter

M: Molar

mM: millimolar

µmol: micromolar

MS: Murashige and Skoog

PCR: polymerase chain reaction

Taq: thermophilus aquaticus

Q-TOF: Quadrupole time-of-flight

Q-TOF-MS: Quadrupole time-of-flight mass spectrometer

RNA: ribonucleic acid

RNase: ribonuclease

rpm: rounds per minute

RT: room temperature

RT-PCR: reverse transcription-polymerase chain reaction

RWC: relative water content

SW: saturated water

TAE: tris-acetate-EDTA

UV: ultraviolet

V: volts

Vol: volume

ASG: acylated sterol glucosides

DAG: diacylglycerol

DGDG: digalactosyldiacylglycerol

MGDG: monogalactosyldiacylglycerol

FA: fatty acid

FAME: fatty acid methyl ester

FFA: free fatty acid

FS: free sterol

PS: phosphatidylserine

PA: phosphatidic acid

PC: phosphatidylcholine

PE: phosphatidylethanolamine

PG: phosphatidylglycerol

PI: phosphatidylinositol

SE: sterol ester

SG: sterol glucosides

SQDG: sulfoquinovosyldiacylglycerol

TAG: triacylglycerol

ASAT1: Sterol acyltransferases 1

EF1a: elongation factor 1 alpha

ELF5: ETS-related transcription factor

TriDGD: tri-digalactosyldiacylglycerol

TeDGD: te-digalactosyldiacylglycerol

ACBP2: acyl-CoA-binding domain-containing protein 2

DGAT1: diacylglycerol acyltransferase 1

DGAT2: diacylglycerol acyltransferase 2

DGAT3: diacylglycerol acyltransferase 3

DGD1: di-galactosyl-diacylglycerol synthase1

FAE1: fatty acid elongation1

MGD1: monogalactosyldiacylglycerol synthase1

KCS1: 3-ketoacyl-CoA synthase 1

KCS5: 3-ketoacyl-CoA synthase 5

KCS18: 3-ketoacyl-CoA synthase 18

KCS20: 3-ketoacyl-CoA synthase 20

SDP1: sugar dependent 1

SFR2: sensitive to freezing 2;

PDAT1: Phospholipid: diacylglycerol acyltransferase1

PDAT2: Phospholipid: diacylglycerol acyltransferase 2

PIS1: phosphatidylinositol synthase 1

PIS2: phosphatidylinositol synthase 2

PLDα1: phospholipase D1

PLDα2: phospholipase D2

PSAT1: Phospholipid: sterol acyltransferases1

LPCAT1: lyso-phosphatidylcholine acyltransferase 1

LPCAT2: lyso-phosphatidylcholine acyltransferase 2

βGAL7: β-galactosidase 7

βGAL9: β-galactosidase 9

βGAL17: β-galactosidase 17

CLO3: Caleosin 3

CLO4: Caleosin 4

CLO6: Caleosin 6

Summary

1. Increasing water scarcity is known as a crucial challenge to sustainable development of the planet. Water scarcity will impact 40% of the world's population at risk of drought by 2030. Most higher plants are unable to survive desiccation to an air-dried state, however, a small group of vascular plants termed "resurrection plants" can revive from a severe cellular water loss. *Oropetium thomaeum* is a compact resurrection plant that has the smallest known genome among the grasses and could survive the extreme water stress such as loss of >95% of cellular water.

2. *Oropetium thomaeum* belongs to the *Chloridoideae* subfamily of Poaceae and 75% of the genome is located in conserved syntenic regions of grass genomes. A proportion (24% of genes) of tandem duplicated genes similar to other grasses are present in *Oropetium thomaeum*.

3. The stomata in *Oropetium thomaeum* leaves are a four-celled structure, a typical character of a C4 plant. The stomata contain more lipids than other surrounding organelles, possibly to help stomatal movement and then support tolerance of the plants during dehydration. With Nile red staining, comparative cellular analyses of desiccated and fully hydrated leaves revealed the accumulation of oil bodies in leaf tissue during desiccation, which is similar to morphological and expression patterns of desiccated seeds.

4. Drought stress triggers the activity of oil bodies-related degrading enzymes (CLO: Caleosin) in *Oropetium thomaeum*, *OtCLO3*, *OtCLO4*, and *OtCLO6* transcript expression levels showed a significant increase during desiccation treatment in leaf tissues, indicating that during drought stress oil bodies are activated by the degradation process, to help organelles get energy to maintain or enhance the ability to resist abiotic stress. However, at the same time, oil bodies accumulated during drought stress in leaf tissues of *Oropetium thomaeum* with similar morphological and expression patterns to desiccated seeds. These changes suggest that the

generation rate of oil bodies is higher than the degradation rate in *Oropetium thomaeum*.

5. SDP1 (SUGAR-DEPENDENT 1, encodes a triacylglycerol lipase) is involved in oil bodies degradation in plants. The transcript level of *OtSDP1* showed a low abundance in leaf tissues of *Oropetium thomaeum* during dehydration and rehydration. In this study, a large accumulation of oil bodies was found in leaf tissues with drought stress, which rapidly decreased after rehydration, indicating that the activity of the degrading enzyme was not positively correlated with transcript level of *Oropetium thomaeum*. This result suggests that *OtSDP1* may also function during the post-transcription.

6. DGAT2 plays a key role in TAG synthesis in leaf tissues of *Oropetium thomaeum* while DGAT1 is the main enzyme for TAG synthesis in seeds of *Arabidopsis thaliana.* Comparison of DGAT2 expression level in other resurrection plants such as *Craterostigma plantagineum* and *Lindernia brevidens*, also showed a significant increase under drought stress (Gasulla *et al.*, 2013; Supplementary Figure 6). These results suggest that DGAT2 is likely to have a predominant role in TAG synthesis in desiccation tolerant plants.

7. Sterol acyltransferases were identified as acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) in plants. PSAT1 is not the key enzyme to limit the sterol metabolic rate in tomato (*Solanum lycopersicum*), and *SlPSAT1* and *SlASAT1* show overlapping but largely complementary models of expression during tomato fruit growth. Banas (2005) concluded PSAT shows the same physiological function in plants as ASAT in animal cells. This statement is consistent with the results in this study. PSAT1 and ASAT also increased or decreased simultaneously in *Arabidopsis thaliana* and *Oropetium thomaeum*, under drought conditions. These results could be a proof that PSAT1 and ASAT1 functions are likely to overlap in a complementary way in *Arabidopsis thaliana* and *Oropetium thomaeum* leaf tissues.

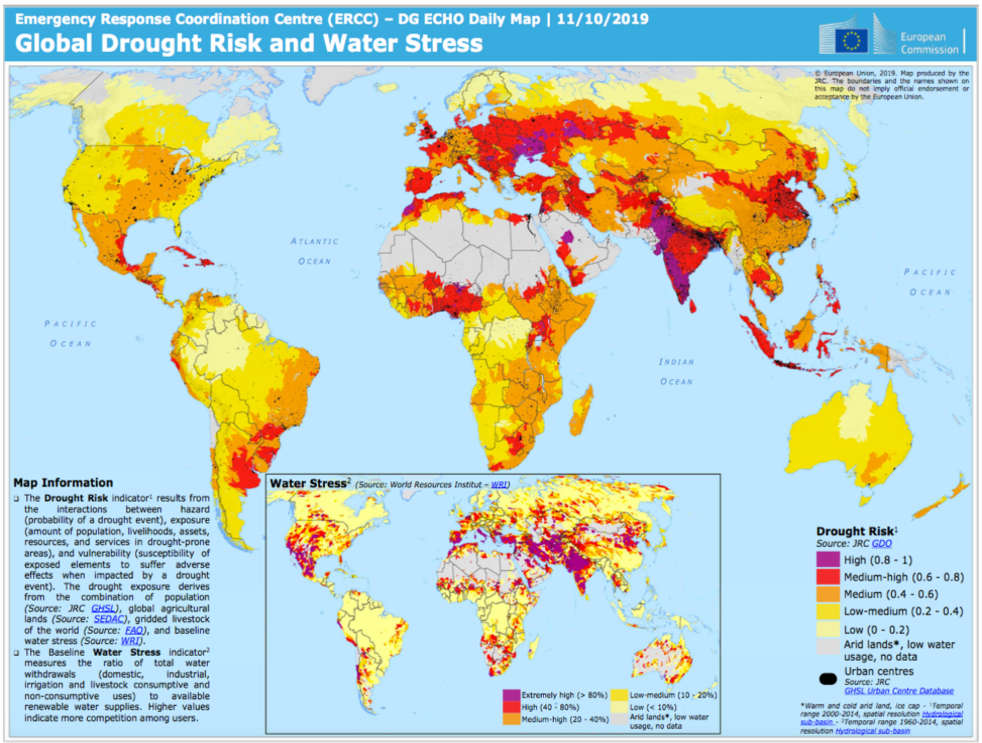
1. Introduction

1.1 Global drought risk and water stress

Water is the most essential substance on earth, all plants and animals must have water to survive (Environmental Health Practitioner Manual, The department of health 2010). The world is faced with a growing number of complex and interconnected challenges. Water is among the top five global risks in terms of impacts, reaching far beyond socio-economic and environmental challenges (World Economic Forum, 2020). Increasing water scarcity is known as a crucial challenge to sustainable development and major cause of conflict, social unrest and alter of traditional migration patterns (Boyer, 1982; Mach, 2017; Tignino and Mach, 2018; WRI, 2019; IOM, 2020; UN-Water, 2020). Global drought impacts on food production, environment homeostasis, electricity production, economy developing, manufacturing, and other social activities, for instance agricultural losses, public water shortages, reduced hydropower supply, and reduced labor or productivity (WRI, 2019; Meza *et al.*, 2020).

Droughts exceed all other natural disasters considering the number of people affected and have promoted some of the world's most severe famines (FAO, 2018; CRED and UNISDR, 2018). Water scarcity already affects every continent. Water use has been growing globally at more than twice the rate of population increases in the last century-An increasing number of regions are reaching the limit at which water services can be sustainably delivered, especially in arid regions (United Nations Water. 2020). At the moment, water is increasingly considered as a method for international cooperation to support food production, livelihoods, ecosystems, climate change, and sustainable development (Farnum, 2018; UN-Water, 2020: UN-Water Analytical Brief on Unconventional Water Resources. Geneva, Switzerland).

The emergency response Coordination Centre - DG ECHO (European Commission's Directorate-General for European Civil Protection and Humanitarian Aid Operations) published a daily map of global drought risks and water stress for 2019.10.11(Figure 1.1), and the world resources institute (WRI) released extremely high-water stress countries in 2019 (Figure 1.2). We are facing severe global drought risks and water stress not only now but also in the future, especially in the southeast of south America, central Europe, south-Central Asia, and southeast of the United States; these are areas with higher populations and extensive crop producing and livestock farming (Carrão *et al.*, 2016; WRI, 2019; FAO, 2019, 2020). According to an analysis released by Washington D.C.-based World Resources Institute's Aqueduct Water Risk Atlas in 2019, 17 countries appear to be approaching a water crisis, and 12 countries are in the Middle East and North Africa, India, Pakistan, Turkmenistan, San Marino, and Botswana (Figure 1.2).



© European Union, 2019. Map produced by the JRC. The boundaries and the names shown on this map do not imply official endorsement or acceptance by the European Union.

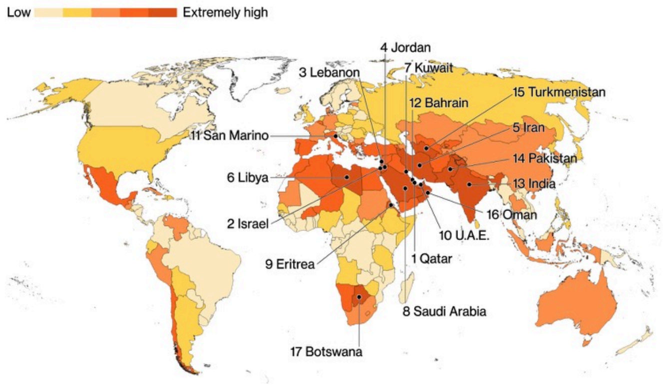
Map Information

- The **Drought Risk** indicator[1] results from the interactions between hazard (probability of a drought event), exposure (amount of population, livelihoods, assets, resources, and services in drought-prone areas), and vulnerability (susceptibility of exposed elements to suffer adverse effects when impacted by a drought event). The drought exposure derives from the combination of population *(Source: JRC GHSL)*, global agricultural lands *(Source: SEDAC)*, gridded livestock of the world *(Source: FAO)*, and baseline water stress *(Source: WRI)*.
- The Baseline **Water Stress** indicator[2] measures the ratio of total water withdrawals (domestic, industrial, irrigation and livestock consumptive and non-consumptive uses) to available renewable water supplies. Higher values indicate more competition among users.

**Warm and cold arid land, ice cap - [1]Temporal range 2000-2014, spatial resolution Hydrological sub-basin - [2]Temporal range 1960-2014, spatial resolution Hydrological sub-basin*

Figure 1.1: The map of global drought risks and water stress in 2019. The Drought Risk indicator results from the interactions between hazard (probability of a drought event), exposure (amount of population, livelihoods, assets, resources, and services in drought-prone areas), and vulnerability (susceptibility of exposed elements to suffer adverse effects when impacted by a drought event). The drought exposure derives from the combination of population (Source: JRC GHSL), global agricultural lands (Source: SEDAC), gridded livestock of the world (Source: FAO), and baseline water stress (Source: WRI). The Baseline Water Stress indicator measures the ratio of total water withdrawals (domestic, industrial, irrigation and livestock consumptive and non-consumptive uses) to

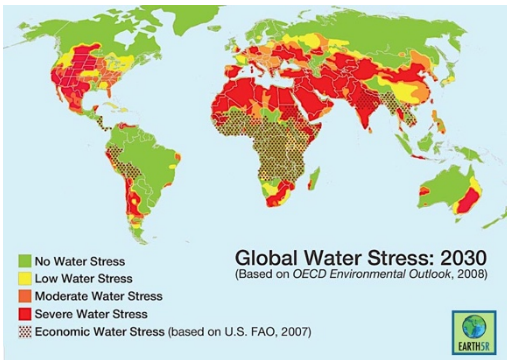
available renewable water supplies. Higher values indicate more competition among users. (Open access: https://reliefweb.int/map/world/global-drought-risk-and-water- stress-dg-echo-daily-map-11102019)

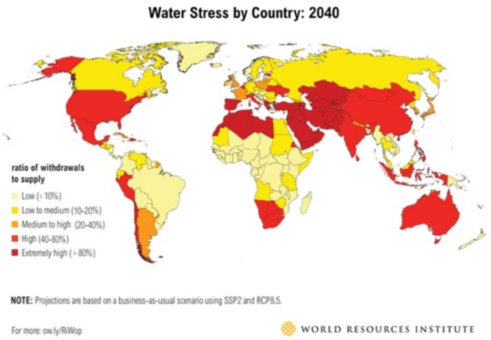


Note: Data on water withdrawal, available water and groundwater are used to calculate baseline water stress.
Source: World Resources Institute's Aqueduct Water Risk Atlas

Figure 1.2: The map of 17 countries which face extremely high-water stress (updated in 2019). Figure taken from WRI (World Resource Institute) Aqueduct. Source: wri.org/aqueduct.

Earth 5R (2008) and World Resources Institute (WRI) (2015) predicted water stress for countries in 2030 and 2040 respectively (Figure 1.3). Climate change projections foresee an increase of drought frequency and intensity for arid regions (WRI, 2015; Carrão et al., 2016). Water scarcity impacts 40% of the world's population, and as many as 700 million people are at risk of being displaced as a result of drought by 2030 (WHO). According to an analysis conducted by the World Resources Institute 2015, nearly one-fifth of all countries worldwide will face water scarcity problems, and Chile, Estonia, Namibia, and Botswana could experience an especially significant increase in water stress by 2040 (Luo *et al.*, 2015).





NOTE: Projections are based on a business-as-usual scenario using SSP2 and RCP8.5.

For more: ow.ly/RiWop

Figure 1.3: A map ranking countries affected by global water stress in 2030 and 2040. Global water stress 2030 data is from Earth 5R, and it is based on the OECD environmental outlook 2008 and U.S. FAO 2007. Global water stress 2040 data are from WRI (World Resources Institute) and the aqueduct project from August 2015, include the exposure of countries to baseline water stress. Projected scores are included for 167 countries under the business-as-usual scenarios. Scores are available for overall stress levels as well as stress levels for industrial, agricultural, and domestic users. (Open access:

https://www.wri.org/resources/data-sets/aqueduct-projected-water-stress-country-rankings)

1.2 Global hunger index in the past 20 years

While many sectors are influenced by drought, agriculture is the mostly heavily affected sector, because of the character of agriculture is highly dependent on water (Dilley *et al.*, 2005; UNDRR, 2019). Drought triggers reduced yields of major crops and contributes to food shortage. Already, yields of major food crops such as maize and wheat are declining due to extreme events, epidemics of plant diseases, and declining water resources. In semi-arid regions, 80 percent or more of year- to-year variation in cereal production can be attributed to climate variability (FAO *et al.*, 2018). The 2019 global hunger index shows that although the global hunger index score decreased from 29.0 to 20.0, but the region of near east & north Africa has higher hunger levels in 2019 than in 2010.

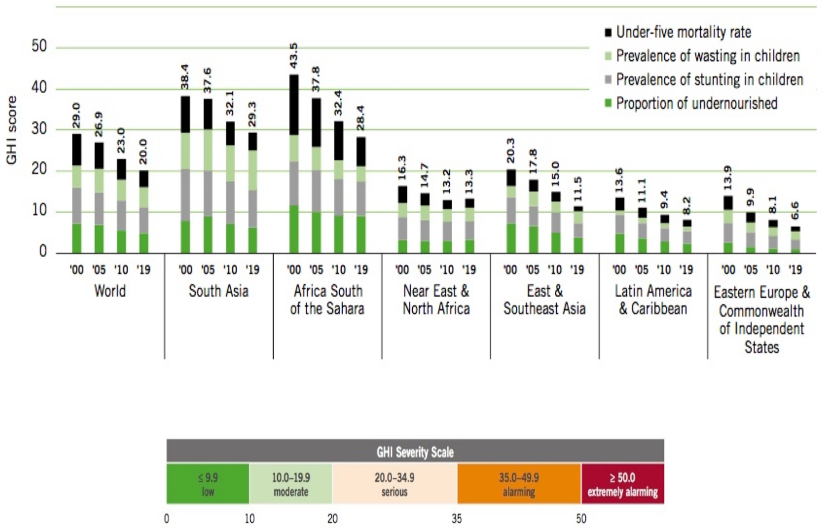


Figure 1.4: Global and regional hunger index scores with components in 2000, 2005, 2010 and 2019.（Grebmer *et al.*, 2019. Data source: UNICEF/WHO/World Bank 2019 is the primary data source,

and WHO 2019a; UNICEF 2019, 2013 and 2009; and MEASURE DHS 2019 are complementary data and FAO）

The world population is expected to be around 10 billion by 2050, massive changes of the global food system are needed (Willett et al., 2019). Future food and nutrition security will depend on adapting to rapid biological evolution created by human-induced changes. Over 40% of calories consumed by humans are obtained from grasses, and the grass family (Poaceae) is possibly the most important plant family regarding to global food safety (Elert, 2014; Van Buren et al., 2017). With changing temperatures and precipitation rates, plant and animal diseases are spreading into new biomes. It requires crops with a stronger ability to adapt to adverse growth environments (Grebmer et al., 2019). Therefore, it is a good approach to study desiccation tolerant plants or resurrection plants, especially a desiccation tolerant grass, which is close to regularly cultivated species, e.g., wheat (Triticum aestivum), rice (Oryza sativa), maize (Zea mays), barley (Hordeum vulgare).

1.3 Desiccation tolerant plants and crops

Desiccation tolerant plants also called “resurrection plants”, are mostly poikilohydrious and herbaceous in nature, which means that their water content adjusts with the relative humidity of the environment (Deeba and Pandey, 2017). These plants are able to stay in the desiccated state until water becomes available and resume full physiological activities (Levitt, 1980; Le and McQueen-Mason, 2006; Costa, 2019). Most higher plants are unable to survive desiccation to an air-dried state (Bartels, 2005; Dinakar and Bartels, 2013). However, a small group of vascular plants termed “resurrection plants” have evolved desiccation tolerance, and they can revive from an air-dried state (Proctor and Tuba, 2002; Oliver *et al.*, 2005 and Moore *et al.*, 2009). Resurrection plants are able to withstand severe water loss, and some are even able to equilibrate the leaves with air to 0% (v/v) relative humidity (Gaff, 1987). This is the severest form of water stress since most protoplasmic water is lost from the cell under these conditions. Although desiccation tolerance in seeds and pollen is common in higher plants (Dickie and Pritchard, 2002; Farmsworth, 2004; Illing *et al.*, 2005), but desiccation tolerance in vegetative tissues is restricted to the unique group of resurrection plants (Porembski and Barthlott, 2000; Bartels and Hussain, 2011; Dinakar and Bartels, 2013).

In the past decades, studies of desiccation tolerance have focused on a few species representing different groups: the dicotyledonous south African *Craterostigma plantagineum* (Bartels *et al.*, 1990, 2005; Norwood *et al.*, 2010; Rodriguez *et al.*, 2010; Giarola and Bartels, 2015; Jung *et al.*, 2019; Giarola *et al.*, 2020), the monocotyledonous species *Sporobolus stapfianus* (Neale *et al.*, 2000; Gaff *et al.*, 2009; Blomstedt *et al.*, 2010; Oliver *et al.*, 2011; Yobi *et al.*, 2017) and the moss *Tortula ruralis* (Oliver *et al.*, 2004, 2010; Naszradi *et al.*, 2007; Schonbeck and Bewley, 2011), and the bryophyte *Selaginella bryopteris* (Liu *et al.*, 2008; Wang *et al.*, 2010; Pandey *et al.*, 2010; Singh, 2015; Deeba, Pandey and Pandey, 2016; Paswan *et al.*, 2017; Singh *et al.*, 2017).

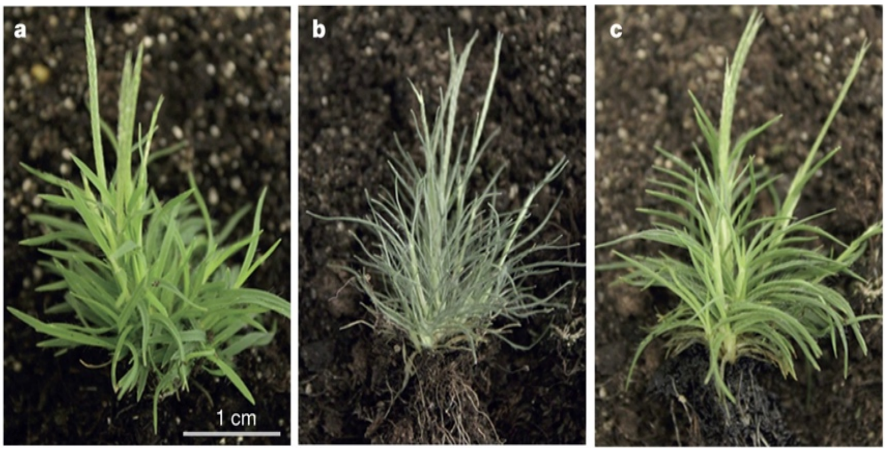


Figure 1.5: a, Well-watered; b, Desiccated (relative water content <5%) after 9 days of drought stress. c, Condition 24 h post-hydration (relative water content >70%).

From: VanBuren *et al.*, Single-molecule sequencing of the desiccation-tolerant grass *Oropetium thomaeum*. Nature 527, 508–511 (2015).

Oropetium thomaeum (L.f.) Trin. (Figure 1.5) is a resurrection plant which can survive the extreme water stress such as loss of >95% of cellular water (*Bartels et al.*, 2002; Van Buren *et al.*, 2015). It has the smallest known genome among the grasses (Jones and Pašakinskiene, 2005) at 90%, 60%, 50%, 30% and 10% the size of Brachypodium (Vogel *et al.*, 2010), rice (Matsumoto *et al.*, 2005), setaria (Bennetzen *et al.*, 2012), sorghum (Paterson *et al.*, 2009) and maize (Schnable *et al.*, 2009) respectively. The next-generation sequencing (NGS) technology, single-molecule real-time sequencing method was used to map genomic data of *Oropetium thomaeum*, which means longer reads of sequences and the first high-quality reference genome from the *Chloridoideae* subfamily and chromosome-scale assembly (VanBuren *et al.*, 2015 and 2018).

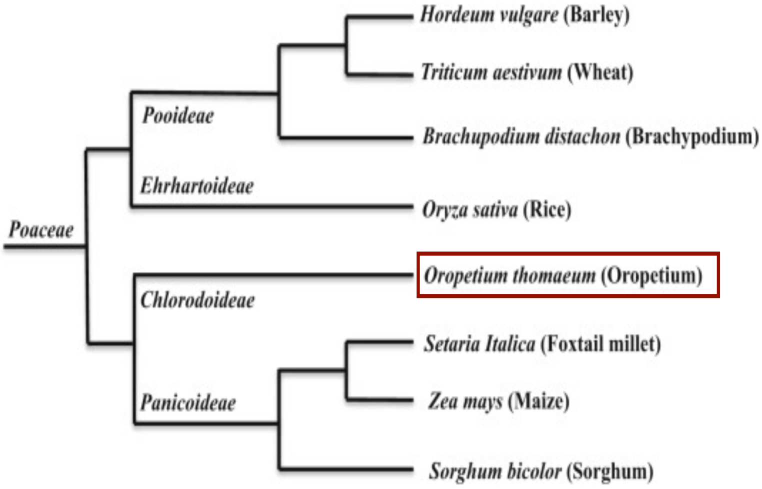


Figure 1.6: Phylogenetic relationships of *Oropetium thomaeum* and some species of grass family. (Liu *et al.*, 2019)

Poaceae, the large family of monocotyledonous plants, are widely known as grasses, including several important cultivated species, like wheat, rice, maize, barley, sorghum, and others. It is commonly accepted that Poaceae is the most important group of crops susceptible to abiotic stress (Landi *et al.*, 2017; Liu *et al.*, 2019). With the completion of the sequencing of various grass genome data, the evolutionary relationship of grasses has become increasingly clear (Paterson *et al.*, 2009; Bennetzen *et al.*, 2012; Schnable *et al.*, 2012; Mascher *et al.*, 2017; Liu *et al.*, 2019). *Oropetium thomaeum* belongs to the *Chloridoideae* subfamily of Poaceae (Figure 1.6) (Liu *et al.*, 2019). Knowing the mechanisms of extreme desiccation tolerance in resurrection plants, *Oropetium thomaeum* likely provides clues for understanding drought tolerance in domesticated cereal crops.

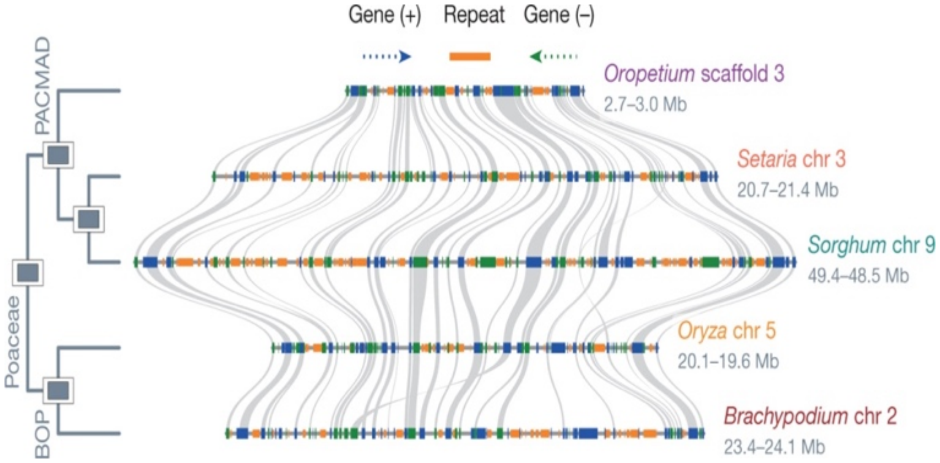


Figure 1.7: Typical micro-collinearity patterns among genomic regions from *Oropetium, Setaria, Sorghum, Oryza* and *Brachypodium.* Colors displayed relative orientations, rectangles indicate gene models. Grey connections show the matching genes. Chr: chromosome. (VanBuren *et al.*, 2015. Single-molecule sequencing of the desiccation tolerant grass *Oropetium thomaeum*).

The complete genomic data of *Oropetium thomaeum* was analyzed and the result showed highly repetitive features with the comparisons of *Setaria*, *Sorghum*, *Oryza* and *Brachypodium* (Figure 1.7). From the perspective of genomic data, 75% of the *Oropetium thomaeum* genome, or 89% of its gene space, is contained in conserved syntenic regions, and the genomic collinearity across grass genomes is extensive, with a high density of orthologous genes spanning much of the euchromatin. *Oropetium thomaeum* has the similar proportion (24% of genes) of tandem duplicated genes with other grasses. Tandem duplicated genes are enriched for gene ontology terms involved in response to abiotic stresses, gene regulation and cellular metabolism (VanBuren *et al.*, 2015 and 2018).

1.4 Oil bodies: energy resources in plants

The stored energy in plant tissues is commonly preserved in the form of carbohydrates or lipids, and occasionally proteins. Plant cells deposit storage resources of carbohydrates, proteins, and neutral lipids in subcellular particles such as starch granules, protein bodies, and oil bodies, respectively (Tzen, 2012). Research has mainly focused on protein bodies (Kawakatsu and Takaiwa, 2010; Tandang-Silvas *et al.*, 2011; Ibl and Stoger, 2012) and starch granules (Smith *et al.*, 1997; Smith, 2001; Ball and Morell, 2003; Pérez and Bertoft, 2010), whereas research on oil bodies was less probably due to technical problems caused by the hydrophobic features of the lipid-storage organelles (Lersten *et al.*, 2006; Quettier and Eastmond, 2009; Tzen, 2012; Cao *et al.*, 2015; Shimada, Takano and Hara-Nishimura, 2015; Van Buren *et al.*, 2017)

Oil bodies also called lipid droplets, lipid bodies, fat bodies, fat droplets, oleosomes, or spherosomes (Frandsen *et al.*, 2001; Farese and Walther, 2009; Pyc *et al.*, 2017) are lipid storage organelles that play a crucial role as an energy resource to fuel germination and early plant growth in a variety of eukaryotic cells (Eastmond, 2006; Quettier and Eastmond, 2009; Shimada and Hara-Nishimura, 2015; Thazar-Poulot *et al.*, 2015; Pyc *et al.*, 2017; Huang, 2018).

1.4.1 The model of plant oil bodies

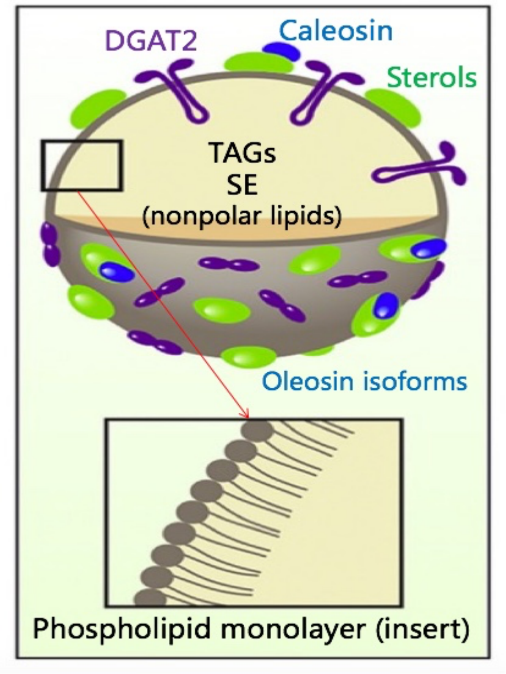


Figure 1.8: Schematic representation of the structure of plant oil bodies. TAGs: triacylglycerols, SE: sterol esters, DGAT2: diacylglycerol acyltransferase, PL: Phospholipids. (Modified from Tzen and Huang, 1992; Frandsen *et al.*, 2001; Farese and Walther, 2009; Pyc *et al.*, 2017).

The structural features of plant oil bodies are the nonpolar lipids of the core [i.e., triacylglycerols (TAGs) and sterol esters (SE)] surrounded by monolayer polar surface lipids [i.e., phospholipid (PL) (insert)], and a variety of lipid-associated proteins decorating the surface of the droplet (Murphy, 2012): diacylglycerol acyltransferase (DGAT2), oleosin isoforms, caleosin isoforms, sterols and others Phospholipids (PL) with the acyl moieties of the PL facing the TAG, and the PL head group exposed to the cytosol (Frandsen *et al.*, 2001). Previous studies on plant oil bodies were mainly focused on oil bodies in food, cosmetics, or other nutrients, *e.g.*, oil bodies in sesame, soybean, avocado, olive, coconut, et al., especially in seeds (Tzen *et al.*, 1993; Ross *et al.*, 1993; Chen *et al.*, 1999; Hsiao *et al.*, 2011; Horn *et al.*, 2013; Cao, 2015; Gidda *et al.*, 2016; Huang, 2018; Dave, 2018). Recently more information has become available on metabolism and development of the metabolic mechanism and the model of plant oil bodies (Tzen and Huang, 1992; Frandsen *et al.*, 2001; Pol *et al.*, 2014; Pyc *et al.*, 2017). The physical feature of oil bodies is determined mainly by their chemical compositions, like the chain length and the degree of unsaturation of fatty acids as well as their distribution among the glycerol backbone positions of triacylglycerols (TAGs) (da Silva *et al.*, 2010; Pocheco *et al.*,2014). Triacylglycerols (TAGs) and sterol esters (SE) are the main storage neutral lipids in oil bodies. (Tzen, 2012).

1.5 Triacylglycerols (TAGs) and Diacylglycerols (DAGs) in plants

1.5.1 Chemical structures of TAGs and DAGs in plants

Various studies demonstrated that triacylglycerols are the major components of leaf oil bodies and they are an important storage lipid in some plant species, fungi, and yeasts, but not in bacteria (Parker and Murphy, 1981; Hudak and Thompson, 1997; Thompson *et al.*, 1998b; Horn *et al.*, 2011; Kopischke *et al.*, 2013; Sandhir, 2014). Normally only small amounts of TAG are found in leaves and are stored in oil bodies (Chapman and Ohlrogge, 2012). Triacylglycerol is composed of three fatty acids esterified to a glycerol molecule and the physical properties of the triacylglycerol are determined by the specific fatty acids esterified to the glycerol moiety and the bonding position: sn-1, sn-2, and sn-3. (Lichtenstein, 2013). TAGs have a glycerol backbone, and they are presumed to be associated with other non-polar lipids such as sterol esters (Kemp and Mercer, 1968; Zinser *et al.*, 1993).

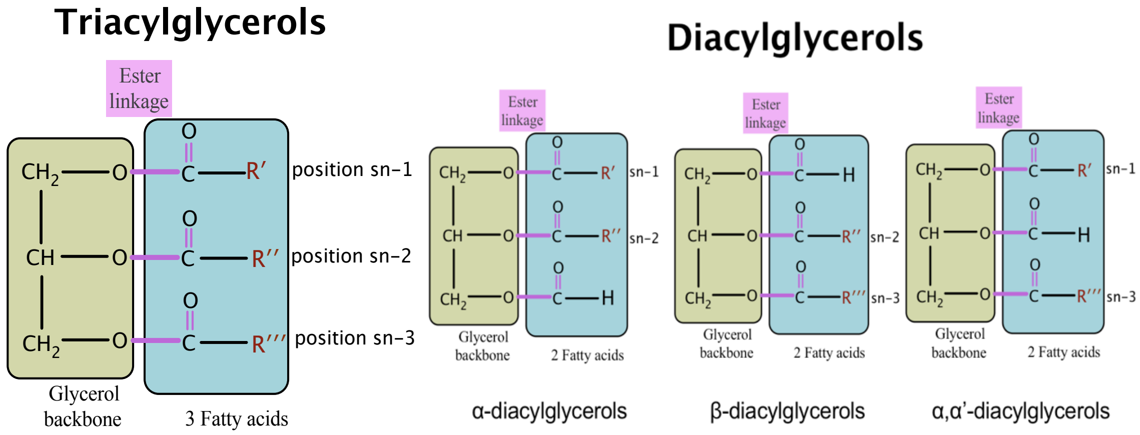


Figure 1.9: Schematic diagram of chemical structures of triacylglycerols and diacylglycerols. R′, R″, and R‴ are an alkyl or an alkenyl, hydrocarbon chain of a fatty acid (general formula R-COOH). sn-1,2- and 2,3-diacylglycerols are named as α, β-diacylglycerols, while sn-1,3-diacylglycerols is represented α, α'-diacylglycerols.

(Modified from Madawala *et al.*, 2011 (Journal of Lipids, Article ID 419809) and "The LipidWeb" https://lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/dg/index.htm).

The neutral lipids diacylglycerols (DAGs) or diglyceride, are abundant lipids of plant membranes, consist of two fatty acid chains bonded to a glycerol molecule with a carbohydrate head group at the sn-3 position. DAGs are involved in many metabolic pathways, they function as components of cellular membranes, as building blocks for glycerolipids, and as lipid second messengers. DAGs can be generated by several reactions, such as neutral triglyceride lipases in the process of lipolysis, phospholipase C phosphatides in the endoplasmic reticulum, and sphingomyelin synthase catalysis. (Eichmann and Lass, 2015; Nicholson and Marangoni, 2019) (Figure 1.9). DAGs are also an important substrate in the biosynthesis of triacylglycerols (TAGs), or phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

1.5.2 Lipolysis of TAGs in plants

Triacylglycerols (TAGs) are chemically inert and highly hydrophobic, but they have an important function, as a source of energy for seed germination, seedling growth, and development of photosynthetic establishment in plants (Chapman *et al.*, 2012; Lipid maps). As it is shown in Figure 1.10, TAGs do not localize to membranes, but accumulate in the cytosol in oil bodies, because of no polar moieties in the structure (Quettier and

Eastmond, 2009; Lichtenstein, 2013). TAGs kept in the oil bodies are degraded to DAGs and fatty acids, and then to MAGs and fatty acids, and then to glycerol by the sequential action of one or more lipases, such as diacylglycerol-O-acyltransferases. Fatty acids are transported into the peroxisomes where they are catalyzed to acyl-CoA and finally used for energy metabolism.

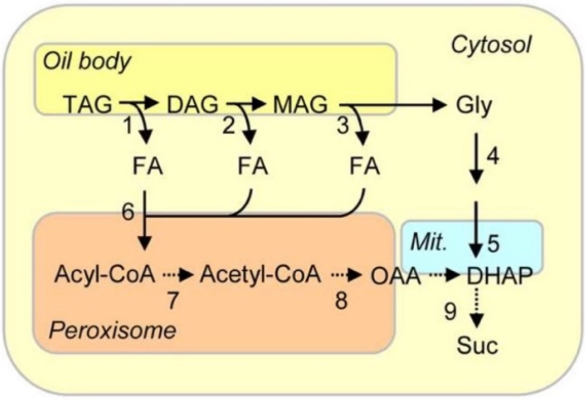


Figure 1.10: A schematic depiction of lipolysis of triacylglycerols (TAGs) following seed germination. Fatty acids (FA), glycerol (Gly), mitochondrion (Mit.), oxaloacetate (OAA), dihydroxyacetone phosphate (DHAP), sucrose (Suc). Arrows with dotted lines denote pathways, several of which are non-linear. (From: Quettier and Eastmond, 2009, Plant Physiology and Biochemistry, Storage oil hydrolysis during early seedling growth.)

1.5.3 Biosynthesis of TAGs in plants

TAG synthesis was first described in higher plants by Barron and Stumpf in 1962, after the illustration of the Kennedy pathway in animal cells (Weiss *et al.*, 1960 and Kennedy, 1961). The Kennedy pathway in plants is an “acyl-CoA-dependent” pathway, which is shown by green arrows in figure 1.11. From G3P (glycerol-3-phosphate) and acyl-CoA to PA (phosphatidic acid), and then assembled to DAG, DAG acylated by DGAT for TAG synthesis, this process also called *de novo* triacylglycerol assembly (Li-Beisson *et al.*, 2010; Chapman and Ohlrogge, 2011; Bates *et al.*, 2013). Another complex TAG biosynthesis pathway in plants is “acyl-CoA-independent” pathway in which PC as the crucial intermediate, direct producing TAG from PC by PDAT (purple arrows) and through PC-derived DAG as the substrates to generate TAG (blue arrows) (Dahlqvist *et al.*, 2000; Bates and Browse, 2012; Bates *et al.*, 2013).

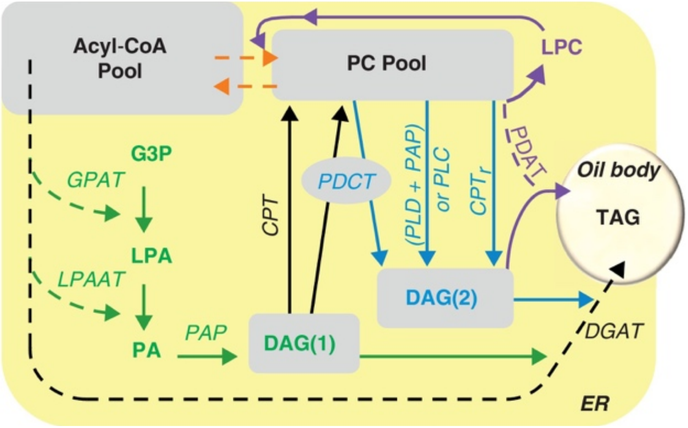


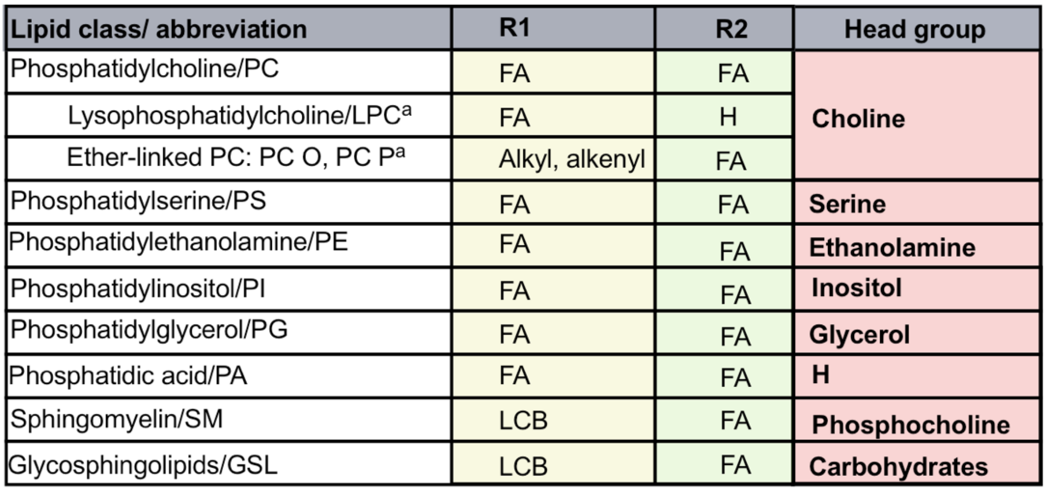
Figure 1.11: Overview of major reactions during TAGs synthesis in the endoplasmic reticulum (ER) of *Arabidopsis thaliana*. TAG: triacylglycerol; DAG: diacylglycerol; PC: phosphatidylcholine; LPC: lyso-phosphatidylcholine; PA: phosphatidic acid; LPA: lyso-phosphatidic acid; G3P: glycerol-3-phosphate; ER: endoplasmic reticulum. Enzymes are in italic, DGAT: diacylglycerol-O-acyltransferases; PDAT: phospholipid: diacylglycerol acyltransferase; PDCT: PC-DAG choline phosphotransferase; PLC: phospholipase C; PLD: phospholipase D; PAP: PA phosphatase; LPAAT: acyl-CoA-LPA acyltransferase; GPAT: acyl-CoA-G3P acyltransferase. (From Bates *et al.*, 2013. Current opinion in plant biology, Biochemical pathways in seed oil synthesis)

In the *de novo* triacylglycerol assembly process, acylation of DAG is the final step of TAG synthesis. This esterification reaction is catalyzed by enzymes called diacylglycerol-O-acyltransferases (DGATs, also named acyl-CoA: DAG acyltransferase). DGATs activity is rate limiting for triacylglycerol accumulation in mammals, plants and microbes (Grisart *et al.*, 2004; Beopoulos *et al.*, 2012; Aymé *et al.*, 2014). There are three classes of DGATs in eukaryotic cells, DGAT1 and DGAT2 are integral membrane proteins and DGAT3 is a soluble protein (McFie *et al.*, 2010; Liu *et al.*, 2011). DGAT1 also called TAG1, is a key enzyme for TAG synthesis and oil accumulation in seeds (Cases *et al.*, 1998; Zhang *et al.*, 2009). Liu *et al.*, 2012 suggested that the *Arabidopsis thaliana DGAT2* gene may exhibit a strong codon bias affecting its expression in yeast (Aymé *et al.*, 2014). PDAT, phospholipid: diacylglycerol acyltransferase, transfers an acyl group from the sn-2 position of PC to the sn-3 hydroxyl of DAG generating TAG (Dahlqvist *et al.*, 2000).

1.6 Biosynthesis of glycerolipids in plant

Glycerolipids are a structurally heterogeneous group of lipids that play pivotal structural and functional roles in cell membranes of bacterial, plants, and animals. They are also essential for maintaining proper fluidity and flexibility of cell membrane. The multiple morphological modifications of lipids help cell membranes for division, differentiation, and adaption (Lipowsky, 2014; Nicolson, 2014; Dawaliby *et al.,* 2016). Glycerolipids have at least one hydrophobic chain linked to a glycerol backbone in an ester or ether linkage and can be divided into glycosylglycerolipids (non-polar) and phospholipids (polar) by the structure of head group. Glycosylglycerolipids carry sugar head groups (MGDG: monogalactosyldiacylglcerol, DGDG: digalactosyldiacylglycerol and SQDG: sulfoquinovosyldiacylglycerol) and phospholipids carry phosphate head groups (PC: phosphatidylcholine; PA: Phosphatidic acid; PG: phosphatigylglycerol; PE: phosphatidylethanolamine; and PI: phosphatidylinositol) (Joyard *et al.*, 1994; Benning and Ohta, 2005; Bittman, 2013).

Table 1.6.1: The most abundant lipid classes in membranes. (From: Scotland and Sandvig, 2019. Nature communication, The role of PS 18:0/18:1 in membrane function.)



Lipid class/ abbreviation	R1	R2	Head group
Phosphatidylcholine/PC	FA	FA	**Choline**
Lysophosphatidylcholine/LPC[a]	FA	H	
Ether-linked PC: PC O, PC P[a]	Alkyl, alkenyl	FA	
Phosphatidylserine/PS	FA	FA	**Serine**
Phosphatidylethanolamine/PE	FA	FA	**Ethanolamine**
Phosphatidylinositol/PI	FA	FA	**Inositol**
Phosphatidylglycerol/PG	FA	FA	**Glycerol**
Phosphatidic acid/PA	FA	FA	**H**
Sphingomyelin/SM	LCB	FA	**Phosphocholine**
Glycosphingolipids/GSL	LCB	FA	**Carbohydrates**

a: Lysolipids and ether-linked lipids may occur in some lipid classes but are for simplicity shown of PC; FA: fatty acyl group; LCB: long-chain base.

1.6.1 Glycosylglycerolipids in plants

Glycosylglycerolipids, also named galactosylglycerides, as the major plastid lipids, are abundant lipids of photosynthetic membranes in photosynthetic bacteria, cyanobacteria, algae, and higher plants (Kobayashi *et al.*, 2007; Gaude *et al.*, 2007; Bittman, 2013; Guschina and Harwood, 2006; 2009 and 2013). Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are the primary lipids of photosynthetic membranes. Another category of glycosylglycerolipids are the plant sulfolipids, sulfoquinovosyldiacylglycerol (SQDG), which are presented abundantly in both photosynthetic and in nonphotosynthetic species. These glycosylglycerolipids are common in photosynthetic membranes of higher plants and are also components of plant leaves and non-photosynthetic tissues such as seeds, fruits, and potato tubers. (Browse and Somerville, 1991; Joyard *et al.*, 1998; Hamed *et al.*, 2005; Guschina and Harwood, 2013; Bittman, 2013). In thylakoid membranes of chloroplasts and cyanobacteria, about 85% of the lipids are MGDG, DGDG, and SQDG (Bittman, 2013).

1.6.2 Phospholipids in plants

Phospholipid, also known as phosphatide or glycerophospholipid, is a type of lipid molecule that contain a phosphorus substance. It is a key component of cell membranes and play an important metabolic role in plants, mammals, and bacteria. The main role of phospholipids is the permeability barrier for cells, participating in signal transduction, and in cell organelles (Hernández and Cooke, 1996; Whitehead and Day, 1997; Andersson *et al.*, 2005; Nakamura, 2017). The chemical structure of phospholipids is a glycerol molecule linked to two fatty acids, and a phosphate group, and these two fatty acids help to form diacylglycerol (DAG). Phospholipids are amphiphilic lipids, because the phosphate head group is hydrophilic (polar “head”) while the fatty acid tails are hydrophobic (non-polar “tails”) They are able to form a monolayer of a polar surface of oil bodies in plants, and also a lipid bilayer as the fundamental molecules of membranes (Murphy, 2012; Nakamura, 2017; Phospholipid: Biochemistry, Encyclopedia Britannica, 2020). Phospholipids are mainly distributed in plasma membranes and mitochondrial envelopes, while they are scarcely found in photosynthetic membranes. For example, the most extraplastidic membranes contain phosphatidylcholine (PC) and phosphatidylethanolamine (PE) as the abundant phospholipids, whereas they are less in thylakoid membranes, with phosphatidylglycerol (PG) as the only phospholipid class and galactolipids as the main components (Joyard *et al.*, 1998).

PC (Phosphatidylcholine), also called 1,2-diacyl-sn-glycero-3-phosphocholine (once named 'lecithin') is the most abundant phospholipid in most eukaryotic cells including yeast that incorporate choline as a “head” (Kroon *et al.*, 2013). It is a key component of biological membranes and can be found in all plant and animal cells while they are absent in the membranes of most bacteria, including *Escherichia coli*. (Biochemistry of lipids, 1991). PC has been proved to play a key role in many important cellular functions, in particular endocytosis and membrane trafficking (Donaldson, 2009). PC is structurally related to PE and has the same function as PE in some biological networks. Because of the same structure part with amino alcohol in the 'head' group. **PE (Phosphatidylethanolamine)**, or 1,2-diacyl-sn-glycero-3-phosphoethanolamine (once named 'cephalin'), is usually the second most abundant phospholipid in plant and animal lipids, and commonly the major lipid element in microbial membranes. PC and PE are the main components of bilayers in membranes and the ratio of PC to PE possibly impacts cellular functions. PE occasionally has the same function as PC in biological processes (Di Bartolomeo *et al.*, 1862; Webb and Green, 1991; Dawaliby *et al.*, 2016; Annibal *et al.*, 2016; Ball *et al.*, 2017). PE can be a precursor of PA to remove the terminal phosphodiester bond to produce PA (Bargmann and Munnilc, 2006). **PA (Phosphatidic acid)** or 1,2-diacyl-sn-glycero-3-phosphate is one of the simplest molecular structures within the phospholipids but act an important intermediate in plant signaling pathways and as precursors for TAGs, and in the synthesis of other glycerophospholipids. It is also closely related to membrane structure and dynamics, and proteins interactions in plants, animals, and organisms because of its unique physicochemical properties. (Munnik, 2001; Ryu, 2004; Wang, 2005; Testerink and Munnik, 2005; Cowan, 2006). PA is not abundant in any living cells, normally it is less than in picomolar concentration. PA is the simplest diacyl-glycerophospholipid and the only one with a phosphomonoester at the head group (Kim and Wang, 2020).

PI (Phosphatidylinositol), 1,2-diacyl-sn-glycero-3-phospho-(1'-myo-inositol), consists of a phosphatidic acid backbone, linked via the phosphate group to inositol. It is a minor phospholipid of the cell membranes, but it is an important membrane constituent and participant in membrane trafficking and signaling in all plants and animal cells. PI is especially significant in microdomains at the shoot apical meristem, pollen tubes, root hairs, etc. (Bella, 2013; Heilmann and Heilmann, 2014; De Matteis and De Camilli, 2015; Choy *et al.*, 2017; Champeyroux *et al.,* 2020; Blunsom and Cockcroft, 2020). **PG (phosphatidylglycerol)** can be found in cell membranes of plants and animals with a low abundance, but it is a major component of membranes in some bacterial. In the photosynthetic membranes of higher plants, such as *Arabidopsis thaliana*, PG is the only phospholipid with a unique characteristic of a high portion of trans3-hexadecenoic acid exclusively located in position sn-2. PG is mainly present in thylakoid membranes and has special functions of photosystem II of plants (Hagio *et al.*, 2002; Mizusawa and Wada, 2012; Kobayashi 2016; Michaud *et al.*, 2017). **PS (Phosphatidylserine),** 1,2-diacyl-sn-glycero-3-phospho-L-serine, is a vital phospholipid but with low abundance in most membranes of animals, plants, and microorganisms. In *Arabidopsis thaliana* leaves, the content of PS is about 0.3% of total phospholipids (Smith *et al.*, 2013). It is one of the essential components of cell membranes in both eukaryotes and prokaryotes, and usually the content of PS is less than 10% of the total phospholipids in eukaryotic cells, while the amount is more than 30% in yeast (*Saccharomyces cerevisiae*) (Lipid maps web). Studies of *Arabidopsis thaliana* show that the fatty acid constitution of PS resembles PE in leaves (The lipid web). PS also participates in cell metabolism, it serves as the precursor of PE in mitochondria for example (Vance and Steenbergen, 2005; Nerlich *et al.*, 2007).

1.6.3 Glycerolipids pathway in plant cell

With the identification and characterization of lipid biosynthesis processes response to biotic and abiotic stresses in *Arabidopsis thaliana*（Welti *et al.*, 2002 and 2007; Shen *et al.,* 2010; Wang *et al.*, 2012; D. Singer *et al.*, 2016; Negi *et al.*, 2018）, *Triticum aestivum* (Narayanan *et al.*, 2016), *Chlamydomonas* (Li-Beisson *et al.*, 2015; Zienkiewicz *et al.*, 2016; Morales-Sánchez *et al.*, 2017; Kim e*t al.*, 2018), *Saccharomyces cerevisiae* (Carman and Han, 2011; Kohlwein *et al.,* 2013; Athenstaedt, 2021; Kohlwein, 2021), soybean (Zhang *et al.*, 2019 and 2020), *Craterostigma plantagineum* (Gasulla *et al.*, 2013), and introgression genotypes of *Lolium multiflorum* and *Festuca arundinacea* (Perlikowski *et al.*, 2016), glycerolipid biosynthesis pathway is elucidated with more details. Lipid metabolism in plant cells is complicated because plant cells are composed of different organelles and discrete subcellular compartments with special lipid compositions. Studies on glycerolipids metabolism mainly focuses on three biosynthetic pathways that are compartmentalized within plastids, mitochondria, and the endoplasmic reticulum (ER) (Roughan and Slack, 1982; Browse *et al.*,1986; Jouhet *et al.*, 2006; Shen *et al.*, 2010; Wang *et al.*, 2012; Singer *et al.*, 2016; Sezgin *et al.*, 2017; Levental *et al.*, 2020). Glycerolipids assembled on ER membranes mostly are membrane phospholipids and substrates to generate TAGs (Ohlrogge and Browse, 1995; Kim *et al.*, 2018; Lavell and Benning, 2019). The majority of glycerolipids synthesized in chloroplasts are converted to galactolipids which are the basic components of photosynthetic membranes (Dörmann and Benning, 2002). Glycerolipids produced in mitochondria possibly are membrane lipids and fatty acids (Zheng *et al.*, 2003; Li-Beisson *et al.*, 2013; Kobayashi, 2016; Harayama and Riezman, 2018). Grasses mainly utilize the ER pathway, while *Arabidopsis thaliana* utilizes both ER pathway and plastid pathway (Petroutsos *et al.*, 2014; Yang *et al.*, 2017; Lavell and Benning, 2019).

As it shown in Figure 1.12, the glycerolipid biosynthesis in *Arabidopsis thaliana* can be divided into three steps. The first step is assembly of PA by attaching two acyl groups to glycerol-3-phosphate, which take places on ER membranes and in plastids. The second step splits to two branches, PA is converted to cytidine diphosphate diacylglycerol (CDP-DAG) and the activated formation of DAG. CDP-DAG is a precursor for the biosynthesis of PI, PG and CL. The third step is assembly of different head groups to glycerol backbone (DAG) and forming glycerolipids. (Williams *et al.*, 2000; Nakamura, 2017; Reszczyńska and Hanaka, 2020). PG can be Produced in three biosynthesis pathways, plastids, ER, and mitochondria. PG is the only phospholipid class synthesized in the plastids, and it is essential for photosynthetic system II in plants (Jones, 2007; Kobayashi and Wada, 2016). PIS (Phosphatidylinositol synthase) is the enzyme responsible for the synthesis of phosphatidylinositol, a crucial phospholipid component of all eukaryotic membranes and the precursor of messenger molecules involved in plant development and function (Thole and Nielsen, 2008; Munnik and Nielsen, 2011; Boss and Im, 2012; Heilmann and Heilmann, 2015). Contrast to mammals and bacteria, photosynthetic membranes in plant cells are mainly consist of non-phosphorus galactolipids. The galactolipids in plant cells are synthesized in chloroplasts (Dörmann *et al.*, 1995 and 1999).

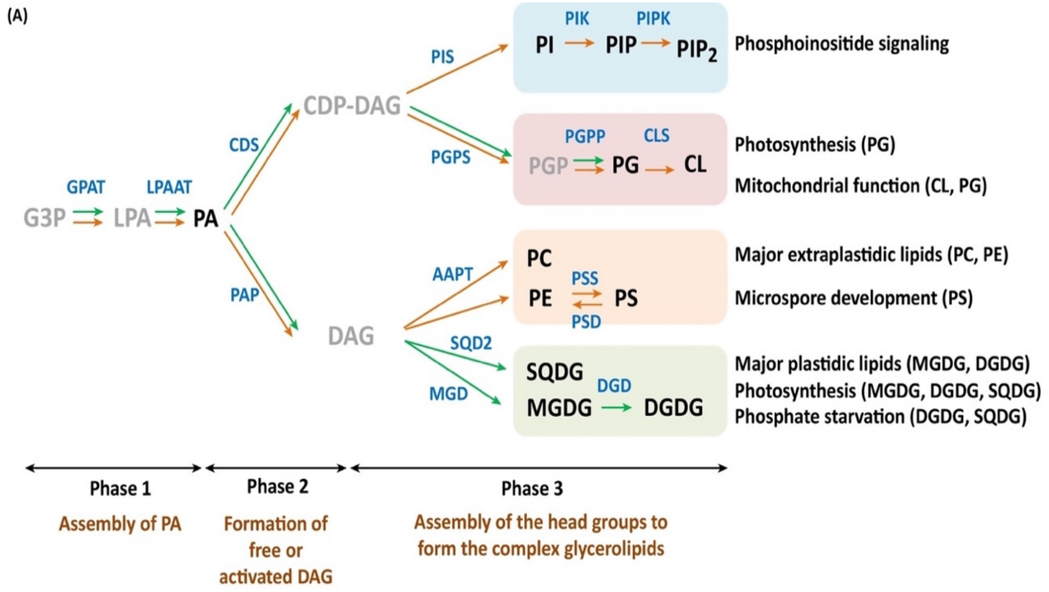


Figure 1.12: A conceptual diagram of glycerolipids biosynthesis in *Arabidopsis thaliana*. The polar glycerolipids are divided into four groups (all but the green category belongs to phospholipids) with the functional aspects described at the right. Black letters, polar glycerolipids end products; blue color shows of enzymes; and gray color is indicating the precursors and intermediates of polar glycerolipids biosynthesis. G3P: glycerol 3-phosphate.

(From: Nakamura, 2017, Trends in Plant Science, Plant phospholipid diversity: emerging functions in metabolism and protein–lipid interactions)

1.7 Biosynthesis of Sterols in plant

1.7.1 Plant sterols

Plant sterols also known as sterolins and phytosterols (comparatively to zoosterols), are present in all plants and almost all living organisms. As the third lipid class, sterols play structural roles as the critical elements of cell membranes and regulate membrane permeability and fluidity (Bouic, 1998; Hartmann, 1998; Clouse 2002; Valitova *et al.*, 2016), and also biological roles as hormone signals in plant growth and resistance to biotic and abiotic stresses in plants (Schaller, 2003 and 2004; Boutté and Grebe, 2009; Griebel and Zeier, 2010; Malinsky *et al.*, 2013; Tapken and Murphy, 2015; Ferrer *et al.*, 2017). Sterols are isoprenoid lipids, made up of a steroid backbone, substituted by an alkyl side chain and a hydroxyl group. Sterol lipids are considered as membrane reinforcers. Plants have complex and diversified sterol compositions (Nes and McKean, 1977; Schaeffer *et al.*, 2002). The free form of free sterols (FS) and conjugated forms of sterol glycosides (SG), acylated sterol glycosides (ASG), and sterol esters (SE) are the predominant sterols in plants. The value of sterols amount is generally constant in plants: about 2–3 mg of total (free and esterified) sterols per gram of dry weight (Schaeffer *et al.*, 2002; Holmberg *et al.*, 2002; Benveniste, 2004). Free sterols are generally the most abundant sterol lipids and mainly exist as 3 forms: campesterol, β-Sitosterol, and stigmasterol (Figure 1.13), while cholesterol, the most important zoosterol, is usually a minor component of plant sterols (Nomura *et al.*, 1999; Wewer *et al.*, 2011; Bot, 2019; Abobucker and Suza, 2019; The lipidweb). Conjugated sterols are ubiquitously found in plants, but their contents highly differ among species and their profile may change in response to developmental and environmental cues (Moreau *et al.*, 2002; Schaller, 2004; Boutté and Grebe, 2009; Ferrer *et al.*, 2017). Plant free sterols and conjugated sterols are involved in membranes adaptation, e.g., the ratio of stigmasterol to β-sitosterol is increased in *Arabidopsis thaliana* leaves with pathogen infection, and the ratio of stigmasterol to β-sitosterol is also related to environmental temperature changes (Griebel and Zeier, 2010; Valitova *et al.*, 2019).

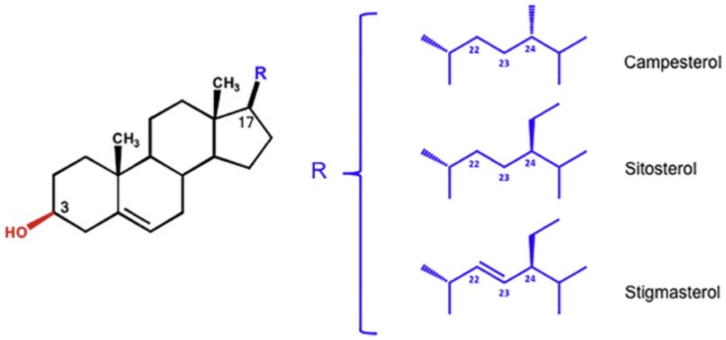


Figure 1.13: Chemical structure of the major free sterols in plants. (From: Ferrer *et al.*, 2017. Emerging roles for conjugated sterols in plants)

1.7.2 Sterol esters

Sterol esters (SE) can be found in all plant tissues, but the amounts and relative content are dependent on plant species and even cell organelles and the growth conditions. Sterol esters possibly present with an abundant amount in pollen grains, senescent leaves, and some seeds, e.g., 35% of phytosterols is SE in *Brassica napus* seed oil (Wu *et al.*, 1999; Sabir *et al.*, 2002; Harker *et al.*, 2003; Bouvier-Navé *et al.*, 2010). Generally, SE are present in plant cells at much lower amounts compared to free sterols, but the sterol components are almost the same (Wewer *et al.*, 2011; Valitova *et al.*, 2016; Rogowska and Szakiel, 2020). SE are inferred to play the role of inert storage forms of sterols, coupled with longer chain fatty acids through the formation of esters, and localized to the oil bodies. Sterol esters are the other non-polar lipids which are presumed to be associated with TAGs in plant oil bodies (Kemp and Mercer, 1968; Zinser *et al.*, 1993). Sterol esters are the key components in cell membrane sterol homeostasis and presumed to constitute a storage pool of sterols during seed germination, leaves senescence and environmental adaptation (Dyas and Goad, 1993; Chang *et al.*, 1997; Schaller, 2004; Bouvier-Navé *et al.*, 2010). Schaller and Ferrer pointed out that sterol esters are promoting oil bodies biogenesis in plants (Schaller, 2004; Ferrer *et al.*, 2017).

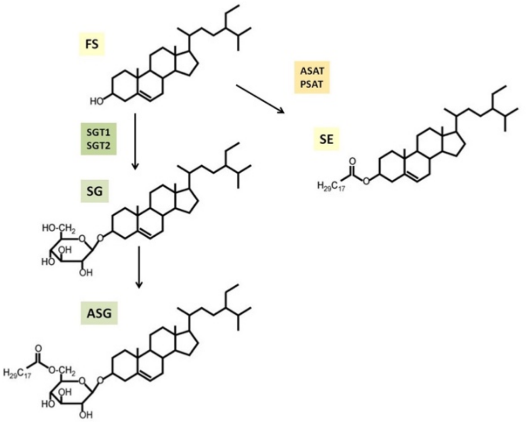


Figure 1.14: Schematic diagram of biosynthesis of conjugated sterols in *Arabidopsis thaliana*. Free sterols (FS) are used as substrates for the synthesis of sterol glucoside (SG) by enzymes SGT1 and SGT2 and for the synthesis of sterol esters (SE) by enzymes ASAT and PSAT. ASG: acylated sterol glucoside; ASAT: acyl-CoA: sterol acyltransferase; PSAT: phospholipid: sterol acyltransferase; SGT: sterol glucoside transferase. (From: Vera Wewer, 2012, PhD dissertation)

The genes of sterol esterification in mammals and yeast have been known for a long time (Jonas, 2000; Temel *et al.*, 2003; Wagner and Daum, 2005; Daum *et al.*, 2007). In plants, sterol acyltransferases were identified as acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) (Ferrer *et al.*, 2017; Korber *et al.*, 2017; A. Lara *et al.*, 2018). PSAT1 and ASAT1 are cloned and characterized in *Arabidopsis thaliana*, AtASAT1 (At3g51970) and AtPSAT1 (At1g04010) (Banaś *et al.*, 2005; Chen *et al.*, 2007). Banas (2005) indicated AtPSAT is the only intracellular enzyme that catalyzing acyl-CoA-independent sterol ester biosynthesis. A. Lara *et al.*, (2018) indicated that that PSAT1 is not the key enzyme to limit sterol metabolic rate in tomato (*Solanum lycopersicum*), and SlPSAT1 and SlASAT1 show overlapping but largely complementary models of expression during tomato fruit growth. Wewer (2011) suggested that the SE increased under phosphate deprivation may represents a stress response in both *Arabidopsis thaliana* leaves and roots (Wewer *et al.*, 2011). A recent study of barley represented those sterols accumulated with heat/drought stresses (Kuczyńska *et al.*, 2019). Desiccation tolerant cultivar of *Oryza sativa* showed higher amounts of sterols and sterol esters than drought susceptible cultivar under drought stress, while the expression level of PSAT exhibited same increase (Kumar *et al.*, 2015 and 2018).

2. Materials and methods

2.1 Materials

2.1.1 Cultivation of plants

Oropetium thomaeum (L.f.) Trin. belongs to the Poaceae and was collected by Dr. Labu Rao in Jodhpur, Rasasthan, India, in 1991 (Bartels and Mattar, 2002). Seeds were collected and cultivated in the greenhouse of the University of Bonn. Seeds were germinated on the surface of propagation substrates (Type VM, http://www.einheitserde.de/). After four weeks, always three seedlings were transferred to a new plant pot with the same substrates. The conditions for the germination of *Oropetium thomaeum* seeds and seedling growth was as follows: 16 h illumination per day with a light intensity of about 150-175 µmol × m^{-2} × S^{-1} and 22 °C during the day and 18 °C during the night, 60-65 % relative humidity.

Craterostigma plantagineum plants were originally collected from South Africa and cultured based on the description by Bartels et al (1990) in the IMBIO Institute of the University of Bonn. Seedlings were cultivated on MS medium for about two weeks, and then transferred to artificial clay substrate. The plants were grown with 16 h illumination of 105-115 µmol × m^{-2} × S^{-1}, and at 22 °C in the light and 18 °C in the dark, and 60-65 % relative humidity in a plant growth chamber.

Lindernia brevidens Skan and *Lindernia subracemosa* De wild plants were collected by Eberhard Fisher from the Taita Hills, Kenya (Phillips *et al.*, 2008) Propagation of plants was according to Dinakar and Bartels (2012). Substrates and vermiculite (3/1, v/v) were mixed as growth medium. The cultivation process was under light intensity of 110-120 µmol m^{-2} Sec^{-1} at 22°C during the day and 18°C during the night, 55-60 % relative humidity in a plant growth chamber. The light/dark cycle was 16/8 hours.

Arabidopsis thaliana (Col-0) plants were germinated and grown on a mix of substrates /vermiculite (3/1, v/v) under 55-60 % relative humidity and white light of approximately 110-120 µmol m^{-2} Sec^{-1} at a day/night temperature of 22 °C/18 °C. Plants were grown under the day/night cycle of 16/8 hours in a plant growth chamber.

The seeds of *Oropetium thomaeum, Craterostigma plantagineum, Lindernia brevidens, Lindernia subracemosa* and *Arabidopsis thaliana* were kept at 4°C, 72 hours for vernalization before sowing.

2.1.2 Chemicals

In this study, chemicals were used from these companies:

AppliChem (Darmstadt, Germany); Roth (Karlsruhe, Germany); Sigma-Aldrich Chemie GmbH (Munich, Germany); Merck (Darmstadt, Germany); Invitrogen/Life Technologies (Karlsruhe, Germany); Bio-rad (Munich, Germany); Thermo Fisher Scientific (Waltham, USA); Fermentas (St. Leon-Rot, Germany); Sigma-Aldrich (Taufkirchen, Germany); J.T. Baker/VWR (Darmstadt, Germany); Grüssing (Filsum, Germany); Agilent (Böblingen, Germany); Spurr's resin (Spurr Low-Viscosity Embedding kit), Sigma-Aldrich, USA.

All solvents for lipid extraction and analysis were with highest purity, "for analysis" or "HPLC grade". Only glasses tools were used for the lipid extraction and analysis which involved a Q-TOF.

2.1.3 Machines and devices

- T3-Thermocycler, Biometra, Göttingen, Germany.
- Imaging system Typhoon Scanner 9200 Variable Mode imager, Amersham Biosciences, Freiburg, Germany.
- Confocal Laser Scanning Microscope ZE2000 with Laser D-eclipse C1, Nikon, Düsseldorf, Germany.

- Electroporation system Genepulser II Electroporator, Bio-Rad, Hercules, USA.
- Olympus FV1000 confocal laser scanning microscope with the FV10-ASW1.7 software, Olympus, Hamburg, Germany.
- Binocular microscope SMZ-800, Nikon, Düsseldorf, Germany.
- HPLC (High Pressure Liquid Chromatography) with Fluorescent Light Detector (FLD) 1100 Series, Agilent, Böblingen, Germany.
- GC-MS (Gas Chromatography- Mass Spectrometry), 6530 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF), Agilent, Böblingen, Germany.
- Water bath, Köttermann Lab Devices (Uetze / Hänigsen, Germany.
- SDS-PAGE Mini gel system, Biometra, Göttingen, Germany.
- Centrifuge 5415R; 5417R; 5810R, Eppendorf, Hamburg, Germany.
- pH Meter, Schott Glas, Mainz, Germany.
- pH Meter, Mettler Toledo, Giessen, Germany.
- Ultracentrifuge, Beckman-Coulter, Krefeld, Germany.
- Typhoon Scanner 9200, Amersham Biosciences, Freiburg, Germany.
- TEM (transmission electron microscopy) Zeiss LEO912 AB TEM, Carl Zeiss, Inc., Oberkochen, Germany.
- Reichert Ultracut S ultramicrotome, Leica, Austria.
- Leica Reichert-jung Ultracut E ultramicrotome, Reichert, England.
- Light Microscopy with lens of LEICA DM750, Leica Microsystems, Wetzlar, Germany.

2.1.4 Software, programs, and online tools

- Phytozome (http://phytozome.jgi.doe.gov/)
- Plant Care (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)
- TAIR-Arabidopsis database (https://www.arabidopsis.org/)
- Fancy GENE (http://bio.ieo.eu/fancygene).

- Sequence alignment (http://multalin.toulouse.inra.fr/multalin/).
- AtGenExpress database
- Image Quant Version 5.2 software
- Image J (https://imagej.nih.gov/ij/ version 1.52n)
- Microsoft Office package 2010
- GraphPad Prism 8 (https://www.graphpad.com/scientific-software/prism/)
- NCBI (https://www.ncbi.nlm.nih.gov/)
- Sequence Reverse complement (https://www.bioinformatics.org/sms/rev_comp.html)
- Oligo 7 (http://www.oligo.net/tutorials.html)
- Oligo Evaluator (http://www.oligoevaluator.com/)

2.1.5 Consumables for lipid extraction

- Glass vials with thread, 8 ml, VWR, Darmstadt, Germany.
- Glass vials without thread, 8 ml, VWR, Darmstadt, Germany.
- Glass vials with thread, 40 ml, Schmidilin, Neuheim, Switzerland.
- Glass Pasteur pipettes 150 mm and 225 mm, Wertheim Brand, Germany.
- PTFE screw caps for 8 ml glass vials, Schott, Mainz, Germany.
- PTFE screw caps for 40 ml glass vials, Schmidlin, Neuheim, Switzerland.
- Teflon septa for screw caps, 13.3 mm and 22.4 mm, Schmidilin, Neuheim, Switzerland.
- SPE columns, strata Si-1 sillica, 55 µm, 70A, 500 mg / 6 ml and 100 mg/1 ml, Macherey and Nagel, Düren, Germany.
- Small vials with screw caps and Teflon septa for GC and LC machine, Macherey and Nagel, Düren, Germany.

2.2 Methods

2.2.1 Plant treatments

2-2.5-month-old *Lindernia brevidens* and *Lindernia subracemosa* (De wild) plants, 2.5-3-month-old *Craterostigma plantagineum* plants, 6-7-week-old *Arabidopsis thaliana*, 3.5-4 months old *Oropetium thomaeum* plants were used to the following treatments:

Plants grown under normal conditions were used as control plants. For stress treatments plants were dehydrated for 7 days and 14 days by withdrawing watering under normal growth conditions, after dehydration plants were rehydrated for 2 days by watering them under the same growth chamber conditions. After each treatment plants were sampled, parts of the samples were immediately used for lipid extraction and sectioning. The other samples were frozen in liquid nitrogen and kept at -80 °C for molecular analysis.

2.2.2 Relative water content

The relative water content (RWC) was calculated according to González and González-Vilar (2001) and Bartels and Mattar (2002). RWC (%) = [(FW- DW)/ (SW-DW)] × 100; FW, DW and SW indicate fresh weight, dry weight after drying at 80 °C for 2 days, and saturated plant weight after submerging plants under water for 36 hours, respectively.

2.2.3 Histological sections

Tissues were cut from leaves of 3.5 months old Oropetium thomaeum plants for sections. Samples were taken from three different treatments, well-watered (Control), dehydrated for 7 days (D-7d) and 14 days (D-14d) plants (Dehydrated).

2.2.3.1 Hand sections

Leaves were cut into small pieces by using a fine foam to help holding samples. Then the sections were stained with different solutions and methods: 0.1% (w/v) methylene blue for 20 min; 1 mg/ml Sudan III in 50% (w/v) glycerol solution heated for 3-5 min; 0.05% chrysoidine solution with heating for 3 min.

Nile red staining was modified according to James *et al.*, 2010; Shimada *et al.*, 2015; Gidda *et al*., 2016 and Zhou *et al.*, 2019.

Plant oil bodies were stained using 5 µg/mL Nile Red in 10% DMSO for 1 min. Samples were excited at 488 nm laser and emission fluorescence signals were collected at around 590 nm and imaged using confocal laser scanning fluorescence microscopy (ZE2000 with Laser D-eclipse C1, Nikon, Düsseldorf, Germany).

2.2.3.2 Semithin sections

Leaves were cut into small pieces and then fixed, dehydrated, embedded in Spurr's resin (Spurr Low-Viscosity Embedding kit), after that, sectioned for semi-thin sectioning and ultra-thin sectioning for optical microscopy and TEM (transmission electron microscopy) respectively.

For fixation, small pieces of leaves were immersed in 50 mM Na-cacodylate buffer (pH 7.0) containing 3 % (v/v) glutaraldehyde, 1 % (v/v) formaldehyde, 0.25 % (w/v) sucrose and 1 % (v/v) DMSO for 3hours at RT. Afterwards, samples were washed in 100 mM Na-cacodylate buffer for 10 min 3 times; then the leaf samples were postfixed in 2 % w/v OsO4 for 2 h at room temperature and washed in deionized water.

Then samples were subsequently dehydrated in a graded series of acetone solutions followed by 15 % (v/v) acetone for 5 min 3 times; 30 % 10 min 3 times; 50 % 10min 3 times; 75 % acetone 45 min; 90 % 15min 3 times; 96% 15 min 3 times; and then 100 % acetone for 15 min 4 times.

Next step, samples were embedded in a graded series of epoxy resin in acetone 1:3(v/v), overnight; and then 1:1(v/v), 4 h; later 3:1(v/v), 4 h, after that samples were put into pure epoxy resin overnight and changed into new pure epoxy resin for 4 h. Then specimens were kept at 70 °C for 7.5 h to polymerize.

Semi-thin sections were done using a Reichert Ultra cut ultramicrotome. Semi-thin sections were stained with 1 % (w/v) toluidine blue solution with heating for 1 min and imaged using a LEICA DM750 microscope (Leica Microsystems, Wetzlar, Germany).

2.2.3.3 Ultrathin sections

Ultrathin sections were cut using a Leica Reichert Ultra E ultramicrotome, 600-900A and 900-1500A metal grids (silver to golden) were used for holding the samples, and then observed by Transmission electron microscope (TEM) Zeiss LEO912 AB (Carl Zeiss, Inc., Oberkochen, Germany).

2.2.4 Lipid extraction

Phospholipids, glycerolipids and sterol lipids extraction for Q-TOF MS analysis from plant leaves was according to Welti *et al.* (2002) and Gasulla *et al.* (2013). Separation and quantification by Q-TOF MS/MS measurements (Wewer *et al.*, 2011; Gasulla *et al.*, 2013; vom Dorp et al., 2013).

2.2.4.1 Extraction of total lipids from plant leaves.

1. Cut 100 mg leaf material from plants and immediately transferred into glass tubes (with Teflon septa) and incubate in boiling water bath for 10-15 min.
2. Remove water completely with a glass pipette.

3. Extract leaf tissue with 1 vol $CHCH_3$: MeOH (1:2, v/v), collect organic phase.
4. Extract leaf tissue with 1 vol $CHCH_3$: MeOH (2:1, v/v), collect organic phase.
5. Extract leaf tissue with 1 vol $CHCH_3$, harvest organic lipid extraction.
6. Repeat 3 times of extraction with 1 vol $CHCH_3$: MeOH (2:1, v/v), harvest organic lipid extraction, until all the remaining plant tissue appeared white.
7. Collect all the organic lipid extractions in a fresh glass vial.
8. To combined chloroform fractions, add 0.75 vol 300 mM NH_4OAc solution, make sure the final ratio of $CHCH_3$: MeOH: NH_4OAc is 2:1:0.75 (v: v: v).
9. Vortex thoroughly and then centrifuge at room temperature for 5 min 2000 rpm.
10. Use a glass pipette to collect the chloroform phase, avoid taking the aqueous phase.
11. Evaporate solvents of lipid extraction with N_2 gas, dissolve in 2 ml of Chloroform and then divided to two 1 ml aliquots.

Separation of phospholipids and glycerolipids

Take 200 µl of the total lipids extract and 20 µl internal standard mixture of phospholipid to evaporate under the stream of air. The internal standard mixture contained in 20 µl: 0.2 nmol of each di14:0-PC, di20:0-PC, di14:0-PE, di20:0-PE, di14:0-PG, di20:0-PG, di14:0-PA and di20:0-PA; 0.03 nmol di14:0-PS and di20:0-PS; 0.3 nmol of 34:0-PI; 0.15 nmol 34:0-MGDG, 0.10 nmol 36:0-MGDG; 0.2 nmol 34:0-DGDG, 0.39 nmol 36:0 DGDG; 0.4 nmol SQDG. Then dilute with 200 µl of Q-TOF solvent (Methanol/Chloroform/300 mM ammonium acetate, 665:300:35, v/v/v) for the measurement of phospholipids and glycerolipids.

Separation of nonpolar lipids

Nonpolar lipids like DAGs and TAGs, conjugated sterols (SE, SG, and ASG) need purification of total lipid extracts. Solid phase extraction with Strata silica columns was used for the separation of nonpolar lipids.

1. Take 500 µl of the extracted lipids solution, add TAG/DAG standard 50 µl, sterol mix standard 50 µl and sphingolipid mix standard 50 ul, free fatty acid standard 100 µl 15:0 fatty acid, later evaporate solvents under a stream of N_2 and dissolve in 1ml 100 % hexane.
2. load the sample on a silica column (500 mg, 6 ml). These columns were washed with 3 vol of hexane before using.
3. Put the columns to the next fresh glass vial and elute the sterol esters with 3 vol hexane/diethyl ether (99/1, v/v). Evaporate this fraction and dissolve in 250 µl Q-TOF-LM and measure the sterol esters with the SE-method using a Q-TOF.
4. Put the columns in the next new tube and rinse the original tube of the lipid extract and elute the triacylglycerol (TAG) with 3 vol of hexane/diethyl ether (95/5, v/v). Evaporate this fraction and dissolve in 100 µl Q-TOF-LM and measure the TAG with the “TAG-method 48-56er” with Q-TOF.
5. Put the columns in the next new tube and rinse the original tube of the lipid extraction and elute the free fatty acid (FFA) with 3 vol of hexane/diethyl ether (92/8, v/v). Evaporate this fraction and dissolve in 100 µl 100% acetonitrile and measure the FFA with the “FFA-method in ESO mode” in Q-TOF.
6. Put the columns to a new tube and rinse the original tube of the lipid extract and elute the diacylglycerol (DAG) with 3 vol 100% diethyl ether. Divide this fraction into

two portions. Evaporate one of these aliquots and dissolve it in 100 µl Q-TOF-LM and measure the DAG with the DAG-method MS/MS with Q-TOF.

7. The other portion is for measurement of free sterols. This fraction has to be derivatized by N-chlorobetainylchloride. Therefore, evaporate this fraction and add 500 µl dichlormethan, a pinch of N-chlorobetainylchlorid and 3 drops of pyridine (this should induce the generation of a little smoke). Leave it at 42 °C for 4 h or overnight. then add 500 µl chloroform, methanol, and synergy-water each, vortex thoroughly and centrifuge at 2000 rpm for 2 min, harvest the lower phase and transfer into another tube. Evaporate and dissolve in 250 µl Q-TOF-LM and measure the betainylated sterols immediately using the method of free sterols CBC mode in Q-TOF.

8. Put the columns to a new tube and rinse the original tube of the lipid extract and elute the sterol glucosides (SG), acylated sterol glucosides (ASG), ceramides (Cer) and glucosylceramides (GICer) and TriDGD and TeDGD with 6 vol of acetone/2-isopropanol (9/1, v/v). Evaporate this fraction and dissolve it in 200 µl Q-TOF-LM and measure TriDGD and TeDGD with the TriDGD-TeDGD -method using a Q-TOF.

2.2.4.2 Extraction of fatty acid methyl ester (FAME) from plant leaves.

FAME reaction (Benning, 1995)

1. Fill lipid extract into a glass vial with a screw cap and evaporate under the stream of air. (Use Teflon septum in caps, not plastic or rubber)

2. Add 1 ml 1N HCL in methanol (stored at 4 °C) with a glass pipette.

3. Add an internal standard of 100 µl of working solution, with the amount of 5 µg. (Pentadecanoic acid,15:0, working solution:50 µg/ml in methanol, 1: 200 of super stock, 10 mg/ml in methanol)

4. Incubate at 80 °C for 20 min for FAME formation from lipid extracts.

5. After cooling to room temperature, add 1 ml 0.9 % (w/v) NaCl and 1ml hexane for FAME formation from conjugated sterols, then shake vigorously.

6. Centrifuge 3 min at 1000 rpm.

7. Collect the upper hexane phase with a pipette to a fresh glass vial.

8. Concentrate FAME in N_2 stream but do not dry it completely (because short and medium chains of FAME 10:0, 12:0, and14:0 are easily blown out).

9. Dissolve FAME in 100 µl hexane and transfer to gas chromatography (GC) tubes for measurements.

FAME measurement: GC-FID (Gas Chromatography-Flame Ionization Detection) was used for detection, and the process was described by Katharina vom Dorp (2015, PhD dissertation)

2.2.5 RNA isolation

Total RNA isolations were conducted according to Valenzuela-Avendaño *et al.* (2005) with a few modifications. Plant tissue was ground to a fine powder by using liquid nitrogen. 100-200 mg ground tissue was added to a 2 ml Eppendorf tube with 1.5 ml of RNA extraction buffer. The homogenate was incubated at RT for 10 min after vortexing and then centrifuged at 10000 rpm for 10 min at room temperature. Then the supernatant was transferred to new tubes and 300 µl of a mixture of chloroform and isoamyl alcohol (24:1, v/v) were added. After shaking vigorously by vortexing for 10 seconds, the tubes were centrifuged at 10000 rpm for 10 min at 4°C. Then the upper aqueous phase was transferred to new tubes, mixed thoroughly with 375 µl isopropanol and a 375 µl solution of sodium chloride and sodium citrate (with the concentration of 1 M and 0.8 M respectively). The mixture was kept at room temperature for 10 min. Then samples were centrifugated at 13000 rpm for 10 min at 4 °C. After that, the supernatant was removed, and the remaining pellet was washed with 1 ml of 70% (v/v) pre-cold ethanol (-20 °C). Samples were centrifuged at 11000 rpm, 4 °C for 10 min, then dried at RT and dissolved with 100 µl sterile H_2O. After this, 167 µl of 4 M LiCl was added and the tubes were incubated on ice for 2 h. Then RNA samples were centrifuged at 12000 rpm for 20 min at 4 °C to obtain the RNA pellet. The pellet was washed twice with 1 ml of 70 % (v/v) pre-cold ethanol (-20 °C), then dried and finally dissolved in 50 µl sterile H_2O.

RNA extraction Buffer: buffer: saturated phenol (62:38, v/v); 0.8 M guanidine thiocyanate; 0.4 M ammonium thiocyanate; 0.1 M, pH 5.0, sodium acetate; 5 % (v/v) glycerol.

Total plant RNA extraction for RNA-seq also used this method. Then RNA was precipitated with ethanol for delivery of samples. 0.1 volume 3 M sodium acetate solution and 3 volumes of 100% ice cold ethanol were added to RNA samples, then vortexed to mix thoroughly and precipitated at -80 °C overnight. Then the RNA samples were delivered to the collaborator, Porf. Vanburen, Michigan State University, USA. RNA sequencing (RNA-seq) libraries were constructed and analyzed. The Illumina TruSeq stranded RNA Sample Preparation Kit (Illumina, San Diego, USA, #RS-122-2001) was used for RNA-seq libraries, and the libraries were sequenced on an Illumina HiSeq2500 machine under single-end 100 nt mode.

2.2.6 Reverse transcription of RNA

3-4 µg of total RNA was mixed with 1 µl 10×DNase I reaction buffer, 1 µl RNase-free DNase I and sterile H_2O to make a total volume 10 µl. The mixture was incubated at 37 °C for 30 min to remove DNA in the samples. 1 µl of 25 mM EDTA was added and the mixture was incubated at 65 °C for 10 min. After briefly spinning down, the mixture was kept on ice.

The first strand cDNA synthesis was performed according to the cDNA synthesis kits of Thermo scientific. 10 µl DNase I-treated RNA was mixed with 1 µl 100 µM oligo-dT 18 primer and 10 µl H_2O. The mixture was incubated for 5 min at 65 °C after a gentle centrifugation. Then samples were put on ice and the following reagents were added: 4 µl 5× first strand buffer, 1 µl RiboLock ™ RNase inhibitor, 2 µl 10 mM dNTPs mix and 1 µl reverse transcriptase. The mixture was incubated at 42 °C for 60 min. The reaction was terminated by heating at 70 °C for 5 min. The mixture was dissolved with 60 µl sterile H_2O and then used for PCR directly or stored at -20 °C.

10× DNase I reaction buffer:

100 mM Tris-HCl pH 7.5 at 25 °C,

1 mM $CaCl_2$,

25 mM $MgCl_2$.

2.2.7 Polymerase chain reaction (PCR) with Taq polymerase

2.2.7.1 PCR system

Thermocycler T3000, Biometra (Göttingen, Germany) was used for PCR in this study.

20 µl PCR reaction was composed of:

2 µl 10× PCR Buffer containing 15 mM $MgCl_2$, 0.4µl 10 mM dNTPs mix, 0.8 µl 10 µM (10 pmol/µl) forward primer and 0.8 µl 10 µM (10 pmol/µl) reverse primer, 0.2 µl Taq polymerase, 14.8 µl H_2O and 1µl cDNA template.

Cycling conditions for PCR were as follows:

1. 95 °C, 5 min for initial denaturing.

2. 95 °C, 30 sec for cycling denaturing.

3. 52-60 °C, 40 sec for annealing (annealing temperature was determined by the GC content of each pair of primers).

4. 72 °C, 60 sec for elongation (normally 1 kb PCR product need 1 min in this step).

Then start recycling for amplification from step 2, 25-36 times.

5. 5 min for final elongation at 72 °C.

6. pause at 4 °C for keeping the samples stable.

6× DNA Loading Dye:

10 mM Tris-HCl pH 7.6 at 25 °C

0.03 % (w/v) xylene cyanol FF

0.03 % (w/v) bromophenol blue

60 % (v/v) glycerol

TE Buffer:

10 mM Tris-HCl pH 8.0 at 25 °C.

1 mM EDTA, and with NaOH adjust pH 8.0.

10× PCR Reacting Buffer:

100 mM Tris-HCl, pH 8.3 at 25°C

500 mM KCl

15 mM $MgCl_2$

0.01% gelatin

2.2.7.2 Primers

Primers were designed with the software Oligo 7 (http://www.oligo.net/tutorials.html). Then the online tool ‘Oligo Evaluator’ was used to check the quality of primers. (http://www.oligoevaluator.com/).

Primers were synthesized by Eurofins MWG Operon (Ebersberg, Germany) and dissolved in TE buffer to a final concentration of 100 μM.

***Oropetium thomaeum* reference genes**

Oropetium thomaeum reference candidate genes in leaves were selected from the *Oropetium thomaeum* transcriptome database (VanBuren et al., 2017 released at NCBI BioProject PRJNA286116). The expression level of these candidate genes was tested with untreated, dehydrated, and rehydrated treatments in *Oropetium thomaeum* leaves. SQ-PCR result of these candidate genes was shown in supplementary. Ot-10692 was identified as the reference gene with the most stable expression level during dehydration and rehydration.

Table 2.1 Primers of reference candidate genes for *Oropetium thomaeum*. These candidates are screened from *Oropetium thomaeum* transcriptome data, with stable expression under untreated, drought-treated, and rehydrated plants.

Name	5'-3' sequence
Ot_10692_fwd	CCGTACTTGCTATCCTCAGACT
Ot_10692_rev	TTCCATCAGGTTCAGGCACTC
Ot_16314_fwd	GATGAGGCAGAAGGAGCAGAT

Name	5'-3' sequence
Ot_16314_rev	GGCGAAGTAGGACAGGATGAT
Ot_07286_fwd	GAGACGGAAGAGGAGAGT
Ot_07286_rev	AACTGAGGACTTGGTAAGC
Ot_02054_fwd	TTGGTCTGGCAGGATGAT
Ot_02054_rev	GTCACCGAATGGAGCAATC
Ot_EF1a_fwd	CGCCGTCCTTATCATCGACTC
Ot_EF1a _rev	CGCCGATCTTGTAGACGTCCT
Ot_UBC1_fwd	CCTGACATCCAGTTGATATGTG
Ot_UBC1_rev	TAACCTCGTATACATTCTTGCC

Table 2.2 Primers of genes (MGD1, DGD1, SFR2, PIS1, PIS2, βGAL7, βGAL9, βGAL17, DGAT2, PLDa1, PLDa2) encoding lipid biosynthesis enzymes of *Oropetium thomaeum.*

Name	5'-3' sequence
Ot_MGD1_fwd	AACTAGCACGACCTGATGCC
Ot_MGD1_rev	ATCCCTCACGGGAGATAGCA
Ot_DGD1_fwd	AAGAGCCTGAGCACCTCAAC
Ot_DGD1_rev	TAGCACCTGACAGTCGCAAG
Ot_SFR2_fwd	AACTTTGCAGCACTTGAGCG
Ot_SFR2_rev	AGCACCAGCACAATACGTCA
Ot_PIS1_fwd	AGGCTGGACCAGGTACCATT
Ot_PIS1_rev	AGGACGACGTCAATGAACCA
Ot_PIS2_fwd	TTTCGATCTGAAGCGTGGCA

Ot_PIS2_rev	ATACGCCGGCTTACATGTGG
Ot_βGAL7_fwd	CAAGGCCGGTTCAGTTTGTG
Ot_βGAL7_rev	CTCCAGCCAGATACTGCGAG
Ot_βGAL9_fwd	CCCGGTCGTTTATCCAACCA
Ot_βGAL9_rev	GCTGATGACTTCCCCTGCTT
Ot_βGAL17_fwd	AGCATCCATTCACGGTGGTC
Ot_βGAL17_rev	AGGTCTAAGGATTGGTGCGG
Ot_DGAT2_fwd	GGACATGGCTTGGATTGG
Ot_DGAT2_rev	CGTAAGGCGATGATGAACT
Ot_PLDa1_fwd	GCTTACCGCCTTCTGTCTGT
Ot_PLDa1_rev	TTCTCGAGGTCAATGGTGGC
Ot_PLDa2_fwd	GACCGTGCTAAGTATCCCCG
Ot_PLDa2_rev	ACCGCTTTTTCTCTCGCTCA

Table 2.3 Primers of genes (MGD1, DGD1, SFR2, PIS1, PIS2, βGAL7, βGAL9, βGAL17, DGAT2, PLDa1, PLDa2) encoding lipid biosynthesis enzymes, and the reference gene ELF5 of *Lindernia. brevidens*.

Name	5'-3' sequence
Lb_ELF5_fwd	CATCAATCGTGAAGGAGAGGA
Lb_ELF5_rev	CTAAACCTTGGCAGCAGCAC
Lb_MGD1_fwd	GCCCTCGTTTGTGAAGCCCGTT
Lb_MGD1_rev	ACCGGGACCAGCCTTAGTAATG
Lb_DGD1_fwd	ATAAACCCGAGTGTCAGCGACGTG
Lb_DGD1_rev	CTGCGGAGGCAACAAACTAAGATC

Lb_SFR2_fwd	GGTCGTGGGGTTTATCCCGATGGC
Lb_SFR2_rev	GGTCGTTGAAGCGGTCGACGGCTA
Lb_PIS1_fwd	CGCTATATGTTTCAAGGACAAGTACC
Lb_PIS1_rev	ACGCAAGTAATATCAAGAAAGGCCAG
Lb_PIS2_fwd	GCTATATGTTTCAAGGACAAGTA
Lb_PIS2_rev	GAACAAACGCAAGTAATATCAAG
Lb_βGAL7_fwd	AGAAGTGTTGTGTTCTTGCTGG
Lb_βGAL7_rev	AGTGCAGCCAAAGAGGAAAC
Lb_βGAL9_fwd	ATTTCCGTTGGGATTGGCGGGATT
Lb_βGAL9_rev	TGACAAACCGTTCCATTTCGTCCT
Lb_βGAL17_fwd	AGATCGTAACACGAAGCTCCATC
Lb_βGAL17_rev	AGAGGTCATGACTAGGCGACTCCA
Lb_DGAT2_fwd	TTCTGCTTCGGTCAGTCCAA
Lb_DGAT2_rev	ATTACTGAATCCTGAGCGGTGT
Lb_PLDa1_fwd	TGAAGAGACGATGGCTCAGA
Lb_PLDa1_rev	TATTAGAGTGGCACCGATGGGGTTGT
Lb_PLDa2_fwd	TGTGAAGAGACGATGGCTCAGA
Lb_PLDa2_rev	ATTTCGTGAACGGGCACGTAAGCCCT

Table 2.4 Primers of PSAT1 and ASAT1 genes in *Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia. brevidens*, *Lindernia subracemosa*, and *Arabidopsis thaliana*

Name	5'-3' sequence
Ot_PSAT1_fwd	GCTGGCTCTAACGACAAG
Ot_PSAT1_rev	AACTCCTTCATCACCTCCT

Ot ASAT1 fwd	CATCTACTCTTACCAGTTCAAG
Ot_ASAT1 _rev	CGTGAAGGAGGAAGAAGG
Cp_ASAT1_fwd	TCGAGCCACAGTTCGACGAG
Cp_ASAT1_rev	AGAACCACGTCACCTCCCAC
Cp_PSAT1_fwd	TGGTGCTACAAGTGGGGACG
Cp_PSAT1_rev	CAGCTCGCGCATTAGGACTG
Lb_ASAT1_fwd	GAGGAGCGGTTGTATCCGAG
Lb_ASAT1_rev	CCAAACCCGAAACCACGAAC
Lb_PSAT1_fwd	ATAATACCGGGGTTCGCGTC
Lb_PSAT1_rev	GGCGGCAATCTCCAGTCATA
Ls_ASAT1_fwd	TTCGTAGTTTCGGGTCTGGC
Ls_ASAT1_rev	CGAGGTCAAAATCCTCCCA
Ls_PSAT1_fwd	TCCGTCATTTATGGGGCCAG
Ls_PSAT1_rev	ACCAGGGTTCCCCTCTACAA
Cp_EF1a_fwd	AGTCAAGTCCGTCGAAATGC
Cp_EF1a_rev	CACTTGGCACCCTTCTTAGC
ELF5_fwd	CATCAATCGTGAAGGAGAGGA
ELF5_rev	CTAAACCTTGGCAGCAGCAC

Table 2.5 Primers of genes (FAE1, PDAT1, PDAT2, LPCAT1, LPCAT2, DGAT1, DGAT3, SDP1, CLO3, CLO4, CLO6, CSPLP) encoding sterol ester enzymes of *Oropetium thomaeum.*

Name	5'-3' sequence

Ot FAE1 fwd	CAAGACCAACATCACGACGC
Ot FAE1 fwd	CAAGACCAACATCACGACGC
Ot FAE1 rev	GACCCGCTTCATCCTAAGCA
Ot PDAT1 fwd	ATGATGCAGCGTGGAAGTGT
Ot PDAT1 rev	AGCTATTTTCGTCCCCACCC
Ot PDAT2 fwd	GGTTCTCAGTGCGGGTTACA
Ot PDAT2 rev	TATACCTGGTCACCGCCAAG
Ot LPCAT1 fwd	TGAGTGGAGATGCATGGAAGG
Ot LPCAT1 rev	CAGCAAAATGGCTACCACAGC
Ot LPCAT2 fwd	TGCGAGCAATTCAGTCCTCC
Ot LPCAT2 rev	TACATGTTGCTCACTCCGCC
Ot DGAT1 fwd	AGCAACCGCAACACGAGATG
Ot DGAT1 rev	CAGAAGACCTGCATGGCTCT
Ot DGAT3 fwd	GAATCCAGTGAGAGTGAATG
Ot DGAT3 rev	CTACGGTGCCAACATCTT
Ot SDP1 fwd	TGCTGTAACTGCCTCTTG
Ot SDP1 rev	CGATACTTGACCTCCATCTC
Ot CLO3 fwd	CAAGGACTCGCAGACCAT
Ot CLO3 rev	GGTAGATGATGCCGTTGTC
Ot CLO4 fwd	CGTCGTCCTCAGTTATCC
Ot CLO4 rev	AGAGCAACAGCCATTCAC
Ot CLO6 fwd	GCGTCGTCTCCATATCAG
Ot CLO6 rev	GAACCTTCCTTCAGCATCA
Ot CSPLP fwd	GGATGCTCGTCTTGCTGAT
Ot CSPLP rev	CGAACTTGATGTCTTGCTCTG

2.2.7.3 Electrophoresis

1 %-1.5 % (w/v) agarose gel electrophoresis was performed to analyze RNA, DNA, and PCR products and to check the quality and amount.

Gel preparation: 0.8-1.5g agarose powder mixed with 60-100 ml of 1× TAE buffer and the mixture was boiled. Then poured into the gel tray and 0.05- 0.1 ml ethidium bromide solution (1 mg/L) was added for staining. Then the gel was left for 30 min at RT for solidification.

Loading: Samples were loaded on the gel and separated by electrophoresis in 1× TAE buffer with 75-100 mA electric current, 100-120 volts, 1kb or 100 mb DNA ladder was used as reference for the DNA fragments.

Photos: gels were visualized under UV-light, and pictures were taken using a Typhoon Scanner 9200 (Amersham Biosciences, Freiburg, Germany).

1× TAE buffer: 40 mM Tris, 20 mM acetate, 1 mM EDTA, pH 8.5

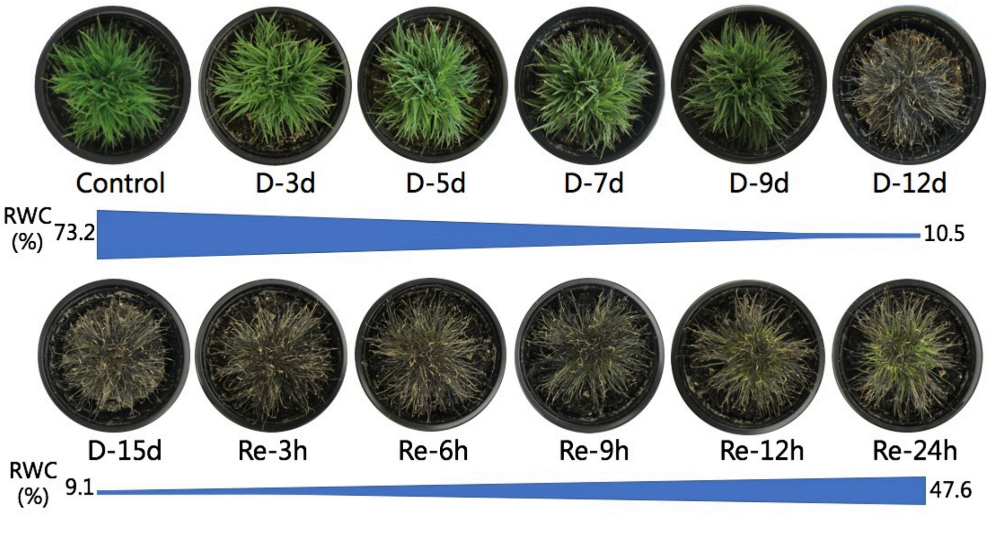
2.2.8 Statistical analysis

In this study, Excel and ANOVA, Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***) were used for the statistical analysis with at least 5 biological repetitions. SPSS Statistics 22.0 (IBM) and GraphPad Prism 8 were used for data analysis and figures.

3. Results

Oropetium thomaeum (L.f.) Trin. (hereon *Oropetium thomaeum*) is a compact resurrection plant that has the smallest known genome among the grasses (Jones and PaSakinskien, 2005, VanBuren *et al.*, 2015). The high-quality genome sequencing information of *Oropetium thomaeum* was published recently and it was obtained using single-molecule real-time sequencing (VanBuren *et al.*, 2015). Until now, only a few analyses focused on the analysis of *Oropetium thomaeum* (Bartels and Mattar, 2002; Jones and PaSakinskien, 2005; VanBuren *et al.,* 2015, 2017 and 2018; Zhang *et al.*, 2018), the information about the physiological characters, like anatomical cell structure or lipid metabolites are still unrevealed. This study mainly analyzed the cell structure and oil bodies accumulations in *Oropetium thomaeum* leaves, and changes of phospholipids, glycerolipids and sterol contents in desiccation tolerant plants (*Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia brevidens*) compared to desiccation sensitive plants (*Lindernia subracemosa*, *Arabidopsis thaliana*), as well as the expression of related genes during the process of dehydration and rehydration.

3.1 Morphological appearances and relative water content (RWC) of the desiccation and rehydration process in Oropetium thomaeum.



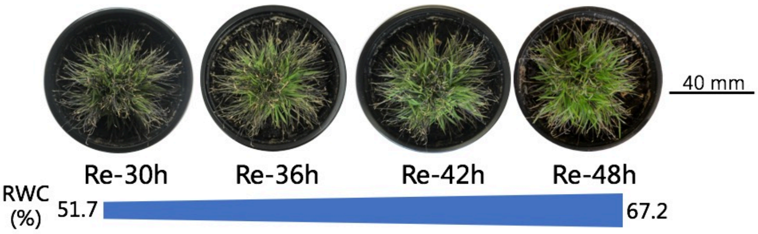


Figure 3.1 *Oropetium thomaeum* from well-watered (Control) to desiccation treatments with 3 days, 5 days, 7 days, 12 days, 15 days dehydration and rehydration treatments with 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 30 hours, 36 hours, 42 hours, and 48 hours post rehydration. Relative water content (RWC, %) of plant leaves are shown by blue bars and numbers.

As one of the desiccation tolerant grasses, *Oropetium thomaeum* leaves turned dry after 12 days of dehydration, at this time point the relative water content (RWC, %) decreased 85.6% (from 73.2 to 10.5). With 15 days of dehydration, the RWC (%) of plant leaves was reduced to 9.1 (about 87.6% lower than the well-watered plant) and then rapidly recovered 65% after 24 hours of rehydration compared with the well-watered plant (47.6/73.2). After 48 hours of re-watering, the RWC (%) of these leaves increased to 67.2%. Compared with the well-watered leaves, the RWC of *Oropetium thomaeum* leaves could recover 91.8% after 2 weeks of dehydration and then 2 days of rehydration (67.2% in 2 days rehydration / 73.2% in well-watered leaves).

3.2 Histological analysis of *Oropetium thomaeum* leaves

3.2.1 Overview of anatomical structures

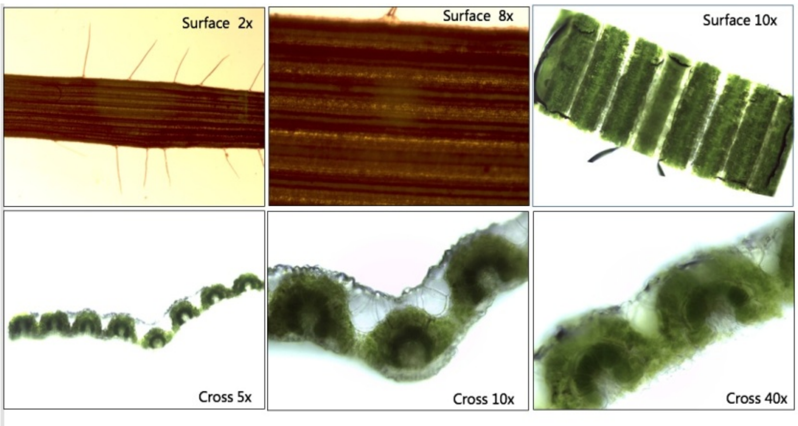


Figure 3.2.1.1 Hand sections from *Oropetium thomaeum* fresh leaves without staining. The magnifications are shown in the pictures.

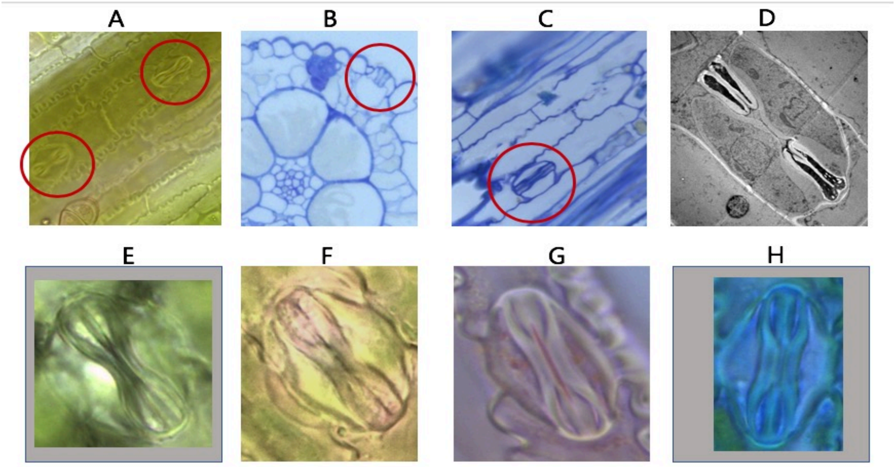


Figure 3.2.1.2 Stomata in well-watered *Oropetium thomaeum* leaf cells. A: surface-section with Sudan III glycerol staining. B and C: cross- and surface- semithin section with toluidine blue staining. D: stomata under transmission electron microscopy. E, F, G, and H: hand sections with different staining solutions, E: without staining, F: CFA staining, G: Sudan III glycerol heating staining, H: methylene blue staining. Stomata are denoted by the red circle in A, B, and C.

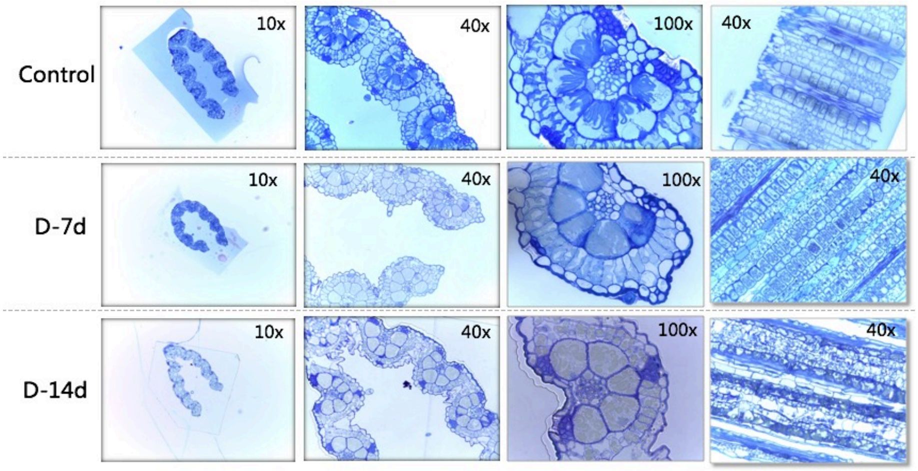


Figure 3.2.1.3 Semithin sections of *Oropetium thomaeum* leaf cells from well-watered plants (Control), dehydrated 7 days plant (D-7d) and dehydrated 14 days plant (D-14d). The magnifications are shown in the pictures. Pictures on the most right are surface-sections, and the others are all cross-sections.

The histological structure of *Oropetium thomaeum* leaves was observed by using a LEICA DM750 microscope (Leica Microsystems, Wetzlar, Germany) for hand sections and semithin sections and a Zeiss LEO912 AB transmission electron microscope (TEM) (Carl Zeiss, Inc., Oberkochen, Germany) for ultrathin sections. The semithin sections were stained with 1% (w/v) toluidine blue. All these sections show the typical anatomy characteristics of the Poaceae C4 plants in *Oropetium thomaeum* leaf cells. The bundle sheath cells are abundant and the surrounding mesophyll cells are with the close arrangement, the chloroplasts can be found in bundle sheath cells and mesophyll cells (Figure 3.2.1.3), and the stomata are four-celled structures (Figure 3.2.1.2) (stomatal biology of Reissig lab, http://raissiglab.org/research/). The most convenient method of lipid staining is using Sudan III reagent (Marcel Locquin and Maurice Langeron, Handbook of microscopy, p235, 1983). The sections in Figure 3.2.1.2, G were stained with Sudan III glycerol with heating for 3 min, stomata show distinct red staining. It means that stomata contain more lipids than other surrounding organelles, possibly because lipids could help stomatal movement and then support plants tolerance with dehydration.

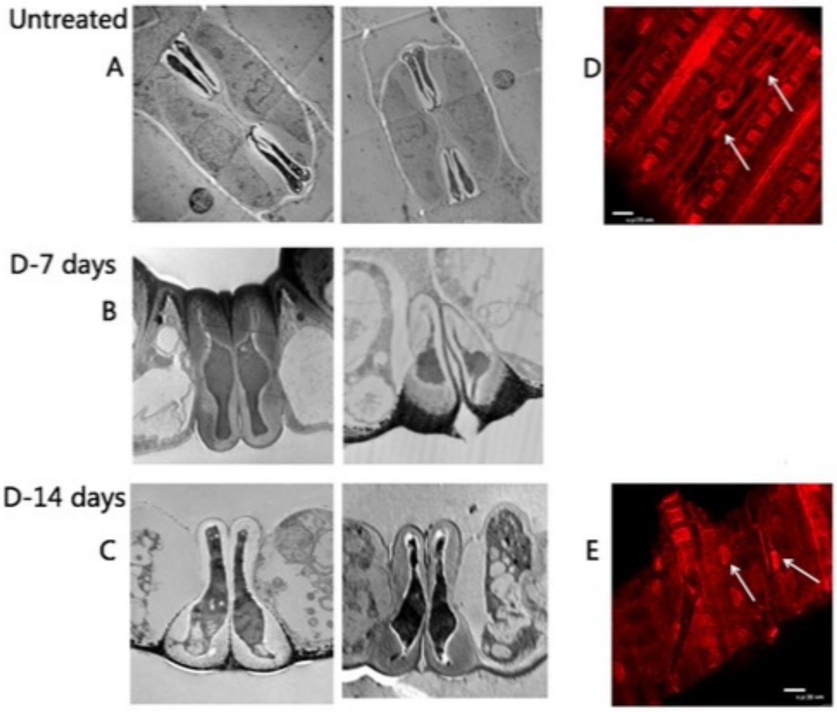


Figure 3.2.1.4 The performance of stomata during dehydration process. *Oropetium thomaeum* leaf cells from untreated plant (Control, A and D), dehydrated 7 days plant (D-7 days, B) and dehydrated 14 days plant (D-14 days, C and E). A, B, and C: ultrathin sections imaged under transmission electron microscope; D and E: Nile Red staining sections (5 µg/ml Nile Red in 10% DMSO for 1 min) imaged under confocal microscope with excitation at 488 nm laser and emission at 590 nm. Stomata are indicated by white arrows in D and E.

As in Figure 3.2.1.4, stomata are regular square shaped in untreated *Oropetium thomaeum* leaves when imaged by high magnification TEM and confocal microscopy (A and D). At the first stage of dehydration (D-7 days), stomata were slightly shrunk but kept their regular shape (B). With increasing dehydration (D-14 days), stomata were squeezed to lentil shape and totally closed (C and E). Nile red staining images showed the same trend during dehydration. Previous research has shown that stomata are an important cell organ for plants to adapt to environmental stress, especially when confronting with drought stress (Kirschbaum *et al*., 1988; Knapp, 1993; Pearcy and Way, 2012; Lawson and Blatt, 2014). The number and size of stomata in plant cells is not necessarily the simplest or best approach to adjust environmental changes, and the idea of stomata to response growth conditions could provide means to both improve the water use inefficiency of plants and, at the same time, to increase the photosynthetic carbon gaining. (Cardon *et al.*, 1994; Allen and Pearcy, 2000; Noe and Giersch, 2004; Smith and Berry, 2013). Stomata in leaf cells showed significant changes of morphology when *Oropetium thomaeum* plants were confronted with drought. This is sufficient evidence to state that *Oropetium thomaeum* is a desiccation tolerant plant.

3.2.2 Anatomy analysis with semithin sections and ultrathin sections

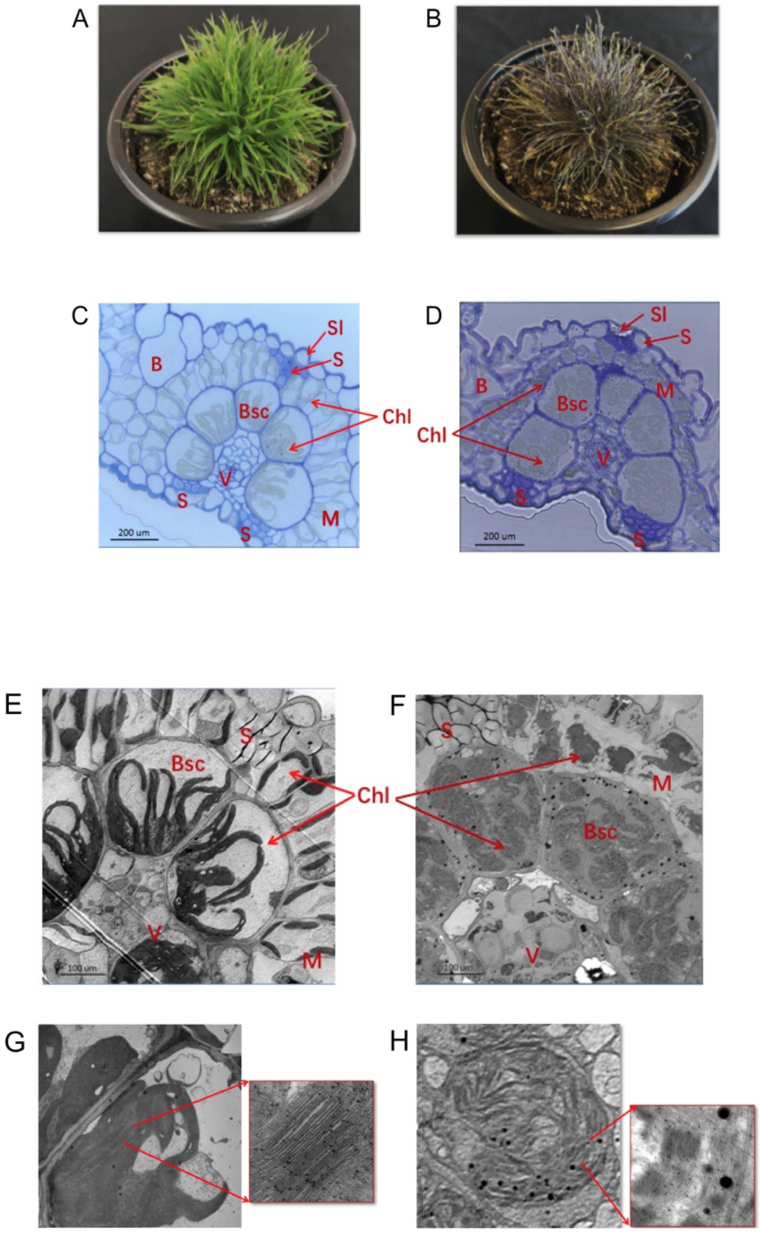


Figure 3.2.2 (A) Well-watered plants. (B) dehydrated 14 days plants (desiccated). Semi-thin section micrographs (C and D) and transmission electron micrographs (E, G, and F, H) of cross sections of *Oropetium thomaeum* leaves from well-watered plants (C, E, and G) and 14 days dehydrated plants (D, F, and H). Enlarged part of bundle sheath cells (G and H). V: vascular tissue; Bsc: bundle sheath cells; Chl: chloroplast; M: mesophyll cells; SI: silicon cells; B: buliform cells; S: sclerenchyma.

(Partial results already published in Plant, Cell and Environment, 2017. Title: Seed desiccation mechanisms co-opted for vegetative desiccation in the resurrection grass *Oropetium thomaeum.* Authors: R. VanBuren, C. Wai, Q. Zhang, X. Song, P. Edger, D. Bryant, T. Micheal, T. Mockler, D. Bartels.

During dehydration, the cell volume shows slightly shrink, the mesophyll cells lose a significant amount of cell volume, but the bundle sheath cells have almost the same volume compared with the untreated plant cells. Vacuole fractionation is obvious in the whole cell, especially in the bundle sheath cells and mesophyll cells (C, E and D, F). Similar desiccation-related changes in leaf morphology were reported in another resurrection grass *Eragrostis nindensis* (Vander Willigen *et al.,* 2004). In the well-watered *Oropetium thomaeum* leaf cell, plastids in the bundle sheath cells are elongated, finger-shaped and the base part aligned to the vascular bundle with a few and small oil dots (G). After dehydration, plastids in the bundle sheath cells are irregularly piled and the size and number of oil dots are increased (H).

In *Oropetium thomaeum* leaf cells (C and D), bulliform cells are large, bubble-shaped epidermal cells that occur in groups on the upper surface of the leaves of many monocots. These cells are present on the adaxial or the upper surface of the leaf. During dehydration, bulliform cells are involved in the folding of leaf tissue to control light intensity and reduce overall water loss (D and F). The same anatomical structures were also found in halophytes (An Integrative Anatomical Study, 2014).

3.3 Accumulation of oil bodies during desiccation

This part is published (2017), Plant Cell and Environment. Title: Seed desiccation mechanisms co-opted for vegetative desiccation in the resurrection grass *Oropetium thomaeum.* Authors: R. VanBuren, C. Wai, Q. Zhang, X. Song, PP. Edger, D. Bryant, T. Micheal, T. Mockler, D. Bartels.

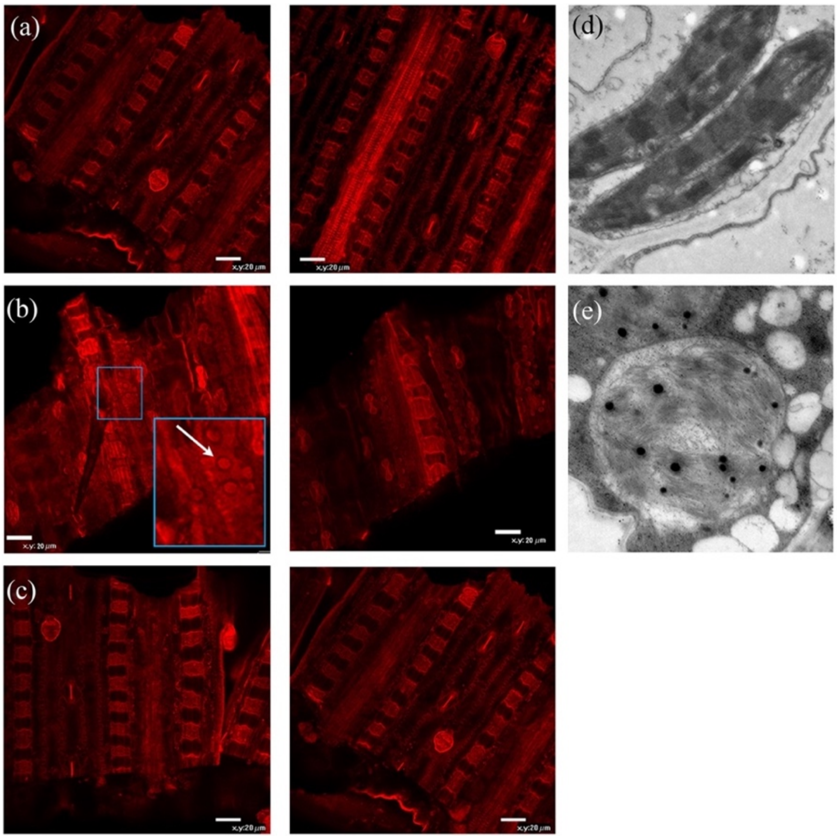


Figure 3.3 Oil bodies accumulation during desiccation in *Oropetium thomaeum*. Nile red stained *Oropetium thomaeum* leaf tissue under well-watered conditions (a), 14 days desiccated (b) and 48 hours post rehydration (c). Oil bodies are denoted by a white arrow in (b). The left and right panels were collected from independent plants. Transmission electron microscopy of *Oropetium thomaeum* chloroplasts in well-watered (d) and 14 d post desiccation (e) leaf tissue.

Comparative cellular analyses of desiccated and fully hydrated leaves revealed the accumulation of oil bodies in leaf tissue during desiccation. Well-watered *Oropetium thomaeum* leaf tissue stained with Nile red contains no detectable oil bodies (a, d). *Oropetium thomaeum* desiccated for 14 days shows an accumulation of lipid bodies dispersed throughout the leaf epidermal cells with concentrations around malformed cell membranes (b, e). Tissues 48 hours post rehydration show some residual oil bodies, but cells largely resemble well-watered cells (c). Together this suggests that oil bodies accumulate during desiccation in *Oropetium thomaeum* leaf tissue with similar morphological and expression patterns to seed desiccation.

3. 4 Transcript expression analysis of genes related to plant oil bodies in *Oropetium thomaeum*

Murphy (2012) illustrated that there are a variety of lipid-associated proteins decorating the surface of plant oil droplets, e.g., diacylglycerol acyltransferase (DGAT2), oleosin isoforms, caleosin isoforms, *et al.* In *Arabidopsis thaliana* cells, oleosin is abundant in seeds and tapetum, whereas oleosin levels are low in leaves (Hu *et al.*, 2002), despite of the fact that leaf cells have oil bodies (Lersten *et al.*, 2006). The working principle of leaf oil bodies in cells is complicated and unclear, but the protein components of leaf oil bodies and functions were revealed in *Arabidopsis thaliana* (Shimada and Hara-Nishimura, 2015). Caleosin contain an oil bodies binding domain and a calcium-binding domain (Chen *et al.*, 1999). Oil bodies proteins in leaves of *Arabidopsis thaliana* were identified: CLO3 (Caleosin 3, At2g33380), CLO4 (Caleosin 4, At1g70670), CLO6 (Caleosin 6, At1g70680), a-DOX1(At3g01420), and SDP1 (Sugar-dependent 1, At5g04040). The best matched sequences between *Arabidopsis thaliana* and *Oropetium thomaeum* were identified from the *Oropetium thomaeum* transcriptome database (VanBuren *et al.,* 2017 released at NCBI: PRJNA286116). The BLAST result of *a-DOX1*(At3g01420) showed a very low similarity between *Oropetium thomaeum* and *Arabidopsis thaliana* sequences. Then this study focused on the analysis of *OtCLO3*, *OtCLO4*, *OtCLO6*, and *OtSDP1*. These cDNA sequences of *Oropetium thomaeum* and *Ot-reference* sequence are listed in supplementary.

The transcript expression levels of CLO3, CLO4, CLO6 and SDP1 in *Oropetium thomaeum* leaves were measured by SQ-PCR, the results and heatmap are shown in Figure 3.4.1. *AtCLO3* and *AtCLO4* are found to be expressed in leaves of *Arabidopsis thaliana* (Shen *et al.*, 2014) and *AtCLO3* is expressed in response to abscisic acid, drought, salt, and stress tolerance (Shimada, 2000; Blée *et al.*, 2014). *OtCLO3* and *OtCLO6* expression levels showed a significant increase during desiccation in *Oropetium* *thomaeum* leaves, and *OtCLO4* transcripts also increased but not as much as *OtCLO3* and *OtCLO6* (Figure 3.4.1).

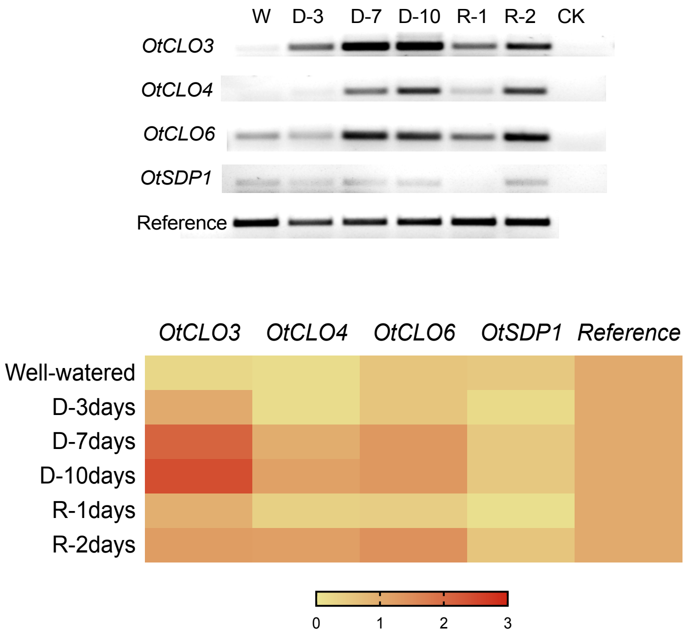


Figure 3.4.1 Expression profiles of genes encoding plant oil bodies enzymes in *Oropetium thomaeum* leaves. W： well-watered；D-3: 3 days dehydrated；D-7: 7 days dehydrated；D-10: 10 days dehydrated； R-1: 1 day rehydrated; R-2: 2 days rehydrated. CLO3: Caleosin 3; CLO4: Caleosin 4; CLO6: Caleosin 6; SDP1: Sugar-dependent 1. *Oropetium thomaeum* reference gene: Ot-10692.

According to the AtGenExpress database and TAIR, *CLO6* (At1g70680) is found ubiquitously expressed in various organs and located in chloroplasts, *SDP1* (sugar-dependent 1) was known as an oil bodies associated protein, which is involved in initiating release of fatty acids from oil bodies in germinating seedlings of *Arabidopsis thaliana* (Athenstaedt and Daum, 2005; Zimmermann *et al.*, 2004; Gronke *et al.*, 2005). Although many germinating oilseeds are known to possess lipase activities associated with oil bodies, but no activity was detected from peanut, soybean, and cucumber (Huang, 1983). The expression level of *OtSDP1* shows a low abundance in leaves, although the abundance is slightly higher in well-watered and 2 days rehydrated plants than the 10 days drought stressed *Oropetium thomaeum* (Figure 3.4.1). The transcript levels of *SDP1* do not correlate positively with enzyme activity in *Arabidopsis thaliana*, suggesting a post-transcriptional regulation (Quettier and Eastmond, 2009). This performance in *Arabidopsis thaliana* gives a clue that *OtSDP1* may also function during the post-transcription.

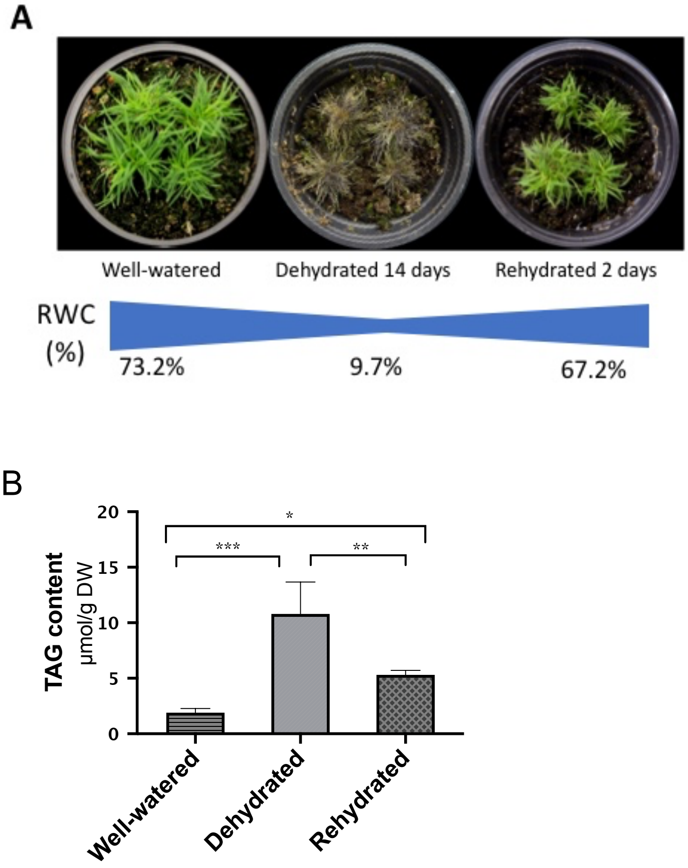
3.5 Plant oil bodies components

The structural features of plant oil bodies are the nonpolar lipids of the core [i.e., triacylglycerols (TAGs) and sterol esters (SE)] surrounded by monolayer polar surface lipids [i.e., phospholipid (PL) (insert)], and proteins decorating the surface (Murphy, 2012). In this study, TAGs, DAGs, SE, galactolipids, phospholipid and the correlated metabolites have been quantified and their related genes were analyzed by transcript expression in *Oropetium thomaeum*; fatty acids and sterol lipids were quantified in *Oropetium thomaeum*, *Craterostigma plantagineum* and *Arabidopsis thaliana*; sterol esters were analyzed in the desiccation tolerant plants: *Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia brevidens*, and in the desiccation sensitive plants, *Lindernia subracemosa* and *Arabidopsis thaliana*. There are already some studies on plant oil bodies and their compositions (Joyard *et al.*, 1998; Slabas *et al.*, 1999; Dörmann and Benning, 2002; Klaus *et al.*, 2002; Phillips *et al.*, 2008; Hernandez *et al.*, 2012; Gasulla *et al.*, 2013; Aymé *et al.*, 2014; VanBuren *et al.*, 2018), but no data of oil bodies compositions have been reported in *Oropetium thomaeum*.

3.5.1 Quantification of nonpolar glycerolipid triacylglycerol (TAG) in *Oropetium thomaeum*

Previous reports demonstrated that triacylglycerols are the major components of leaf oil bodies and an important storage lipid in some plant species (Parker and Murphy, 1981; Hudak and Thompson, 1997; Thompson *et al.*, 1998b; Horn *et al.*, 2011; Kopischke *et al.*, 2013), but normally only small amounts of TAG are found in leaves and are stored in oil bodies (Chapman and Ohlrogge, 2012). However, in *Arabidopsis thaliana* leaves TAGs accumulate under drought stress, osmotic stress, or freezing stress to accommodate a shrinking organelle and to encounter the loss of membrane integrity and cell death (Moellering *et al.,* 2010).

Oropetium thomaeum plants and the relative water contents are shown in Figure 3.5.1A under the treatments of well-watered, 14 days dehydration and 2 days rehydration. The TAG and the molecular species of TAG in *Oropetium thomaeum* leaves were analyzed by Q-TOF MS/MS, and the TAG contents are shown in Figure 3.5.1B, the molecular species contents of TAG are shown in Figure 3.5.1 C and D.



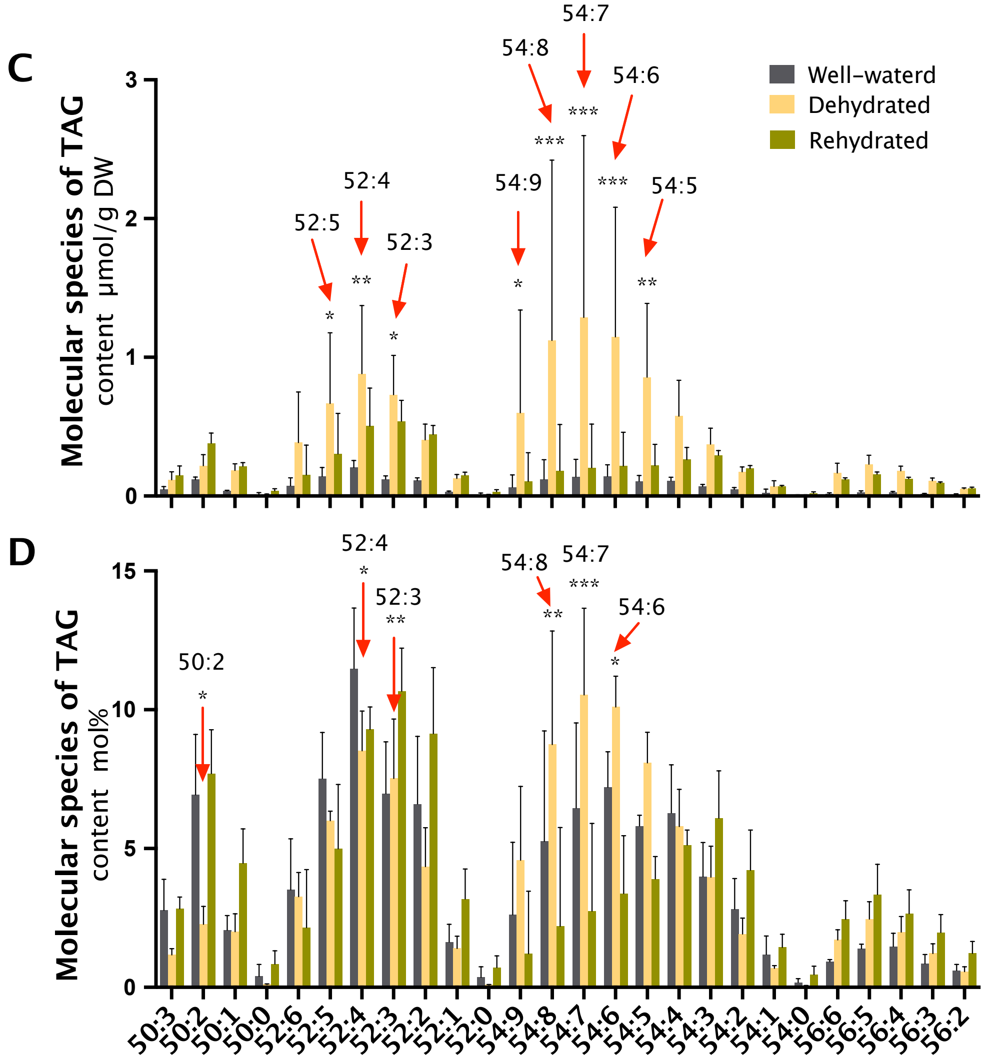


Figure 3.5.1 A: Appearance of well-watered, 14 days dehydrated, and 2 days rehydrated plants. **B:** total content of triacylglycerol in *Oropetium thomaeum* leaves of well-watered, 14 days dehydrated, and 2 days rehydrated leaves. **C and D:** Molecular species content of TAG in *Oropetium thomaeum* leaves. Well-watered with grey bars; Dehydrated, 14 days desiccated with yellow bars, and Rehydrated, 2 days rehydrated with green bars. TAG content was measured by Q-TOF MS/MS. DW, dry weight. Data are presented as mean and standard deviation of five measurements is given. Asterisks indicate values that are significantly different from the control (well-watered), according to Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***).

Fresh *Oropetium thomaeum* leaves were collected from well-watered, 14 days dehydrated, and 2 days rehydrated plants. The content of TAG significantly increased during dehydration, from 1.9 μmol/g (DW) to 10.8 μmol/g (DW), and after 48 hours rehydration, the TAG content decreased a lot, but still above the level of untreated plants (Figure 3.5.1 B). TAGs measurements by Q-TOF MS/MS analysis revealed the accumulation of all TAG molecular species in *Oropetium thomaeum* leaves after dehydration and rehydration treatments. The molecular species of 54:x and 52:x showed a remarkable increase during dehydration and decreased a lot after dehydration. The contents of 50:x and 56:x containing TAG are lower than 52:x and 54:x during the whole process (Figure 3.5.1 C). The mol% content of TAG indicated that 52:x and 54:x are the main components of TAG molecular species, and 52:4, 54:7 are the most abundant molecular species in well-watered and dehydrated *Oropetium thomaeum* leaves respectively. Almost all mol % of 54:x containing TAG increased after 14 days dehydration, whereas 52:x and 50:x molecular species showed the opposite trend (Figure 3.5.1 D).

Transcript expression analysis of triacylglycerol (TAG) related genes in *Oropetium thomaeum*

Diacylglycerol acyltransferases (DGATs) are acyl-CoA dependent enzymes, which catalyze the last step and the only committed step of the triacylglycerol (TAG) synthesis (Jako *et al.*, 2001; Grisart *et al.*, 2004; Beopoulos *et al.*, 2012; Aymé *et al.*, 2014). DGATs have been suggested to be one of the rate-limiting enzymes in plant lipid accumulation (Ichihara *et al.*, 1988; Xu *et al.*, 2012). DGATs have been classified to three different classes in *Arabidopsis thaliana: At2g19450*, *At3g51520* and *At1g48300*. DGAT1 also called TAG1, is a key enzyme to triacylglycerol (TAG) synthesis and oil accumulation in seeds (Cases *et al.*, 1998; Zhang *et al.*, 2009). The best matched sequences were found through BLAST from *Oropetium thomaeum* transcriptome database (VanBuren *et al.*, 2017 released at NCBI: PRJNA286116).

The transcript expression levels of DGAT1, DGAT2 and DGAT3 in *Oropetium thomaeum* leaves were analyzed by SQ-PCR under well-watered, dehydrated, and rehydrated treatments, the results and heatmap were shown in Figure 3.5.2.

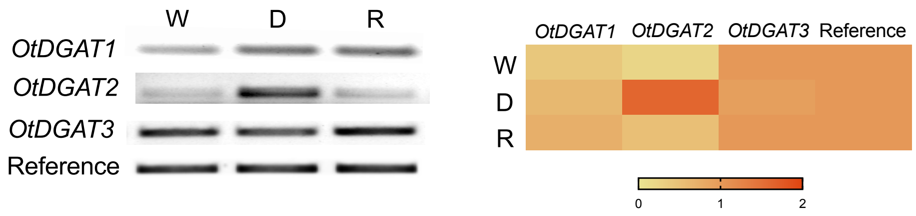


Figure 3.5.2 Expression profiles of DGATs in *Oropetium thomaeum* leaves. W： well-watered；D: 14 days dehydrated；R: 2 days rehydrated. DGAT1: Diacylglycerol acyltransferases 1; DGAT2: Diacylglycerol acyltransferases 2; DGAT3: Diacylglycerol acyltransferases 3; Reference gene: Ot-10692. The cDNA sequences of OtDGAT1, OtDGAT2, and OtDGAT3 are listed in supplementary.

In *Oropetium thomaeum* leaves, the expression level of the *OtDGAT*1 transcript is lower than *OtDGAT2* and *OtDGAT3,* with a slight increase after 14 days of dehydration. *AtDGAT2* was confirmed to have diacylglycerol acyltransferase activity in *Arabidopsis thaliana* (Aymé *et al.*, 2014). The transcriptional expression level of *OtDGAT2* showed a fast accumulation after dehydration, and then rapidly decreased after watering in *Oropetium thomaeum* leaves. *OtDGAT3* (diacylglycerol acyltransferase 3) is described as plants cytosolic enzyme synthesizing triacylglycerol. DGAT3 is highly and ubiquitously expressed in *Arabidopsis thaliana* and could therefore fulfill housekeeping functions, e.g., acyl regulation of exchanges between TAGs and the cytosolic acyl-CoA pool (Hernandez *et al.*, 2012). In *Oropetium thomaeum*, DGAT3 also showed a stable expression during the 2 weeks of dehydration and the re-watering period. In this study *OtDGAT3* is also considered as a candidate for a housekeeping gene, however as shown in the heatmap of Figure 3.5.2, the *OtDGAT3* expression level slightly decreased after14 days of dehydration and then almost recovered to the same level of untreated plants.

Phospholipid: diacylglycerol acyltransferase 1 (PDAT1) is a major enzyme involved in TAG accumulation in yeast (Oelkers *et al.*, 2002). PDAT1 and DGAT1 have overlapping functions during plant development in *Arabidopsis thaliana* (Zhang *et al.*, 2009). PDAT1 (*AT5G13640*) and PDAT2 (*AT3G44830*) have been identified in

Arabidopsis thaliana (Stahl *et al.*, 2004). The transcript expression levels of PDAT1 and PDAT2 in *Oropetium thomaeum* leaves were analyzed by SQ-PCR under well-watered, dehydrated, and rehydrated treatments, and the results and heatmap were shown in Figure 3.5.3.

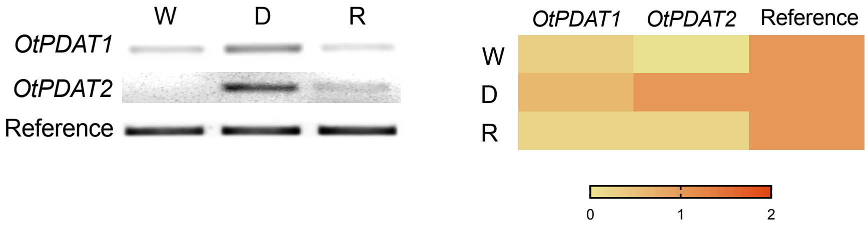


Figure 3.5.3 Expression profiles of PDATs in *Oropetium thomaeum* leaves. W： well-watered；D: 14 days dehydrated；R: 2 days rehydrated. PDAT1: Phospholipid: diacylglycerol acyltransferase 1; PDAT2: Phospholipid: diacylglycerol acyltransferase 2; *Oropetium thomaeum* Reference gene: Ot-10692. The cDNA sequences of OtPDAT1 and OtPDAT2 are listed in supplementary.

OtPDAT1 and *OtPDAT2* transcripts were up-regulated during dehydration and down-regulated during re-watering, and the expression level of well-watered and rehydrated plants are almost the same. The level of *OtPDAT2* transcripts was hardly detectable in well-watered *Oropetium thomaeum* leaves. From this observation we could suggest that drought stress significantly induced *OtPDAT1* and *OtPDAT2* expression, especially on *OtPDAT2*.

3.5.2 Quantification of nonpolar glycerolipid diacylglycerol (DAG) in *Oropetium thomaeum*

In plant oil bioengineering, DAG can be utilized for TAG synthesis by acyl-CoA: diacylglycerol acyltransferase (DGAT), DAG provides two of the three FA in TAG (Li-Beisson *et al.*, 2010; Bates and Browse, 2012). The ligation of glycerol-3-phosphate with acyl-CoA to generate DAG and then acetylation to TAG is also referred to as Kennedy pathway (Weiss and Kennedy, 1956; Weiss *et al.*, 1960).

The DAG in *Oropetium thomaeum* leaves were analyzed by Q-TOF MS/MS under well-watered, dehydrated, and rehydrated treatments, and the DAG contents are shown in Figure 3.5.4A, the molecular species contents of DAG were shown in Figure 3.5. B and C.

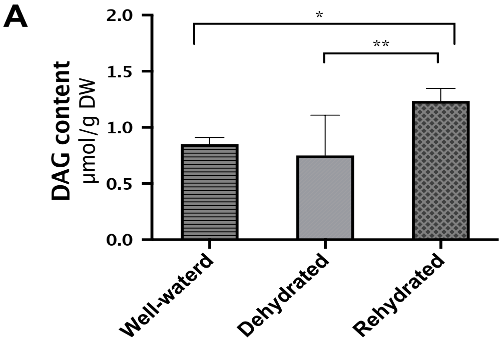
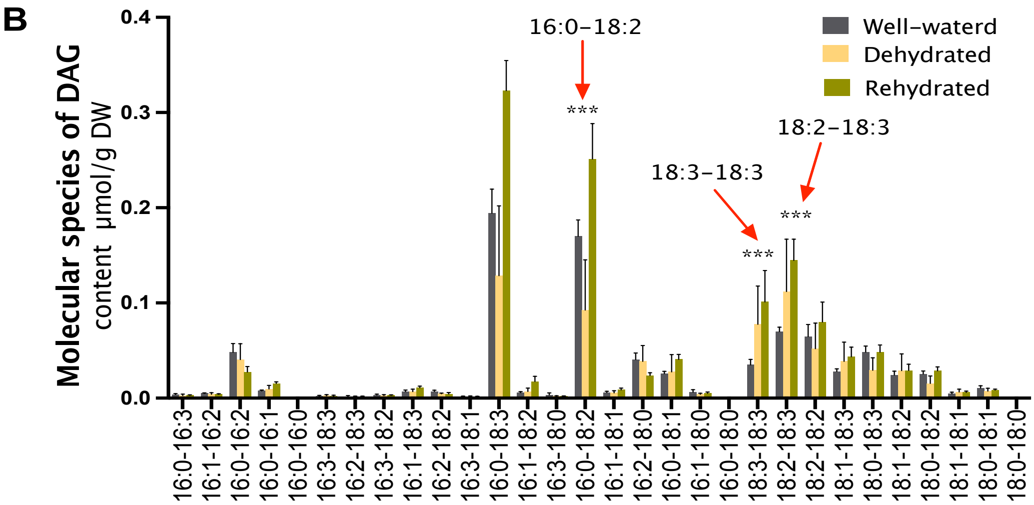


Figure 3.5.4 A: Total content of diacylglycerol in *Oropetium thomaeum* leaves of well-watered, 14 days dehydrated, and 2 days rehydrated plants.



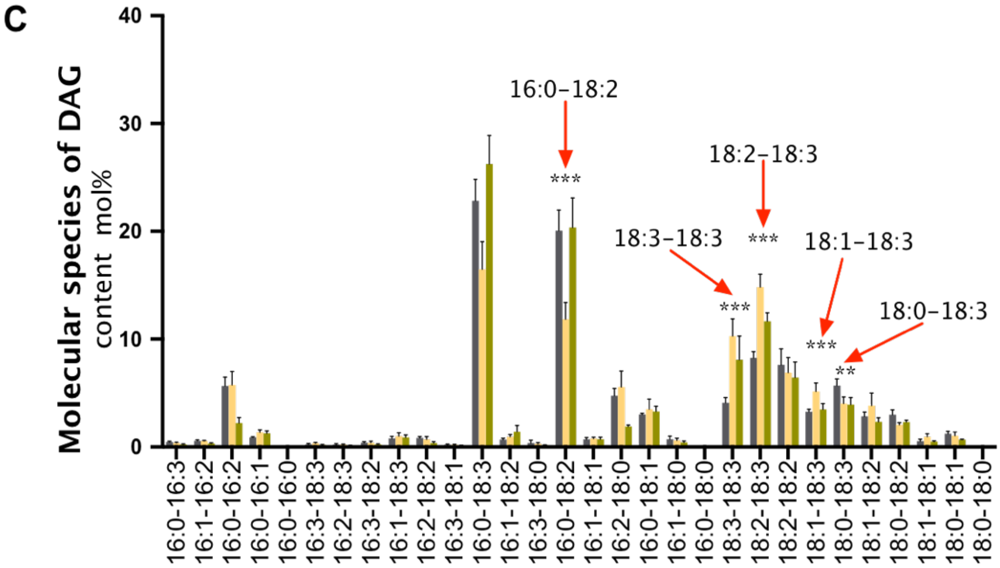


Figure 3.5.4 B and C: Molecular species content of DAG in *Oropetium thomaeum* leaves. Well-watered with grey bars； Dehydrated, 14 days desiccated with yellow bars, and Rehydrated, 2 days rehydrated with green bars. DAG content was measured by Q-TOF MS/MS. DW, dry weight. Data are presented as mean and standard deviation of five measurements. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***).

While the TAG content was notably increased under dehydration stress, the amounts of the nonpolar glycerolipids DAG did not significantly change in *Oropetium thomaeum* leaves. In this study, both total TAG and DAG contents increased after 48 hours of rehydration compared to well-watered and dehydrated leaf samples (Figure 3.5.4 A). The analysis of the fatty acid composition showed that 16:0-18:2 and 16:0-18:3 are the most abundant molecular species of DAG in well-watered and rehydrated *Oropetium thomaeum* leaves. With the desiccation treatment, 16:0-18:2 content is the only one composition which showed a significant decrease, while 18:x-18:3 molecular species significantly accumulated in *Oropetium thomaeum* leaves. 16:x-16:x are the compounds with the lowest abundances in DAG, about 6.5% of 16:0-16:2 and 1.5% of 16:0-16:1 (Figure 3.5.4 B and C).

3.5.3 Quantification of phospholipids (PL) in *Oropetium thomaeum*

Phospholipids (PL) are the major components of all cell membranes and play a key role in the communication between the extra- and intracellular space (Hernández and Cooke, 1996; Whitehead and Day, 1997; Andersson *et al.*, 2005). Phospholipids form a monolayer of a polar surface of oil bodies in plants (Murphy, 2012). The chemical structure of phospholipids is a glycerol molecule linked to two fatty acids, and a phosphate group, and these two fatty acids can help to form diacylglycerol (DAG).

Phospholipids in *Oropetium thomaeum* leaves were measured by Q-TOF MS/MS and shown in Figure 3.5.5.

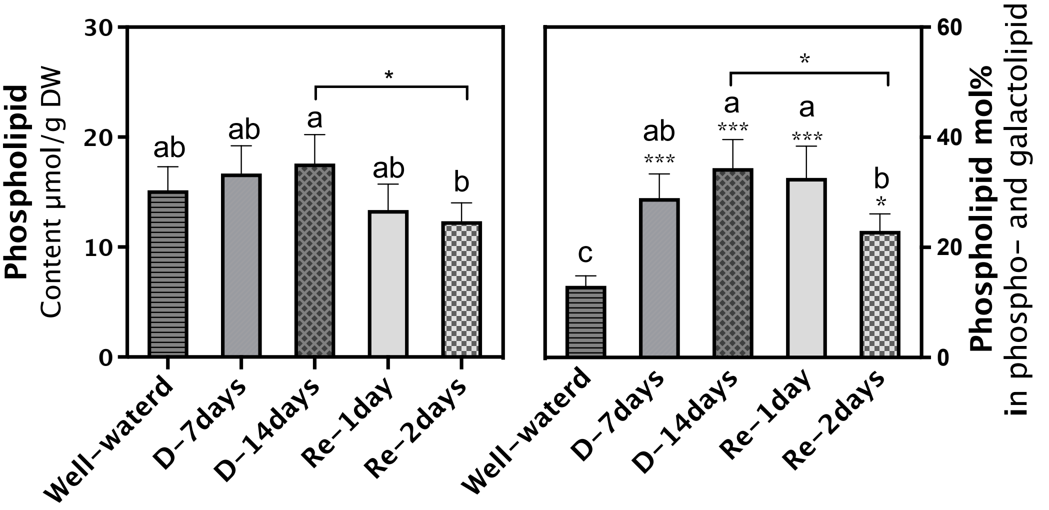
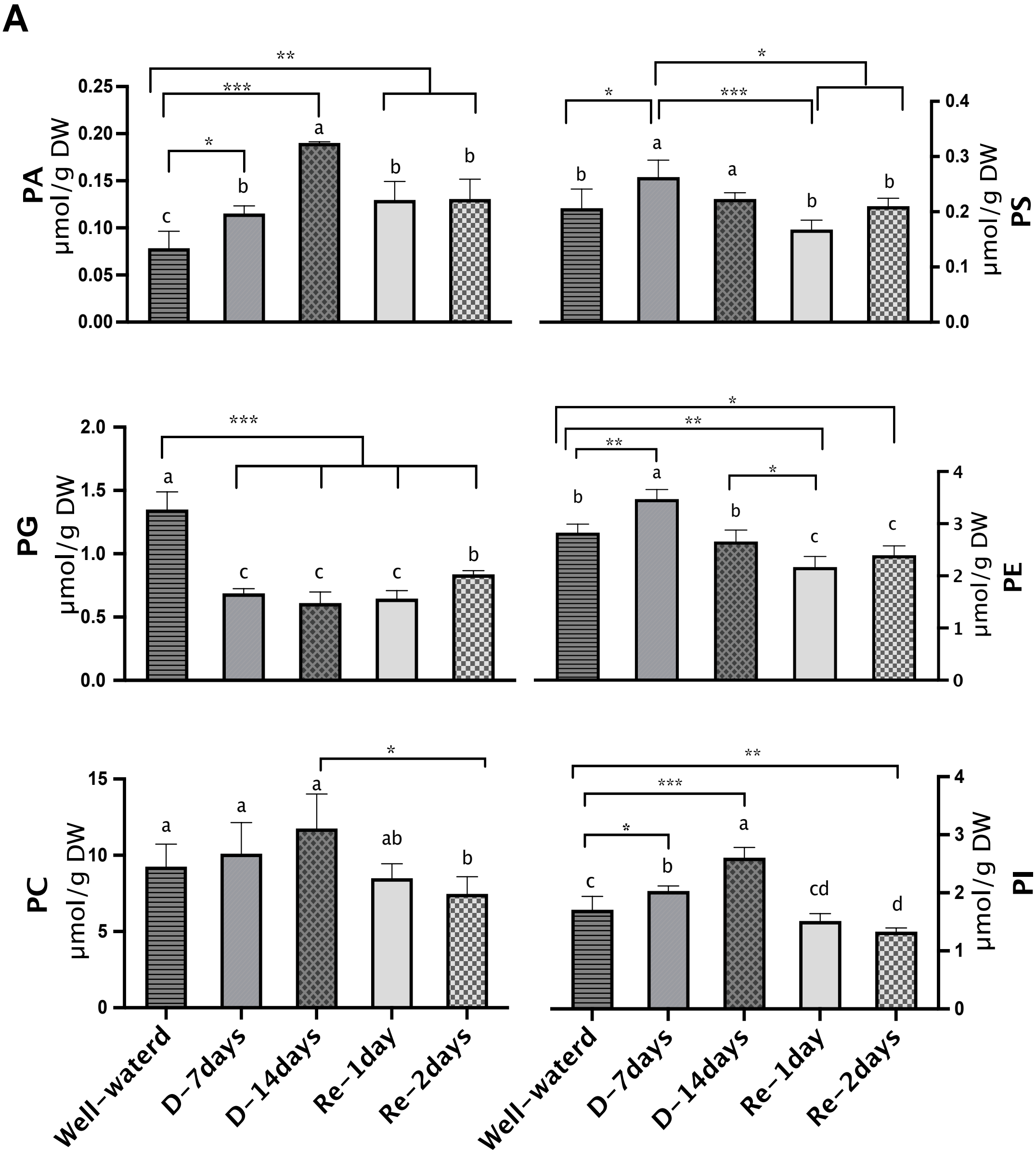


Figure 3.5.5: Phospholipid contents and phospholipid distribution of phospho-/galactolipid in *Oropetium thomaeum* leaves of well-watered, 7 days dehydrated, 14 days dehydrated, 1 day rehydrated and 2 days rehydrated. Phospholipid content was measured by Q-TOF MS/MS. PL, phospholipids; DW, dry weight. Data are present as mean and standard deviation from six biological replicates. Asterisks symbols indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***).

Gasulla *et al.* (2013) analyzed the lipid metabolism in the desiccation tolerant plant, *Craterostigma plantagineum* and revealed that a constant level of total polar lipids at approximately 100 nmol/mg dry weight was maintained during dehydration and rehydration. In this study, *Oropetium thomaeum* showed a stable content of phospholipids during dehydration but a decrease after rehydration. The mol percent of phospholipids in phospho- and galactolipids rapidly accumulated in *Oropetium thomaeum* leaves during dehydration, and then showed a decrease current during rehydration. (Figure. 3.5.5).



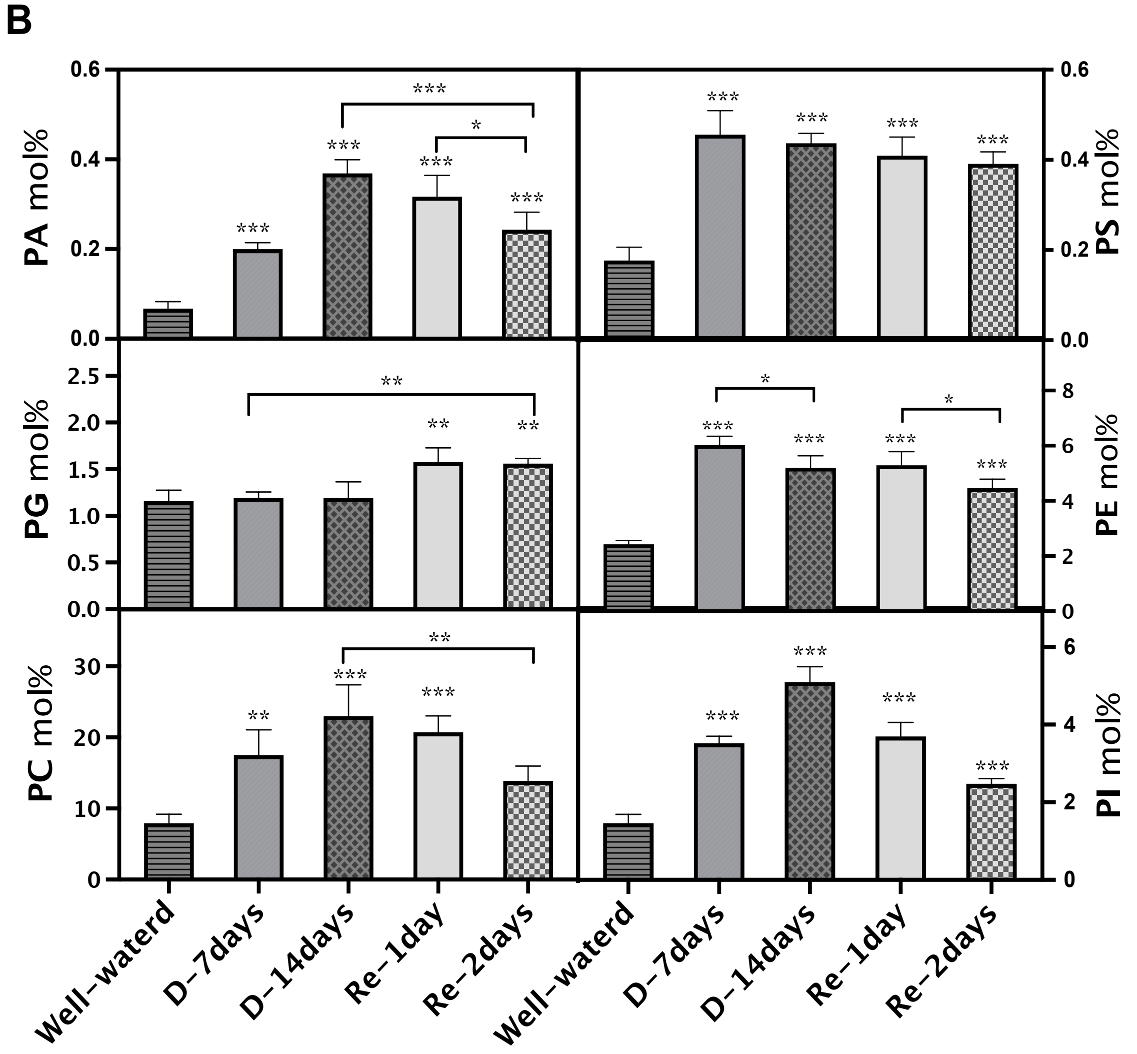


Figure 3.5.6 A: PA, PS, PG, PE, PC and PI content in *Oropetium thomaeum* leaves of well-watered, 7 days dehydrated, 14 days dehydrated, 1 day rehydrated and 2 days rehydrated. **B:** The mol% of PA, PS, PG, PE, PC and PI content in phospholipid of *Oropetium thomaeum* leaves of well-watered, 7 days dehydrated, 14 days dehydrated, 1 day rehydrated and 2 days rehydrated. PA, PS, PG, PE, PC and PI content was measured by Q-TOF MS/MS. PA, phosphatidic acid; PG, phosphatidylglycerol; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DW, dry weight. Data are presented as mean and standard deviation from six biological replicates. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.05 (*), *P*<0.025 (**); *P*<0.01 (***).

Although the total phospholipid content is stable during drought stress, the lipid composition of phospholipids changed (Figure 3.5.6 A and B). Desiccation induced significant accumulation of PA, PS, PE, PI, and a notable decrease of PG content in *Oropetium thomaeum* leaves. Compared with the well-watered plant, PC, PE, PG and PI content were reduced, PA content increased, and PS did not change after rehydration (Figure 3.5.6 A). The mol percent content of almost all these phospholipids showed an accumulation during rehydration, except for PG, which stayed at the same level during dehydration and then significantly increased after re-watering (Figure 3.5.6 B). It is generally accepted that PC is a vital phospholipid in plant membranes. In *Oropetium thomaeum* leaves, PC is the most abundant component among phospholipids, about 9 % in well-watered *Oropetium thomaeum* leaves and it significantly increased to 17 % and 23% after 7- and 14-days of dehydration respectively. The PC content showed the same trend with the total phospholipids in phosphor- and galactolipids, stable during dehydration and a decrease after rehydration (Figure 3.5.5 and 3.5.6 B, PC).

Transcript expression analysis of phospholipid-related genes in *Oropetium thomaeum*

Phospholipase D (PLD) was firstly identified in plants (Hanahan and Chaikoff, 1947). The most widely studied PLD activity cleaves the membrane lipid phosphatidylcholine (PC) to yield free choline and phosphatidic acid (PA) (Ali, 2013). PLDα is the most universal form of PLD and can be found in many plant tissues (Pappan *et al.*, 1997). Phosphatidylinositol synthase (PIS) is the enzyme responsible for the synthesis of phosphatidylinositol. The isolated *Oropetium thomaeum* cDNAs were identified from the transcriptome (VanBuren *et al.*, 2017 released at NCBI: PRJNA286116; these are the best matches of the *Oropetium thomaeum* sequences to *Arabidopsis thaliana* PLDα1 (*AT3G15730*), PLDα2 (*AT1G52570*), PIS1 (*AT1G68000*), PIS2 (*AT4G38570*), LPCAT1 (*AT1G12640*), and LPCAT2 (*AT1G63050*).

The transcript expression levels of PLDα1, PLDα2 PIS1, PIS2, LPCAT1 and LPCAT2 in *Oropetium thomaeum* leaves were analyzed by SQ-PCR under well-watered, dehydrated, and rehydrated treatments, and the results are shown in Figure 3.5.7.

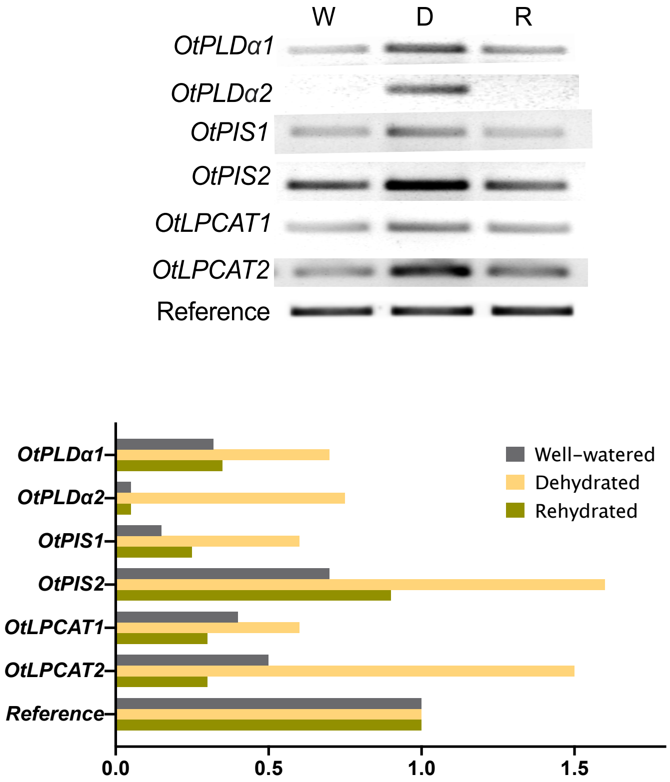


Figure 3.5.7 Expression profiles of phospholipid synthase genes in *Oropetium thomaeum* leaves. W：well-watered with grey bars；D: 14 days dehydrated with yellow bars；R: 2 days rehydrated with green bars. PLDα1, Phospholipase D alpha1; PLDα2, Phospholipase D alpha2; PIS1, Phosphatidylinositol synthase 1; PIS2, Phosphatidylinositol synthase 2; LPCAT1, lyso-phosphatidylcholine acyltransferase 1; LPCAT2, lyso-phosphatidylcholine acyltransferase 2; Reference, Ot-10692. The cDNAs sequences of OtPLDα1, OtPLDα2, OtPIS1, OtPIS2, OtLPCAT1 and OtLPCAT2 are listed in supplementary.

Previous research showed that activation of PLD and PIS is triggered by various biotic and abiotic stresses in plants, including drought stress (Frank *et al*., 2000; Liu *et al*., 2013). The transcript levels of *OtPLDα1*, *OtPLDα2*, *OtPIS1* and *OtPIS2* were upregulated in response to dehydration, after 2 days of rehydration *OtPLDα1* and *OtPLDα2* levels declined to the level in untreated plants, while *OtPIS1* showed a lower transcript level and *OtPIS2* displayed a higher transcript level than well-watered plants. There are two genes identified in *Arabidopsis thaliana* LPCAT1 and LPCAT2 which encode chloroplast localized LPCATs which are involved in the direct incorporation of fatty acids into PC through acyl editing (Karki *et al.*, 2019). In this study, water deprivation induced the upregulating of *OtLPCAT1* and *OtLPCAT2* (Figure 3.5.7), this trend correlated with the PC content which accumulated in response to drought stress in *Oropetium thomaeum* leaves (Figure 3.5.6 A, PC).

3.5.4 Quantification of galactolipids MGDG, DGDG, SQDG in *Oropetium thomaeum*

Galactolipids are glycolipids with galactose as a sugar group. Galactolipids are the principal category of lipids in the membranes of plastids and are indispensable for photosynthetic reactions (Kobayashi *et al.*, 2007). Unlike many bacteria, animals, and yeast, galactolipids such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) are commonly found in many plant species (Gaude *et al.*, 2007). The galactolipids MGDG and DGDG constitute about 75% of the total membrane lipids in leaves and therefore outnumber phospholipids (Joyard *et al.*, 1998; Slabas *et al.*, 1999; Dörmann and Benning, 2002). The high abundance of glycoglycerolipids in thylakoids of leaves contributes to plant photosynthesis (Douce and Joyard, 1998).

Galactolipids, MGDG, DGDG and SQDG, contents in *Oropetium thomaeum* leaves were analyzed by Q-TOF MS/MS and shown in Figure 3.5.8.

During dehydration and rehydration, the amount of galactolipids significantly decreased in *Oropetium thomaeum* leaves (A, B and C). The content of MGDG showed the biggest change from 62.9 µmol/g in untreated plant leaves and then rapidly decreased to 5 µmol/g within 14 days of dehydration, after 2 days re-watering recovered to 22.8 µmol/g (Figure 3.5.8 A). MGDG is the most abundant compound in phospho-/galactolipids, it continuously decreased with drought stress, from 54.5% in well-watered to 27.1% in 7 days dehydrated and 9.4% in 14 days dehydrated *Oropetium thomaeum* leaves, and rapidly increased to 33.6% after 1-day rehydration and 40.8% with 2 days rehydration (Figure 3.5.8 D). DGDG is one of the most abundant lipids but showed the reverse trend to MDGD in mol percent content, from 29.4% under non-stress growth conditions to 49.1% after dehydration and then recovered to the basal level after rehydration (Figure 3.5.8 D and E). SQDG contents show the same trend as DGDG after dehydration and rehydration treatment (Figure 3.5.8 B and C). But the total content of SQDG in well-watered and rehydrated *Oropetium thomaeum* leaves is only one tenth of MGDG and one sixth of DGDG.

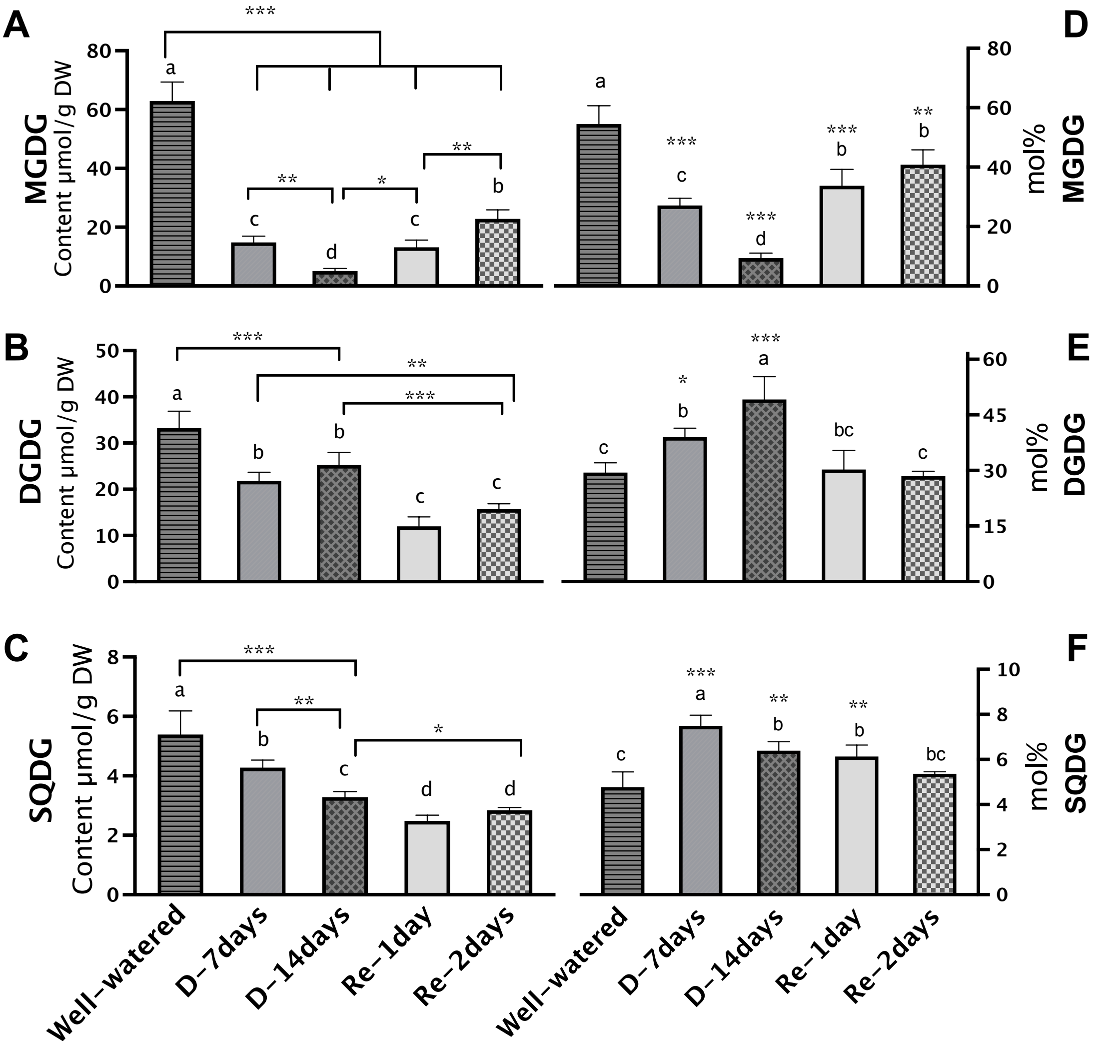


Figure 3.5.8 MGDG, DGDG and SQDG content (A, B and C) and mol percent (D, E and F) of phospho-/galactolipid in *Oropetium thomaeum* leaves of well-watered, 7 days dehydrated, 14 days

dehydrated, 1 day rehydrated and 2 days rehydrated. Galactolipid content was measured by Q-TOF MS/MS. MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DW, dry weight. Data are presented as mean and standard deviation from six biological replicates. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.05 (*), *P*<0.025 (**); *P*<0.01 (***).

Transcript expression analysis of galactolipid synthesis-related genes in *Oropetium thomaeum*

The transcript expression levels of MGD1, DGD1, SFR2, βGAL7, βGAL9and βGAL17 in *Oropetium thomaeum* leaves were analyzed by SQ-PCR under well-watered, dehydrated, and rehydrated treatments, and the results and heatmap were shown in Figure 3.5.9.

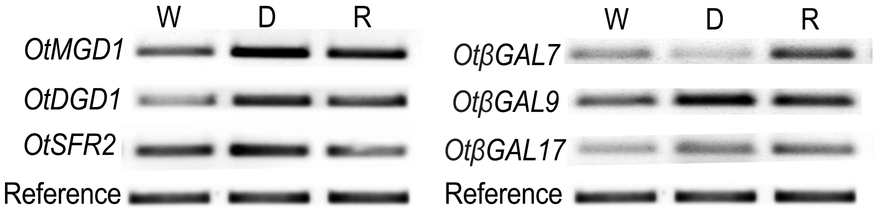


Figure 3.5.9 Expression profiles of galactolipid synthase genes in *Oropetium thomaeum* leaves. W： well-watered；D: 14 days dehydrated；R: 2 days rehydrated. MGD1, monogalactosyldiacylglycerol synthase1; DGD1, digalactosyldiacylglycerol synthase1; SFR2, sensitive to freezing2; βGAL7, βGAL9 and βGAL17: beta-galactosidase 7, 9 and 17; Reference, Ot-10692. The cDNAs sequences of OtMGD1, OtDGD1, OtSFR2, OtβGAL7, OtβGAL9, OtβGAL17, and Ot-Reference are listed in supplementary.

In plants, galactolipid synthesis is initiated by MGDG synthases (MGDs), which transfer a galactosyl residue to diacylglycerol (DAG) to form MGDG. MGDG may be further galactosylated by DGDG synthases (DGDs). MGD1 is a major galactolipid synthase in plants (Dörmann *et al.*, 1999; Rocha *et al.*, 2013 and 2016) and DGD1 represents the key synthase activity in *Arabidopsis thaliana* chloroplasts (Härtel *et al.*, 2000 and 2001). Galactolipid galactosyltransferase SFR2 is involved in oligogalactolipid synthesis and contributed to freezing and other abiotic stress tolerance in *Arabidopsis thaliana* (Thorlby *et al.*, 2004; Barnes *et al.*, 2016 and 2019). Here *Oropetium thomaeum* cDNA sequences of *OtMGD1*, *OtDGD1* and *OtSFR2* were matched to *AtMGD1* (*AT4G31780*), *AtDGD1* (*AT3G11670*) and SFR2 (*AT3G06510*). The SQ-PCR analysis showed that *OtMGD1* and *OtDGD1* transcript expression level were induced by dehydration and declined during rehydration (Figure 3.5.9), which were opposite to the contents of MGDG and DGDG which decreased in desiccated leaves (Figure 3.5.8). The *OtSFR2* expression level was up-regulated during dehydration and down-regulated after rehydration.

Beta-galactosidase (βGAL) is an important type of glycosidase enzyme, which catalyzes the hydrolysis of MGDG. βGAL7 (AT5G20710), βGAL9 (AT2G32810) and βGAL17 (AT1G72990) were the best matched sequences through BLAST algorithm with the *Oropetium thomaeum* transcript database. *OtβGAL7* transcript expression level showed a decrease during dehydration and then started to increase with re-watering. *OtβGAL9* rapidly accumulated during drought stress and then showed a slight decrease after rehydration. The transcript expression level of *OtβGAL17* was lower than *βGAL7 and βGAL9* in well-watered *Oropetium thomaeum* leaves.

3.5.5 Distribution of galactolipids and phospholipids in *Oropetium thomaeum* during drought stress and re-watering.

Galactolipids, MGDG, DGDG and SQDG, and phospholipids, PI, PG, PA, PC, PS and PE were analyzed by Q-TOF MS/MS in *Oropetium thomaeum* leaves under well-watered, dehydrated, and rehydrated treatments, and the mol% contents are shown in Figure 3.5.10.

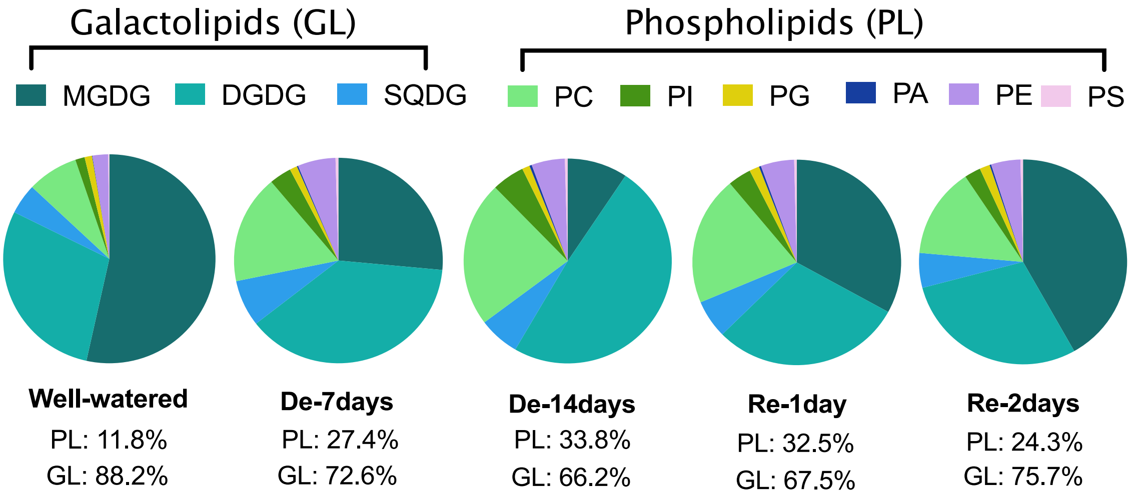


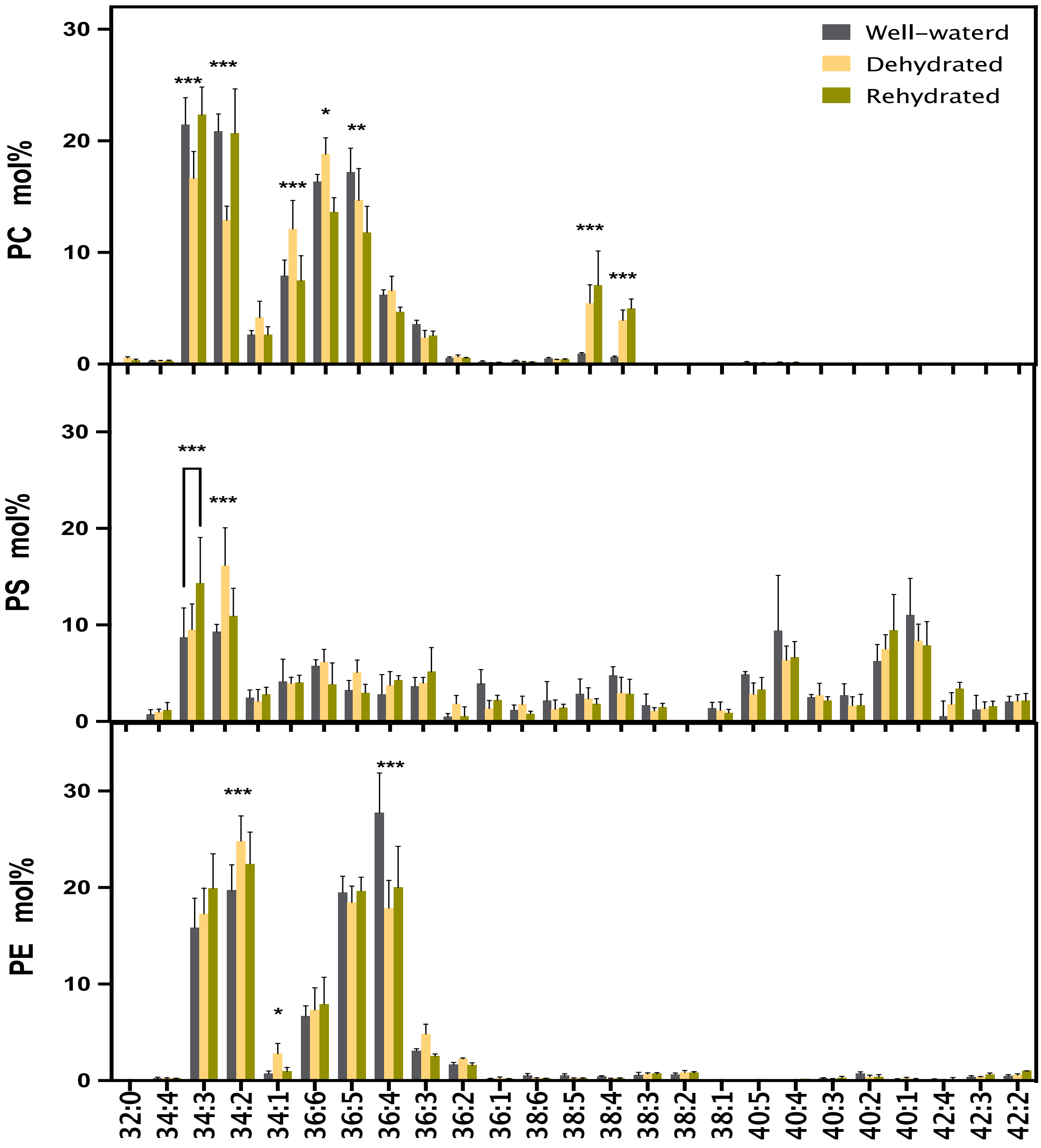
Figure 3.5.10 The distribution of galactolipids and phospholipids changes in mol% during dehydration and rehydration in *Oropetium thomaeum* leaves. De-7days, 7 days dehydrated; De-14days, 14 days dehydrated; Re-1day, 1 day rehydrated; Re-2days, 2 days rehydrated. GL, galactolipids; PL, phospholipids; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PA, phosphatidic acid; PG, phosphatidylglycerol; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol. Galactolipid and phospholipid contents were measured by Q-TOF MS/MS. Data are presented as mean from six biological replicates.

The amount of galactolipids decreased about a quarter compared with the untreated samples during drought stress, whereas phospholipids increased to triple times of well-watered plants. After rehydration, the amount of galactolipids recovered to 85.8% (75.7% to 88.2%) and phospholipids increased 105.9% (24.3% to 11.8%) of the well-watered leaves. The amount of MGDG was decreased during dehydration and rapidly increased after rehydration, while the amounts of DGDG, SQDG, PC, PI and PE quickly accumulated during drought stress and declined after re-watering. PG, PA, and PS are the minor lipids in *Oropetium thomaeum* leaves, of which the amounts are stable from well-watered to dehydration and re-watering treatments.

Analysis of the fatty acid composition of galactolipids and phospholipids in *Oropetium thomaeum*

The fatty acids' composition of MGDG, DGDG, SQDG, PI, PG, PA, PC, PS and PE were analyzed by Q-TOF-MS/MS under well-watered, dehydrated, and rehydrated treatments (Figure 3.5.11).

The most abundant fatty acid of MGDG and DGDG is 36: x. The mol percent content of 36:6 was decreased while 36:5 and 36:4 quickly accumulated during dehydration in MGDG and DGDG. 36:x and 34:x are the major components of SQDG, PC, PA, PE, and PG in *Oropetium thomaeum* leaves. The mol percent content of 34:3 was distinctly increased and 34:2 was clearly decreased during dehydration in SQDG, PG, PI, and PA. In this study only PG presented 32:x and showed a significant gain after 14 days of dehydration. PC is the main component of phospholipids (Figure 3.5.10), and here 34:x content was considerably decreased while 36:x and 38:x contents were increased during dehydration. PS is the only one which is ranged in almost all fatty acid compositions except 30:2 and 38:2. The most abundant of 40:x fatty acid was found in PS. PE showed the opposite trend as PC during dehydration and rehydration, with an increase of 34:x and a reduction of 36:x (Figure 3.5.11).


Well–waterd
Dehydrated
Rehydrated
PC mol%
PS mol%
PE mol%
0
10
20
30
***
**
*
32:0
34:4
34:3
34:2
34:1
36:6
36:5
36:4
36:3
36:2
36:1
38:6
38:5
38:4
38:3
38:2
38:1
40:5
40:4
40:3
40:2
40:1
42:4
42:3
42:2

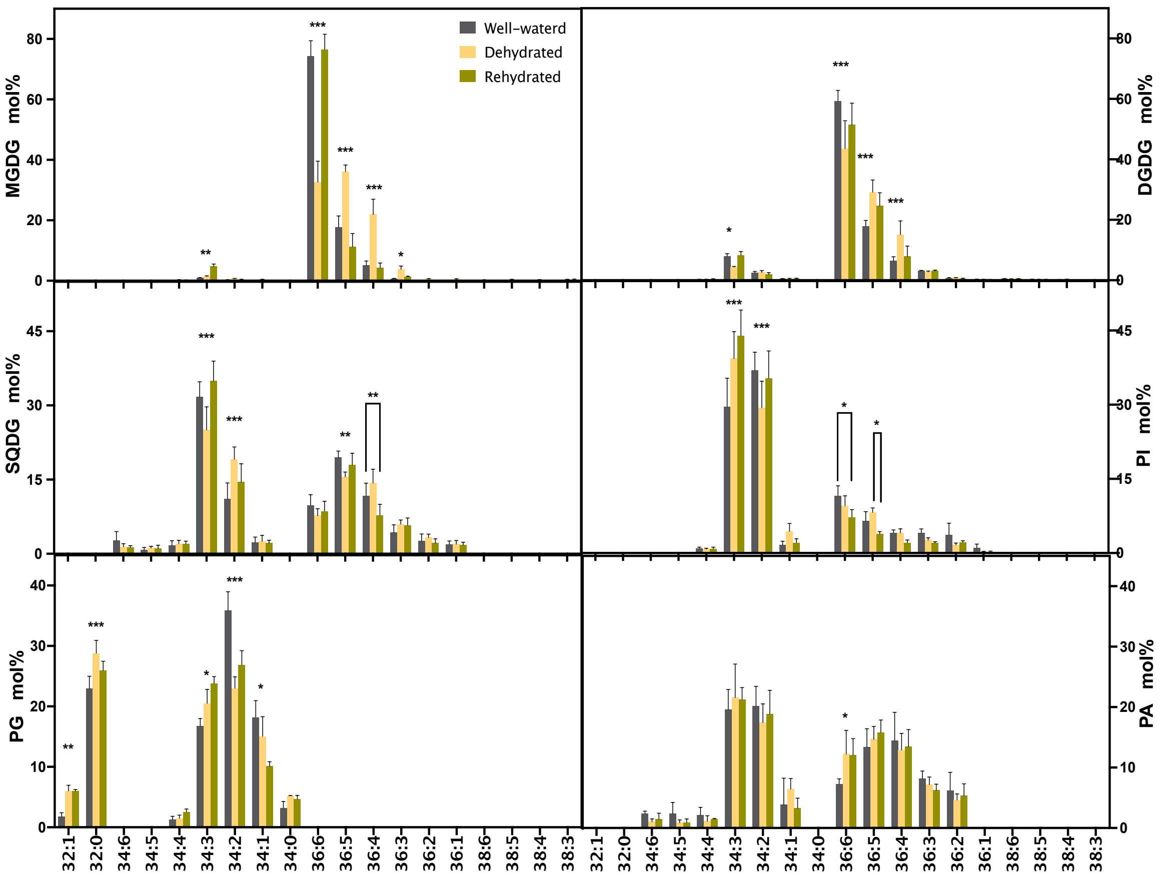


Figure 3.5.11 The fatty acids' composition of galactolipids and phospholipids changes in mol% during dehydration and rehydration of *Oropetium thomaeum* leaves. De-7days, 7 days dehydrated；De-14days, 14 days dehydrated molecular species composition of polar lipids in *Oropetium thomaeum*. Molecular species of polar lipids were measured in *Oropetium thomaeum* leaves of well-watered (W, grey baes), 14 days desiccated (D, yellow bars) and 2 days rehydrated (R, green bars). *Oropetium thomaeum* plants shows in Figure 3.5.1. PC, phosphatidylcholine; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiaylglycerol; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol. Data are presented as mean and standard deviation from six biological replicates. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.05 (*), *P*<0.025 (**); *P*<0.01 (***).

3.5.6 Quantification of sterol lipids in *Oropetium thomaeum*, *Craterostigma plantagineum*, and *Arabidopsis thaliana*

Plant sterols and sterolins, also known as phytosterols, are present in all plants (Bouic, 1998). Sterol lipids are considered as membrane reinforcers. As the third lipid class, sterols regulate biological metabolism and sustain the domain structure of cell membranes (Schaeffer *et al.* 2003; Ribeiro *et al*. 2007; Dufourc, 2008). The most abundant sterol lipid is free sterol in many species of plant leaves. Sterol esters (SEs) are coupled with longer chain fatty acids through the formation of esters, but sterol esters are present in plant cells at much lower amounts compared to free sterols (Valitova *et al.*, 2016). In this study, we compared the sterol lipids and the contents in desiccation tolerant plants, *Oropetium thomaeum* and *Craterostigma plantagineum* and in the desiccation sensitive plant, *Arabidopsis thaliana*.

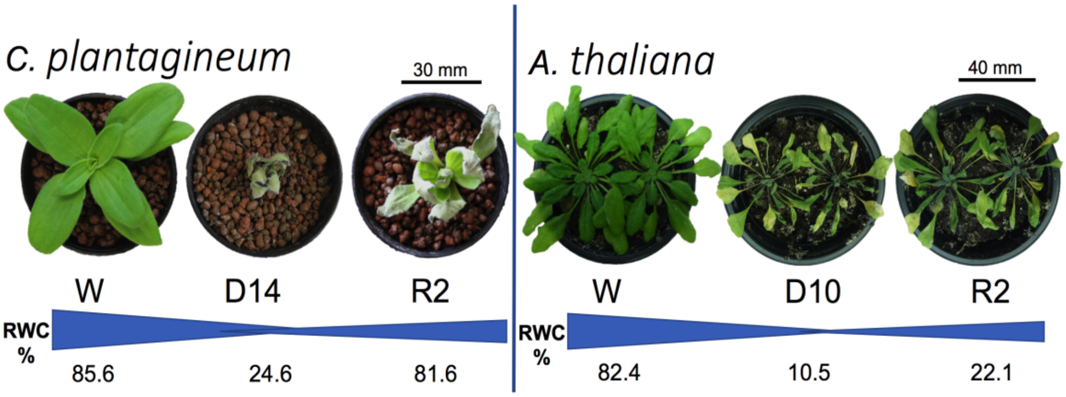


Figure 3.5.12 Appearance of *Craterostigma plantagineum* and *Arabidopsis thaliana* with relative water content under well-watered (W), 14 days dehydrated (D14), and 2 days rehydrated (R2) and well-watered (W), 10 days dehydrated (D10), and 2 days rehydrated (R2). The RWC (%) data are presented as means from six biological samples.

As a resurrection plant, *Craterostigma plantagineum* lost 71.3% of cellular water after 14 days of dehydration and recovered to 95.3% of the well-watered situation after rehydration, while *Arabidopsis thaliana* lost more cellular water (87.3% of well-watered plant) after dehydration for 10 days, and slightly increased to 22.1% of RWC within 2 days of rehydration.

Sterol lipids in *Oropetium thomaeum*, *Craterostigma plantagineum* and *Arabidopsis thaliana* leaves were analyzed by Q-TOF MS/MS, the total sterol lipids contents are shown in Figure 3.5.13A and the compositions of sterol lipids are shown in Figure 3.5.13B.

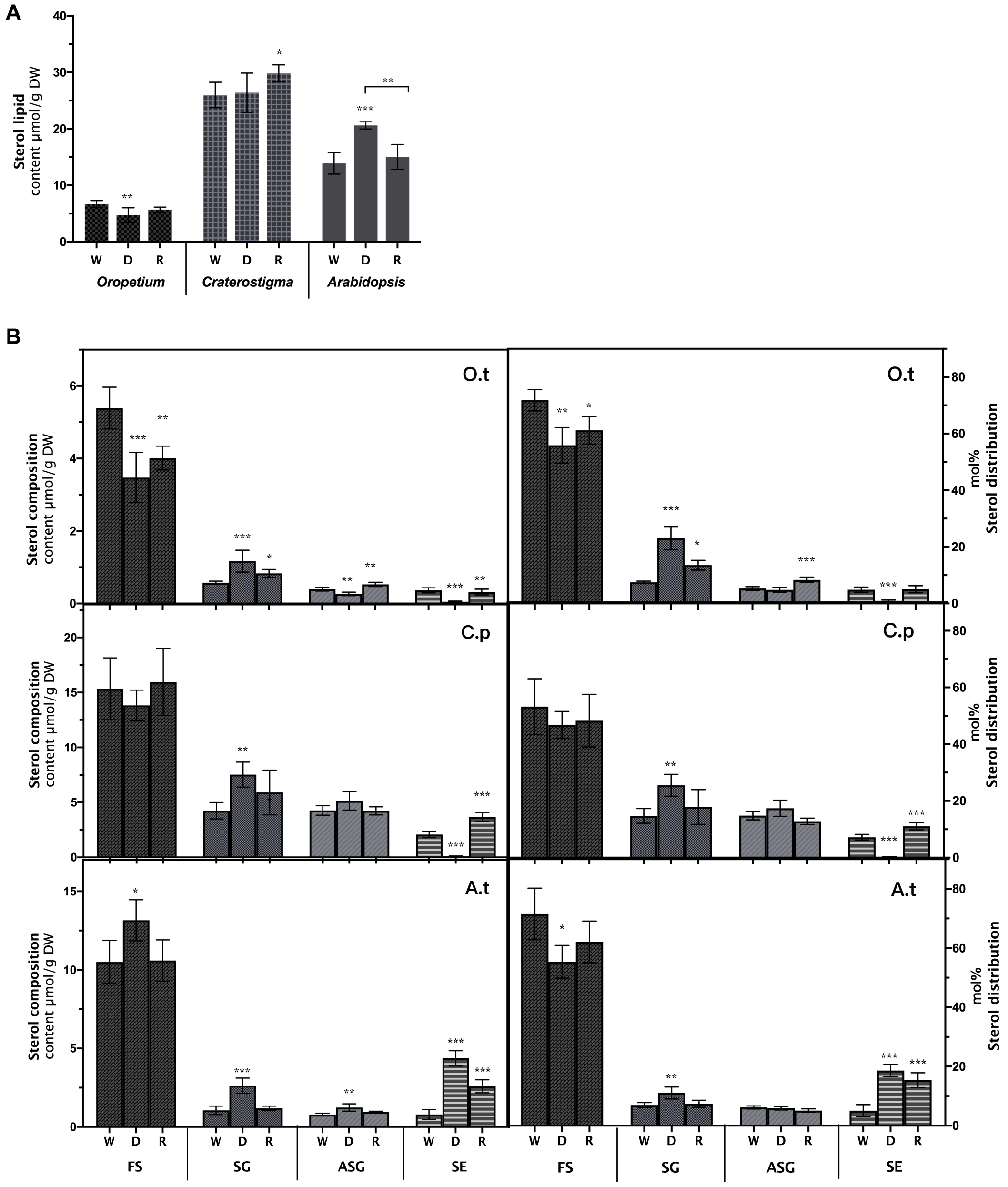


Figure 3.5.13 A: Total content of sterol lipids in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Arabidopsis thaliana* (A.t) leaves under different treatments. **B:** Sterol composition contents and sterol composition distribution in *Oropetium thomaeum* (O.t),

Craterostigma plantagineum (C.p) and *Arabidopsis thaliana* (A.t) leaves. Treatments for desiccation tolerant plant, *Oropetium thomaeum* and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments for the desiccation sensitive plant, *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). Sterol lipid contents were measured by Q-TOF MS/MS. FS, free sterols; SG, sterol glycosides; ASG, acylated sterol glycosides; SE, sterol ester; DW, dry weight. Data are mean values and standard deviation of five measurements. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***).

The total sterol lipid contents of *Oropetium thomaeum* are significantly reduced after dehydration, from 6.73 μmol/g dry weight to 4.78 μmol/g dry weight, and then increased to the same level of the untreated plants after 48 hours of rehydration. The desiccation sensitive plant, *Arabidopsis thaliana* showed a significant increase during drought stress and decreased after rehydration. The other desiccation tolerant plant, *Craterostigma plantagineum* presented a stable level of total sterol content (from 26.04 μmol/g dry weight to 26.45 μmol/g dry weight) during 14 days of water deprivation and 2 days of re-watering (Figure 3.5.13 A).

Plants have complex and diversified sterol compositions (Nes and McKean, 1977). The free form of free sterols (FS) and conjugated forms of sterol glycosides (SG), acylated sterol glycosides (ASG), and sterol esters (SE) are the predominant sterols in plants. Conjugated sterols are ubiquitously found in plants, but their contents highly differ among species and their profile may change in response to developmental and environmental cues (Ferrer et al., 2017). Figure 3.5.13 B shows that the majority of sterols were free sterol, about 80% (mol%) in well-watered plant leaves of *Oropetium thomaeum* and *Arabidopsis thaliana* which decreased to 71.3% and 69.9% after water deprivation for 14 days respectively. Sterol glycosides (SG) accumulated after dehydration in all these three plants. Sterol ester is the least abundant component of sterol lipids in *Oropetium thomaeum* and *Craterostigma plantagineum* leaves. The sterol ester (SE) content was dramatically decreased in *Oropetium thomaeum* and *Craterostigma plantagineum* leaves after dehydration but increased in *Arabidopsis thaliana* leaves (Figure 3.5.13 B).

Composition of sterol lipids of FS (free sterols), SG (sterol glycosides), and ASG (acylated sterol glycosides) in *Oropetium thomaeum*, *Craterostigma plantagineum* and *Arabidopsis thaliana*.

Sterols are precursors of brassinosteroids, which regulate plant growth and development and participate in transmembrane signal transduction. The predominant sterols in plants are campesterol, stigmasterol, and β-sitosterol (Valitova *et al*., 2016). It is widely known that cholesterol is commonly found in animal cells, but in recent years with the improvement of cholesterol detection methods, small amounts of cholesterol have also been found in plant cell membranes and in surface lipids of leaves (Schaeffer *et al*., 2001; Behrman and Gopalan, 2005). In this study, desiccation tolerant plant, *Oropetium thomaeum* and *Craterostigma plantagineum*, desiccation sensitive plant, *Arabidopsis thaliana* seedlings were treated with water deprivation and re-watering to analyze sterol compositions. Free sterols (FS), sterol glycosides (SG), and acylated sterol glycosides (ASG) contents were measured by Q-TOF MS/MS and shown in Figure 3.5.14.

Figure 3.5.14 shows that sitosterol is the most abundant compound of free sterols, sterol glycosides and acylated sterol glycosides in all leaf samples during dehydration and rehydration. The content of cholesterol is minute in plant leaves and hardly detectable in sterol glycosides (SG). Under drought stress, campesterol, stigmasterol and sitosterol of SG and ASG accumulated in all leaf samples (B and C). The content of cholesterol, campesterol, stigmasterol and sitosterol of FS were reduced in *Oropetium thomaeum* and *Craterostigma plantagineum* leaves, while *Arabidopsis thaliana* showed the reverse change with a slight increase of all these four compounds during dehydration (A). The content of campesterol, stigmasterol and sitosterol in ASG increased in *Craterostigma plantagineum* and *Arabidopsis thaliana*, whereas they decreased in *Oropetium thomaeum* during dehydration (C). The composition of free sterols did not show significant changes in *Craterostigma plantagineum* and *Arabidopsis thaliana* during dehydration and rehydration (A).

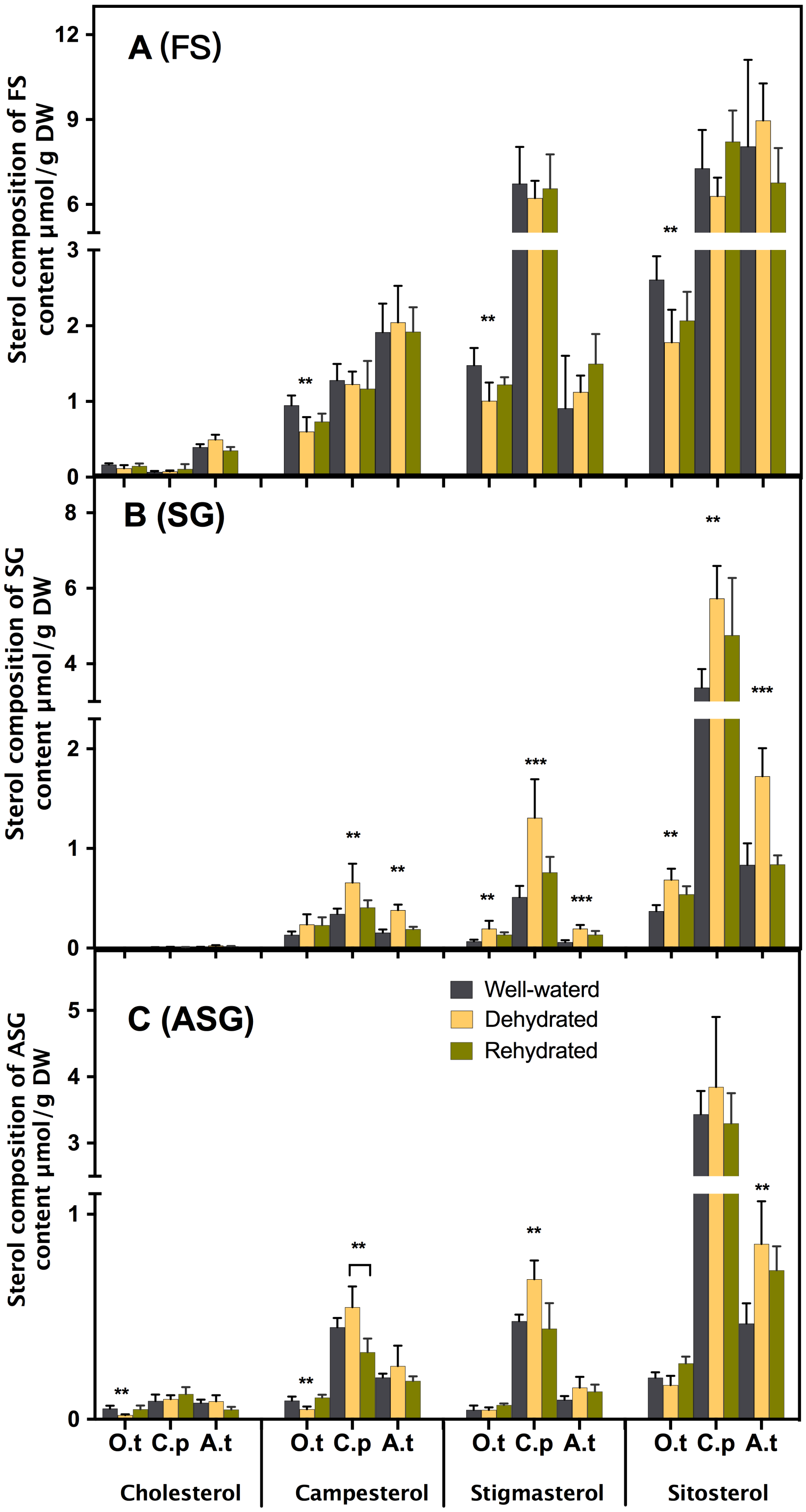


Figure 3.5.14 Sterol composition of free sterols **(A, FS)**, sterol glycosides **(B, SG),** and acylated sterol glycosides **(C, ASG)** in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Arabidopsis thaliana* (A.t) leaves under different treatments. Treatments for desiccation tolerant plant,

Oropetium thomaeum and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments for desiccation sensitive plant, *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). Sterol compositions contents were measured by Q-TOF MS/MS. FS, free sterols; SG, sterol glycosides; ASG, acylated sterol glycosides; DW, dry weight. Data are mean and standard deviation of five measurements. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.025 (**); *P*<0.01 (***).

3.5.7 Quantification of fatty acids in *Oropetium thomaeum*, *Craterostigma plantagineum*, and *Arabidopsis thaliana.*

The contents of fatty acid methyl ester (FAME) were measured by GC-FID in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Arabidopsis thaliana* (A.t) leaves under well-watered, dehydrated, and rehydrated treatments (Figure 3.5.15).

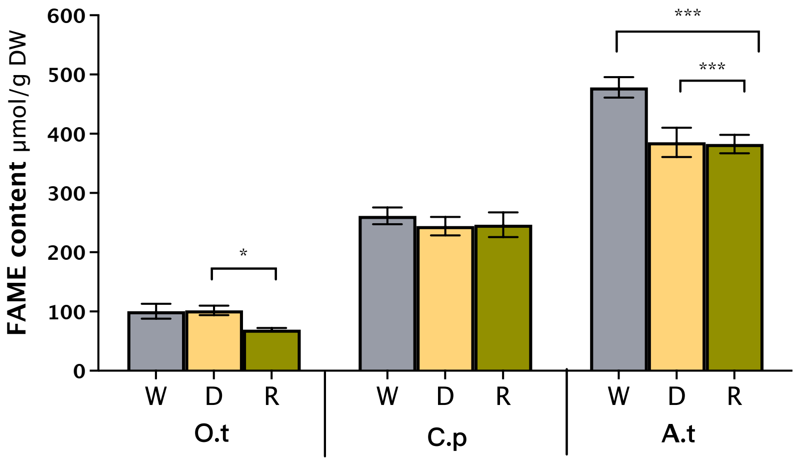


Figure 3.5.15 Fatty acid methyl esters content in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Arabidopsis thaliana* (A.t) leaves under different treatments. Treatments for desiccation tolerant plant, *Oropetium thomaeum* and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments for desiccation sensitive plant, *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). Fatty acid methyl esters content was measured by GC-FID. DW, dry weight. Data are mean values and standard deviation of six measurements.

Fatty acids are usually analyzed by gas chromatography or other techniques after conversion to the corresponding methyl esters, FAME (fatty acid methyl esters) (Browse *et al.*, 1985). Fatty acid methyl esters (FAME) are a type of fatty acid esters that are derived by transesterification of fats with methanol. The total content of FAME differs in plant species, 485.7 µmol/g dry weight in *Arabidopsis thaliana*, 261.4 µmol/g dry weight in *Craterostigma plantagineum* and 100.6 µmol/g dry weight in *Oropetium thomaeum*. The FAME content in desiccation tolerant plants, *Oropetium thomaeum* and *Craterostigma plantagineum,* kept stable during dehydration, while the desiccation sensitive plants, *Arabidopsis thaliana* showed a significant decrease with drought stress. *Craterostigma plantagineum* showed a constant content of FAME through the whole process of 14 days dehydration and 2 days rehydration treatments (Figure 3.5.15).

The molecular compositions of fatty acid methyl ester (FAME) contents were analyzed by GC-FID and shown in Figure 3.5.16.

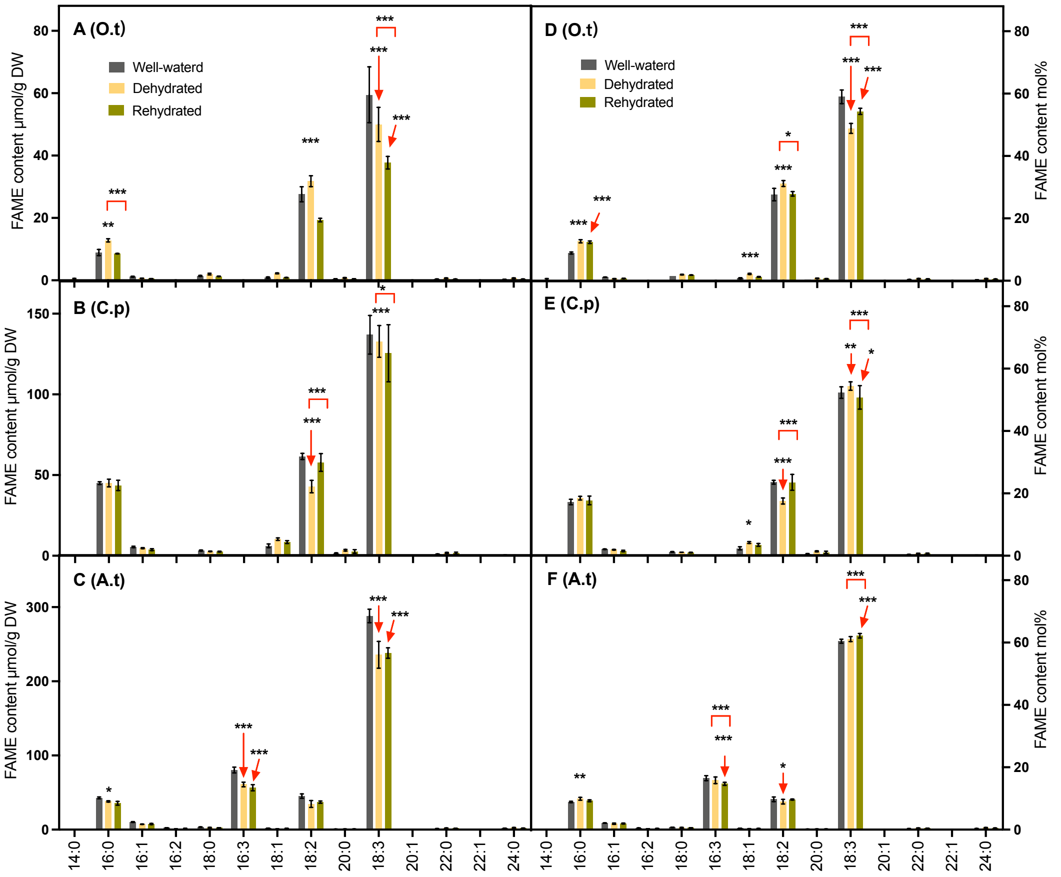
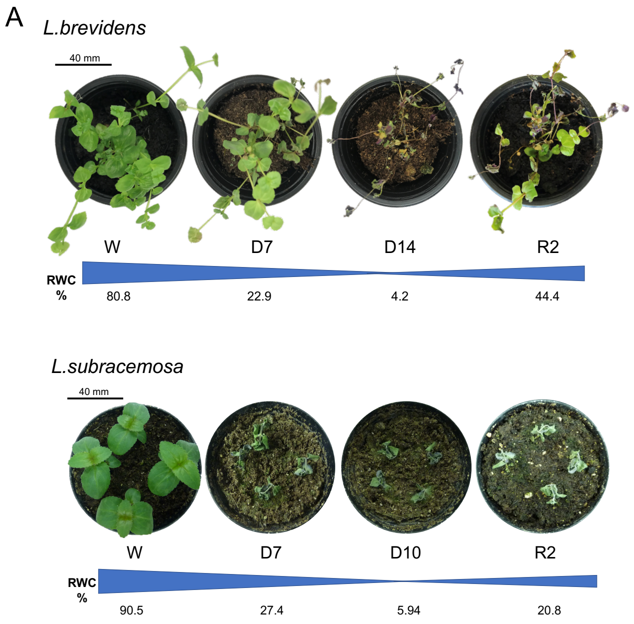


Figure 3.5.16 The molecular compositions of fatty acid methyl ester contents in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Arabidopsis thaliana* (A.t) leaves under different treatments. Treatments for desiccation tolerant plant, *Oropetium thomaeum* and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments for the desiccation sensitive plant, *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). FAME molecular compositions contents were measured by GC-FID. DW, dry weight. Data are mean values and standard deviation of six measurements.

Plant oils that are used for human consumption are comprised essentially of only five main fatty acids, the saturated palmitic (C16:0) and stearic acids (C18:0), monounsaturated oleic acid (C18:1), and the polyunsaturated linoleic (C18:2) and α-linolenic acids (C18:3) (Singh *et al.*, 2005). The analysis of fatty acids molecular compositions was shown in Figure 3.5.16. The most abundant molecular species composition of fatty acid was 18:3, the content of 18:3 was significantly decreased with water deprived in all these three plant species (A, B and C), while the mol percent of 18:3 reduced in *Oropetium thomaeum* (D)*,* but increased in *Craterostigma plantagineum* (E), and kept stable in *Arabidopsis thaliana* leaves (F). The fatty acid composition 18:2 was the second major composition in *Oropetium thomaeum* and *Craterostigma plantagineum*. During dehydration, the fatty acid of 18:2 accumulated in *Oropetium thomaeum* and declined in *Craterostigma plantagineum* and *Arabidopsis thaliana.* The fatty acid composition 16:3 was hardly detected in *Oropetium thomaeum* and *Craterostigma plantagineum* leaves, whereas 16:3 represented as the second abundant molecular species in *Arabidopsis thaliana*.

3.5.8 Quantification of sterol ester (SE) in Oropetium thomaeum, Craterostigma plantagineum, Lindernia breviden, Lindernia subracemosa, and Arabidopsis thaliana

In this study, we compared the sterol ester contents and their compositions in the desiccation tolerant plants (*Oropetium thomaeum, Craterostigma plantagineum and Lindernia brevidens*) and in the desiccation sensitive plants (*Lindernia subracemosa and Arabidopsis thaliana*). The appearance of the plants and the relative water content data are shown in Figure 3.5.1A, Figure 3.5.12 and Figure 3.5.17. *Lindernia brevidens* and *Lindernia subracemosa* are closely related species but show different desiccation tolerance. Parallel sampling of leaf tissue was done during desiccation and rehydration time courses in *Lindernia brevidens* and *Lindernia subracemosa*. Parallel sampling between species allowed us to distinguish between genes involved in general dehydration responses and those related specifically to desiccation tolerance. Sampling ranged from mild dehydration stress (relative water content [RWC] 53–56%; 3 d) through to severe dehydration (RWC 23–27%; 7 d) and desiccation (RWC 6–9%; 10 and 14 d), followed by 24 and 48 h of rehydration (Figure3.6.17). RWC was 53 to 56% at day 3 and fell below 10% after 10 d of dehydration in both species (Figure 3.5.17 B). *Lindernia subracemosa* plants were largely dead upon rehydration, and *Lindernia brevidens* plants were mostly viable and physiologically active at 48 h post rehydration (RWC 44%) (Figure 3.5.17A).



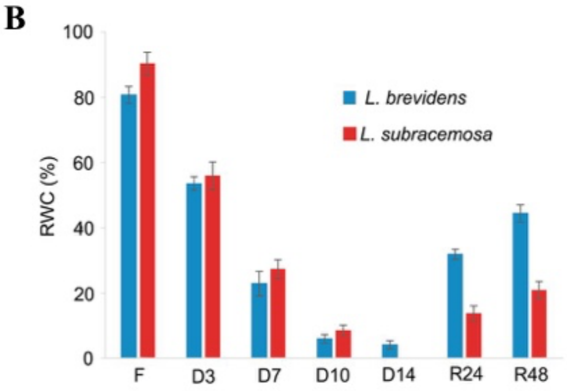


Figure 3.5.17: Appearance of *Lindernia brevidens* and *Lindernia subracemosa* with relative water content under well-watered (W), 14 days dehydrated (D14) of *Lindernia brevidens*, 10 days dehydrated (D10) of *Lindernia subracemosa*, and 2 days rehydrated (R2). RWC: relative water content.

(Figure 3.5.17 was already published. VanBuren et al., Plant Cell 2018; 30: 2943-2958)

It is widely accepted that sterol esters perform a major role in cell membrane sterol homeostasis (Schaller, 2004). Sterol esters are the other non-polar lipids which are presumed to be associated with TAGs in plant oil bodies (Kemp and Mercer, 1968; Zinser *et al.*, 1993). As shown in Figure 3.5.18 A, the SE content rapidly declined during drying in *Oropetium thomaeum* leaves, this is opposite to the contents of TAGs (Figure 3.5.1 B). Sterol esters are substituted with an acyl chain at the C3 position, which does not participate in membrane formation due to the lack of a polar head group. This may be the reason why the content of sterol ester (SE) is so much lower than TAGs and phospholipids.

The sterol ester (SE) contents were analyzed by Q-TOF MS/MS in *Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia brevidens*, *Lindernia subracemosa* and *Arabidopsis thaliana* leaves under well-watered, dehydrated, and rehydrated (Figure 3.5.18 A).

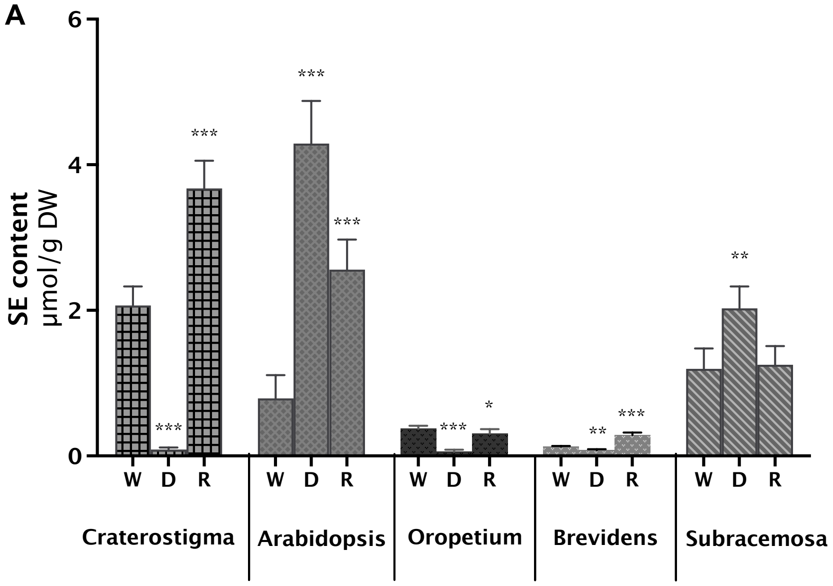


Figure 3.5.18 A: Total sterol ester content in *Oropetium thomaeum*, *Craterostigma plantagineum*, *Arabidopsis thaliana*, *Lindernia brevidens* and Lindernia subracemosa leaves of well-watered (W), dehydrated (D) and rehydrated (R) treatment. SE, sterol esters; DW, dry weight. Data are mean and standard deviation of five measurements. Asterisks symbols indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.05 (*), *P*<0.025 (**); *P*<0.01 (***).

Sterol esters are found in all plant tissues, their relative content usually differs among organs and tissues and may change in response to developmental and environmental factors. Under normal growth condition, the SE content in *Craterostigma plantagineum*, *Arabidopsis thaliana*, *Lindernia brevidens* and *Lindernia subracemosa* were around 6 times, two times and three times more, respectively compared to the SE content in *Oropetium thomaeum* leaves. During dehydration, sterol esters are significantly reduced in the desiccation tolerant plants, *Oropetium thomaeum*, *Craterostigma plantagineum* and *Lindernia brevidens* leaves, whereas SE content was significantly increased in leaves of the desiccation sensitive plants, *Lindernia subracemosa* and *Arabidopsis thaliana* (Figure 3.5.18 A). After re-watering the plants, SE content in *Craterostigma plantagineum* and *Lindernia brevidens* recovered fast and accumulated to a higher level than in well-watered plants, while the SE content in *Arabidopsis thaliana* and *Lindernia subracemosa* rapidly declined with rehydration.

The fatty acid molecular species of SE was analyzed by Q-TOF MS/MS in *Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia brevidens*, *Lindernia subracemosa* and *Arabidopsis thaliana* leaves under different treatments (Figure 3.5.18 B).

Under normal growth condition, 18:3 and 18:2 were the most abundant fatty acids in these 5 plant species. In *Craterostigma plantagineum* and *Lindernia subracemosa* the 18:1 content was much higher than in well-watered *Oropetium thomaeum*, *Lindernia brevidens* and *Arabidopsis thaliana*. The contents of 18:3 and 18:2 rapidly declined in *Oropetium thomaeum* and *Craterostigma plantagineum* (desiccation tolerant plants), whereas *Arabidopsis thaliana* and *Lindernia subracemosa* (desiccation sensitive plants) showed the opposite changes during dehydration. *Lindernia brevidens* kept a stable 18:2 content and a slight increase of 18:3 during dehydration. After rehydration, the 18:x content continuously declined in *Oropetium thomaeum*, which showed a significantly lower content than in well-watered plants. But in *Craterostigma plantagineum*, *Lindernia brevidens* and *Arabidopsis thaliana*, the 18:x fatty acids content in rehydrated plants was higher than in untreated samples. Although only a few 16:0 fatty acids were found in sterol esters, it represented the same trend as 18:3 and 18:2 fatty acids during dehydration and rehydration in all these 5 plant species.

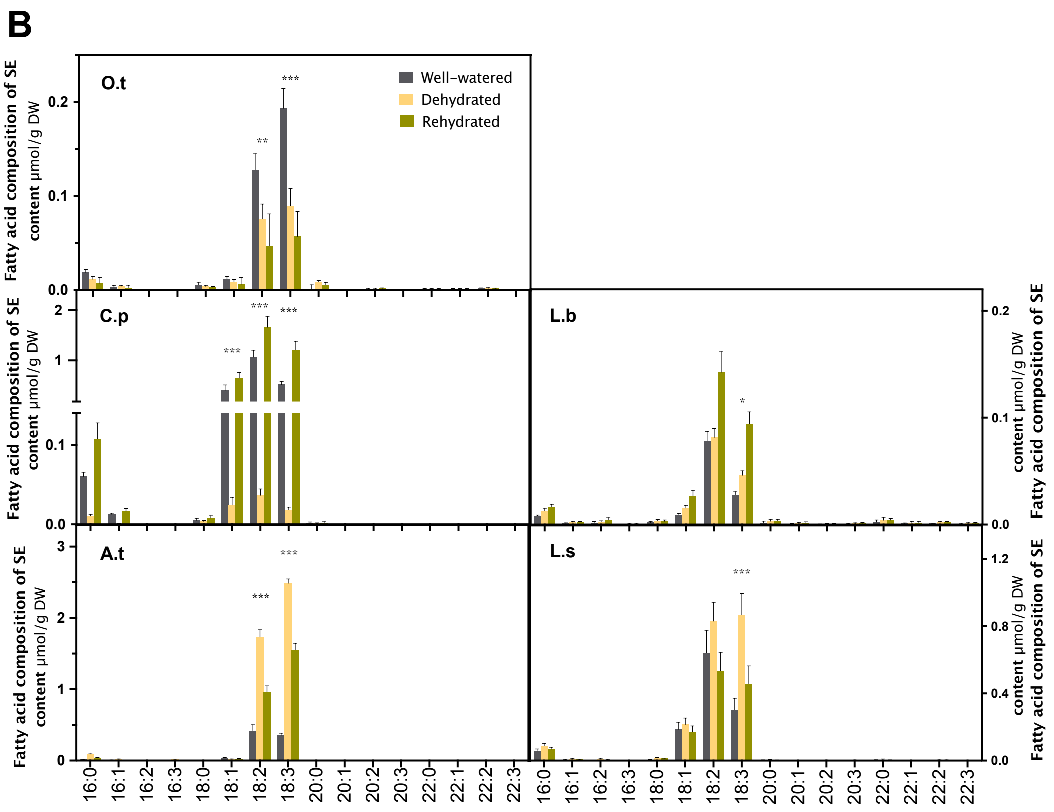


Figure 3.5.18 B: The fatty acid molecular species of sterol ester composition content in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p) and *Lindernia brevidens* (L.b), *Lindernia subracemosa* (L.s) and *Arabidopsis thaliana* (A.t) under different treatments. Treatments for desiccation tolerant plant, *Oropetium thomaeum* and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments for desiccation sensitive plant, *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). Sterol ester molecular composition content was measured by Q-TOF MS/MS. DW: dry weight. Data are mean and standard deviation of five measurements. Asterisks symbols indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, $P<0.025$ (**); $P<0.01$ (***).

Components of sterol esters

The sterol compositions of SE were analyzed by Q-TOF MS/MS in *Oropetium thomaeum*, *Craterostigma plantagineum*, *Lindernia brevidens*, *Lindernia subracemosa* and *Arabidopsis thaliana* leaves under different treatments (Figure 3.5.19).

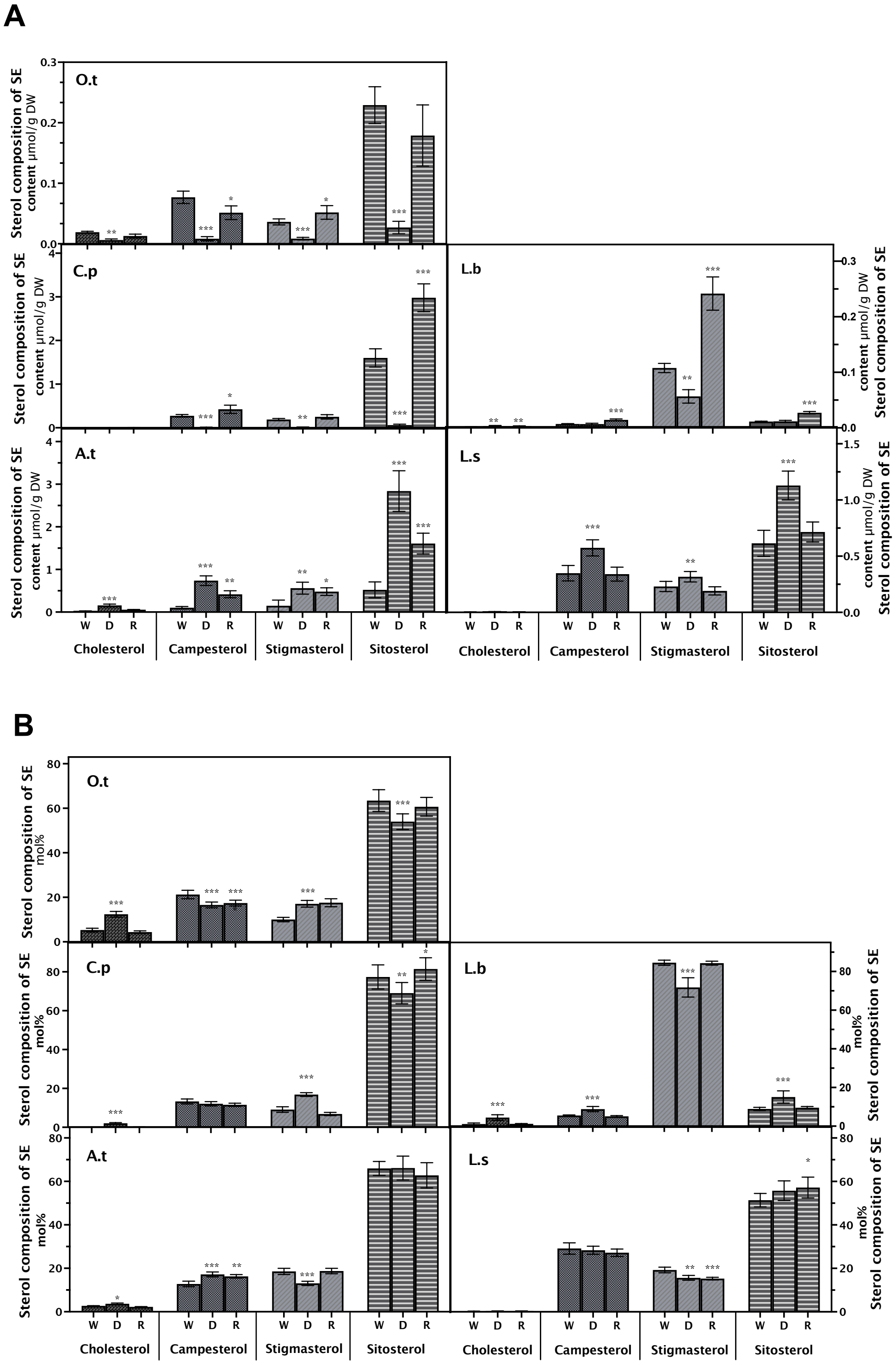


Figure 3.5.19 A and B: Sterol compositions contents of sterol ester (SE) were analyzed in *Oropetium thomaeum* (O.t), *Craterostigma plantagineum* (C.p), *Lindernia brevidens* (L.b), *Lindernia subracemosa* (L.s) and *Arabidopsis thaliana* (A.t) leaves under different treatments. Treatments for the desiccation tolerant plants, *Oropetium thomaeum, Lindernia brevidens* and *Craterostigma plantagineum,* are well-watered (W), 14 days dehydrated (D) and 2 days rehydrated (R). Treatments

for the desiccation sensitive plants, *Lindernia subracemosa* and *Arabidopsis thaliana*, are well-watered (W), 10 days dehydrated (D) and 2 days rehydrated (R). The sterol ester content was measured by Q-TOF MS/MS. SE: sterol esters; DW: dry weight. Data are mean values and standard deviations of five measurements. Asterisks indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, $P<0.05$ (*), $P<0.025$ (**); $P<0.01$ (***).

The contents of campesterol, stigmasterol and sitosterol of SE were significantly decreased in leaves of *Oropetium thomaeum, Craterostigma plantagineum* and *Lindernia brevidens* (desiccation tolerance) and increased in *Arabidopsis thaliana* and *Lindernia subracemosa* (desiccation sensitive). Cholesterol was hardly detected in *Lindernia subracemosa* and *Craterostigma plantagineum* leaves whereas in *Oropetium thomaeum* and *Lindernia brevidens*, it changed during dehydration and rehydration. Behrman and Gopalan (2005) illustrated that cholesterol occurs as a component of plant membranes and as part of surface lipids in leaves where it is sometimes the major sterol. The cholesterol amount is generally small relative to the content of total lipids, if expressed as percent. Here, in *Oropetium thomaeum* leaves, the mol percent of cholesterol of sterol esters is about 5% in well-watered and rehydrated plants and increased to 12.3% after 14 days of drought stress. Sitosterol (beta-sitosterol) is one of several phytosterols (plant sterols) with chemical structure is similar to cholesterol. It is widely distributed throughout the plant kingdom, and it is known to be involved in the stabilization of cell membranes (Bouic *et al.*, 1999; Sayeed *et al.*, 2016). Sitosterol was found as the most abundant component in leaves of *Oropetium thomaeum, Craterostigma plantagineum, Arabidopsis thaliana* and *Lindernia subracemosa,* it comprised about 60%-70% of the total sterol esters (Figure 3.5.19 B). The contents of sitosterol in *Oropetium thomaeum* and *Craterostigma plantagineum* were reduced during dehydration, while it rapidly accumulated in *Arabidopsis thaliana* and *Lindernia subracemosa* (Figure 3.5.19 A). Although the mass content of sitosterol changed remarkably during dehydration but the mol percent content of sitosterol was stable in *Arabidopsis thaliana* and *Lindernia subracemosa.* In *Lindernia brevidens* leaves, the mol percent of stigmasterol was about 84% in well-watered and rehydrated leaves and decreased to 72.8% after 14 days of dehydration (Figure 3.5.19 B).

Transcript expression analysis of sterol ester synthesis-related genes in desiccation tolerant and desiccation sensitive plant leaves

The synthesis of sterol ester is catalyzed by a group of enzymes collectively known as sterol acyltransferases, acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) (Korber *et al*., 2017; A. Lara *et al*., 2018). Acyl-CoA: sterol acyltransferases1 and phospholipid: sterol acyltransferases1 in *Arabidopsis thaliana (AtASAT1* and *AtPSAT1*) have been cloned and characterized in plants (Banas *et al*., 2005; Chen *et al*., 2007). *AtPSAT1* and *AtASAT1* sequences were aligned with transcriptome of *Oropetium thomaeum* (Ot), *Craterostigma plantagineum* (Cp), *Lindernia brevidens* (Lb) and *Lindernia subracemosa* (Ls), and the transcript expression results were shown in Figure 3.5.20.

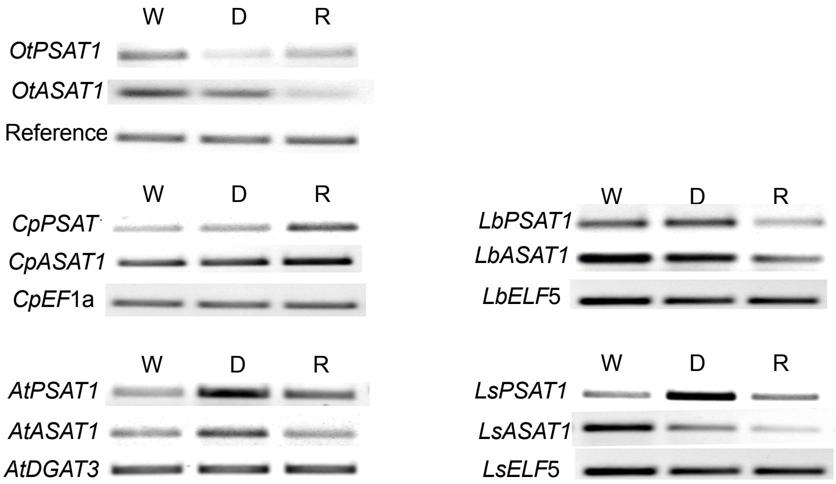


Figure 3.5.20 Transcript expression profiles of sterol ester synthase genes in *Oropetium thomaeum* (Ot), *Craterostigma plantagineum* (Cp), *Lindernia brevidens* (Lb), *Lindernia subracemosa* (Ls) and *Arabidopsis thaliana* (At). W： well-watered；D: 14 days dehydrated of Ot, Cp, Lb and 10 days dehydrated of Ls, AT；R: 2 days rehydrated. PSAT1, Phospholipid: sterol acyltransferase; ASAT1, Acyl-CoA sterol acyltransferase 1; EIF5: Eukaryotic initiation factor 5; EF1α, Elongation factor 1α; DGAT3, Diacylglycerol acyltransferases 3; Reference, Ot-10692. All these sequences are listed in supplementary.

Phospholipid: sterol acyltransferase (PSAT) is the primary enzyme synthesizing sterol esters in *Arabidopsis thaliana*. The PSAT is the only intracellular enzyme which is known so far to catalyze an acyl-CoA-independent (a phospholipid-dependent) sterol ester formation, and the predominance of PSAT1 over ASAT1 (Acyl-CoA sterol acyltransferase1) in controlling the free sterol content of rosette leaves (Pierrette Aesterification in yeast and plants (Ferrer *et al.*, 2017). The expression of PSAT1 and ASAT transcripts was low in *Oropetium thomaeum*, this correlates with the sterol ester content which is shown in Figure 3.5.18 A. The expression level of PSAT1 was induced by drought stress in the desiccation sensitive plant, *Arabidopsis thaliana* and *Lindernia subracemosa*, whereas the desiccation tolerant plant, *Oropetium thomaeum* showed a significant decrease. The expression level of PSAT1 and ASAT1 did not show significant changes *in Craterostigma plantagineum* and *Lindernia brevidens* during dehydration, but after rehydration PSAT1 and ASAT1 are down-regulated in *Lindernia brevidens* and up-regulated in *Craterostigma plantagineum* (Figure 3.5.20).

4. Discussion

4.1 Severe drought stress in the future and desiccation tolerant plants

According to IWMI (International Water Management Institute) one-third of the population will undergo severe water shortage living conditions by the year 2025 (www.iwmi.org). Water scarcity will impact 40% of the world's population, and as many as 700 million people are at risk of drought by 2030 (WHO, 2020). The World Resources Institute (WRI) predicted that nearly one-fifth of all countries worldwide will face water scarcity problems and experience an especially significant increase of drought stress by 2040 (WRI, 2015; Luo et al., 2015). Drought stress is one of the most impacting factors which alter seriously plant physiology, finally leading to the decline of crop productivity. (Dilley et al., 2005; UNDRR, 2019). Global drought impacts on growth of plants, environmental homeostasis, economic developments, agricultural losses, public water shortages, reduced labor, and productivity (World Resources Institute, 2019; Meza et al., 2020). Agriculture is the mostly heavily affected sector of drought stress. Over 40% of calories consumed by humans are obtained from grasses, and the grass family (Poaceae) is possibly the most important plant family regarding global food safety (Elert, 2014; Van Buren et al., 2017; Food and Agriculture Organization of the United Nations, 2018). As it shows in Figure 1.6 and 1.7, with the completion of genomic sequencing and evolutionary relationship analysis of grasses, e.g. Oropetium thomaeum, barley, wheat, brachypodium, millet, maize, rice, and sorghum, Oropetium thomaeum is clearly shown to belong to the Chloridoideae subfamily of Poaceae and 75% of the genome is located in the conserved syntenic regions of grass genomes (Paterson et al., 2009; Bennetzen et al., 2012; Schnable et al., 2012; Mascher et al., 2017; VanBuren et al., 2015 and 2018; Liu et al., 2019). As a resurrection plant, Oropetium thomaeum could survive extreme water stress such as loss of >95% of cellular water (Bartels et al., 2002; Van Buren et al., 2015).

In this study, relative water contents (RWC) were compared in desiccation tolerant plant leaves and desiccation sensitive plant leaves. Here, the RWC calculation was the most common one, using saturated water content in plants (Turner, 1981; González and

González-Vilar, 2001). *Oropetium thomaeum* showed 87.6% of relative water content (RWC) loss in leaves after 15 days of dehydration and recovered to 91.8% of RWC in 2 days after re-watering (Figure 3.1). Another resurrection plant *Craterostigma plantagineum* lost 71.3% of RWC after 14 days of dehydration and recovered to 95.3% of the well-watered situation after rehydration (Figure 3.5.12). Gasulla *et al.*, 2013 showed that *Craterostigma plantagineum* could survive 93.4% of relative water loss with dehydration treatment and recovered to 96.1 of relative water content of untreated plants. *Lindernia brevidens* plants showed 94.8% of cellular water loss after 14 days of drought stress (from 80.8% to 4.4%) and were mostly viable and physiologically active at 2 days post rehydration (recovered to 45% of cellular water of well-watered plants) (Figure 3.5.17A). The desiccation sensitive plants, *Arabidopsis thaliana* displayed around 80% relative water content (Bouchabke *et al.*, 2008 and Khalatbari *et al.*, 2020), but *Arabidopsis thaliana* and *Lindernia subracemosa* were largely dead after 10 days of dehydration and subsequent re-watering (Figure 3.5.12 and Figure 3.5.17A).

4.2 Resurrection plant morphological response to drought stress

Response of drought stress in plants is usually displayed in a set of morphological, anatomical, physiological, molecular and biochemical changes, mainly addressed to limit the loss of water by transpiration with the attempt to increase the plant water use efficiency (Levitt, 1980; Le and McQueen-Mason, 2006; Gigon *et al.*, 2004; Torres-Franklin *et al.*, 2007; Costa, 2019). Cellular structures were observed with well-watered *Oropetium thomaeum* and 14 days dehydrated plant leaves using electron microscopy (Figure 3.2.2). Vacuole fractionation is obvious in the whole cell, especially in the bundle sheath cells and mesophyll cells under drought stress. The same desiccation-related changes in leaf morphology were reported for another resurrection grass *Eragrostis nindensis* (Vander Willigen *et al.*, 2004). After dehydration, bulliform cells are involved in the folding of leaf tissues, most possibly to control light intensity and reduce overall water loss ((Figure 3.2.2 D and F). The same anatomical structures were also found in halophytes (MN Grigore, L Ivanescu, C Toma, Book: An Integrative Anatomical Study, 2014.)

Chaves (2011) and Kapoor (2020) made the point that stomata closure is one of the first consistent reactions observed under drought stress, this results in photosynthetic adjustments to enhance the plant defense barriers against abiotic stress. The histological structure of *Oropetium thomaeum* leaves shows the stomata are a four-celled structure, also called Dumb-bell shape, while Kidney shape (two-celled structure) stomata are found in C3 plants (Figure 3.2.1.2 and Figure 3.2.1.4; Jones *et al.*, 2003 and 2005; Shtein *et al.*, 2017). This more complex form of stomata, in contrast to two-celled structures, is found in most C4 plants, e.g., wheat, rice, maize, and barley. Four-celled structures of stomata can function better through enhancing plant water use efficiency and photosynthetic carbon gain (Kirschbaum *et al.*, 1988; Knapp, 1993; Cardon *et al.*, 1994; Allen and Pearcy, 2000; Noe and Giersch, 2004; Pearcy and Way, 2012; Smith and Berry, 2013; Lawson and Blatt, 2014). Rui and Anderson, 2016 and Shtein *et al.*, 2017 indicated that cellulose and xyloglucan are required for proper stomata function, however in this study, as shown in Figure 3.2.1.4, localization of lipids followed the changes of the stomata structure during stomata closure when plants confront drought stress. Lipids are an important component of the subcellular and cellular membranes and thus play an important role in membrane permeability, therefore analyzing lipid changes in plant cells is a key factor to reveal the mechanism of desiccation tolerant plants.

4.3 Plant oil bodies: energy resources

Plant cells deposit storage resources of carbohydrates, proteins, and neutral lipids in subcellular particles such as starch granules, protein bodies, and oil bodies, respectively (Tzen, 2012). Oil bodies are largely seed-specific lipid organelles that store triacylglycerols as food reserves to fuel germination and early plant growth in a variety of eukaryotic cells (Eastmond, 2006; Quettier and Eastmond, 2009; Shimada and Hara-Nishimura, 2015; Thazar-Poulot *et al.*, 2015; Pyc *et al.*, 2017; Huang, 2018). The membrane of oil bodies is filled with oleosins, structural proteins that prevent coalescence during seed desiccation. *Arabidopsis thaliana* mutant lines with suppressed oleosins contain unusually large oil bodies and have delayed germination (Siloto *et al.* 2006). Oleosins are also associated with freezing tolerance in seeds, and they likely protect membrane integrity during freeze-thaw cycles (Shimada *et al.* 2008). Oleosins are conserved across grasses with rice, sorghum, Brachypodium and maize each having six members (Vanburen *et al.*, 2017), although *Arabidopsis thaliana* has undergone significant expansion with 19 members (Huang & Huang 2015).

VanBuren (2018) identified oleosin transcripts from the *Lindernia brevidens* (desiccation tolerant) and *Lindernia subracemosa* (desiccation sensitive) transcriptome and found that six oleosin genes in *Lindernia brevidens* and three oleosin genes in *Lindernia subracemosa* were upregulated in desiccating tissues. Oil bodies in plants function to store neutral oil (Lersten *et al.*, 2006). Oil bodies are largely absent from well-watered *Oropetium thomaeum* leaves but are highly abundant in desiccated leaf tissues. During desiccation, oil bodies may function as a high-density energy source used during rehydration, mirroring their role in seeds (Huang, 1992), or they may have acquired new roles such as membrane repair or preventing coalescence of the cell membrane during desiccation. Oleosins maintain oil body size and prevent their coalescence during seed desiccation (Siloto *et al.*, 2006). Oleosins are among the most highly expressed genes in desiccated *Oropetium thomaeum* tissues, and they may also prevent thylakoid, mitochondrial and cell membrane coalescence during extended desiccation in addition to their established role in oil body stability. Oil bodies accumulate in the xylem vessels of the resurrection plant *Myrothamnus* (Schneider *et al.*, 1999).

Besides oleosins, membranes of oil bodies are also filled with caleosin, which contain an oil body binding domain and a calcium-binding domain (Chen *et al.*, 1999), Poxleitner (2016) suggested that caleosins function in oil body degradation. VanBuren (2018) analyzed the *Lindernia brevidens* and *Lindernia subracemosa* transcriptome, four caleosin genes and three caleosin genes were identified respectively, and no caleosin gene is upregulated in *Lindernia subracemosa* upon desiccation treatment. While in

Oropetium thomaeum the expression of the caleosin transcripts *OtCLO3*, *OtCLO4*, and *OtCLO6* showed a significant increase during desiccation treatment in plant leaf tissues (Figure 3.4.1), indicating that during drought stress oil bodies are activated by the degradation process, to help organelles get energy to maintain or enhance the ability to resist abiotic stress. However, at the same time, oil bodies accumulated during drought stress in leaf tissues of *Oropetium thomaeum* with similar morphological and expression patterns to desiccated seeds. These changes suggest that the generation rate of oil bodies is higher than the degradation rate in *Oropetium thomaeum*.

Quettier and Eastmond (2009) demonstrated that the transcript levels of the oil body-associated transcript SDP1 (SUGAR-DEPENDENT 1, encodes a triacylglycerol lipase) do not correlate positively with enzyme activity in *Arabidopsis thaliana*, suggesting a post-transcriptional regulation. The expression level of SDP1 in *Oropetium thomaeum* leaves also shows a low abundance during the whole process of dehydration and rehydration treatments, thus *OtSDP1* may also be regulated post transcriptionally (Figure 4.1, SDP 1 marked by the green square).

4.4 Changes of phospholipids and galactolipids during drought stress

Compared to desiccation sensitive plants, desiccation tolerant plants have the ability of maintaining a better cellular homeostasis when coping with water deprivation (Vieira da Silva *et al.*, 1974; Gigon *et al.*, 2004; Wolf *et al.*, 2012). Lipids are pivotal membrane elements, within drought stress the lipid composition changes to help maintaining membrane stabilization and integrity (Gigon *et al.*, 2004; Torres-Franklin *et al.*, 2007). Sakurai (2006) indicated monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) constitute ~80% of total thylakoid lipids. Dörmann proposed MGDG and DGDG constitute about 75% of the total membrane lipids in leaves and therefore outnumber phospholipids *(*Joyard *et al.,* 1998*;* Slabas *et al.,* 1999; Dörmann and Benning, 2002*)*. The high abundance of glycoglycerolipids in thylakoids of leaves contributes to plant photosynthesis (Douce and Joyard, 1998). In this study, MGDG and

DGDG compose 83.9% of membrane lipids in hydrated *Oropetium thomaeum* leaves and decreased to 58.5% during 14 days of dehydration.

MGDG is the most abundant thylakoid membrane lipid of green plants, the percentage content is about ~50% (Garab *et al.*, 2000; Dlouhy *et al.*, 2020; Reszczynska and Hanaka, 2020). Reduction of MGDG is a typical response to resist osmotic stress arising by salinity, drought or freezing in *Arabidopsis thaliana* (Welti *et al.*, 2002; Gigon *et al.*, 2004). Gasulla (2013) measured MGDG in *Craterostigma plantagineum,* and showed dehydration led to an almost complete loss of 96% of MGDG content. *Oropetium thomaeum* showed an extreme decrease of 92% from 62.93 to 5.03 µmol/g DW after 14 days dehydration in this study, and then with 48 hours rehydration, MGDG content recovered to 22.84 µmol/g DW, 36.3% of non-dehydrated leaf tissues (Figure 3.5.8 and 3.5.10). Desiccation tolerant plants, *Craterostigma plantagineum* and *Oropetium thomaeum* show extreme (over 92%) reduction of MDGD content when plants encounter water-deficit compared to *Arabidopsis thaliana* (23%)*,* for which the MGDG reduction is mainly attributed to 36:6 molecular species (Figure 3.5.11)

Dörmann (2002) and Demé (2014) inferred that the ratio of MGDG/DGDG is crucial for the structural stability of the thylakoid membrane in plants. Previous research concluded that dehydration responses show a decrease of galactolipids with respect to phospholipids, and the ratio of DGDG to MGDG is increased, mainly originating from the decline of MGDG, the major chloroplast lipid (Stevanovic *et al.*, 1992; Quartacci *et al.*, 1997; Gigon *et al.*, 2004; Torres-Franklin *et al.,* 2007). In this study, the mol% ratio of DGDG to MGDG increased about ten times from non-stressed to 14 days of dehydrated *Oropetium thomaeum*, from 0.539 to 5.22 (Figure 3.5.8), suggesting drought stress triggers a high-level ratio of DGDG to MGDG for adaptation in *Oropetium thomaeum* leaves.

Torres-Franklin (2007) analyzed two cultivars of cowpea, which show different resistant ability for dehydration, and hypothesized that dehydration prompts DGDG accumulation in membranes of leaves, aiming to enhance plant tolerance to arid environments. The DGDG content in the resurrection plant, *Craterostigma plantagineum,* increased during desiccation, from 21.6 to 28.4 nmol/mg DW (Gasulla *et al.*, 2013) In this study, the mol % of DGDG showed an increase when the water deficit level goes up, from 29.4% under non-stress growth conditions to 49.1% after dehydration, then dropped rapidly with subsequent rewatering in *Oropetium thomaeum* leaves (Figure 3.5.8).

Arabidopsis thaliana total lipids progressively decreased with dehydration in leaf tissues (Gigon *et al.*, 2004). The lipid classes (DGDG, MGDG, PG, PC, and PE) content in soybean leaves showed a significant reduction after 0.5-hour of salt treatment (Liu *et al.*, 2021). *Craterostigma plantagineum* keeps a constant level of polar lipids during dehydration and rehydration treatments (Gasulla *et al.*, 2013). *Oropetium thomaeum* shows a stable phospholipid content during 7-days and 14-days dehydration treatment, and 48 hours rehydration. However, the total polar lipid content is significantly reduced by dehydration in leaf tissues of *Oropetium thomaeum.* (Figure 3.5.5 and supplementary Figure 1).

The lipid distribution in *Arabidopsis thaliana* is relatively stable during the initial drought stress stage (Gigon *et al.*, 2004). Despite the accumulation of phosphatidylglycerol (PG), phosphatidic acid (PA), and phosphatidylinositol (PI) contents in response to dehydration and rehydration treatments, but the distribution of PG, PA, and PS in *Oropetium thomaeum* leaves remains constant (Figure 3.5.6 and 3.5.10). The same results have been found in *Craterostigma plantagineum* when plants encounter drought stress (Gasulla *et al.*, 2013), or when soybeans are confronted with salt stress (Liu *et al.*, 2021).

4.5 Changes of plant sterols when facing drought stress

Plant sterols are an integral part of lipids in membrane structures and they are involved in plant metabolic processes, e.g., maintaining cell integrity and fluidity, increasing plant tolerance, and assisting cell signaling (Schaller, 2004; Simons and Sampaio, 2011; Rogowska and Szakiel, 2020). Valitova (2019) observed that cold stress induced high levels of sterols in wheat seedlings. In this study here, *Arabidopsis thaliana* also shows a significant increase of sterol lipid content after dehydration. But the desiccation tolerant plants show different changes, *Oropetium thomaeum* presents a decrease of sterol content, and *Craterostigma plantagineum* keeps a stable level in the response to drought stress. *Oropetium thomaeum* and *Craterostigma plantagineum*, as desiccation tolerant plants both can survive after a severe drought stress but display different total sterol contents during dehydration. This is possibly caused by the total sterol amount in plant leaves, the sterol amount in *Oropetium thomaeum* is about 1/4 of *Craterostigma plantagineum*, and 1/2 of *Arabidopsis thaliana* (Figure 3.5.13). This result is different from previous studies which indicated that the amount of sterol is generally constant in plants (Bawankar *et al.*, 2014). This result suggests *Craterostigma plantagineum* has higher levels of sterol and a stronger ability to maintain a balance of sterol lipids than *Oropetium thomaeum* and *Arabidopsis thaliana* in response to dehydration.

The ratio of various forms of sterol determines the adaptability of plants to environmental changes (Griebel and Zeier, 2010; Ferrer *et al.*, 2017; Du *et al.*, 2022). The ratio of campesterol to sitosterol should be kept in a balance to meet the growth requirement and membrane integrity in *Arabidopsis thaliana* (Schaeffer *et al.*, 2002). In this study, *Oropetium thomaeum*, *Craterostigma plantagineum*, and *Arabidopsis thaliana* maintain a stable level of campesterol: sitosterol ratio during dehydration and rehydration of plants (Supplementary Figure 2).

Griebel and Zeier (2010) verified bacterial and fungal pathogen infection triggers the conversion of sitosterol to stigmasterol and it can influence cell membrane properties to modulate plant metabolic signaling. In this study, during dehydration, the ratio of stigmasterol to sitosterol in total sterol lipids remains stable (from 0.53 to 0.45 in *Oropetium thomaeum*, from 0.51 to 0.52 in *Craterostigma plantagineum*, and from 0.11 to 0.14 in *Arabidopsis thaliana*) (Supplementary Figure 3). The ratio of stigmasterol to sitosterol in sterol ester changes significantly when plants are confronted with drought stress. *Oropetium thomaeum* and *Craterostigma plantagineum* (desiccation tolerant plants) show a double increase, whereas *Arabidopsis thaliana* (desiccation sensitive plant) shows a significant decrease (Supplementary Figure 4). This result suggests that some plants could keep a stable level of the ratio of total stigmasterol to sitosterol in response to dehydration, but a change of the ratio of stigmasterol to sitosterol occurs in sterol esters during drought-promoted stress between desiccation tolerant plants and desiccation sensitive plants.

4.6 Pathways of lipid metabolism in *Oropetium thomaeum*

Formation of oil bodies: The leaf tissues of *Oropetium thomaeum* was observed under a confocal microscope and an electron microscope (Results part: Figure 3.2.1.4 and 3.3). Drought stress triggers the activity of oil bodies-related degrading enzymes in *Oropetium thomaeum*, OtCLO3, OtCLO4, and OtCLO6 expression levels showed a significant increase during desiccation treatment in plant leaf tissues, indicating that with drought stress oil bodies are activated by a degradation process, to help organelles to get energy to maintain or enhance the ability to resist abiotic stress. However, at the same time, oil bodies accumulated during drought stress in leaf tissues of *Oropetium thomaeum* with similar morphological and expression patterns to seed desiccation. These contrasting changes suggest that efficiency of generation of oil bodies is higher than the degradation rate in *Oropetium thomaeum.*

Oil bodies–associated protein (SDP1) is involved in initiating release of fatty acids from oil bodies in germinating seedlings of Arabidopsis *thaliana* (Athenstaedt and Daum, 2005; Zimmermann *et al.*, 2004; Gronke *et al.*, 2005). SDP1 participates in oil body degradation, but the transcript level showed a low abundance in leaf tissues of *Oropetium thomaeum* during dehydration and rehydration, which is consistent with reports for peanut, soybean, and cucumber (Huang, 1983). Quettier and Eastmond (2009) indicated that the transcript levels of SDP1 do not correlate positively with enzyme activity in *Arabidopsis thaliana*, suggesting a post-transcriptional regulation, this performance in *Arabidopsis thaliana* gives a clue that *OtSDP1* may also function during the post-transcription.

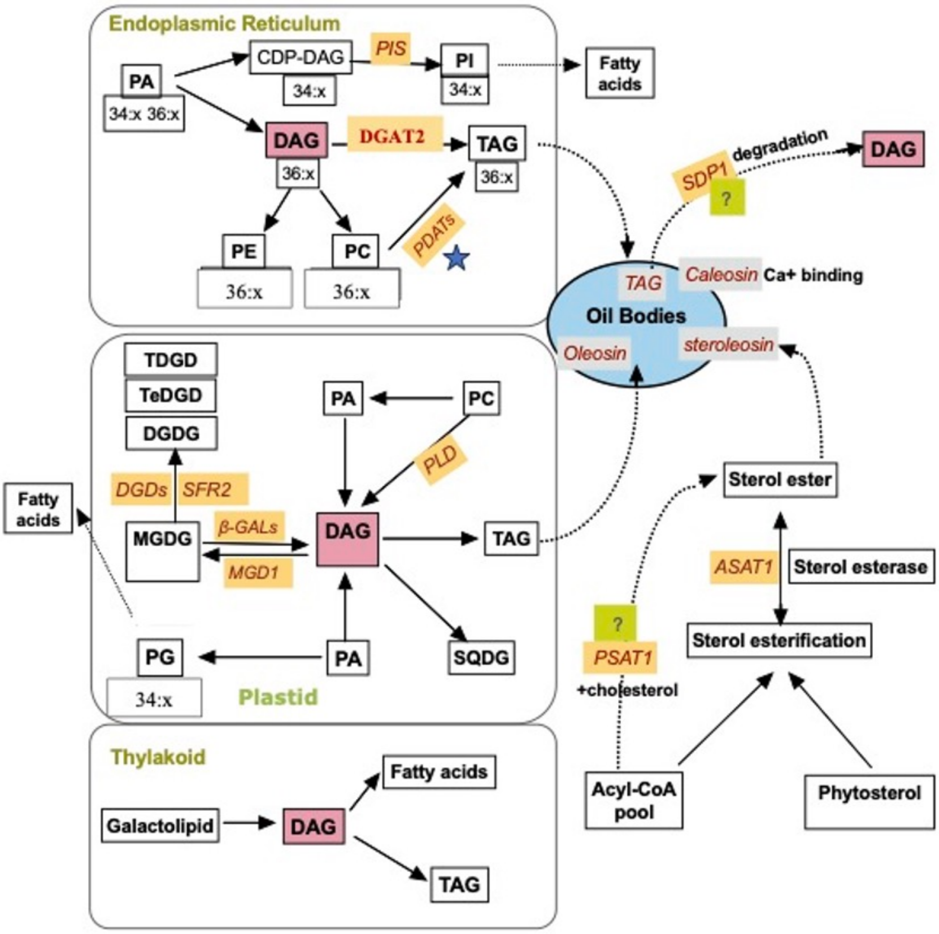


Figure 4.1 The proposed phospholipid pathway, galactolipid pathway, and phytosterol pathway in *Oropetium thomaeum* during drought stress. TAG: Triacylglycerol; DAG: Diacylglycerol; CDP-DAG：Cytidine diphosphate diacylglycerol; DGATs: Diacylglycerol acyltransferases; PA: Phosphatidic acid; PI: phosphatidylinositol; PE phosphatidylethanolamine; PIS: phosphatidylinositol synthases; PDATs: phospholipid: diacylglycerol acyltransferases; MGD1: monogalactosyldiacylglycerol synthase1; DGDs: digalactosyldiacylglycerol synthases; SFR2: sensitive to freezing 2; β-GALs: β-galactosidases; MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol; PG: PLD: phospholipase D; TGDG: trigalactosyldiacylglycerol; SDP1: sugar-dependent1; PSAT1: phospholipid: sterol O-acyltransferase 1; ASAT1: acyl-coA: sterol-O-acyltransferase 1.

TAG synthesis: Acyl-CoA: diacylglycerol acyltransferases (DGATs) play a key role in TAG assembly by transesterification in plants. Most plants have two or more unrelated *DGATs* genes, like *DGAT1* and *DGAT2*, but principally choose one gene to activate formation of oil bodies, however, the reason is still unclear. (Regmi, 2018). A study in *Arabidopsis thaliana* seeds shows that DGAT1 is the main enzyme for TAG synthesis. This study revealed three genes for *Oropetium thomaeum* which has DGAT1, DGAT2 and DGAT3, but the DGAT1 activity was almost undetectable, even during the dehydration and rehydration process. The expression abundance of DGAT2 significantly increased during dehydration stress and recovered to a level of untreated plants after 48 hours of rehydration. DGAT3 kept a stable level under dehydration treatment (Figure 3.5.2). This result indicates that *DGAT2* plays a key role in TAG synthesis in leaf tissues of *Oropetium thomaeum* (Figure 4.1). Comparison of the expression level of *DGAT2* in other resurrection plants like *Craterostigma plantagineum* and *Lindernia brevidens*, show a significant increase under drought stress (Gasulla *et al.*, 2013； Supplementary Figure 6). These results suggest that DGAT2 is likely to have a predominant role in TAG synthesis in desiccation tolerant plants.

Phospholipids: diacylglycerol acyltransferase 1 (PDAT1): “Acyl-CoA-independent” pathway for TAG synthesis is directly producing TAG from PC by PDAT (Figure 4.1 marked by blue star). DGAT1-PDAT1 have overlapping functions during vigorous growth in *Arabidopsis thaliana* (Zhang *et al.*, 2009). In this study, DGAT2 is the key enzyme for TAG synthesis during drought stress in *Oropetium thomaeum*, at the same time, the expression levels of PDAT1 and PDAT2 are up-regulated during dehydration and down-regulated during re-watering, whereas the expression levels in well-watered and rehydrated plants are almost the same. The level of OtPDAT2 transcripts was hardly detectable in well-watered *Oropetium thomaeum* leaves. This observation suggests that drought stress significantly induced OtPDAT1 and OtPDAT2 expression, especially OtPDAT2.

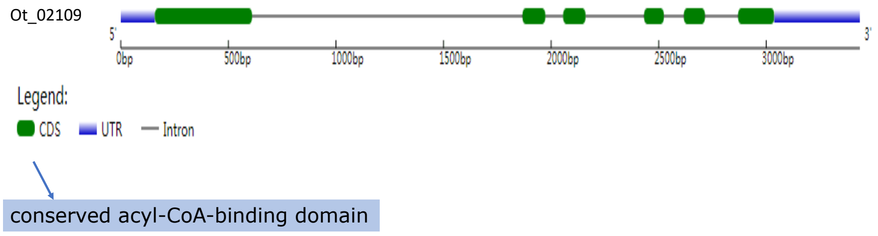
SE (Sterol ester) synthesis: PSAT1 and ASAT1: Sterol esters have a primary role in membrane sterol homeostasis and represent a storage pool of sterols to maintain the stable function of cell membranes (Schaller, 2004; Bouvier-Navé *et al.*, 2010). Sterol acyltransferases were identified as acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) in plants. (Ferrer *et al.*, 2017; Korber *et al.*, 2017; A. Lara *et al.*, 2018). Research shows that high levels of sterol negatively affect plant cells during growth and development (Shimada *et al.*, 2019 and 2020). Sterol acyltransferases were identified as acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) in plants. (Ferrer *et al*., 2017; Korber *et al.*, 2017; A. Lara *et al.*, 2018). PSAT1 has a key role in maintaining free sterol homeostasis in plant cell membranes (Bouvier-Navé *et al.*, 2010). Lara (2018) reported that that PSAT1 is not the key enzyme to limit sterol metabolic rates in tomato (*Solanum lycopersicum*), and SlPSAT1 and SlASAT1 show overlapping but largely complementary models of expression during tomato fruit growth. Banas (2005) concluded that PSAT shows the same physiological function in plants as ASAT in animal cells. This statement is consistent with this study. PSAT1 and ASAT also increased or decreased simultaneously in *Arabidopsis thaliana* and *Oropetium thomaeum,* under drought-induced conditions. These results could be a proof that PSAT1 and ASAT1 are likely to overlap in their functions in *Arabidopsis thaliana* and *Oropetium thomaeum* leaf tissues.

Future perspectives

1. Acyl-CoA: sterol acyltransferases (ASAT) and phospholipid: sterol acyltransferases (PSAT) are key enzymes to maintain sterol ester homeostasis in some plant cells. SI-PSAT1 (Tomato) and At-PSAT1 (*Arabidopsis thaliana*) sequences were found sharing 75% sequence identity and the presence of an N-terminal signal peptide with fully consistent segments. Sl-ASAT1 is a true ortholog of At-ASAT1, and ASAT1 seems to act a general feature of sterol ester metabolism in plants (Chen *et al.,* 2007; Lara *et al*., 2018). Cloning and functional characterization of the sterol acyltransferases PSAT1 and ASAT1 in *Oropetium thomaeum* will be a first step to learn more about the relevance of sterol esters which are significantly reduced by dehydration, while *Arabidopsis thaliana* shows rapid accumulation of sterol esters.

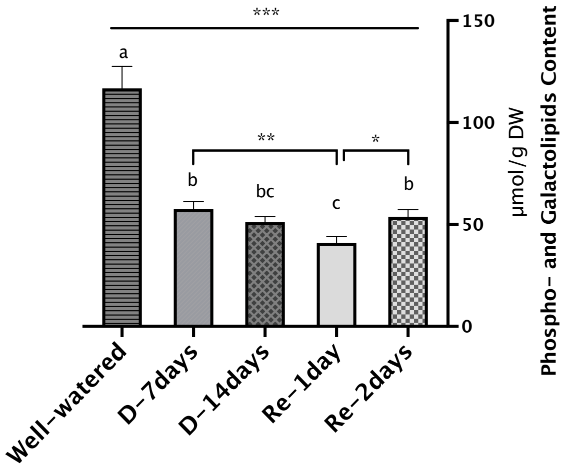
2. Acyl-CoA-binding protein2 (ACBP2) plays a role in drought tolerance (Du *et al.*, 2010, 2013; Xue *et al.*, 2014), ACBP2 overexpression in transgenic *Arabidopsis thaliana*

seedlings enhance plant drought tolerance (Du *et al*., 2013). *Oropetium thomaeum* sequence 02109, here named Ot-ACBP2, shares six segments of conserved acyl-CoA-binding domains with *Arabidopsis thaliana* ACBP2 sequence (This gene structure is shown below). Cloning the ACBP2 promoter and analyzing ACBP2 protein expression will help to understand the role of ACBP2 in drought tolerance in *Oropetium thomaeum*.

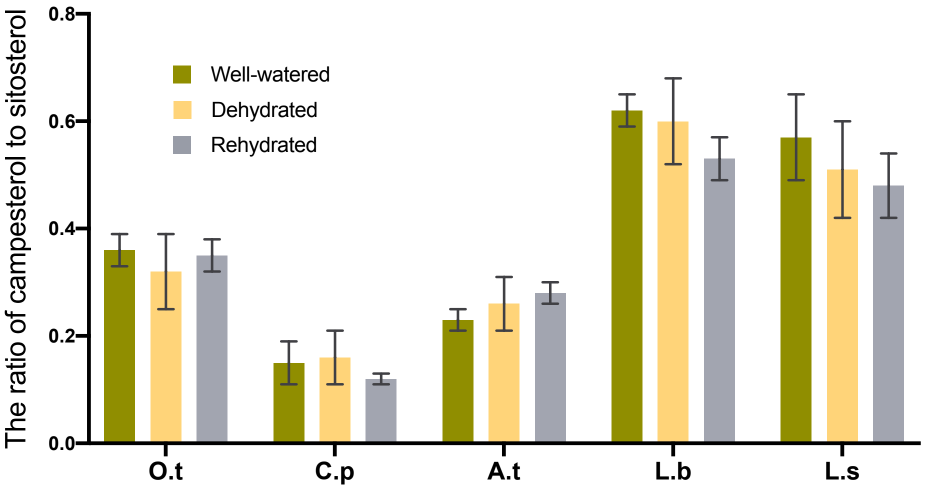


3. Oil bodies are widely recognized as cytoplasmic organelles, and recently they have also been found inside the nucleus (Layerenza *et al*., 2013; Uzbekov and Roingeard, 2013; Ohsaki *et al.,* 2017). Biogenesis and function of oil bodies are largely evolutionary conserved in plants, yeasts, and mammals (Kent *et al.,* 2012). However, there is currently no study on the subcellular localization of oil bodies in *Oropetium thomaeum*. Therefore, it will be of interest to study subcellular localization of oil bodies and intracellular trafficking in *Oropetium thomaeum* leaves to get a further understanding of the basic mechanisms by which oil bodies homeostasis play a role in response to environmental stress.

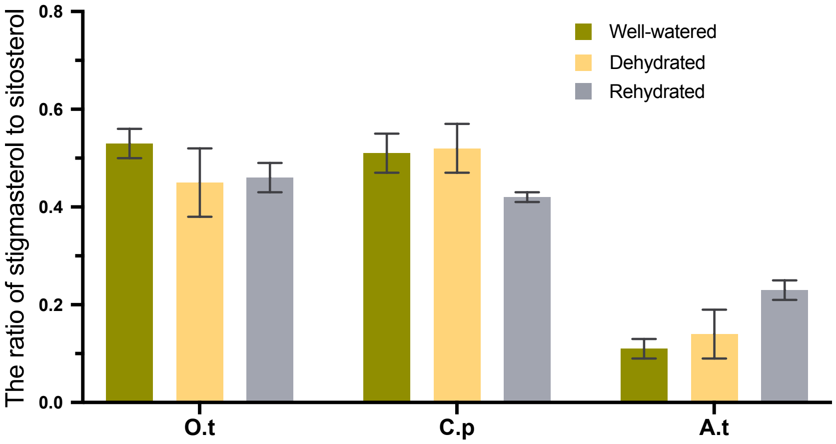
5. Supplementary data



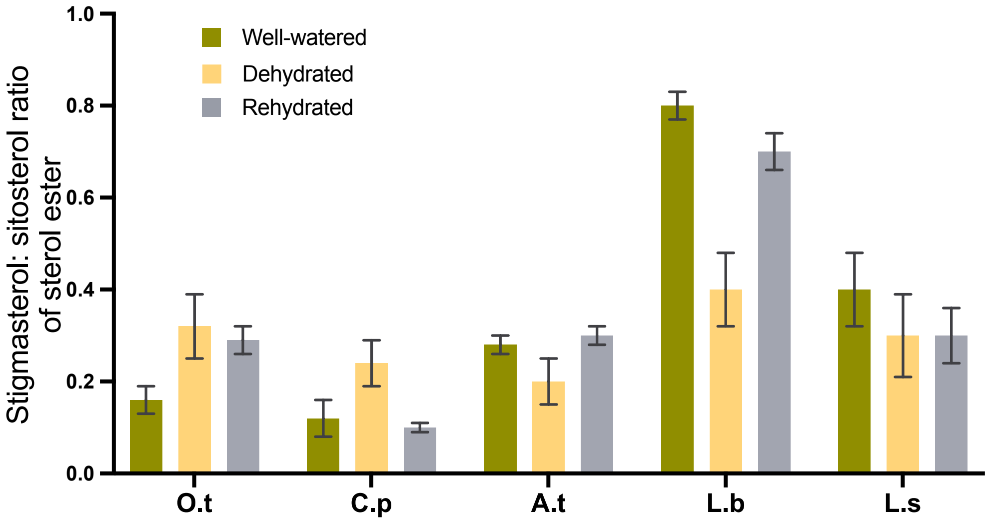
Supplementary Figure 1: Phospho-and galactolipids content in *Oropetium thomaeum* leaves of well-watered, 7 days dehydrated, 14 days dehydrated, 1 day rehydrated and 2 days rehydrated. Phospholipid content was measured by Q-TOF MS/MS. DW, dry weight. Data are present as mean and standard deviation from six biological replicates. Asterisks symbols indicate values that are significantly different from the control, according to Tukey's multiple comparisons test, *P*<0.05 (*), *P*<0.025 (**); *P*<0.01 (***).



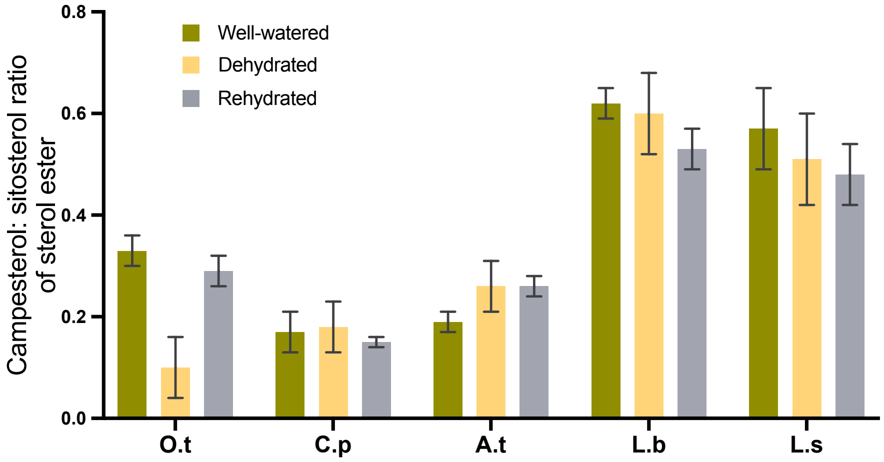
Supplementary Figure 2: The ratio of campesterol to sitosterol in plants leaves of well-watered, dehydrated, and rehydrated. Desiccation tolerance plants: O.t (*Oropetium thomaeum)*, C.p (*Craterostigma plantagineum*), and L.b (*Lindernia brevidens*) were treated with 14 days dehydration, and desiccation sensitive plants: A.t (*Arabidopsis thaliana*) and L.s (*Lindernia subracemosa*) were treated with 10 days dehydration. Rehydrated plants were treatment with 2 days rehydration. Campesterol and sitosterol contents were measured by Q-TOF MS/MS. Data are present as mean and standard deviation from six biological replicates.



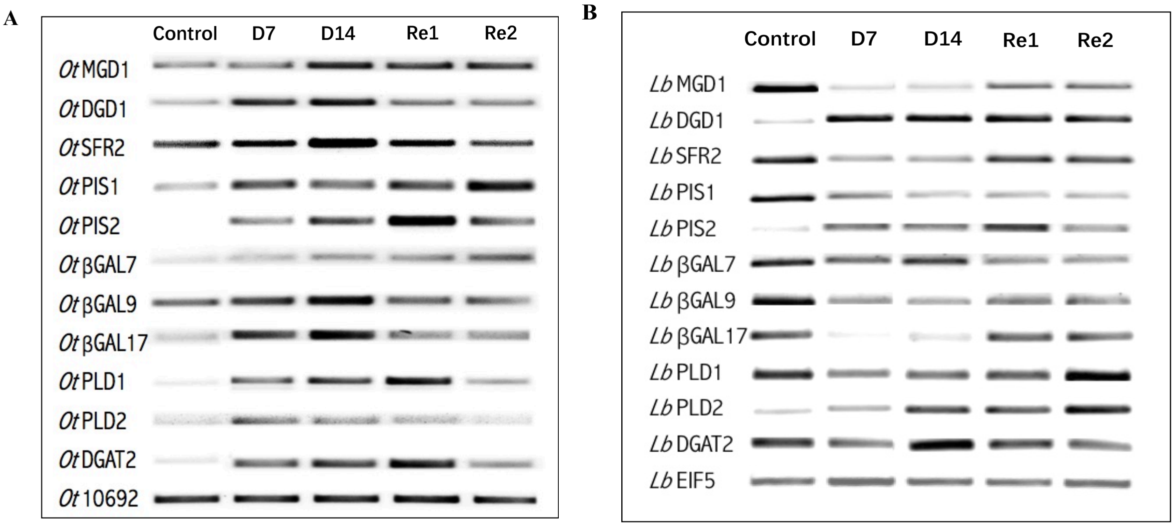
Supplementary Figure 3: The ratio of stigmasterol to sitosterol in plants leaves of well-watered, dehydrated, and rehydrated. Desiccation tolerance plants: O.t (*Oropetium thomaeum)* and C.p (*Craterostigma plantagineum*) were treated with 14 days dehydration, and desiccation sensitive plants: A.t (*Arabidopsis thaliana*) were treated with 10 days dehydration. Rehydrated plants were treatment with 2 days rehydration. stigmasterol and sitosterol contents were measured by Q-TOF MS/MS. Data are present as mean and standard deviation from six biological replicates.



Supplementary Figure 4: Stigmasterol to sitosterol ratio in sterol ester of well-watered, dehydrated, and rehydrated. Desiccation tolerance plants leaves: O.t (*Oropetium thomaeum)*, C.p (*Craterostigma plantagineum*), and L.b (*Lindernia brevidens*) were treated with 14 days dehydration, and desiccation sensitive plants: A.t (*Arabidopsis thaliana*) and L.s (*Lindernia subracemosa*) were treated with 10 days dehydration. Rehydrated plants were treatment with 2 days rehydration. Stigmasterol and sitosterol contents were measured by Q-TOF MS/MS. Data are present as mean and standard deviation from six biological replicates.



Supplementary Figure 5: Campesterol to sitosterol ratio in sterol ester of well-watered, dehydrated and rehydrated. Desiccation tolerance plants leaves: O.t (*Oropetium thomaeum)*, C.p (*Craterostigma plantagineum*), and L.b (*Lindernia brevidens*) were treated with 14 days dehydration, and desiccation sensitive plants: A.t (*Arabidopsis thaliana*) and L.s (*Lindernia subracemosa*) were treated with 10 days dehydration. Rehydrated plants were treatment with 2 days rehydration. Campesterol and sitosterol contents were measured by Q-TOF MS/MS. Data are present as mean and standard deviation from six biological replicates.



Supplementary Figure 6: Transcripts expression profiles of genes involved in lipid biosynthesis. A: Ot (*Oropetium thomaeum)*; B: Lb (*Lindernia brevidens*). Control; D7: with 14 days dehydration; D14: with 14 days dehydration, Re1: with 1-day rehydration; Re2: with 2 days rehydration. Total RNA was isolated from samples of three biological replicates. MGD1: monogalactosyldiacylglycerol synthase1; DGD1: digalactosyldiacylglycerol synthase1: SFR2: sensitive to freezing 2; PIS1: PI synthase1; PIS2:PI synthase 2;βGAL7,βGAL9,βGAL17: β-galactosidase 7, 9 and 17; DGAT2: diacylglycerol O-acyltransferase; PLD1: phospholipase D1; PLD2: phospholipase D 2; Ot-10692 and Lb EIF5 as reference of RT-PCR.

Gene sequences

>Oropetium_02109: ACBP2

ACAATAACTGAAACACTACTAGAAGATAATCCGGATTTCCCTGATTTCCAGTCTTATACTAAGAACCCCAATCCAGTTTTTTGTAAAAAAAGAAA
AGTTTATTTCCCTAAGATTATTTATTTGCATACTCAGAGTTTATTTTAGTGGTTGCTTCTCATTGAACAGTATAATCCATTTATCCGAAAAAATGCTT
CAGTTTGTTACATGCAGATATTAGTATGCAAGTCAGATAACCATTTTGTGTTGGATGATTGCAATTGTTTTTGTTTTGCACGGGCAATTAACGAATG
AAGCACTGATCAAAGATGGAGTGGTTGGCTGTAATTTCGCTGCCTGATAACTTGTTTCACCAGATCGATTCAAATCCATTACTGATCAGCCACGAC
CGCTTCACAGAACCGATGAAGAACTGGTAGCAAAAAAGCAAGGGTAGCATTATGTGGAAGGGAGCGCAGCCCATTACATTTGACGAAATCATAC
ACTATACTAGTTGACAATAAAAGGAACAACACGCACAACTACAACTACTAGGCACAAACCCAAAGTATCGGCCAGAATACGCAAAAAACAAGCA
TTGAAGACACAAACGTTAGAGATGAGTAAGAGGAAACAAATTCTTGCTACAACTTTATCACATGCTATCAAAAACGTTACTTATAAAACTGATAT
ATTGTTATTGTATATTATAGCTTCAGTATTCGTAGGGTGCTTTATTGTACCATTTTAGTTATACTATTTAGGTATTGTAGCAAATAATCATTTGAGTT
GATTTTACATCCTTTTTTAGTCAGTTATTATAAATAAACTCGAGCGAAACTCATTACAACAATTCGATCGAACTACAAAGTGTGTGTTGTTGCCTCG
CTAACAGAGGTTGTAAATCATTACCTCTTAATTTGCTATTGATATTGAAAAAAATTGTAATCTAGATTATTTGTATCCATTGTATTCGACTACTTAA
AACTATTTTATGGTTTTAATTTTTTATTCTTGTTATTTTTATTTACACTCTATTTTTTTTGTTTATGTTTTCAGAAAATAAATTTTGATAATTTTCTACT
ATAAAAATTAGTGATGTTTTTGATAAATATATATGAAGTAGTAATAAATTTGAAAATTTTACAATACAAAAAATAAAAGTATGAATCCTCTATTGT
AAATAATAATAATTTCTCTCAAAAATTAATGGAAGGGAGCATAGAGTATACTCTTTTTCAAACTATATGAAAGTGGATAGGCATGGAGACTAGTT
GAGGCCAACTTTTACTTTTATTTAATTATTGTATCATTGATTTTTAGCTTTAATTTTGACCATTGCTCCTCCTCCACGTGGTGCAAAACTTATTTAAA
GATATGTTCATAATAAAATTATTTACTAGTAAACTAAGTTAAATATAAAATCTAATCAGCATAGGATTTTTAGAGAGGGAGTTGAATTTTGTTGAG
GATGGACACACAATTTAAGAGAAAACTGAAAGGTATTAAAAAGTAGCACGTGAAATGAAAAGATACTTTTGTTTTGTTCTACACAACACCATAGC
AAAAGGGCCACAAAAGGCTTTCCTATGTTATTAGTTTCCTTATGTAGCATGAAGCTCTTTGCTATGCAAATGTAGCAGGCGTGCAAGGATGGAGCC
ACCACAATGTTTCTGCTTCAATTAGTTATTGCAGGCCGATAAAAAAAAACAGTTATTGTAGGAAGAAGACTCAGATGCTTACATTAAATTAATAAT
TGTTTCATGAAGCTTCTCAAAAAAATATGTTAAGAATTGTTTAATAAAAAAGATATTAAACATTACTCCCTTCATTTTGTTTTGGTTATCGTTATAT
CGTTAGGACAACTAAAATATACTTGTGTCTAGTGTAATTTTCTGTCAGAAGGGACACACAAAGATGAAAGGAGGAGTATGTAGCATGAAGCTTCT
ATTCTGTGCCAAAATCGTGTGTGATCCTATTCTGTGCGTCATGGTTCAGGTCTTCACTCTTCAGGCGCCCTCTCCACATTGTCATTTTATCCTTTGCC
CCTTACGTTTTGGTCTTTTTCACTTTCTAACCCCCATCCCCCTTGCCCAGTTTCCCAGTCTTCCCACCTGACCCAACTCAGCAGCTCTCACCGCCGCC
GTCCCCATCTAGGTCACCACCTCGGCGGCGTCGACC

>Ot-10692-reference gene (|length=1563|F_exp=261.21|D_exp=250.24|R_exp=268.64)

TCCGCATCCCAGCCCACGGTACACCGCCCTCAGCCAACACTATCCCCGAGGTCGCCTGCCCCTGCCCGTCACCGCCGCGAGGGGGTCAGCGCTAT
AAGTATCCGACCTCGCCGGAACCCTACCCTTCGAGAACCCCTTCTCCGCCGCTACCCAAGCTCTCGCCCTTGTACCCCACGCCGTCTGCTGCTGCT
AGAGAGCTTCTATTCCGGCGACATCAAGAGAGCTAGCAGCAGTAGCGGCCCCTATTGTCCCACGCCCGTCCGCCGATGCCGCACCTCGAGGGAGC
CGACCGGATCGCCGCCGGGTGGGCGGCGACGGACGCTGAGCGCCCCGGGGAGCTTCGTAGAAGCGGCTCCTCCAGCCGCCTCAACGCGCAGGCG
CCGGAGTTCGTGCCGCGCGGGCCGCCGGCGGCAGTGGTGCCACCGCCGCCCGTGATCCGCGTGTTCACCGCGCCTCCACCGCCGCCGCGCGCGTC
TTTCTTTGCGGCGCCTCCGCCCCCGCCACCCCAGTTCGAGTACTATGCGCCGGTGGGGGGACGCGGTGGATTTGCCGCCAAGGAGCCGAAGCTGG
AGCCACAGCCGGAGCCTGAGGTGGAGAACCCGGTGGCGCTGAACACGGAGCCGCTGGTTGAAGGGCTAGCTGACGAGGTCGTGCACAAGATCAC
CAAGCAGGTGGAGTACTATTTTAGTGATATAAACCTGGCTACCACTGAACATTTGATGAGATTCATTAGCAAGGACCCTGAGGGATATGGTTGTA
AGTGAGGATGGGAAAAGAGTGAAGCGTCAGGAGCCATTTACTGAATCTGATTTGCAGGAGCTCCAGTCCCGAATTATTGTTGCAGAAAATCTTCC
TGGTGACCCTTCCTACCAGATTCTTAAGAAGATATTTTCAGCTGTCGGGAGTGTGATTTCTATCCGTACTTGCTATCCTCAGACTCCAAATGGCACT
GGTCCTGCTACTAACAGATCTGCAAAGCTTGATATGCTTTTTGCTAACAAGCTTCATGCTTTTGTGGAATATGAGACTCCTGAGGATGCTGAGAAG
GCAATTTTGGAGCTCAATGATGAGAAGAACTGGAGGAATGGGCTCCGAGTCCGGCTGTTGAATACTTGCACGACAAAGGGGGAGGGCAAAGGCA
AAAAGGGAGTGCCTGAACCTGATGGAAATGGCGAGGAAGATGTCTCCACATCGAACCAATCAACTGAGAAACAGCTTGAAGAATCCTCTGAACT
CTTAGATGTATTGCCAGAACACTTGGTTCCAAGACGTGCCAAAGGACGTGGACGTGGCAGAGGGCGAGGGCGTGGGCGTGGTTACCAGCAGTAC
AACAACAATCAGCACCACCAGAACAATCAGCACTACAATCACCACGGCAACAACCATCATGTTGGGACTCCACCAAATAACCAGCCAATCAAAC
CTGAACAGCACCAGCAGCTGCCAATTGGGGCCACCAAGCAACCTCCAGGCCCGCGCATGCCTGATGGCACTCGAGGTTTCACCATGGGCAGAGGC
AAGCCGCTAACCATTTTTCCTGGTTTGTGCGCTGTTGCTGAGTAA

>Ot-CLO3 (Oropetium_20150105_12086|length=624|F_exp=10.88|D_exp=967.02|R_exp=340.45

ATGTCGCCCGTGGAGGCCAAGGACTCGCAGACCATCGTCGCGCTGCAGTCGCCGGTGACCGTGATGCGCCCCGTCCGCGGCGACCTCGAGGAGCA
CGTGCCAAAGCCATATCTGGCGAGGGCTCTGGCGGCGCCGGACATCTACCACCCGGACGGCACGACGGACGAGTACCACAGGCACAACAACATG

AGCGTCTTGCAGCAGCACGTCGCCTTCTTTGACCGCGACGACAACGGCATCATCTACCCGTGGGAGACCTACGCCGGATCGCGCGCGCTTGGATT
CAACGTGATCATGTCTTTCATGATCGCGTTCATCGTGAACGTGACCATGAGCTACCCGACCCTGCCTGGCTGGCTGCCGAATCCTCTGTTCCCGAT
CTACGTGCACAACATCCACAAGAGCAAGCACGGGAGTGACTCGGGGACCTACGACAACGAGGGCAGGTTTATGCCGGTGAATTTCGAGAATATG
TTCAGCAAGTACGCCCGCACGTCCCCCGACCGGCTCACCTACAAGGAGCTCTGGTCCATGACCGAGGGATTCCGTGAAGCCCTCGATTTCTACGG
CTGCCTCTTCGAGTATGTCGTCAGGCAGCGCGAGCAGCACGCCAAGATGTCATAG

>Ot-CLO4 (Oropetium_20150105_00540|length=1062|F_exp=5.71|D_exp=318.65|R_exp=502.50

CTGCGAGCAATAGGGTGCAGTTTCCCAGTATCCTTGCTTTTCGCTTTCTTCATCAACCTCGCCCTCAGTTACCCCACTCAGCCGGGTTGGTTACCTT
CTCCTTTGCTCTCCATCCACATAAAAAACATCCACAAGACTAAGCACGGGAGTGATTCTGAAACCTACGACACTGAAGGGAGGTTTGATCCATCG
AAATTTGATGCTATATTTAGCAAGTATGGCCGAACCATTCCCAACGCCTTGACGGAAGATGAGCTGAATTTGATGCTTAAAGGAAACCGCAATTT
ATATGATTTCTTTGGCTGGATCGCCGCCGTTGGCGAATGGAAGCTACTCTATAGTGTGGCAAAGGATAAGGATGGCCTATTGCAACGAGAAACTG
CGCAGGAGACGGTGGCACCCCACGCGCCGGTCACGAGGCAGCGGAGGCTCAACCCCGAATTGGAGGAGCACCTTCCTAAGCCATATCTCGCGAG
GGCTCTTGAGGCGGTGGACCCGAGCCACCCGCAGGGGACCAAGGACCGTGACCCGCGCGGGATGACAGTGCTTCAGCAGCACGTAGCCTTCTTCG
ACCGCAATGGCGACGGCATTATCTACCCATGGGAGACGTTTGAAGGCTTGCGAGCAATAGGGTGCGGGATCCCTGTATCCTTCATGGGCTCATTC
GCGATCCACGTCGTCCTCAGTTATCCCACTCAGCCGGGGTGGTTGCCTTCCCCTCTGCTTTCCATCCATATAAAGAACATCCACATGGGTAAGCAC
GGCAGTGATTCTGAATCCTATGATACTGAAGGAAGGTTTGATCCAGCGAAATTCGATGCTATATTTACCAAATATGGTCTAACTCATAAAAACGCT
TTGACGAGAGAGGAGGTGTCGGCGATGCTTAAAGGAAATCGCAACATGCATGATTTCATGGGCTGGGCTTTTGCCGCTGGTGAATGGCTGTTGCT
CTACAACGTGGCGAAGGATAAAGATGGCCTGTTGCAAAGAGAAACTGTCAGGGGTTGTTTCGATGGCAGTCTATTTGAGCGACTGGAAGAAAAA
AGGAAATCCTCGTAA

>Ot-CLO6 (Oropetium_20150105_04115|length=1488|F_exp=21.46|D_exp=24.25|R_exp=20.47

ATGACGGAGCTGCAGAAGCACGTGGCGTTCTTCGACCGCGACCACGACGGCATCATCACCTTCGACGAGACGTACCAAGGTGAGAGAACCAACA
GGCGGCTCCGGCTCGGCTCTGCCGCGACGACCGCCAAAGCGAGCGCAGCACTTATCAACGCCGGCCTTGGACCCAAGACCAGACCGGATAATGC
AAACTCATTGTCATCGAGTATGGATATCTACATAGTGAACATCCACAAAGGGATTCACGGGAGCGATACAGGCGCATACGACGCTCAAGGAAGTT
TCGTTCCTGCAAAGCTGGATGAAATGTTCGCCAAGCACGGGAAAACCGTACCTGATGCCATGACCAAAGACGAGGTCGACGAGATGCTCAAGGC
TAACCGAGACAACATGGACGTCAGAGGATGGCTTGCAGCTTCGTCGGAGTGGAACTTGCTGTACAAGCTCGCCAAGGACAAAGATGGGTATCTGC
GCAAGGGTCGCGCGGCGGCGTACGGCGGATACGGGGAGGCCGCCGGCATGACGGACCTGCAGAAGCACGCGGCGTTCTTCGACAGCAACGGCGA
CGGCGTCGTCTCCATATCAGAGACGTACGATGCGTTCCGGGCTCTCGGATTCGGAGTTGGCATGTCCAGCGTCAGTGCCGCTTTCATCAATGGCGC
CCTTGCTAGCAAGACCAGACCTGAAAATGCAACGTCATCGCACCTGGACATCTACATAGAGAACATCTATAAAGGGATCCATGGAAGTGATTCAG
GCGCCTATGATGCTGAAGGAAGGTTCGTTCAGGAAAAATTCGATGCAATATTTGCCAAGCACGCCAAAACTGTCCCAGACGCTCTGACATCAGAT
GAGATCGACGAGTTGCTCCAAGCAGACAGACAGCCTGGTGACTACCAAGGATGGGTTGGAGCAACATCGGAGTGGAGGATACTGTACAGCCTCG
GCAAGGACAAGGACGGCCTTCTTCGCAAGGACGTCGCGAGAGGCGTCTACGACGGGAGCCTGTTCCACGAATTGGTGCAGGTGAGGGGATCCGG
TGATAAGTGAAAATTGACGTACTACTAGCGATTTCGATTGGGATAAGGATTCGACGCTGAAGTCTGAACAATGCAGAGGACTGGAATTGGACGCT
GAAGTCTGAATCTGAACAATGAAAATGTGGCGTCAAAGCAACAAGTGGCGGTGTGAACGGGTGCGAACGGAATCTGCAGATAGGACGATTGCTT
TAATGTGTCTTCTTGTCTCCATTCGTGGAGTTCGATGGTTCTGTACAGTTGCCCATGGATTATAGGATACTACTGTATTCGAGTGACTCTCGTGAAT
CTGTCACGTACCTTTGTCATCTTGTTGTCCTTGTCTATATGCTAATCTTCCATCGTTGCAGATTTCGTGGTTCTCTTCATCCAGTATGCTTCTGATGTT
AACTGATCCACGGAGCCGAACCAAATACCCTTCTCAATTTGTTTCGTTTGTTTGTATTAGCTG

>Ot-SDP1（Oropetium_20150105_17063A transcript offset:0 AED:0.02 eAED:0.02 QI:0|0|0|1|1|1|4|0|826

ATGGAAGATTCAGGGGAAGCAATCGTCGGGGCCTTCAGGATCGGGCCGTCGACGCTGCTCGGCCGCGGGGTCGCTATCCGCGTGCTCCTGTTCAA
CTACCTATGGCGTCTGCGGGCGCGCGCGTACGCCGCCATCTCGCGCGTGCGAAGCACGACGCTGCCAGCGGTGTCTTCCTGGCTGCACCTCAGGA
ACACCCATGGCGTCCTCCTGGTGGTCTTCCTCTTTGTGCTCTCGTTGAGGAAACTCTCGGGTGCGCGGTCACTGGCGGCGGTGGCGCGCCGGCGTA
GGCTGTGCCAGAAGATTATGCGGCATGCGGCCACCTACGAGGACTGGGCGCGCGCTGCCAAGGTGCTCGACAGATTGTCCGAGCACATAAATGA
GGCCGATCTCTATGACGAGGAGCTGATCAGGAATAGGCTGGAGGAGCTCCGAAGGCGGAGGGAGGAAGGGTCGCTCCGGGATGTGGTGTTCTGT
ATGCGCGGCGATCTCGTCAGGAACTTGGGTAACATGTGTAATCCCGAGCTTCATAAGGGCAGGCTGGAGGTTCCCAAGCTTATACAAGATTACAT
TAATGAGGTTTCTGCTCAACTGAAAATGGTGTGTGAATCTGATTCCGAGGAGGTACTTTTGGAAGAGAAGCTTGCCTTTGTTCAGGAAACCAGGC
ATGCCTTTGGGAGGACAGCGCTACTCTTAAGTGGAGGTGCTTCACTAGGGTCATTCCATGTAGGTGTAGTAAAAACATTGGTTGAGCATAAGCTTC
TGCCTAGAATAATAGCTGGGTCTAGCGTTGGTTCCATCATATGCTCAATTGTTGCTACCCGAACATGGCCTGAGATTGAGAGCTTCTTTATGGACT
CATTACAGACCTTGCAGTTCTTTGATAGGATAGGTGGGATTTTTGCAGTTATGAGGCGGGTCACGACTTATGGTGCACTACATGATATTAGCCAGA

TGCAAAGGCTTTTGAGGGATCTCACAAGTAACCTAACATTTCAAGAGGCTTACGACATCACTGGCCGTGTTCTTGGAATCACTGTTTGCTCACCTA

GGAAAAATGAGCCCCCACGCTGTCTCAACTATCTGACATCACCGCATGTTGTTATTTGGAGTGCTGTAACTGCCTCTTGTGCCTTCCCTGGGCTCTT

TGAAGCTCAGGAATTGATGGCAAAGGATAGATTCGGCAACATTGTTCCCTTCCATGCACCCTTTTCCACAGACCCTGAAGAAGGTCCTCGAGCATC

AAAGCGCCGTTGGAGAGATGGGAGTTTGGAGATGGATTTGCCCATGATGCAAATCAAGGAGTTGTTCAATGTGAATCATTTTATTGTAAGCCAAA

CTAATCCTCACATTTCTCCACTCCTTCGGATGAAAGAGCTTGTTCGAACGTATGGAGGCCGTTTTGCTGGAAAGCTTGCTCGTCTTGCTGAGATGG

AGGTCAAGTATCGATGTCACCAAATTCTAGAGATTGGGCTTCCACTGGGAGGACTTGCAAAGTTGTTTGCTCAGGACTGGGAAGGTGACGTCACA

ATGGTTATGCCAGCAACAGTAGCTCAGTACTTGAAGATTATACAGAATCCGACGTATGCGGAACTCCAGATGGCTGCCAACCAAGGTCGCAGGTG

CACATGGGAGAAGCTCTCTGCCATTAGAGCCAACTGTGCTATTGAACTTGCATTGGATGAATCCATTGCAGTTTTAAATCACAAGCGGAGGCTAA

AAAGAAGCATTGAAAGGACAGAAGCAGCTTCCCGGGGACATGCTAACTATGTTAGAACCAGAACACCAAGGAGGGTACCATCATGGAGCTGTAT

CAGTCGAGAGAACTCTTCAGAATCTCTCTTGGAAGATATTGTTGCAGCTGCTACTTCATCCACGCACCAAGGTGCTGTCCTTGTCGCACCAAATAT

TTCTCACCATAATCGGCACAATTCCTATGATGGAAGTGAGAGTGAATCAGAGACCATTGACCTCAATTGTTGGACAAGGAGTGGTGGGCCTCTAA

TGAGGACTTCATCAGCTGACAAGTTCATCAATTTCATTCATAATCTTGAGCTTGACACTGAGTTCAGTAGGAGCTATACAGTAGAGGATGATACTA

TAGGCATTTTATCAGAATCAGCCTTACCTAACAATCCACACCTTACCAATAGTTCTGGGACCGAGTCCTGCAGCACGGGGAACAATAGAGCCAGT

CAATCTTCTACTCCGACAAGCATTGCTGTTTCTGAAGGAGATTTTCTGCAACCTGAGAGGACTACTGACGGTATTTTACTGAACATTGTGAAAAGA

GATGATATGCTTTTTCACAACGGTCCGACTGAACTGGCTCAAAGCTCCTCTGCAGAATTGTGTGTGGAAACTTGCGACGCCACCTCAGGATCAGAT

TGTGCTGAAGATAACAATGATGTAGCTGGCTCGAGCAACCCCTCAGCAGTTGATCCTGCTGATTTTATAACTTCACAGCCATCATCAGTAGATTAG

>Ot-DGAT1(Oropetium_20150105_17615|length=648|F_exp=0.89|D_exp=2.09|R_exp=0.00)

ATGGCCCCACCCCAGTCCATGGCCTCAGCGGCCTCCGATCGGGCCGACGGGGGCGAACCCGGCTCGGCCCTCCGCCTCCGCCGCGCACCGTCCGC

CGACGCCGGCGAACAGTCCGCAGGCCTGCAGGGGAACGTTGAGCCGCACCCACCGCAGCACCAGGAGCAGCAACCGCAACACGAGATGTTCTGC

TATCGTGCCTCGGCGCCCGCGCACCGCCGCGTCAAGGAGAGCCCCCTCAGCTCGGACGCCATCTTACGCCAGAGCCATGCAGGTCTTCTGAATCT

ATGCATTGTTGTACTTATTGCGGTGAACAGCAGACTCATTATTGAGAATTTAATGAAGTCTGTGAATTTCAGCCTTACTTTACCAATTTTCCCACTT

GCTGCACTCGTAGCTGAGAAGCTTATTCAAAGAAAGTTCATTAGTGAAAATGGAGTTACATATGGTAACTCTATCGATCCTGAGATTATGAAGGA

CCCAACCTTTAAAAGTCTTGTGCTAAACATTCTTGCTGAACTACTACGTTTTGGTGATCGTGAATTTTATAAGGACTGGTGGAACGCCAAAACAGT

TGAAGAGATATGCATCGCTGTGCCATGCCACATTTTCAAGTTCTGGGCATTTATTGGAATCATGTTTCAGGTATAG

>Ot-DGAT2 (Oropetium_20150105_23487|length=693|F_exp=15.61|D_exp=42.11|R_exp=25.16

ATGGGGATTCTTTTAGTCATCAGGTTGGGTGATGCTAGTTGTTCTGTTTTTGCAGTGTTCGGTTATGAGCCGCACTCTGTCCTGCCCATAGGGCTGT

CAGCGCTTGCTGACCTTGTGGGATTCTTGCCTTTGACTAAAATCAAGATCCTTGCGAGCAGCGCTGTGTTCTACACCCCCTTCTTGAGGCAGATTTG

GACATGGCTTGGATTGGTCCCTGCAACAAGGAAGAATTTCTACACTTACCTTGAGGCGGGCTACAGTTGTATCATTGTGCCTGGTGGTGTAAGGGA

AATGCTTCATATGGATCGTGAATCAGAGGTTGCTTTCCTTAAATCTAGAAAAGGGTTTGTTAAGATAGCTATGCAGTCTGGCAGTCCGTTGGTCCC

TGTTTTCTGTTTTGGACAGAGCTATGCATACAAGTGGTGGCGGCCTGGTGACAAATTGTTTGTCATCATTTCCAAAGCAGTTAAATTTACTCCTATT

ATCTTTTGGGGAAGATATGGAACACCATTCCCCTTTGCAAGACCGATGCATGTAGTGGTGGGTAGACCAATTGAGGTTCATAAAAACGCACAGCC

TACAATTGATGAGATAAATGAAGTTCATGAGCAGTTCATCATCGCCTTACGAGAGCTATTTGAAAAGTACAAGGCCAAAGCTGGATATCCTGGCC

TCCATCTAAGAGTCCTATGA

>Ot-DGAT3 (Oropetium_20150105_15462|length=1197|F_exp=233.68|D_exp=112.26|R_exp=279.21

ATGGACCTCACCGGCGTCGCGGCGCTCCGGCGGTCCCTGCCGTCGGCGTCCCCGACTGGCGCGCGGGGGAGGAGGCGGCCGTCCAGGGTGTCCTG

TTGCATCCGCGGCCGGTTCGCGGAGGAAGGGCACCTCAAGTACTACGAGGAAGGGGCGGCGCCGCGGAAGGCGGCGGTGGAGGCGGTGGCGAG

GGACCTGGCTAAGATCCATGCCATGGGGCTCGTCGCCGGGGACGCGGCCAAGGAGAAGGTCCTCTCGGAAGCCACTGATCTTCTGCTTCAGGCGT

TGGACCAGATGAAGGATGATGAGTACATCATGAAGAAGGTCCGGAAAGAGGAGAAGGCTGCCATGAAATCTCTGAAGCAGCAGCAGAAGGAAG

CAAAGAAGGCAGCTGCCATGATGAGCTGTGAAGACGGATCCTCATCTGAATCAAGCGAGAGTGATTGCGAGGAGGAACAAATCATGAGAATTGG

GCANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCGAGAAGGCTGCGATG

AAAGCCATNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTATGGCCG

CCGTGAATGGATGCAGGGATGAAGACAGCTCATCGGAATCCAGTGAGAGTGAATGTGAGGGGGAAGTGGTCAGAATGAGCCGCTGCGCTACAAT

CACCACCGCACCTGAAGAGTCAGCTCCCAGCACGGTCTTTCCAATTATAATTCCTCAGATTCCTGAATCCGTGTCACCAGATCCATCACAAGAGCA

TCGGGTCTCCTCAGAGCCTCCTGCATCTGCAACACAACGCACCACCAGCACTGCAGTAGTTGCGATGCCAATGGCCAACAGAATCGAGGTCTGCA

TGGGGGGCAAGTGCAAGAAATCAGGGTCACTCGCTCTTCTGCAGGAGTTCGAGAAGGAGGTGGGCACTGAAGGCGCAGTTGTTGGCTGCAAGTG

CCTGGGCAAGTGCGGGCTAGGCCCGAACGTGCGGCTGCGGAGCGAGAGCTCAGTGGAAGGTTCTGTAAAGAAGAACCCTCTCTACATTGGTGTA
GGGTTAGAAGATGTTGGCACCGTAGTGGCAGGCCTCTTTGGGGACGGTGATCTGGGCGTGACGCCTGCTTAG

>Ot-PDAT1 (Oropetium_20150105_05749|length=1791|F_exp=53.06|D_exp=30.47|R_exp=26.48)

ATGTCGCTCCTGCGGCGGCGGAAACAGCCGCAGCGCGAGGAGCCACCGCCGCCCTCGGACGGGTCGGACCACGACGATGACAAGGGCGGCGACA
AGAGGGGCAAGCAACCGCCGCCCCCGCCGTCCAAGGAGGCGACGCGGCGGACCAAGGCCAAATATTCGTGCGTGGACACCTGCTGCTGGCTGGT
CGGGATCGTGTGCTCCGTGTGGTGGCTGCTGCTCTTCCTCTACAACGCGATGCCGGCCTCGTTCCCGCAATACGTCACCGAAGCCATCACGGGGCC
GCTCCCTGACCCGCCCGGGGTCAAGCTGCAGAAGGAGGGGCTGCGCGTCAAGCACCCCGTCGTGTTCGTCCCCGGAATCGTCACCGGGGGGCTCG
AGCTCTGGGAGGGGCACCAGTGCGCCGAAGGGCTCTTCCGAAAGCGGCTATGGGGCGGCACATTTGGTGACGTCTACAAGAGACCTTTGTGCTGG
GTTGAACATATGTCATTGGACAATGAAACTGGATTAGACAAACCAGGAATAAGGGTGAGGCCAGTCACAGGTCTTGTCGCAGCAGACTACTTTGT
CCCTGGATACTTTGTTTGGGCAGTCTTAATTGCCAATTTAGCACGTATTGGATATGAAGAAAAGTCCATGTACATGGCTGCATATGATTGGAGGTT
GTCTTTCCAGAATACTGAGAGAATTAGTTCTATGTTACAGTTTCTTGTTACATACAGAGCTATTGCACCTGAGTTTCTGGACTCCGACTTCCTTGGA
CTTCAGACTTTGCGCCATTTAATGCGTATGACTCGTACATGGGATTCAACGATGTCAATGATTCCCAAGGGTGGTGATACTATCTGGGGAGGTTTT
GATTGGTCTCCAGAAGATGGTTTTGAGTGTAAAGCTAATAAGCGAAAAAACAATGATTCTGATATCTCTAACGGGGAAAATGCCAAGGTTCAACC
AGAGCCTGTTAACTATGGAAGAATGGTCTCTTTTGGTAAAGAAGTAGCAGAAGCTCCCTCTTCAGAAATTGAACAGATAGAATTTCGAGATGCTG
TTAAAGGCCGTAATATTGCTCAGTCAAATACATCATGTCGGGATATCTGGACTGAGTATCAGGAATTAGGGTGGGGTGGGATAAAGGCAGTTGCA
GACTACAAAGTTTACACTGCTGGTTCCATCATAGACCTCCTTCATTTTGTTGCTCCAAGGATGATGCAGCGTGGAAGTGTTCACTTTTCATATGGAA
TCGCTGATAACTTGGATGATCCAAAGTACAAGCATTACAAATATTGGTCAAACCCGCTGGAAACAAAGTTACCAAAGGCACCTGATATGGAAATA
TTTTCAATGTATGGAGTAGGTATTCCTACTGAAAGAGCTTATGTGTATAAGTTGGCTCAACAGGCAGAGTGTTATATACCTTTCCAGATTGACACT
TCTGCTGAGGGTGGGGACGAAAATAGCTGTCTGAAGGGAGGTGTTTACTTAACTAACGGCGATGAAACTATACCGGTTCTCAGTGCGGGTTACAT
GTGTGCAAAAGGATGGCGTGGGAAAACCCGTTTCAACCCTTCTGGCAGCAAGACTTACCTGAGAGAATATAATCATTCCCCACCCTCAACTCTTTT
GGAAGGTCGGGGTACTCAGAGTGGTGCACATGTTGATATTATGGGCAACTTCGCTTTGATTGAAGACGTCATCAGAATAGCTGCCGGAGCTACTG
GAGAGGAACTTGGCGGTGACCAGGTATATTCTGATATATTTAAATGGTCAGAGAAAATTAAACTGAAATTATGA

>Oropetium_20150105_05339A transcript offset:0 AED:0.21 eAED:0.21 QI:0|0.61|0.78|1|0.76|0.71|14|664|311

ATGGGAGTATGGTACAATGGTTTATTGATGGGCAAGCTTCATTCGAAGCTATTGCTTCCTCTATTGAGGAAGCTAAATCAGAGATATTTATCACGG
GCTGGTGGCTTTGCCCTGAGTTGTATCTTCGACGCCCATTCAAACATCATGGGTCATCTAGGCTTGATGCTCTGCTGGAAGCAAGAGCTAAGGAGG
GTGTACAGGTCACACCATGAGAAGATTGTGATTGTTGATAATCAAGTATGTTATGTTGGAGGCCTTGACTTGTGTTTTGGTCGCTACGATAATCCT
GAACACAAAGTTACTGATTCGCCTCCTCTGATATGGCCAGGGAAGGATTACTACAACCCCAGGGAATCTGAGCCCAATTCTTGGGAGGACACAAT
GAAAGATGAGTTGGACCGTGCTAAGTATCCCCGCATGCCTTGGCATGATGTCCAGTGTGTTCTCTACGGTCCACCTTGTCGTGATGTAGCAAGACA
TTTTGTTCAGCGCTGGAACTATGCAAAGAGGAACAAAGCTTTAAATGAGCAAGCAATTCCCTTACTGATGCCTCATCACCACATGAACCAATTTTT
CATATCGGGCCTGTCAGGAGATGATACAATTAAAAACCGTGTATTGGAAGCATTGTACAGGCGTATTCTTCGAGCTGAGCGAGAGAAAAAGCGGT
TCAAAGCCATCGTAGTCATACCTCTTTTACCTGGTTTTCAGGGAGGTATTGATGATGGTGGAGCTGCATCAATTTATGTTCATAGCAAGTTGATGA
TAATTGATGACCGCATCACATTGATTGGTTCAGCCAACATAAATGATAGAAGCTTGCTTGGATCAAGGGATTCTGAGATTAGCCGCATCATGGATC
CTATTGATGATTCAACATTCAAAAACATCTGGATGGCTACTGCAAAGGGTCCAATTCCGTCAGAGCTTTGCTAATTTGAAGGACAAAATTGGCCAC
ACTACAATTGATTTGGGTGTCGCCCAAGAGAAACTGGATACCTACCAAGATGGTGATCTGAAAGCTACTGACCCAATGGATAGATTGCGGTTAGT
CAGAGGTCACCTTGTTTCTTTCCCTTTGGATTTCATGTGCCAAGAGGACTTGAGACCATATTTCAGCGAAAGCGAGTATTACACATCTCCACAAGT
TTTCCATTAGTTTTTGTTTCATGGAAAATTATCCCGTAGGAGGCTAGGATCTCGATTGTACATACTGCAAGGATGAGCTTTGTAGATGTTTTACATA
TGATCGATCGTAGAGAGGAAATGAAAATAAAAGGAAAATACTAGTATCATACAATTATAGAGAGATTACAGGTATAGTTTTCTAAAAAGAAAGG
ATACATGTGTAGCTCGTTCATCAGACTTCTTTTGGGGGCAATACAACACTCCTGCTCGTACATCAATTGTCAATGCAAACAACAGAGTCAAATTAA
TTCTTTTTACATTTTCTTTGGCGACAAATTTGGACGCATCTTCCTTGCTGTTGTAATGGAAACTATTGTAACATTGTATGCACCTATTGTTCCTTGTT
TCATGCGTAGCTGTCAAGTTCATGATGGAACCCAAAAACAAAAAAGTTTGTGTACGTATTACAGGA

>Ot-PDAT2 (Oropetium_20150105_05749|length=1791|F_exp=53.06|D_exp=30.47|R_exp=26.48)

ATGTCGCTCCTGCGGCGGCGGAAACAGCCGCAGCGCGAGGAGCCACCGCCGCCCTCGGACGGGTCGGACCACGACGATGACAAGGGCGGCGACA
AGAGGGGCAAGCAACCGCCGCCCCCGCCGTCCAAGGAGGCGACGCGGCGGACCAAGGCCAAATATTCGTGCGTGGACACCTGCTGCTGGCTGGT
CGGGATCGTGTGCTCCGTGTGGTGGCTGCTGCTCTTCCTCTACAACGCGATGCCGGCCTCGTTCCCGCAATACGTCACCGAAGCCATCACGGGGCC
GCTCCCTGACCCGCCCGGGGTCAAGCTGCAGAAGGAGGGGCTGCGCGTCAAGCACCCCGTCGTGTTCGTCCCCGGAATCGTCACCGGGGGGCTCG

AGCTCTGGGAGGGGCACCAGTGCGCCGAAGGGCTCTTCCGAAAGCGGCTATGGGGCGGCACATTTGGTGACGTCTACAAGAGACCTTTGTGCTGG
GTTGAACATATGTCATTGGACAATGAAACTGGATTAGACAAACCAGGAATAAGGGTGAGGCCAGTCACAGGTCTTGTCGCAGCAGACTACTTTGT
CCCTGGATACTTTGTTTGGGCAGTCTTAATTGCCAATTTAGCACGTATTGGATATGAAGAAAAGTCCATGTACATGGCTGCATATGATTGGAGGTT
GTCTTTCCAGAATACTGAGAGAATTAGTTCTATGTTACAGTTTCTTGTTACATACAGAGCTATTGCACCTGAGTTTCTGGACTCCGACTTCCTTGGA
CTTCAGACTTTGCGCCATTTAATGCGTATGACTCGTACATGGGATTCAACGATGTCAATGATTCCCAAGGGTGGTGATACTATCTGGGGAGGTTTT
GATTGGTCTCCAGAAGATGGTTTTGAGTGTAAAGCTAATAAGCGAAAAAACAATGATTCTGATATCTCTAACGGGGAAAATGCCAAGGTTCAACC
AGAGCCTGTTAACTATGGAAGAATGGTCTCTTTTGGTAAAGAAGTAGCAGAAGCTCCCTCTTCAGAAATTGAACAGATAGAATTTCGAGATGCTG
TTAAAGGCCGTAATATTGCTCAGTCAAATACATCATGTCGGGATATCTGGACTGAGTATCAGGAATTAGGGTGGGGTGGGATAAAGGCAGTTGCA
GACTACAAAGTTTACACTGCTGGTTCCATCATAGACCTCCTTCATTTTGTTGCTCCAAGGATGATGCAGCGTGGAAGTGTTCACTTTTCATATGGAA
TCGCTGATAACTTGGATGATCCAAAGTACAAGCATTACAAATATTGGTCAAACCCGCTGGAAACAAAGTTACCAAAGGCACCTGATATGGAAATA
TTTTCAATGTATGGAGTAGGTATTCCTACTGAAAGAGCTTATGTGTATAAGTTGGCTCAACAGGCAGAGTGTTATATACCTTTCCAGATTGACACT
TCTGCTGAGGGTGGGGACGAAAATAGCTGTCTGAAGGGAGGTGTTTACTTAACTAACGGCGATGAAACTATACCGGTTCTCAGTGCGGGTTACAT
GTGTGCAAAAGGATGGCGTGGGAAAACCCGTTTCAACCCTTCTGGCAGCAAGACTTACCTGAGAGAATATAATCATTCCCCACCCTCAACTCTTTT
GGAAGGTCGGGGTACTCAGAGTGGTGCACATGTTGATATTATGGGCAACTTCGCTTTGATTGAAGACGTCATCAGAATAGCTGCCGGAGCTACTG
GAGAGGAACTTGGCGGTGACCAGGTATATTCTGATATATTTAAATGGTCAGAGAAAATTAAACTGAAATTATGA

>Ot-LPCAT1 (Oropetium_20150105_21151|length=1857|F_exp=89.81|D_exp=85.74|R_exp=50.19

CTCAGAGGGAGCTCGCATCCGAACGCAACACATCGCTCGCGCGAGCAGACCAAATCCTCTCCCCGCATCTGGGCGGCCTCCTCCCCCACGGCGGC
GACGACGATGGGGCTGGAGATGGAGGCCATGGCGGCGTCTATTGGCGTGTCGGTGCCCGTGTTCCGCTTCCTGCTCTGCTTCGCGGCCACAATCCC
GACGGGCCTCCTGTGGCGCGCGGTGCCCGGTACCACGGGGCGCCACCTCTACGCGGGGCTCACGGGCGCCGTGCTCTCCTACGTCTCCTTCGGGG
CCACGTCCAACCTCCTCTTCGTCATCCCCATGACGCTCGGCTACCTCGCCATGCTCCTCTGCCGCCGCCACGCGGGGCTTATCACCTTCCTCGGCGC
CTTCGGCTTCCTCATCGCATGCCATGTATACTACATGAGTGGAGATGCATGGAAGGAGGGAGGCATCGATGCAACTGGTGCTTTAATGGTCTTAAC
ACTGAAAGTCATTTCGTGTGCAATAAACTACAGTGATGGCTTCTTGAAGGAAGAGGGTTTACGTGATGCTCAGAAAAAGTACCGATTGACTAAGC
TTCCTTCGCTAATTGAATATTTTGGATACTGCCTCTGCTGTGGTAGCCATTTTGCTGGACCAGTATATGAGACGAAAGATTATCTTGAATGGACTGA
AAGGAAAGGACTATGGGCTAGCCCAACTCCATCACCATTGCTACCTACTTTACGTGCTCTGGTTCAGGCTGGTATATGCATGGGGTTATATTTATA
TCTGTCACCTAAATTCCCACTCTCAAGGTTTAATGAGCCCCTATATTATGAATGGGGTTTCTGGCACCGGCTCTTCTATCAGTACATGTCTGGCTTT
ACTGCTCGTTGGAAATATTACTTCATATGGTCAATCTCAGAAGCTGCAATTATTATTTCTGGTCTGGGATTTTCTGGTTGGTCGGATTCTTCTCCCC
CAAAACCCAAGTGGGATCGTGCAAAGAATGTTGATGTCTTAGGTGTTGAATTAGCTAGAAGTGCAGTTCAGCTGCCCCTTGTATGGAATATTCAA
GTGAGCACATGGCTACGATACTATGTGTATGAGAGGCTAATCCAGAAAGGAAAGAGACCAGGTTTCCTTCAGTTGCTGGGTACACAGACAGTCAG
TGCCATCTGGCATGGACTGTATCCTGGATATATGATATTCTTTGTTCAATCAGCATTGATGATAAATGGTTCACGAGTTATATACAGATGGCAGCA
AGCTGCGAGCAATTCAGTCCTCCGCAATATACTGGCTTTCCTAAATTTTGCATATACTTTGCTGGTGCTCAACTACTCATGCATTGGCTTCCAGGTA
TTGAGCTTCAAGGAAACCTTGGCATCGTACCAGAGCGTATACTATGTTGGGACGATTATTCCCATAGTATGTCTCGTGCTGGGCTCTGTGATGAAG
CCAGCAAGGCCTGTGAAGCCCAAGGTTCGGAAGGCGGAGTGAGCAACATGTATTGAAATGATGACTACGCTGAAGAAAGATGGAGAAGCTTCAA
TGACGATAACGACAAGTTAGTATTTGTTTATTTATTCACTCTTTTGGAACAGTGTCAAAGTTAGCAGAGCACGAGGTTTATGTGGTGGCCCCAGCT
CTCATACTTTGGAAATCCATTGTGATTGACCCATCACCGAAACGTGACACATTTGTGGTTTGCTACGATTTAATTGCCTACAACATGCAGGAAAGG
ATTTTGCTTGATGACAAAAGGATCCCTTTTTGCTCATAGTCATATGTTAGTAGTCTTCTTGCTGACTGAATCTAAAAAACATTTGCTTTTTGGACCG
AGTTGTGAATGCGCGTGATCTCGGCACCTCACGC

>Ot-LPCAT2 (Oropetium_20150105_21151|length=1857|F_exp=89.81|D_exp=85.74|R_exp=50.19

CTCAGAGGGAGCTCGCATCCGAACGCAACACATCGCTCGCGCGAGCAGACCAAATCCTCTCCCCGCATCTGGGCGGCCTCCTCCCCCACGGCGGC
GACGACGATGGGGCTGGAGATGGAGGCCATGGCGGCGTCTATTGGCGTGTCGGTGCCCGTGTTCCGCTTCCTGCTCTGCTTCGCGGCCACAATCCC
GACGGGCCTCCTGTGGCGCGCGGTGCCCGGTACCACGGGGCGCCACCTCTACGCGGGGCTCACGGGCGCCGTGCTCTCCTACGTCTCCTTCGGGG
CCACGTCCAACCTCCTCTTCGTCATCCCCATGACGCTCGGCTACCTCGCCATGCTCCTCTGCCGCCGCCACGCGGGGCTTATCACCTTCCTCGGCGC
CTTCGGCTTCCTCATCGCATGCCATGTATACTACATGAGTGGAGATGCATGGAAGGAGGGAGGCATCGATGCAACTGGTGCTTTAATGGTCTTAAC
ACTGAAAGTCATTTCGTGTGCAATAAACTACAGTGATGGCTTCTTGAAGGAAGAGGGTTTACGTGATGCTCAGAAAAAGTACCGATTGACTAAGC
TTCCTTCGCTAATTGAATATTTTGGATACTGCCTCTGCTGTGGTAGCCATTTTGCTGGACCAGTATATGAGACGAAAGATTATCTTGAATGGACTGA
AAGGAAAGGACTATGGGCTAGCCCAACTCCATCACCATTGCTACCTACTTTACGTGCTCTGGTTCAGGCTGGTATATGCATGGGGTTATATTTATA
TCTGTCACCTAAATTCCCACTCTCAAGGTTTAATGAGCCCCTATATTATGAATGGGGTTTCTGGCACCGGCTCTTCTATCAGTACATGTCTGGCTTT

ACTGCTCGTTGGAAATATTACTTCATATGGTCAATCTCAGAAGCTGCAATTATTATTTCTGGTCTGGGATTTTCTGGTTGGTCGGATTCTTCTCCCC
CAAAACCCAAGTGGGATCGTGCAAAGAATGTTGATGTCTTAGGTGTTGAATTAGCTAGAAGTGCAGTTCAGCTGCCCCTTGTATGGAATATTCAA
GTGAGCACATGGCTACGATACTATGTGTATGAGAGGCTAATCCAGAAAGGAAAGAGACCAGGTTTCCTTCAGTTGCTGGGTACACAGACAGTCAG
TGCCATCTGGCATGGACTGTATCCTGGATATATGATATTCTTTGTTCAATCAGCATTGATGATAAATGGTTCACGAGTTATATACAGATGGCAGCA
AGCTGCGAGCAATTCAGTCCTCCGCAATATACTGGCTTTCCTAAATTTTGCATATACTTTGCTGGTGCTCAACTACTCATGCATTGGCTTCCAGGTA
TTGAGCTTCAAGGAAACCTTGGCATCGTACCAGAGCGTATACTATGTTGGGACGATTATTCCCATAGTATGTCTCGTGCTGGGCTCTGTGATGAAG
CCAGCAAGGCCTGTGAAGCCCAAGGTTCGGAAGGCGGAGTGAGCAACATGTATTGAAATGATGACTACGCTGAAGAAAGATGGAGAAGCTTCAA
TGACGATAACGACAAGTTAGTATTTGTTTATTTATTCACTCTTTTGGAACAGTGTCAAAGTTAGCAGAGCACGAGGTTTATGTGGTGGCCCCAGCT
CTCATACTTTGGAAATCCATTGTGATTGACCCATCACCGAAACGTGACACATTTGTGGTTTGCTACGATTTAATTGCCTACAACATGCAGGAAAGG
ATTTTGCTTGATGACAAAAGGATCCCTTTTTGCTCATAGTCATATGTTAGTAGTCTTCTTGCTGACTGAATCTAAAAAACATTTGCTTTTTGGACCG
AGTTGTGAATGCGCGTGATCTCGGCACCTCACGC

>Ot-PLDa1 (Oropetium_20150105_07247A transcript)

TTGGGGAACCAAACCGACCCGGTAAGCCTCCCTTAACCCTTTTTGCTCTCCTCTCTCATCTCCTTCATTAAGCTCCTCCCACTCCCAGATCTGCTGC
GACCGCTTCTCCCCCCCATTTCTCCGTGCGGTCTGCTCTGGTTTCGTTTCATACTCTTTGCGGCGCTCGATCCGCGGAGCCTTGCCCGCCTCCCTTGC
TTACCGCCTTCTGTCTGTGACTGGTGATTACTGATTAGGCTGGTGGTAGCGAGGACGAGAAGGAAAGGAGGAGGGGAGACATGGCTCAGATCTTG
CTCCACGGCACGCTCCACGTCACCATCTTCGAGGCCGAGTCGCTCTCCAACCCGCATCGCGCCAGCGGCAGCGCCCCCAAGTTCATCCGCAAGGG
GATCGAGGACACCGTCGGCGTGGGCAAAGGAAGCAGCAAGATATATGCCACCATTGACCTCGAGAAGGCCCGCGTCGGGCGTACCCGGATGCTA
AGCAACGAGCCCGTGAACCCCCGCTGGTACGAGTCCTTCCACGTCTACTGCGCCCACCTGGCTGCCGACGTCATCTTCACCGTCAAGGTCGCCCAG
GCCATCGGGGCCTCGCTGATCGGGAGGGCGTACCTGCCGGTCCAAGAGCTCATGAGCGGTGAGGAGATCGATAAATGGCTCGAGATCTGCGACG
AGAACCGGGAGCCCGTTGGTGAAAGCAAGATTCACGTCAAGCTGCAGTACTTTGAGGTGTCCAAGGACCGCAACTGGGCGAGGGGCGTCCGCAG
CACCAAGTACCCTGGTGTTCCCTACACCTTTTTCTCGCAGAGGCAAGGGTGCAAGGTCACTCTGTACCAGGACGCTCATGTCCCCGACAACTTCGT
TCCCAGGATCCCGCTTGCTGATGGGAAGGACTACGAGCCGCACAGGTGCTGGGAGGACATCTTTGATGCCATCAGCAACGCGAAGCATCTGATCT
ACATCACCGGCTGGTCTGTGTACACTGAAATCACCTTGGTCAGGGATACCAAGCGGCCGAAGCCTGGAGGAGATGTTACTCTTGGAGAGTTGCTC
AAGAGGAAGGCCAGTGAAGGCGTCCGGGTCCTTATGCTGGTGTGGGATGATAGGACCTCAGTTGGATTGCTCAAGAGGGATGGCTTGATGGCCAC
CCATGATGAAGAGACTGCAAATTTCTTCCATGACACAGATGTCCACTGCGTTCTCTGCCCACGCAACCCTGATGATTCAGGCAGCATTGTTCAGGA
TCTGCAGATATCAACTATGTTCACCCACCATCAGAAGATTGTAGTGGTTGACCATGAGATGCCAAACCGGGGCTCCGAGCAAAGGAGGATAGTCA
GCTTTGTTGGTGGCATTGACCTGTGCGATGGAAGGTATGACACCCAGTATCACTCCTTGTTTAGGACACTTGACACGGTCCACCATGATGACTTCC
ACCAGCCAAACTTTACGACTGCATCAATCACGAAAGGTGGTCCGAGGGAGCCATGGCATGATATCCATTCGCGGCTAGAAGGGCCAATTGCTTGG
GATGTTCTTTACAATTTTGAGCAGAGATGGAGAAAGCAGGGTGGTAAGGATCTCCTTGTACATCTCAGGGATCTTGCCGACATTATTATCCCTCCT
TCTCCTGTCATGTTCCCAGAGGACAAGGAAACATGGAACGTTCAGCTCTTCAGATCCATTGATGGTGGTGCCGCTTTTGGCTTCCCTGATACTCCT
GAAGAAGCTGCACGTGCAGGACTTGTTAGTGGAAAGGATCAGATCATTGACAGGAGCATCCAGGACGCGTACATACATGCTATCCGGAGGGCAA
AGAATTTCATCTATATCGAGAACCAGTATTTCCTTGGAAGTTCATATTGCTGGAAGCCTGACGGCATCAAGCCAGAAGAAATTGGTGCTCTTCACT
TGATCCCCAAGGAGCTCTCACTGAAGATTGTCAGCAAGATCGAAGCTGGGGAACGATTTACTGTTTATGTTGTGGTGCCAATGTGGCCTGAGGGT
GTTCCAGAAAGCGCTTCTGTTCAAGCAATCCTTGACTGGCAAAGGAGAACAATGGATATGATGTACACTGACATCTCTCAAGCCCTCCAAGCAAA
AGGAATTGAAGCAAACCCCAAGGATTACCTTACTTTCTTCTGCCTAGGTAACCGTGAGGTGAAGCATGAGGGTGAATATGTACCTCAGGAACAGC
CAGAACCTGATACTGATTACATCCGTGCTCAAGAGGCTAGGAGGTTCATGATCTACGTTCACACCAAAATGATGATAGTTGATGATGAATACATT
ATTATCGGGTCTGCCAACATCAACCAGAGGTCCATGGATGGCGCTAGGGACTCTGAAATTGCCATGGGAGCATACCAGCCATACTACCTGTCGAC
CAGGCAGCCAGCCCGTGGCCAGATCCATGGCTTCCGTATGTCGCTGTGGTATGAGCACCTTGGAATGCTGGACGACGTGTTCCAGAACCCTGAGA
GCTTGGAATGCGTGCGGAAGGTGAACAAGATCGCAGAGAAGTACTGGGACATGTATTCCAGCGATGACCTGGAGCAGGACCTCCCTGGACACCT
GCTTAGCTACCCCATCGGCGTTAGCGAAGATGGCACTGTCACAGAGCTGCCAGGGATGGAGTTCTTCCCCGACACCCGCGCCCGTATTCTGGGTG
GCAAGTCAGATTACCTCCCGCCCATCCTCACCACATAGTGGCAGCAGCAAGAATCACGATGTCTTTCTCTGTCTGGCCTCAGTTTGATATCGTTGT
GTTTTCAGATATTTCAGTTCAGATAGTGTTGCTATAAACCACTCAGGAGGTAATAATAAGTGCAGTGTGCATGGTTGGGATGATAGGAGGAAGGA
TGGTGTTGATGTTTACTAATATACTGCATGCAGATTTTCGTATCCATGTATGCTAGCTTGTATATGGCTGGCTGCGTTGCAGTGCCATTTGTAAGAT
AGACTGAAGGTCTGAAGCACTTACGTGATTTCCTGTTTCAGCTGTTTGTGCTGTTCGTTATTTAATTGTTTATGCTGCTTCCATCTTATTTTTGACTT
GTTGTACCGTCGCTGTACACACATTATCGTTCAGGGAAGGCCTGAGATAAAAAAAAAAAAAACCTTACAA

>Ot-PLDa2 (Oropetium_20150105_05339A transcript)

ATGGGAGTATGGTACAATGGTTTATTGATGGGCAAGCTTCATTCGAAGCTATTGCTTCCTCTATTGAGGAAGCTAAATCAGAGATATTTATCACGG
GCTGGTGGCTTTGCCCTGAGTTGTATCTTCGACGCCCATTCAAACATCATGGGTCATCTAGGCTTGATGCTCTGCTGGAAGCAAGAGCTAAGGAGG
GTGTACAGGTCACACCATGAGAAGATTGTGATTGTTGATAATCAAGTATGTTATGTTGGAGGCCTTGACTTGTGTTTTGGTCGCTACGATAATCCT
GAACACAAAGTTACTGATTCGCCTCCTCTGATATGGCCAGGGAAGGATTACTACAACCCCAGGGAATCTGAGCCCAATTCTTGGGAGGACACAAT
GAAAGATGAGTTGGACCGTGCTAAGTATCCCCGCATGCCTTGGCATGATGTCCAGTGTGTTCTCTACGGTCCACCTTGTCGTGATGTAGCAAGACA
TTTTGTTCAGCGCTGGAACTATGCAAAGAGGAACAAAGCTTTAAATGAGCAAGCAATTCCCTTACTGATGCCTCATCACCACATGAACCAATTTTT
CATATCGGGCCTGTCAGGAGATGATACAATTAAAAACCGTGTATTGGAAGCATTGTACAGGCGTATTCTTCGAGCTGAGCGAGAGAAAAAGCGGT
TCAAAGCCATCGTAGTCATACCTCTTTTACCTGGTTTTCAGGGAGGTATTGATGATGGTGGAGCTGCATCAATTTATGTTCATAGCAAGTTGATGA
TAATTGATGACCGCATCACATTGATTGGTTCAGCCAACATAAATGATAGAAGCTTGCTTGGATCAAGGGATTCTGAGATTAGCCGCATCATGGATC
CTATTGATGATTCAACATTCAAAAACATCTGGATGGCTACTGCAAAGGGTCCAATTCCGTCAGAGCTTTGCTAATTTGAAGGACAAAATTGGCCAC
ACTACAATTGATTTGGGTGTCGCCCAAGAGAAACTGGATACCTACCAAGATGGTGATCTGAAAGCTACTGACCCAATGGATAGATTGCGGTTAGT
CAGAGGTCACCTTGTTTCTTTCCCTTTGGATTTCATGTGCCAAGAGGACTTGAGACCATATTTCAGCGAAAGCGAGTATTACACATCTCCACAAGT
TTTCCATTAGTTTTTGTTTCATGGAAAATTATCCCGTAGGAGGCTAGGATCTCGATTGTACATACTGCAAGGATGAGCTTTGTAGATGTTTTACATA
TGATCGATCGTAGAGAGGAAATGAAAATAAAAGGAAAATACTAGTATCATACAATTATAGAGAGATTACAGGTATAGTTTTCTAAAAAGAAAGG
ATACATGTGTAGCTCGTTCATCAGACTTCTTTTGGGGGCAATACAACACTCCTGCTCGTACATCAATTGTCAATGCAAACAACAGAGTCAAATTAA
TTCTTTTTACATTTTCTTTGGCGACAAATTTGGACGCATCTTCCTTGCTGTTGTAATGGAAACTATTGTAACATTGTATGCACCTATTGTTCCTTGTT
TCATGCGTAGCTGTCAAGTTCATGATGGAACCCAAAAACAAAAAAGTTTGTGTACGTATTACAGGA

>Ot-GAL7 (Oropetium_20150105_00738A transcript)

TCAGTCCACACCGCATTCTCTTTAGCTACCCCGCTCGCTCGCCCACTCCACCCCCTAATACCGGGCGAGCCGCGAGCGCGCGGTGCTCTCTCTGTTC
CTTTATTCCTCTTCCTCCTCCTCGGCCGGCAGCCAGGCGACCGCGGCGGCGGCGGCAATGGCCATGCCGCGCGCGGGCACGCGTACGCTGCTTCTT
CTTCCAGTAGCCGCTGCGGTGCTGCTGCTCTGCCTCGCGGGCGGCGCGCGGGCCACGAACGTGACGTACGACCACCGCGCCATCGTCATCGACGG
CGTGCGCCGCGTGCTCGTCTCCGGCTCCATCCACTACCCGCGGAGCACCCCCGACATGTGGCCGGGGCTGATCCAGAAGGCCAAGGACGGCGGCC
TGGACGTCATCGAGACGTACGTCTTCTGGGACATCCACGAGCCCTACGACTTCGAGGGCCGCAAGGACCTGACGGCGTTCGTGAAGGCCGTCGCT
GACGCCGGCCTCTACGTGCACCTCCGCATCGGGCCCTACGTCTGCGCCGAGTGGAACTACGGGGGCTTCCCGTTATGGCTGCACTTCATCCCGGGG
ATCAAGTTCCGCACCGACAACGAACCGTTCAAGGCGGAGATGCAGCGGTTCACGACCAAGGTAGTGGACACGATGAAGGGGGCGGGGCTGTACG
CCTCGCAGGGCGGGCCCATCATCCTCTCCCAGATCGAGAACGAGTACGGCAACATCGACTCGGCCTACGGCGCCGCCGGGAAGGCGTACATCCGC
TGGGCCGCCGGCATGGCCGTCTCCCTCGACACCGGCGTGCCATGGGTCATGTGCCAGCAGGCCGACGCGCCCGAACCAATGATCAACACCTGCAA
CGGGTTCTACTGCGACCAGTTCACGCCCAACTCGGCGAGCAAGCCCAAAATGTGGACCGAGAACTGGAGCGGCTGGTTCCTTTCCTTCGGCGGCG
CCGTTCCCTACCGCCCGGTCGAGGACCTCGCATTCGCCGTCGCGCGATTCTACCAGCGCGGCGGCACGTTCCAGAACTACTACATGTATCATGGCG
GGACCAACTTCGACCGCAGCTCCGGGGGGCCCTTCATCGCCACGAGCTACGACTATGATGCCCCGATCGACGAGTACGGACTGGTCAGGCAACCA
AAGTGGGGCCACTTAAGGGATGTACACAAGGCAATAAAGCTCTGTGAGCCAGCACTCATAGCAACCGATCCATCATACATTTCACTAGGTCAAAA
TGCTGAGGCGCACGTATACAAGGCCGGTTCAGTTTGTGCAGCATTCCTGGCCAACATAGATGGTCAATCCGATAAAACTGTCACCTTCAATGGCA
AGACATATAAACTCCCTGCATGGTCCGTCAGTATCCTCCCCGATTGCAAGAATGTAGTACTTAACACAGCTCAGGTCAATTCTCAGGTGGTAAGTT
CAGAGATGAGGTACTTGCAATCCAGCACCGAAGCATTAGATGATTCATTCGTCACACCAGAACTCGCAGTATCTGGCTGGAGCTATGCCATAGAG
CCTGTTGGAATCACAAAGGATAATTCATTGACAAAACCTGGATTGATGGAGCAGATAAACACCACAGCTGATGCCAGTGATTTCCTCTGGTACTC
AACAAGTGTCACCATTAAAGGTGACGAACCATATCTAAATGGTAGCAATACCAATCTGCTTGTAAACTCACTAGGGCACGTCCTTCAGGTCTACA
TCAATGGTAAATTTGCAGGAAGTGCTCAAGGTAGTGCTAGCAGCTCGCTCATCTCATTCCAGAAGCCTATTACACTTGTGCCTGGGAAGAACAAA
ATAGATCTTTTGAGTGCAACAGTTGGGCTGTCGAACTATGGTGCATTTTTTGATTTGGTAGGTGCTGGAATCACTGGGCCAGTAAAGCTGAGTGGA
CCAAATGGCGCGCTCGATCTGTCTTCTGTAGATTGGACATACCAGGTTGGACTTAAAGGAGAGGACTTGCACCTTTATGATCCTTCAGAGGCCTCT
CCAGAATGGGTTTCAGCCAATGCTTACCCCATAAATCAACCACTGATTTGGTGTAAGACCAAGTTCACAGCACCTGCTGGTGATGATCCAGTTGCC
ATAGACTTCACGGGAATGGGAAAAGGTGAGGCATGGGTGAACGGACAGAGCATTGGGCGATACTGGCCAACAAACCTGGCTCCACAAAGCGGCT
GTGTTAATTCGTGCAACTACAGAGGCGCCTACAGTTCAAACAAATGCCTCAAAAAATGCGGCCAACCATCACAGACTCTGTATCACGTGCCCCGG
TCGTTTATCCAACCAGGCAGCAACGATCTCGTCCTTTTCGAGCAGTTTGGTGGTGACCCAGGCAAGATATCCTTTGTAACAAAGCAGACAGGAAG
TGTGTGCGCACATGTGTCAGAGGCACATCCGCCCCAAATCGATAGCTGGTTCTCTCCCCAACAGAAGGTGCAAAGATCTGGACCTGCACTTCGCCT
GGAATGCCCGAAAGCAGGGGAAGTCATCAGCAGCATCAAGTTTGCAAGCTTCGGAACACCAAGCGGCACATGTGGAAGTTACAGCCATGGGGAA
TGCAGCAGCTCTCAGGCTCTTGCAATTGTTCAGGAGGCCTGCATTGGAGTGAACAGCTGCAGTGTGCCAGTGTCGTCAAAGAACTTGGGTGAACC
ATGCAGTGGAGTCACAAAAAGCCTTGCCGTCGAAGCTGCATGCTCATAACCTGTAAATGTTAGCTAGTAGAAAGCAAAATCGGTTAGAGAAGGTG

AAACCTTCTCTGCGTAGGAAACAAGGAATAAATGATTAAAAGTTCCAATGCTGGCCAAAAGGAATAAATATTCCCAAAAAGTTACCTGCAAGCTA
TTACTAGATGTTAGCTTAGGATTGTGATTGTTAAGATGATAAATTTATCCATCTTGGCAAAGATGATAATCTTTCATGAAATGTTTTCGAAATTCAA
TTTTAGAAGAGAAAAAGGGAATACTTTGCATTTTAATCGAGTTGAATGATACATAC

>Ot-GAL9 (Oropetium_20150105_00738A transcript)

TCAGTCCACACCGCATTCTCTTTAGCTACCCCGCTCGCTCGCCCACTCCACCCCCTAATACCGGGCGAGCCGCGAGCGCGCGGTGCTCTCTCTGTTC
CTTTATTCCTCTTCCTCCTCCTCGGCCGGCAGCCAGGCGACCGCGGCGGCGGCGGCAATGGCCATGCCGCGCGCGGGCACGCGTACGCTGCTTCTT
CTTCCAGTAGCCGCTGCGGTGCTGCTGCTCTGCCTCGCGGGCGGCGCGCGGGCCACGAACGTGACGTACGACCACCGCGCCATCGTCATCGACGG
CGTGCGCCGCGTGCTCGTCTCCGGCTCCATCCACTACCCGCGGAGCACCCCCGACATGTGGCCGGGGCTGATCCAGAAGGCCAAGGACGGCGGCC
TGGACGTCATCGAGACGTACGTCTTCTGGGACATCCACGAGCCCTACGACTTCGAGGGCCGCAAGGACCTGACGGCGTTCGTGAAGGCCGTCGCT
GACGCCGGCCTCTACGTGCACCTCCGCATCGGGCCCTACGTCTGCGCCGAGTGGAACTACGGGGGCTTCCCGTTATGGCTGCACTTCATCCCGGGG
ATCAAGTTCCGCACCGACAACGAACCGTTCAAGGCGGAGATGCAGCGGTTCACGACCAAGGTAGTGGACACGATGAAGGGGGCGGGGCTGTACG
CCTCGCAGGGCGGGCCCATCATCCTCTCCCAGATCGAGAACGAGTACGGCAACATCGACTCGGCCTACGGCGCCGCCGGGAAGGCGTACATCCGC
TGGGCCGCCGGCATGGCCGTCTCCCTCGACACCGGCGTGCCATGGGTCATGTGCCAGCAGGCCGACGCGCCCGAACCAATGATCAACACCTGCAA
CGGGTTCTACTGCGACCAGTTCACGCCCAACTCGGCGAGCAAGCCCAAAATGTGGACCGAGAACTGGAGCGGCTGGTTCCTTTCCTTCGGCGGCG
CCGTTCCCTACCGCCCGGTCGAGGACCTCGCATTCGCCGTCGCGCGATTCTACCAGCGCGGCGGCACGTTCCAGAACTACTACATGTATCATGGCG
GGACCAACTTCGACCGCAGCTCCGGGGGGCCCTTCATCGCCACGAGCTACGACTATGATGCCCCGATCGACGAGTACGGACTGGTCAGGCAACCA
AAGTGGGGCCACTTAAGGGATGTACACAAGGCAATAAAGCTCTGTGAGCCAGCACTCATAGCAACCGATCCATCATACATTTCACTAGGTCAAAA
TGCTGAGGCGCACGTATACAAGGCCGGTTCAGTTTGTGCAGCATTCCTGGCCAACATAGATGGTCAATCCGATAAAACTGTCACCTTCAATGGCA
AGACATATAAACTCCCTGCATGGTCCGTCAGTATCCTCCCCGATTGCAAGAATGTAGTACTTAACACAGCTCAGGTCAATTCTCAGGTGGTAAGTT
CAGAGATGAGGTACTTGCAATCCAGCACCGAAGCATTAGATGATTCATTCGTCACACCAGAACTCGCAGTATCTGGCTGGAGCTATGCCATAGAG
CCTGTTGGAATCACAAAGGATAATTCATTGACAAAACCTGGATTGATGGAGCAGATAAACACCACAGCTGATGCCAGTGATTTCCTCTGGTACTC
AACAAGTGTCACCATTAAAGGTGACGAACCATATCTAAATGGTAGCAATACCAATCTGCTTGTAAACTCACTAGGGCACGTCCTTCAGGTCTACA
TCAATGGTAAATTTGCAGGAAGTGCTCAAGGTAGTGCTAGCAGCTCGCTCATCTCATTCCAGAAGCCTATTACACTTGTGCCTGGGAAGAACAAA
ATAGATCTTTTGAGTGCAACAGTTGGGCTGTCGAACTATGGTGCATTTTTTGATTTGGTAGGTGCTGGAATCACTGGGCCAGTAAAGCTGAGTGGA
CCAAATGGCGCGCTCGATCTGTCTTCTGTAGATTGGACATACCAGGTTGGACTTAAAGGAGAGGACTTGCACCTTTATGATCCTTCAGAGGCCTCT
CCAGAATGGGTTTCAGCCAATGCTTACCCCATAAATCAACCACTGATTTGGTGTAAGACCAAGTTCACAGCACCTGCTGGTGATGATCCAGTTGCC
ATAGACTTCACGGGAATGGGAAAAGGTGAGGCATGGGTGAACGGACAGAGCATTGGGCGATACTGGCCAACAAACCTGGCTCCACAAAGCGGCT
GTGTTAATTCGTGCAACTACAGAGGCGCCTACAGTTCAAACAAATGCCTCAAAAAATGCGGCCAACCATCACAGACTCTGTATCACGTGCCCCGG
TCGTTTATCCAACCAGGCAGCAACGATCTCGTCCTTTTCGAGCAGTTTGGTGGTGACCCAGGCAAGATATCCTTTGTAACAAAGCAGACAGGAAG
TGTGTGCGCACATGTGTCAGAGGCACATCCGCCCCAAATCGATAGCTGGTTCTCTCCCCAACAGAAGGTGCAAAGATCTGGACCTGCACTTCGCCT
GGAATGCCCGAAAGCAGGGGAAGTCATCAGCAGCATCAAGTTTGCAAGCTTCGGAACACCAAGCGGCACATGTGGAAGTTACAGCCATGGGGAA
TGCAGCAGCTCTCAGGCTCTTGCAATTGTTCAGGAGGCCTGCATTGGAGTGAACAGCTGCAGTGTGCCAGTGTCGTCAAAGAACTTGGGTGAACC
ATGCAGTGGAGTCACAAAAAGCCTTGCCGTCGAAGCTGCATGCTCATAACCTGTAAATGTTAGCTAGTAGAAAGCAAAATCGGTTAGAGAAGGTG
AAACCTTCTCTGCGTAGGAAACAAGGAATAAATGATTAAAAGTTCCAATGCTGGCCAAAAGGAATAAATATTCCCAAAAAGTTACCTGCAAGCTA
TTACTAGATGTTAGCTTAGGATTGTGATTGTTAAGATGATAAATTTATCCATCTTGGCAAAGATGATAATCTTTCATGAAATGTTTTCGAAATTCAA
TTTTAGAAGAGAAAAAGGGAATACTTTGCATTTTAATCGAGTTGAATGATACATAC

>Ot-GAL17 (Oropetium_20150105_03684A transcript)

ATGCAGTACTGGAAAGACCGCCTTTTAAGAGCAAAAGCCCTGGGCTTGAACACAATACAAACATATGTACCTTGGAATTTGCATGAGCCAGATCC
GGAGAGGTGGGAGTTCACAGGTTTTGCAGATATTGAATCATATCTGAGACTTACTCAAGAACTAGAAATGCTGGTCATGCTTCGTGCAGGCCCAT
ACATATGTGGAGAATGGGACTTGGGGGGTTTTCCACCTTGGTTGCTCAGCATAGAACCTTGCCTTAAACTGCGGTCATCAGATTCAGCCTATCTTT
CCTTGATTGAAAATGAATTTGGTTCGTTTGGAGATGACAAGAATTACCTTCATTCTCTAGTTGAACTTGCCAGAAGACATCTCGGAAATGACATTA
TTCTGTATACTGTTCTCTTCTCTTATACAACTGATGGAGGTGCCATTGGTAATTTAAGGAACGGGTCAATTCCTCAAGATAATGTTTTCGCAGCTGT
TGATTTTGAAACTGGGTCCAGTCCATGGCCAATATTCAGATTACAAAAGTACTACAATTTGCCAGGAAAATCAGCCCCTTTAACTTCGGAATTCTA
TACCGGATGGTTAACACACTGGGGTGAAAGCATCGCGACAACAGATGCCACAAGTACAGCTAAAGCCCTTAAAGCTATTTTGTGCCACAACGACA
TTGAAAAAGCAAATTATGGACTTGTGAAACTACAAAAGGTTGCTTCTTTATTCGACATCATTGATAATATCAGTGATCCACTGAAAGTAACTGTTG
CAGAGCATCCTTTGTATATGGAACAGATAGGCCAGGTACATGACCGAGCTCAGGTGTTTGTTTCATGCTCGGCTGCTGGTGTCAGAAACCCAGTAT

ATGCCGGTGTGATTGAAAGATGGTCTAACAAGGCACTAGAAATTCCAAATTTAAGCTGTTCATCTAATACCACCTTATACATCCTGGTGGAAAAC
ATGGGTCGTGTAAATTATGGGTCATATATTTTTGACCGGAAGGGAATACTATCACCTGTTCAAGTAGATGGCATTCCTCTCCATCATTGGAAAATG
TTTCCGCTCTCATTCAATGCACTGGACAACCTTCCAAAACTCCAACTGATCAGTCAGATGCCTGATGCTAGATCTAGTAAAGCATCCATTCACGGT
GGTCCAAAGAAGAAATTGCAAGAATTGTCATTCCATTCAAATGGTATGTCTCTGCATCCAAATGTTTCTGTGAGCAGCAAAATGCTCATCACTGCA
CGTGGTCCACAGTGTGCTCTTTATGTTCCCGCACCAATCCTTAGACCTGGAGACAACATTGTTGTAATCTTTGAGCTGCATGGGCCAAACCCTGAG
CACACTATCAACTTTGTCGAAGATCCAGATTTCACATGTGGTTCGAAGCAATGA

>Ot-PIS1 (Oropetium_20150105_05856A transcript)

ATGCCCGCGCCGGCTGCGGAGCCGGGGCTCCCCGCCGCCTCCTTCCTCTGCGTTCCGTTCCCGCTACTCTCCTCGCCTCTACCAACCGCGGCTATCT
CCGCCTCCCCTGCCCTTCCGTCCTCCTACCATGCCTCCCTCCTCCCGCGCTCGCGCGGTGGCCCCCGCGCGGCTCTCTCTGTTGCCACGTCCGTCCC
TGGCCCGGCATCAACCGCCGCTGGGCGGCTTCACCGGATGTGGAGCGAGTTCGCGCACTTCGTGCGGCTGCACGGTAATCAAATCGCGCCGCTCG
GGTTCGCGTCCCTGGGTTTGGGTTCGGGCGGCGGGGGCGGGGCCGGTTACGCCAGCGGGGGAGGCGGGGGAGGGGGTGGCGGGGATGTAGACGC
GGTGTCCGAGGAGGAGGCCGTGGCGCGGGCCGAGGCGCCGAAGAAGGTGCTGATCCTCATGAGCGACACGGGCGGCGGCCACCGCGCGTCAGCC
GAGGCCATCAAGGCCGCCTTCATGCAGGAGTTTGGCGATGACTACCAGGATGAACTGAGAAGAGAGTTAGGACTAGATGAAGATCTGCCTACTGT
CCTATTGATGGGTGGAGGTGAAGGGATGGGCCCTATTGAGGCCACTGCTAGGGCGCTTGGTGACACCTTGTATGATGAAAACCTGGGGGAACCCA
TCGGTCAAATACTTATAATTTGTGGACGTAATAAGAAATTGACTAATCGGTTGCAGTCAATAAACTGGAAAGTACCAGTTCAGGTAAAGGGTTTT
GTTACAAAGATGGAAGAATGTATGGGTGCTTGTGATTGTATAATTACTAAGGCAGGGCCGGGTACAATTGCAGAGGCGATGATTCGTGGCTTGCC
AATTATTTTAAATGATTATATTGCTGGGCAGGAAGCTGGCAATGTTCCATACGTTGTTGAAAATGGGTGCGGGAAATTTTCAAAATCCCCAAAACA
GATTGCAAAGATAGTCGCCGATTGGTTCGGCCCTAGGTCAGATGAGCTCCAAGTTATGTCCCAGAACTGCCTGAAACTAGCACGACCTGATGCCG
TGTTTAAAATCGTTCATGATCTGCACGAGTTGGTCAGGCAAAAATGTTTTGTACCTCAATATGCTTATACAATCTAATGGTTCATTATCTTCATTTT
GAGGTTACTTCTCCATTTGTTTTCATGCGGCACTAGTTATAGTTGATTGGGATGATTGGCAGTATTAATTGATTTCACAAGTCTGCAATAGAAAATG
TTTGAGCGCTGTAATTTTTCCTTTGTGAAATAGTTTTAATACTGTCTTTGGAACTTTCTTAGATTACTCGTATTTAACATGCTAGTCTTTTTATGATG
ACGTTCTACTTGGCATGCTGTCTGCTGTGCTATCTCCCGTGAGGGATAGTCAAGCCTGGATCAACTATTGTTACTGTAACACAGTAATACTGTATG
ATACTGATCTTTTACGAGTACTCCTATATACGATGCTGCTTAGCTGTGACAGTATTCTGAATATACTTTGACGTTTTGGAAGGTCTGTAAATTGTTC
TTCGAGAGTTTGTGATAGGATGGCATGGATTTCTTCAAATCATTATAATAGTCTCCCACGGGAGGGTGCGAT

>Ot-PIS2 (Oropetium_20150105_08732A transcript)

CTCTGATCCGCGCCTTCATCCCGCCACCGCCCTCCGGCCCCCGACGCCGACGCCGCTGAAGTGGGCAGTGTTATTTCACTCTTCTACGCAACCGCT
GCATCTCTCCGCCTCGCTGCTCTGCCTGCCACCCGTCCCCTCCGCCGGCTCGCTGGCCTCCCGGTGTACGAATTCGCCAGTTCAGGAGCTGGCTCCA
GGCTAGGACTCCTCCAGCTGGACCAGCTCTAGTCCTCCGCTGGCATCCAGGTGACGGCAGCCCACGTCCCTGGCTGGATCAAGGGTGACTCCAGG
AACCCCAAAAGTGTTCTACCGGCATTAATTTAAGGGAGGAGCCACTGTCTTCAAGCACCTAATTACTCTTGATCCTAGAAGACACTAGTACACAA
CCACCTGCTTGGAAAATGCCGTCAGTTTATCTTTACGTCCCTAATGTGATTGGGTATTTCAGGATCATCATAAATGTCATTGCATTTGCTGGCTGCT
ACTCTAATAGGTCCCTCTTTGCCATTCTGTACTTCTTCAGCTTTGTCCTTGATGGTGTGGATGGTTGGTTTGCAGGAAGTTTAATCAAGCTCATATT
GTTATTCAGGGTCAGCACTGCTTGTCTGTTGGCTCTGCTCTCACAGTTTTACAGGTAGAAAGTAAGACTAGCCACAAGGATGTGAAGCACACAGG
CAATTGGCTTTTGAAATTGTATTATGGATACAGGCCATTCATGACCTTCTGTTGTGTGTCTTGTGAGGCAAAAACAGCTGCAGACGCATGCGTTAT
TTTCGATCTGAAGCGTGGCAAGTGAAGCAGGAAGGCACCGTTTTCATTCGTATTTGACATAACCAAGGAAACAGTCCGTCAGACGCGTACACCAA
GGTTCATACAGTATAGTGGGTACACTTTGCACGCGCCTTGACATCTCTCTGATTCTGATCCGTGAGGTAAGAAAAGCAAAACCGGAGGGGAAAAT
GCGTGAGGACAAAACGGAGATTGTGAGACTGTTTGTCAGTATGTTCCATTATACAATGCTAATGTGATCATAATGTTATATAGGATTGTCTTTTTG
AGTTGTTTTGAATTGTTGCAAACCACATGTAAGCCGGCGTATCACCTTGGGCGCGGCATATGTTTCCACATTCACTGCCCGAACTCAGAGAAGAAA
ACGGTGAACATTCC

>Ot-SFR2 (Oropetium_20150105_08544A transcript)

CGCCACTCCTATCCATTCGACGCTTCTGACTTGTGCACGAAAGAGAAAAAGAGACAGTTCTCAGAAAGAAAAAAAAAAAAAGGAGATTGGAAAG
CGCGGAGGATTGCCGCGGTAACTACGGAAATGCCACTCCCGGCCTTCCTGGCGGCGGCCGCGAAGCTCGCCGTCCTGGTGGCCGCCGCCGCCACG
GCGGCTAACGCGGTCTCCTACTCGCGGTACCGTCGCCGCCACCTCCGCCGCCTCCTCAACCCCATCGACGAGTCCGCTGACCCCGTCGCTGACTTC
CGCGCCTTGCCCCACTCCGCCGCCGCAGACTCCGACTCCTCCGCAGAGGATGAGAGCTTCTTCTTCGGGCTATCCACCGCCCCTGCACATGTTGAA
GACAGGCTGGAAGACGCCTGGCTTCAGTTTGCAAAGGAGCAGTCCTGCAACGAGAAGGAGGCCGTGCGTGACCAGAAGCCGGCAAATGCGTTGA
TGGCATCTTCTGGTGGCGATGGAGGCTCCCATGTAGCTTCAAGGACCAAAGGGGACGAAAAGGTGGAGGATGGGAAGAAAAGGAAGCCAATCAA
GGTAGCCATGGAGGCCATGGTCAGGGGGTTTGAAGCTTTTGTTGAGGATGTAGAGTCCGGTGGTAGCGAGAATTGCAGTCACAATGTTGCAGCTT

GGCACAATGTTCCTTGCCCGCACGAGAGACTTAAATTTTGGTCTGATCCTGATACTGAGTTGAAACTTGCTAAGGACACTGGCATCAGTGTTTTTC
GCCTGGGGATAGATTGGACAAGGGTAATGCCTAAGGAACCAAATGAAGATTTCAAGAGCTCAGTCAACTTTGCAGCACTTGAGCGGTACAAATG
GATCATTCAAAGAGTTCATGAATATGGCATGAAAGTTATGCTGACGCTGTTCCATCACTCACTTCCTCCTTGGGCTGGACGATACGGGGGATGGAA
AGAGGAAAGAACTGTTAACTACTTCTTGGATTTTGTGAGGCTTGTTGTCGACTGTGTATCAGATTTGGTGGACTACTGGGTGATTTTCAACGAACC
TCATGTATTTGTAATGATGACGTATTGTGCTGGTGCTTGGCCTGGTGGAGACCCTAATACTCTTGAAGTAGCGACATCGGCTTTGCCAACTGGTGT
ATATAACCAAGCATTGTATTTGATGGCTATTGCACATGCTGAAGCCTATGACTATATACACTTGAAAAGAAAAAATGCAAGGAAGCCCATCGTTG
GTGTTGCTCATCATGTATCATTTACAAGGCCATATGGTCTTTTTGATGTTGCGGCTGTCACACTAGCTAATTCGTTGACCCTTTTCCCTTTCATAGAT
AGCATATGTGATAAATTGGACTTCATTGGTATCAACTACTATGGGCAGGAGGTGATATCAGGACCTGGCCTAAAGCTTGTGGATACTGATGAATA
CAGTGAATCTGGTCGTGGTGTTTATCCCGATGGGTTGTTTCGTATCCTGATTAAGTTCAATGAACGATACAAGAGCTTAAATATACCTTTTATCATT
ACTGAAAATGGAGTTTCTGATGAGACCGATTTGATTCGGAAGCCATACATTCTGGAGCACCTTTTAGCCATATATGCTGCAATCATCATGGGTGTG
CGTGTGCTTGGTTATATGTTCTGGACAACATCGGATAATTGGGAATGGGCTGATGGCTACGGTCCAAAGTTTGGGCTTGTTGCTGTTGATCGTGCT
AACAACCTAGCACGGAAACCACGGCCTTCATACTATTTATTCTCCAAGGTAGTTACAACTGGGAAGATTACAAGACAGGATAGAGCACTGGCTTG
GAGGGAACTGAAACAAGCTGCCCTTCAAAAGAAAACACACCCATTTTTCAGGGCAGTAGATAAACATGGTCGGATGTATGCAGTAGTTGTAAGCC
CTTTTCAGCTTAAGAAGTATCCATTTTTTCTGCCTCCACTGAAGCATCACGGCTCACTCTTTGCCTATATAGGAGGCCTCGATCGACCTATTCAACG
ACCTTTTGTATTGCGGGATTGGCGATTTGGTCATTATGAAATGGAAGGGTTGCAGGATCCTTTAAGTCGCTTTTTAAGATCCATTGTTTCACCAATT
TCGCCCAATAAGAAGATTCATTACATTGAAGATGATGCAGATCTTGGAATCTCAAGAGCAGCCAAGGACACTGCAACACTGTGGCTTGCTTGTCC
GTGA

>Ot-PSAT1 (Oropetium_20150105_04609)

GTCCGTTCCAACCAGAACGACACCGCCAGCCAGCTCGCCCCGTCGCCCGCCCGTGACGATGCCTCCAAGGCGGCGCCGGCTCGGCGACCTCGCCT
TGGCGGCGGTGCTCGCGGCGTTGGCGACGGTGGTGGCCGCCGTCGCGGGACTGCCCGGAGCCGACGGCACCGCCGAGTTCGATTACCGGAAGCT
GTCGGGGATTATCATCCCGGGCTTCGCGTCCACGCAGCTGCGCGCGTGGTCGGTGCTGGACTGCCCTTACTCGCCGTTCGACTTCAACCCCCTTGA
CTCCGTATGGCTCGACACCGGAAAGACAGACCATCCGGAATGCAAATCGAGGCCTGATAGTGGACTTTCCGCAATTACAGAGCTGGATCCTGGTT
ATATAACAGATGTAACTGAGAACATGACGTCAATCATGGAGTGTGGAAAGCCAACACTATTGTCATTTTCCGCCAGAGAAGTTTCAGATGGTACT
TTGTTTAAAACAATAGAGGATTATGACCCTCAGAGCAAGGCACTTCTATATCAGCTTGAGAAATCAGGTAATTCTGTTGCTGGAAAGCCTAACACT
ATCAGTGGTGATGGGACGGTTTCCTACAACTCCCTCTCATGGTGCAAGAATTGGCTTGGGCCAAAAGTGAACATAACAAGGGCTCCACAGGCAGA
ACATGATGGTTCTGACATGCAAACACGCATGAATCTTGAGCACCATCATGGCCAGGATATACTTCCAAGCATGACAAGAGCTCCACATGTGAAGT
ATATAACCTACTATGAAGATGCTGAAGGGTACAAGGTGACTCTGAACGTGTACGACCTGAGCAACGGCCTCGCGCGGCAGCTCTCCACCTCCTTC
CTCGGCAAGCCCATCGAGGCCATCTGGCACACGGGCGTTGTGGTGTACGGGACCGAGTACTACTTCGGTGGCGGGATCCAGTCGTCGCCGGCGGG
TACGACGCAGTACGGGCGGCCGATGCGGACGGTGGAGCTCGGGGTCACGCACATCCCGCGCGAGGTGTTCGAGGACTACCTCCGCGACATCGCG
CCACGGTACACGGCCGAGACCTACCGCCTGCTCACCCACAACTGCAACAACTTCAGCAACGAGGTGGCGCAGTTCCTCGTCGGCGTCGGGATCCC
GGAATACATCCTCAACCTCCCGTCCGAGGTCATGTCCAGCCCCATGGGCCCGCTCATCATGCCAATGATCCAGAACCTCGAGGCCACGCTGCGTA
ACAACACGCCGCCGGAGACCACGCAGTTCGTGCCCACCCCGGCGTCAGTCTCCGCGCCGTCCGCGCTCAAGAAGGCCGCCGCGCCTAGTCCGGCT
GCGGCCGCTGGCTCTAACGACAAGCGGGAAGCCCCGGCACCGGCAGCCGAGGACAAGGCTGCGAAGGAAACGACGGCTGCTGCCACTCCTGCTG
CCGCTGACCCGCTCGGCAGCGCGAGGGGAAAGGTGCAGGAGGAGGTGATGAAGGAGTTCGCGGCGCTCATGGCGAGCGGGACGCTGCGGGCCA
GCGAGGCGGCGGCGCTGGCGATGCGCCGGGTCATGGAGCGCCACGGCGACGGCGCCACAATGCAGCAGAGCTAAGATGATATATGGATACGAAT
ACGATGCACTTGACAGCGCGTCGGCGCTTCATTTCCAGCTGAGTTAGAATTTTCTCCGCTGGGACATCAGGCTGCTCCTGCTGCCTTATATGACAA
AATGGGACTTTTTACCTGTCTGTATGTTAAATTGTGACCGGACTGCCCATCTATTGGATGATTAATTTGACAAGATACTCTGCGAGATTTATGTTCT
GCAAGGGTCTCTGATATATGCATGTTTTATAATACGGAATTTTTTCTTGTCAATCGACCTCTAAATCCAAAAGGCTCAATGGGCGTCTTTTGTTCGT
TGTTGATGGAGTTTTTTTTTTTCCAGCACAGAAAAAACGTAGTTTAATCTGTGCTGCCATCAGGAAGGCTAAGAGTGTGTGTCAGAGAGAGAGTAA
ACAAGAGGGAGGGGATTAGGGTCATCTGAGATCTTGATGGAGGTTACTCCATGAATTTTGTTTTGTTGTCAGCTATTCGGTACGCAGTCTGCGGCT
CATATATGCGGTACAGTATCCCATAAATAGTGAAACCGTCGCTACAATACTTCTTCGGATTTGCTATATAGCCCGTCGGACCTTAGGGTGGGGGAT
TTTTTGGGTGCTCTGGTGAGCTCCGGGTTTAGAAAGGGGTTTGGTGCGACGGATGGACCGGCGATGGGCGGCGGAAGGCGGTGCGGCGAGGCGCT
CTGGGCGGCGTCGGAGCCGCAGGCGATGGGTGGGGCTGGCGGCGGGGGAGCTTAAGCCAAGAATTTAGCACAGCGGGGGAACGGAAGGCGGAC
CAGACGTCGGACGTCGCCGGGGACGACGGTCGGAGGGAGGTAGGACGGTGAGCCCTGCCGCGCGCGAGAGACGGTGTTTGTGCTGCCCGCTATG
CGTGAGACCAAAGGTTGAAGACGAAAGAGCTACCCTCCGATGTAATATCAACGGTCCTGCTTGGTCGACTGAAACGAATCGTTTTTCAGCCGAC
CGATAATGAGAACGTCCCATACCTTTTTTTTGCTACAAATAGCTCTCACTTTTTTTTTTCAAAAATACGTAAGAGAGTTAGGTATCTATGTGCTCTC
ACGGAACATTGTGATAGGAGCTATGGTAGAATACTAAAATTTTCACCTCCGCTTGGGGGATCCGATTCCAATGGGAGATGATGGAGCAAACCTCG

TCAGGATTCAATCGATTTCTCTAGTTGGAGTTTTAGGATTTAAAATTTAATCAGTGAATTATTAGTTCTTCCTTATATCTGGTATCTGTAATTTTATG
CCTGCTTGAAAATGGTGTCGAATTGAATC

>Ot-ASAT1 (Oropetium_20150105_11126|length=1923|F_exp=2.56|D_exp=2.47|R_exp=4.73

ATGGCGACCAAGCTGATGGACGGCGAGCTCGGCAGCCTCGTGATGGTCTCCGCGGCGGCGTGGGCGGCAATGTCCTACGCCCGCCTCGCCGCAGC
GCGCCTCCGACCGGGCGCGCCTCGCCTCGCCGCGCTCCTGCCAGTGGTCGCGGTTCTCTACGCCGTCCCTTTCGCGTTCTCGACCACCACGTTCCGC
GGCACCTCCGCCTTCTTGCTCACCTGGCTGGGCTCCTTCAAGCTGCTCCTCCTCGCCGCCGGCCAGGGGCCCCTCGACCCAGCCCTTTCTATCCCTC
ACTTCGTCTGCTCGGCCTTGCTCCCCGTCAAGCTCCGCACCTCCGCCGCCAAAGAAAAGATCCCGGTCATCGTCGCCGGGGGCCGTGCCCCAGGCA
GGATGCTCCTCTGCGGCACCGTCATCCCGGTGATCATCTACTCTTACCAGTTCAAGAGCGCCATGAATCCGTACCAGCTCCTCGCCCTCTACACCC
TACACATCTACTTCTCGCTGGATCTCCTGCTGGCCACCGTGCACGCCGTGATCCACGACCTGCTGGGGATGGAGATGGAGCCACAGGTGGACCGC
CCGTGGCTGGCGTCGTCGCTGCGGGAGTTCTGGGGCCGGCGGTGGAACCTCATGGTGCCGTCCATCCTCCGGCCGTCGGTGTTCCGCCCCGTGCGC
GCGCGCCTTGGCGTCGCCGCCGGCGTGCTGGCCACGTTCCTCGTCTCCGGGCTCATGCACGAGCTCATATTCTACTACCTCATGCGGAGCAAGCCC
ACCGGCCAGGTGACCGCCTTCTTCCTCCTTCACGGGGCGTGCGCGGCGGCGGAGGGATGGTGGGCGTCGCACGCAGGGTGGTGGAGGCCGCCGC
GGGCCGCCGCCGTGCCGCTGACGCTGGCATTCGTGGCCGGGACGGCGTTCTGGCTCTTCTTCCCTGCCATGATCAAGGGCGGCCTCGACGAGATG
GCGCTGCACGAGTGCCAGGGCATGGTGGCGCTCATGGAGCAGGTCGGCCGGCGGCTCGCCGGCGCTACAGATCTCGTCTCCGCACGCGGCACCGC
CGCCTTTTTCCTCGTCTGGCTCGGCGAGTTCAAGCTCCTGCTGCTCGCGTCCNNNNNNNNNNNNNNNNNNNNNNNNNNNATCCGCTCCCATTCG
TGTTCACGGGGGCGCTGCCGGTCAAGCTCCTTCGTCACAAGAGGCCTGCTGATCACGGCGCCGATGCCGCCGTCAAGACGACCAAGACCGCCGCC
GCTGCTGCTCTGCCTTTCCTGTCGTCGAGCATCAAGCTCGCCGTCATGGTGGCCCTGGCCTACGTGTTCCGACGCAAGGACCAGATGCACCCGTAC
GTGACCTCCACCCTGTACGCCGTCTTCATCTACTGCAACCTCGACTTCGTCCTGCCGTGCGTCGCCGCGGTGGGCAGGGCGGCGCTGGGCATGGAG
CTGGAGCCGCAGTTCGACCGGCCGTACCTCTCGGCCTCGCTGGGGGAGTTCTGGGGCCGGCGCTGGAACCTCATGGTCTCGGCGGTGCTCCGCCC
CTCCGTCTACGCCCCCGTCCGCGCGCGCCTCGGCGCCGCCGCCGCCGTGCTCACCACGTTCCTCGCGTCGGGGCTCATGCACGAGGTGGTCGTGTA
CTACGTCACCTTCCGGCCGCCGACGGGGGAGATCACCGCGTTCTTCGCGCTGCACGGCGCCTCCCTGTGCGCCGAAAAGTGGTGGTGCGCCCGGA
CCCGGAGGTTCGGAGGCCATGCGCCGCCAAGAGCTGTGGCCGCGCCGCTCGTCCTGGCGTACGTGGCCGCCACGGCGTTCTGGCTCTTCTTCCCCG
CGCTCTTCGGGGGCGGGATGGACGACCGATTCATCGCCGAGATGAACGCGTTACTGTCCGCCGTCGTGGATTCCGGAGGGCGACTGCTCCGGTGG
GCTAGGAGCTAG

>Cp-PSAT1 (V2_contig_9910)

CATGATGATCCAGTTCTGAATCCTTTAACTCCTTGGGATAGACCACCTCTGAAGAACATCTTCTGTATCTATGGAACAGATTCGAGAACTGAGGTT
GGTTACTATTTTGCACCAAGTGGAAAGCCTTATCCGGATAACTGGATGATTACAGATGTCATATATGAAGTAGAAGGATCTCTATATTCCAGGTCA
GGGAATCTTGTAGAAGGGAACCCTGGTGCTACAAGTGGGGACGATACGGTTCCATACCATTCCCTCTCTTGGTGCAAGAATTGGCTCGGGTCAAA
AGTTAACATAACGAGGGCTCCCCAGTCAGAACACGACGGTTCAGACGTTCAAGTGCAACTAAATGTGGACCGTGAGCCCGAAATGGACATAGTTC
CCAACATGACGAGATCACCTAGAGTGAAGTACATAACCTACTACGAAGACTCTGAAAGTCTACAGGGAAAGAGGACTGCAGTTTGGGAGATTGA
CAAAGCTAACCATAGGAACATCGTAAGGTCTTCAGTCCTAATGCGCGAGCTGTGGCTCGAGACATGGCACGATACACATCCAGACAAAAAAACC
AAATTTGTTACGAAAGCTACTCGCGGTCCTCTTAGGGATGACGACTGCTATTGGGACTATGGAAAAGCACGGTGTGCTTGGCCCGAGTATTGTGA
ATACAGGTATCTATTTGGAGATGTTCACCTAGGGCAGAGCTGTAGGATAAAGAACACTCCGGCTGACCTATTGACACGTTATGTGTAATGTAAGTT
TGCATATGGCTTATTAGTTATTACCTTAGCTTATCTCAGGCTAAGAATTTTGTGAGAAAACATTCATTACATTACATAGCCTAAAAAACACACCAA
GAACAAGACTCTGTTCTGACTCTTTGCATTACATTTTAACCGATACGCCTCGAAAACCTTTTCCTCAAACTCGCCACGACGCATATACGCAAAAAA
AAAAGAGAAAAAAATCCCGTCTTTATTGACCTAACACCTAACGACGGGCCACCCGAAAAAAAAAAAAAAAAAAAAAAAACTAT

>Cp-ASAT1 (V2_contig_28022)

CCTCCTTCCACCTATGTGGGCCGACAACCTTCTACCTCGTTTGGCTCGCTAACTTCAAACTCCTCCTATTCGCCTTCAATAAGGGACCCCTCTTCTC
AACCCCACCTCTCAACTTGCTCCGCTTCGTCGCCGTAGCCCTACTCCCCATCAAGATCGCCGGAAACGCCGACGAACACCGTCAAGAAGAGCCCC
AGCGCCGGCGCCGCCGTGCGTACTCGTTTCTTCTTTATCTGGTCAAGTCCGCGGCGCTGGCGGCTATCGTGGGTCTGTACAGGTACAGAGAGCTCA
TGAGCCGTTACGTGCTGCTGGCCCTTTACTGCTGCCACGTGTACCTCGGCGTCGAGCTCGTCCTAGCCGTGACGGCGGTTCCCGTCCGAGCCATCT
TAGGGCTGGAAATCGAGCCACAGTTCGACGAGCCGTACTTGGCCACGTCGCTTCAAGATTTCTGGGGCCATAGGTGGAATCTCATGGTGAGCAGT
ATTCTACGGTCGACGGTGTACGGCCCCGTCCGATCTCTTTGCGGCCGTACGGCTGGGTTGCTGGCGACTTTCTTGGTCTCAGGGCTGATGCACGAG
GTGATTTATTACTACGTGTCGCGTGCGAGTCCCACGTGGGAGGTGACGTGGTTCTTTGTCCTCCATGGGTTTTGTCTGGCCGTCGAGGTATGGCTG
AAGGAGAGGTTGGGCGGGCGGCGGTGGAGGTTGAATAGGGTTGTTTCTGGGATTCTCACGG

>**Lb-PSAT1** (018442-RA transcript)

CGGTCATTAAGCAAATAAATCCCCCAGGCCCAAAACCTCGATCCCATTCCCATGCTACAGTACATAGCTACCCCCCATCATCCGACTTGCTAAAGT
TTTTATCCTGGAAAAGGGCAATGAGAGGCTGCCACGTAGCACTCGTCGCCGCCGCGTACCTCGCGATTTTCCACCCGGCGTACGGCGGCGATTCA
GGTCTCGACCAAAGCAAGCTCTCCGGGATAATAATACCGGGGTTCGCGTCCACGCAGCTACGCGCCTGGTCTATCTTGGATTGCCCGTACTCTCCT
CTCGATTTCAACCCTCTCGATTTGGTCTGGCTCGATACCACTAAGCTACTATCTGCGGTAAATTGTTGGCTCAAGTGTATGCTGTTAGACCCATACA
ACCAAACGGATCATTCGGAATGCAAATCGCGTCCAGATAGTGGCCCCCTTTCTTCAGTATGGAAAGAATGGCTCAAGTGGTGCATTGAGTTTGGC
ATTGAGGCTAATGCTATTATTGCTGTTCCATATGACTGGAGATTGCCGCCTTCTAAACTTGAGGATCGGGACCTTTATTTTCACAAATTGAAGCTTA
TTTTCGAAACTGCTCTTAAGCTTCGAGGTGGGCCAACTCTTGTTTTTGCTCACTCATTGGGCAACAATGTCTTCAGATACTTCTTGGAATGGTTAAA
ATTAGAAATCGCTCCTAAGCAGTACATGAAGTGGCTGGACGACCATATTCATGCCTACTTTGCCGTTGGTGCTCCACTACTTGGGTCAGTTGAAAC
CATCAAAGGAACGCTATCCGGAGTTACATTTGGTCTCCCTGTTTCAGAGGGAACTGCTCGATTGATGTTCAATTCATTTGGTTCTTCTGTATGGATG
ATGCCATTTACGAAACACTGTAGAGCAGAGAATCTATACCTGAAGCATTTTTCTTCTGGGATAAGAAAGGGGCATTTCGCTTACCATTGCAGCAA
GCATGAATTTGAAGCCAACTACTCTGGATGGCCAACGAATGTAGTCAACGTGGAGATCCCATCAAGTAGAGGGGATGAAGCTTATCCGTCATTTA
TGGGGCCGGGTCAGGTCAATGTTTCTGACATGGATTATGGATTGCCAACTCAGCTCTCCTTTTCGGCTCGTGAAATAGCTGATGGGACATTCTTTA
GAGCTATAAATGATTATGATCCTGACAGTCATAGAGTCTTGCACCTTTTAGAGAAGTTATATCATGATGATCCAGTTCTGAATCCTTTGACTCCTTG
GGATAGACCACCTCTGAAGAACATCTTCTGTATTTATGGAACAGATATGAGAACTGAGGTTGGTTACTATTTTGCACCAAGTGGAAAGCCGTATCC
TGATAACTGGATGATTACGGATGTAATATATGAATTAGAAGGATCTCTATACTCCAGGTCAGGGAATCTTGTGGAGGGGAACCCTGGTGCCACAA
GCGGGGACGAGACGGTTCCATACCATTCCCTCTCTTGGTGCAAGAATTGGCTTGGGTCAAAAGTGAACATAACAAGGGCGCCCCAGTCAGAGCAC
GATGGCTCAGATGTTCAAGTGCAGTTAAATGTTGATCGTGAGCCTGAAATGGATATATTTCCTAACATGACGAAAAAGTCCCCCAGGGTGAAGTA
CATAACTTACTACGAAGACTCTGAAAGTCTACCGGGAAAGAGGACTGCAGTTTGGGAGATTGACAAAGCTAATCATAGGAACATTGTAAGGTCTT
CGGCCTTGATGCGTGAGCTGTGGCTCGAGACATGGCACGATACACATCCAGACAAAAAATCCAAATTTGTTACAAAAGCTACTCGTGGTCCTCTT
AGGGACGACGATTGCTATTGGGACTACGGAAAAGCACGGTGTGCTTGGCCCGAGTATTGCGAATATAGGTATCTATTTGGAGATGTCCACCTTGG
GCAGAGCTGTAGGATAAAGGACACTTCGGCTAACCTATTGTTGCGTTATGTGTAAGTTCTCAGGATCTCATTCATTACACATTAGTGTCGTAATTC
ATTTCATTCACAATAAAGAGGTACTTCCACAACTATATCAATCTAAACAAAACTAGAAAACAGTGGTACCACTCAAGAATTTGTGAACTTTTCACC
GTTGTATAAAATGTGAACTTGTCGGACGAATTGCATACAAATAAATAGAGTTGGTCCATAA

>**Ls-PSAT1** (013445-RA transcript)

GCGACAGATCTCTCCCATCATTCGACTTGCAAACGTTTCTGAACTTTTATCTGGGAAAAGGTCAATGAGAGGCTGCCACGTCGCGCTCGTCGCCGC
CGCGTACCTTCTGATTCTCCCGCCAGCGTGCGGCGGCGAGTCAGGTCTCGACCACAGTAAGCTGTCCGGGATAATAATTCCGGGGTTCGCGTCCAC
GCAGCTGCGCGCGTGGTCTATCTTGGATTGCCCTTACTCTCCTCTTGATTTTAACCCTCTCGATTTGGTCTGGCTCGATACTACTAAGCTACTATCTG
CCGTGAATTGTTGGCTTAAGTGTATGCTCTTAGATCCATATAACCAAACGGATCATTCAGAATGCAAATCACGTCCAGATAGTGGTCCCCTTTCTT
CAGTATGGAAAGAATGGCTTAAGTGGTGCATTGAGTTTGGCATTGAACCTAATGCTATTATTGCTGTTCCATATGACTGGAGATTACCGCCTTCCA
AACTTGAGGATCGGGACCTTTATTTTCACAAATTGAAGCTTATTTTTGAGACTGCTCTTAAGCTTCGAGGGGGGCCAACTCTTGTTTTTGCTCACTC
ATTGGGCAACAATGTCTTCCGATACTTCTTGGAATGGTTAAAATTAGAAATCGCTCCTAAGCAGTACTTGAAATGGCTAGACGAACATATTCATGC
CTACTTTGCTGTTGGAGCTCCACTACTTGGGTCAGTTGAAACTGTCAAAGCAACATTATCGGGATTTACATTTGGTCTGCCTGTCTCAGAGGGAAC
TGCTCGATTGATGTTCAATTCATTTGGTTCTTCTGTATGGATGATGCCATTTTCAAAACACTGTAGAGCAGAGAACTTATACCTGAAGCATTTTTCT
TCTGGGATAAGAAAGGGGCATCTCGCATACCATTGCAGCAAGCATGAATTTGAAGCCAACTACTCTGGATGGCCAACGAACATAGTCAATGTCGA
GATCCCATCAAGTAGAGGTATGAAACTCAGCTCGTCAGCTTTAAGATTTAATATGCTAACGCATATGCATTACAATACCTCTCCAGGGGATGAAG
CTTATCCGTCATTTATGGGGCCAGTTCAGCTCAATGTTTCGGACATGGATTATGGACTACCAACTCAGATGTCCTTTTCGGCTCGTGAAATAGCCG
ATGGGACATTCTTTAGAGCTATAAATGATTATGATCCCGACAGTCATAGAGTCTTGCACCTTTTAGAGAAGTCATATCATGATGATCCGGTTCTGA
ATCCTTTAACTCCTTGGGATAGACCACCTCTGAAGAACATCTTCTGTATTTATGGAACAGATTCGAGAACTGAGGTTGGTTACTATTTTGCGCCAA
GTGGAAAACCTTATCCTGATAACTGGATGATTACGGATGTAATATATGAAGTAGAAGGGTCTCTATATTCCAGGTCGGGGAATCTTGTAGAGGGG
AACCCTGGTGCTTCAAGCGGGGATGAGACGGTTCCATACCATTCCCTCTCTTGGTGCAAGAATTGGCTTGGGTCAAAAGTGAACATAACGAGGGC
TCCCCAGTCAGAACACGATGGTTCAGATGTTCAAGTGCATTTAAATGTGGATCGTGAGCCCGAAATGGATGTAGTTCCCAACATGACGAGATCCC
CTAGAGTTAAGTACATAACCTACTACGAAGACTCTGAAAGTCTACCCGGAAAGAGGACTGCAGTTTGGGAGATTGACAAAGCTAACCACAGGAA
CATTGTAAGGTCTTCAGTCTTGATGCGAGAGCTATGGCTCGAGACATGGCACGATACTCATCCCGACAAAAAAACCAAATTTGTTACGAAAGGGC
TCAATTGGCTGAGTCTCTTCAAGAACCTCAGCAGCTACAAAACATTCAGCAGCTTCCAATGA

6. References

Aymé, L., Arragain, S., Canonge, M., Baud, S., Touati, N., Bimai, O., Jagic, F., Louis-Mondésir, C., Briozzo, P., Fontecave, M., Chardot, T., 2018. Arabidopsis thaliana DGAT3 is a [2Fe-2S] protein involved in TAG biosynthesis. Sci. Rep. 8, 1–10. https://doi.org/10.1038/s41598-018-35545-7

Aymé, L., Baud, S., Dubreucq, B., Joffre, F., Chardot, T., 2014. Function and localization of the Arabidopsis thaliana diacylglycerol acyltransferase DGAT2 expressed in yeast. PLoS One 9, 1–9. https://doi.org/10.1371/journal.pone.0092237

Ball, S.G., Morell, M.K., 2003. From Bacterial Glycogen to Starch: Understanding the Biogenesis of the Plant Starch Granule. Annu. Rev. Plant Biol. 54, 207–233. https://doi.org/10.1146/annurev.arplant.54.031902.134927

Barnes, A.C., Benning, C., Roston, R.L., 2016. Chloroplast membrane remodeling during freezing stress is accompanied by cytoplasmic acidification activating SENSITIVE TO FREEZING2. Plant Physiol. 171, 2140–2149. https://doi.org/10.1104/pp.16.00286

Barnes, A.C., Elowsky, C.G., Roston, R.L., 2019. An Arabidopsis protoplast isolation method reduces cytosolic acidification and activation of the chloroplast stress sensor SENSITIVE TO FREEZING 2. Plant Signal. Behav. 14, e1629270. https://doi.org/10.1080/15592324.2019.1629270

Barron, E.J., Stumpf, P.K., 1962. Fat metabolism in higher plants XIX. The biosynthesis of triglycerides by avocado-mesocarp enzymes. BBA - Biochim. Biophys. Acta 60, 329–337. https://doi.org/10.1016/0006-3002(62)90408-0

Bartels, D., 2005. Desiccation tolerance studied in the resurrection plant Craterostigma plantagineum. Integr. Comp. Biol. 45, 696–701. https://doi.org/10.1093/icb/45.5.696

Bates, P.D., Browse, J., 2012. The significance of different diacylgycerol synthesis pathways on plant oil composition and bioengineering. Front. Plant Sci. 3. https://doi.org/10.3389/fpls.2012.00147

Beller, M., Thiel, K., Thul, P.J., Jäckle, H., 2010. Lipid droplets: A dynamic organelle moves into focus. FEBS Lett. 584, 2176–2182. https://doi.org/10.1016/j.febslet.2010.03.022

Bennetzen, J.L., Schmutz, J., Wang, H., Percifield, R., Hawkins, J., Pontaroli, A.C., Estep, M., Feng, L., Vaughn, J.N., Grimwood, J., Jenkins, J., Barry, K., Lindquist, E., Hellsten, U., Deshpande, S., Wang, X., Wu, X., Mitros, T., Triplett, J., Yang, X., Ye, C.Y., Mauro-Herrera, M., Wang, L., Li, P., Sharma, M., Sharma, R., Ronald, P.C., Panaud, O., Kellogg, E.A., Brutnell, T.P., Doust, A.N., Tuskan, G.A., Rokhsar, D., Devos, K.M., 2012. Reference genome sequence of the model plant Setaria. Nat. Biotechnol. 30, 555–561. https://doi.org/10.1038/nbt.2196

Bernacchia, G., Salamini, F., Bartels, D,，1996. Molecular Characterization of the Rehydration Process in the Resurrection PIant Craterostigma plantagineum. Plant Physiol,111: 1043-1 050. https:// doi: 10.1104/pp.111.4.1043.

Blée, E., Boachon, B., Burcklen, M., Le Gaé, M., Abdulsamie, H., Heintz, D., Ehlting, J., Herrfurth, C., Feussner, I., Bessoule, J.J., 2014. The Reductase Activity of the Arabidopsis Caleosin RESPONSIVE TO DESSICATION20 Mediates Gibberellin-Dependent Flowering Time, Abscisic acid sensitivity, And Tolerance to Oxidative Stress1[w]. Plant Physiol. 166, 109–124. https://doi.org/10.1104/pp.114.245316

Blomstedt, C.K., Griffiths, C.A., Fredericks, D.P., Hamill, J.D., Gaff, D.F., Neale, A.D., 2010. The resurrection plant Sporobolus stapfianus: An unlikely model for engineering enhanced plant biomass? Plant Growth Regul. 62, 217–232. https://doi.org/10.1007/s10725-010-9485-6

Bouic, P.J.D., Clark, A., Lamprecht, J., Freestone, M., Pool, E.J., Liebenberg, R.W., Kotze, D., Van Jaarsveld, P.P., 1999. The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: Inhibition of post marathon immune suppression and inflammation. Int. J. Sports Med. 20, 258–262. https://doi.org/10.1055/s-2007-971127

Cao, Y., Zhao, L., Ying, Y., Kong, X., Hua, Y., Chen, Y., 2015. The characterization of soybean oil body integral oleosin isoforms and the effects of alkaline pH on them. Food Chem. 177, 288–294. https://doi.org/10.1016/j.foodchem.2015.01.052

Carrão, H., Naumann, G., Barbosa, P., 2016. Mapping global patterns of drought risk: An empirical framework based on sub-national estimates of hazard, exposure and vulnerability. Glob. Environ. Chang. 39, 108–124. https://doi.org/10.1016/j.gloenvcha.2016.04.012

Chen, J.C.F., Tsai, C.C.Y., Tzen, J.T.C., 1999. Cloning and secondary structure analysis of caleosin, a unique calcium-binding protein in oil bodies of plant seeds. Plant Cell Physiol. 40, 1079–1086. https://doi.org/10.1093/oxfordjournals.pcp.a029490

De Kroon, A.I.P.M., Rijken, P.J., De Smet, C.H., 2013. Checks and balances in membrane phospholipid class and acyl chain homeostasis, the yeast perspective. Prog. Lipid Res. 52, 374–394. https://doi.org/10.1016/j.plipres.2013.04.006

Deeba, F., Pandey, A.K., Pandey, V., 2016. Organ specific proteomic dissection of selaginella bryopteris undergoing dehydration and rehydration. Front. Plant Sci. 7, 1–20. https://doi.org/10.3389/fpls.2016.00425

Deeba, F., Pandey, V., 2017. Adaptive mechanisms of desiccation tolerance in resurrection plants, Plant Adaptation Strategies in Changing Environment. https://doi.org/10.1007/978-981-10-6744-0_2

Devers, E.A., Wewer, V., Dombrink, I., Dörmann, P., Hölzl, G., 2011. A processive glycosyltransferase involved in glycolipid synthesis during phosphate deprivation in Mesorhizobium loti. J. Bacteriol. 193, 1377–1384. https://doi.org/10.1128/JB.00768-10

Dinakar, C., Bartels, D., 2013. Desiccation tolerance in resurrection plants: New insights from transcriptome, proteome, and metabolome analysis. Front. Plant Sci. 4, 1–14. https://doi.org/10.3389/fpls.2013.00482

Dörmann, P., Balbo, I., Benning, C., 2008. The role of phytosterols in plant adaptation to temperature. Plant Signal. Behav. 3, 133–134. https://doi.org/10.4161/psb.3.2.5051

Deeba, F., Pandey, A.K., Pandey, V., 2016. Organ specific proteomic dissection of selaginella bryopteris undergoing dehydration and rehydration. Front. Plant Sci. 7, 1–20. https://doi.org/10.3389/fpls.2016.00425

Deeba, F., Pandey, V., 2017. Adaptive mechanisms of desiccation tolerance in resurrection plants, Plant Adaptation Strategies in Changing Environment. https://doi.org/10.1007/978-981-10-6744-0_2

Devers, E.A., Wewer, V., Dombrink, I., Dörmann, P., Hölzl, G., 2011. A processive glycosyltransferase involved in glycolipid synthesis during phosphate deprivation in Mesorhizobium loti. J. Bacteriol. 193, 1377–1384. https://doi.org/10.1128/JB.00768-10

Dlouhý O, Kurasová I, Karlický V, Javornik U, Šket P, Petrova NZ, Krumova SB, Plavec J, Ughy B, Špunda V, Garab G. Modulation of non-bilayer lipid phases and the structure and functions of thylakoid membranes: effects on the water-soluble enzyme violaxanthin de-epoxidase. Sci Rep. 2020 Jul 20;10(1):11959. https://doi: 10.1038/s41598-020-68854-x

Eastmond, P.J., 2006. Sugar-dependent1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell 18, 665–675. https://doi.org/10.1105/tpc.105.040543

Eichmann, T.O., Lass, A., 2015. DAG tales: The multiple faces of diacylglycerol - Stereochemistry, metabolism, and signaling. Cell. Mol. Life Sci. 72, 3931–3952. https://doi.org/10.1007/s00018-015-1982-3

Farese, R. V., Walther, T.C., 2009. Lipid Droplets Finally Get a Little R-E-S-P-E-C-T. Cell 139, 855–860. https://doi.org/10.1016/j.cell.2009.11.005

Ferrer, A., Altabella, T., Arró, M., Boronat, A., 2017. Emerging roles for conjugated sterols in plants. Prog. Lipid Res. 67, 27–37. https://doi.org/10.1016/j.plipres.2017.06.002

Frandsen, G.I., Mundy, J., Tzen, J.T.C., 2001. Oil bodies and their associated proteins, oleosin and caleosin. Physiol. Plant. 112, 301–307. https://doi.org/10.1034/j.1399-3054.2001.1120301.x

Gaff, D.F., Blomstedt, C.K., Neale, A.D., Le, T.N., Hamill, J.D., Ghasempour, H.R., 2009. Sporobolus stapfianus, a model desiccation-tolerant grass. Funct. Plant Biol. 36, 589–599. https://doi.org/10.1071/FP08166

Garab G, Lohner K, Laggner P, Farkas T. Self-regulation of the lipid content of membranes by non-bilayer lipids: a hypothesis. Trends Plant Sci. 2000 Nov;5(11):489-94. https://doi: 10.1016/s1360-1385(00)01767-2

Gasulla, F., Vom Dorp, K., Dombrink, I., Zähringer, U., Gisch, N., Dörmann, P., Bartels, D., 2013. The role of lipid metabolism in the acquisition of desiccation tolerance in Craterostigma plantagineum: A comparative approach. Plant J. 75, 726–741. https://doi.org/10.1111/tpj.12241

Giarola, V., Chen, P., Dulitz, S.J., König, M., Manduzio, S., Bartels, D., 2020. The dehydration- and ABA-inducible germin-like protein CpGLP1 from Craterostigma plantagineum has SOD activity and may contribute to cell wall integrity during desiccation. Planta 252, 1–13. https://doi.org/10.1007/s00425-020-03485-0

Gidda, S.K., Park, S., Pyc, M., Yurchenko, O., Cai, Y., Wu, P., Andrews, D.W., Chapman, K.D., Dyer, J.M., Mullen, R.T., 2016. Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. Plant Physiol. 170, 2052–2071. https://doi.org/10.1104/pp.15.01977

González L., González-Vilar M., 2001. Determination of Relative Water Content. In: Reigosa Roger M.J. (eds) Handbook of Plant Ecophysiology Techniques. Springer, Dordrecht. https://doi.org/10.1007/0-306-48057-3_14 Online ISBN: 978-0-306-48057-7

Grigore MN, Ivanescu L, Toma C, Book: An Integrative Anatomical Study,

Heilmann, I., 2016. Phosphoinositide signaling in plant development. Dev. 143, 2044–2055. https://doi.org/10.1242/dev.136432

Hemmerlin, A., Harwood, J.L., Bach, T.J., 2012. A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? Prog. Lipid Res. 51, 95–148. https://doi.org/10.1016/j.plipres.2011.12.001

Hsiao, E.S.L., Tzen, J.T.C., 2011. Ubiquitination of oleosin-H and caleosin in sesame oil bodies after seed germination. Plant Physiol. Biochem. 49, 77–81. https://doi.org/10.1016/j.plaphy.2010.10.001

Huang, A.H.C., 1992. Oil bodies and oleosins in seeds. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 177–200. https://doi.org/10.1146/annurev.pp.43.060192.001141

Huang, M. Der, Huang, A.H.C., 2015. Bioinformatics reveal five lineages of oleosins and the mechanism of lineage evolution related to structure/function from green algae to seed plants. Plant Physiol. 169, 453–470. https://doi.org/10.1104/pp.15.00634

Hubbe, M.A., Chandra, R.P., Dogu, D., Van Velzen, S.T.J., 2019. Analytical staining review. BioResources 14, 7387–7464.

Ibl, V., Stoger, E., 2012. The formation, function and fate of protein storage compartments in seeds. Protoplasma 249, 379–392. https://doi.org/10.1007/s00709-011-0288-z

Jones, N., Pašakinskiene, I., 2005. Genome conflict in the gramineae. New Phytol. 165, 391–410. https://doi.org/10.1111/j.1469-8137.2004.01225.x

Joyard, J., Teyssier, E., Miège, C., Berny-Seigneurin, D., Marèchal, E., Block, M.A., Dorne, A.J., Rolland, N., Ajlani, G., Douce, R., 1998. The biochemical machinery of plastid envelope membranes. Plant Physiol. 118, 715–723. https://doi.org/10.1104/pp.118.3.715

June, C., Page, S.E.E.L., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T.A., Minx, P., Reily, A.D., Courtney, L., Kruchowski, S.S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., Rock, S.M., Belter, E., Du, F., Kim, K., Abbott, R.M., Cotton, M., Levy, A., Marchetto, P., Ochoa, K., Jackson, S.M., Gillam, B., Chen, W., Yan, L., Higginbotham, J., Cardenas, M., Waligorski, J., Applebaum, E., Phelps, L., Falcone, J., Kanchi, K., Thane, T., Scimone, A., Thane, N., Henke, J., Wang, T., Ruppert, J., Shah, N., Rotter, K., Hodges, J., Ingenthron, E., Cordes, M., Kohlberg, S., Sgro, J., Delgado, B., Mead, K., Chinwalla, A., Leonard, S., Crouse, K., Collura, K., Kudrna, D., Currie, J., He, R., Angelova, A., Rajasekar, S., Mueller, T., Lomeli, R., Scara, G., Ko, A., Delaney, K., Wissotski, M., Lopez, G., Campos, D., Braidotti, M., Ashley, E., Golser, W., Kim, H., Lee, S., Lin, J., Dujmic, Z., Kim, W., Talag, J., Zuccolo, A., Fan, C., Sebastian, A., Kramer, M., Spiegel, L., Nascimento, L., Zutavern, T., Miller, B., Ambroise, C., Muller, S., Spooner, W., Narechania, A., Ren, L., Wei, S., Kumari, S., 2012. The B73 Maize Genome: Complexity, Diversity, and Dynamics. Science (80-.). 326, 1112–1115.

Jung, N.U., Giarola, V., Chen, P., Knox, J.P., Bartels, D., 2019. Craterostigma plantagineum cell wall composition is remodelled during desiccation and the glycine-rich protein CpGRP1 interacts with pectins through clustered arginines. Plant J. 100, 661–676. https://doi.org/10.1111/tpj.14479

Kawakatsu, T., Takaiwa, F., 2010. Cereal seed storage protein synthesis: Fundamental processes for recombinant protein production in cereal grains. Plant Biotechnol. J. 8, 939–953. https://doi.org/10.1111/j.1467-7652.2010.00559.x

Kelly, A.A., Froehlich, J.E., Dörmann, P., 2003. Disruption of the Two Digalactosyldiacylglycerol Synthase Genes. Plant Cell 15, 2694–2706. https://doi.org/10.1105/tpc.016675.amount

Kemp, R.J., Mercer, E.I., 1968. The sterol esters of maize seedlings. Biochem. J. 110, 111–118. https://doi.org/10.1042/bj1100111

Klaus, D., Härtel, H., Fitzpatrick, L.M., Froehlich, J.E., Hubert, J., Benning, C., Dörmann, P., 2002. Digalactosyldiacylglycerol synthesis in chloroplasts of the Arabidopsis dgd1 mutant. Plant Physiol. 128, 885–895. https://doi.org/10.1104/pp.010780

Kobayashi, K., & Wada, H. 2016. Role of Lipids in Chloroplast Biogenesis. Sub-cellular biochemistry, 86, 103–125. https://doi.org/10.1007/978-3-319-25979-6_5

Landi, S., Hausman, J.F., Guerriero, G., Esposito, S., 2017. Poaceae vs. Abiotic stress: Focus on drought and salt stress, recent insights and perspectives. Front. Plant Sci. 8, 01214. https://doi.org/10.3389/fpls.2017.01214

Lawson, T., Blatt, M.R., 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol. 164, 1556–1570. https://doi.org/10.1104/pp.114.237107

Layerenza JP, Gonzalez P, Garcia de Bravo MM, Polo MP, Sisti MS, Ves-Losada A., 2013. Nuclear lipid droplets: a novel nuclear domain. Biochim Biophys 1831:327–340. [PubMed: 23098923]

Le, T.N., McQueen-Mason, S.J., 2006. Desiccation-tolerant plants in dry environments. Rev. Environ. Sci. Biotechnol. 5, 269–279. https://doi.org/10.1007/s11157-006-0015-y

Lersten, N.R., Czlapinski, A.R., Curtis, J.D., Freckmann, R., Horner, H.T., 2006. Oil bodies in leaf mesophyll cells of angiosperms: Overview and a selected survey. Am. J. Bot. 93, 1731–1739. https://doi.org/10.3732/ajb.93.12.1731

Leyland, B., Boussiba, S., Khozin-Goldberg, I., 2020. A Review of Diatom Lipid Droplets. Biology (Basel). 9, 38. https://doi.org/10.3390/biology9020038

Lin, L.J., Tai, S.S.K., Peng, C.C., Tzen, J.T.C., 2002. Erratum: Steroleosin, a sterol-binding dehydrogenase in seed oil bodies (Plant Physiology (2002) 128 (1200-1211)). Plant Physiol. 129, 1930. https://doi.org/10.1104/pp.010982

Liping, W., Shen, W., Kazachkov, M., Chen, G., Chen, Q., Carlsson, A.S., Stymne, S., Weselake, R.J., Zou, J., 2012. Metabolic interactions between the lands cycle and the kennedy pathway of glycerolipid synthesis in arabidopsis developing seedsw. Plant Cell 24, 4652–4669. https://doi.org/10.1105/tpc.112.104604

Lippold, F., vom Dorp, K., Abraham, M., Hölzl, G., Wewer, V., Yilmaz, J.L., Lager, I., Montandon, C., Besagni, C., Kessler, F., Stymne, S., Dörmann, P., 2012. Fatty acid phytyl ester synthesis in chloroplasts of Arabidopsis. Plant Cell 24, 2001–2014. https://doi.org/10.1105/tpc.112.095588

Liu, H., Osborne, C.P., 2015. Water relations traits of C4 grasses depend on phylogenetic lineage, photosynthetic pathway, and habitat water availability. J. Exp. Bot. 66, 761–773. https://doi.org/10.1093/jxb/eru430

Liu, Z., Fan, M., Li, C., Xu, J.H., 2019. Dynamic gene amplification and function diversification of grass-specific O-methyltransferase gene family. Genomics 111, 687–695. https://doi.org/10.1016/j.ygeno.2018.04.005

Lung, S.C., Liao, P., Yeung, E.C., Hsiao, A.S., Xue, Y., Chye, M.L., 2017. Acyl-CoA-binding protein ACBP1 modulates sterol synthesis during embryogenesis. Plant Physiol. 174, 1420–1435. https://doi.org/10.1104/pp.17.00412

Mascher, M., Gundlach, H., Himmelbach, A., Beier, S., Twardziok, S.O., Wicker, T., Radchuk, V., Dockter, C., Hedley, P.E., Russell, J., Bayer, M., Ramsay, L., Liu, H., Haberer, G., Zhang, X.Q., Zhang, Q., Barrero, R.A., Li, L., Taudien, S., Groth, M., Felder, M., Hastie, A., Šimková, H., Stanková, H., Vrána, J., Chan, S., Munõz-Amatriaín, M., Ounit, R., Wanamaker, S., Bolser, D., Colmsee, C., Schmutzer, T., Aliyeva-Schnorr, L., Grasso, S., Tanskanen, J., Chailyan, A., Sampath, D., Heavens, D., Clissold, L., Cao, S., Chapman, B., Dai, F., Han, Y., Li, H., Li, X., Lin, C., McCooke, J.K., Tan, C., Wang, P., Wang, S., Yin, S., Zhou, G., Poland, J.A., Bellgard, M.I., Borisjuk, L., Houben, A., Doleael, J., Ayling, S., Lonardi, S., Kersey, P., Langridge, P., Muehlbauer, G.J., Clark, M.D., Caccamo, M., Schulman, A.H., Mayer, K.F.X., Platzer, M., Close, T.J., Scholz, U., Hansson, M., Zhang, G., Braumann, I., Spannagl, M., Li, C., Waugh, R., Stein, N., 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature 544, 427–433. https://doi.org/10.1038/nature22043

Matsumoto, T., Wu, J., Kanamori, H., Katayose, Y., Fujisawa, M., Namiki, N., Mizuno, H., Yamamoto, K., Antonio, B.A., Baba, T., Sakata, K., Nagamura, Y., Aoki, H., Arikawa, K., Arita, K., Bito, T., Chiden, Y., Fujitsuka, N., Fukunaka, R., Hamada, M., Harada, C., Hayashi, A., Hijishita, S., Honda, M., Hosokawa, S., Ichikawa, Y., Idonuma, A., Iijima, M., Ikeda, M., Ikeno, M., Ito, K., Ito, S., Ito, T., Ito, Yuichi, Ito, Yukiyo, Iwabuchi, A., Kamiya, K., Karasawa, W., Kurita, K., Katagiri, S., Kikuta, A., Kobayashi, H., Kobayashi, N., MacHita, K., Maehara, T., Masukawa, M., Mizubayashi, T., Mukai, Y., Nagasaki, H., Nagata, Y., Naito, S., Nakashima, M., Nakama, Y., Nakamichi, Y., Nakamura, M., Meguro, A., Negishi, M., Ohta, I., Ohta, T., Okamoto, M., Ono, N., Saji, S., Sakaguchi, M., Sakai, K., Shibata, M., Shimokawa, T., Song, J., Takazaki, Y., Terasawa, K., Tsugane, M., Tsuji, K., Ueda, S., Waki, K., Yamagata, H., Yamamoto, M., Yamamoto, S., Yamane, H., Yoshiki, S., Yoshihara, R., Yukawa, K., Zhong, H., Yano, M., Sasaki, T., Yuan, Q., Ouyang, S., Liu, J., Jones, K.M., Gansberger, K., Moffat, K., Hill, J., Bera, J., Fadrosh, D., Jin, S., Johri, S., Kim, M., Overton, L., Reardon, M., Tsitrin, T., Vuong, H., Weaver, B., Ciecko, A., Tallon, L., Jackson, J., Pai, G., Van Aken, S., Utterback, T., Reidmuller, S., Feldblyum, T., Hsiao, J., Zismann, V., Iobst, S., De Vazeille, A.R., Buell, C.R., Ying, K., Li, Y., Lu, T., Huang, Y., Zhao, Q., Feng, Q., Zhang, L., Zhu, J., Weng, Q., Mu, J., Lu, Y., Fan, D., Liu, Y., Guan, J., Zhang, Yujun, Yu, S., Liu, X., Zhang, Yu, Hong, G., Han, B., Choisne, N., Demange, N., Orjeda, G., Samain, S., Cattolico, L., Pelletier, E., Couloux, A., Segurens, B., Wincker, P., D'Hont, A., Scarpelli, C., Weissenbach, J., Salanoubat, M., Quetier, F., Yu, Y., Kim, H.R., Rambo, T., Currie, J., Collura, K., Luo, M., Yang, T.J., Ammiraju, J.S.S., Engler, F., Soderlund, C., Wing, R.A., Palmer, L.E., De La Bastide, M., Spiegel, L., Nascimento, L., Zutavern, T., O'Shaughnessy, A., Dike, S., Dedhia, N., Preston, R., Balija, V., McCombie, W.R., Chow, T.Y., Chen, H.H., Chung, M.C., Chen, C.S., Shaw, J.F., Wu, H.P., Hsiao, K.J., Chao, Y.T., Chu, M.K., Cheng, C.H., Hour, A.L., Lee, P.F., Lin, S.J., Lin, Y.C., Liou, J.Y., Liu, S.M., Hsing, Y.I., Raghuvanshi, S., Mohanty, A., Bharti, A.K., Gaur, A., Gupta, V., Kumar, D., Ravi, V., Vij, S., Kapur, A., Khurana, Parul, Khurana, Paramjit, Khurana, J.P., Tyagi, A.K., Gaikwad, K., Singh, A., Dalal, V., Srivastava, S., Dixit, A., Pal, A.K., Ghazi, I.A., Yadav, M., Pandit, A., Bhargava, A., Sureshbabu, K., Batra, K., Sharma, T.R., Mohapatra, T., Singh, N.K., Messing, J., Nelson, A.B., Fuks, G., Kavchok, S., Keizer, G., Llaca, E.L.V., Song, R., Tanyolac, B., Young, S., Ho, K., Hahn, J.H., Sangsakoo, G., Vanavichit, A., De Mattos, L.A.T., Zimmer, P.D., Malone, G., Dellagostin, O., De Oliveira, A.C., Bevan, M., Bancroft, I., Minx, P., Cordum, H., Wilson, R., Cheng, Z., Jin, W., Jiang, J., Leong, S.A., Iwama, H., Gojobori, T., Itoh, T., Niimura, Y., Fujii, Y., Habara, T., Sakai, H., Sato, Y., Wilson, G., Kumar, K., McCouch, S., Juretic, N., Hoen, D., Wright, S., Bruskiewich, R., Bureau, T., Miyao, A., Hirochika, H., Nishikawa, T., Kadowaki, K.I., Sugiura, M., Burr, B., 2005. The map-based sequence of the rice genome. Nature 436, 793–800. https://doi.org/10.1038/nature03895

Mayer, K.F.X., Marcussen, T., Sandve, S.R., Heier, L., Pfeifer, M., Kugler, K.G., Zhan, B., Spannagl, M., Pfeifer, M., Jakobsen, K.S., Wulff, B.B.H., Steuernagel, B., Olsen, O.-A., 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome Ancient hybridizations among the ancestral genomes of bread wheat Genome interplay in the grain transcriptome of hexaploid bread wheat Structural and functional pa. Science 345, 1250092.

Meza, I., Siebert, S., Döll, P., Kusche, J., Herbert, C., Rezaei, E.E., Nouri, H., Gerdener, H., Popat, E., Frischen, J., Naumann, G., Vogt, J. V., Walz, Y., Sebesvari, Z., Hagenlocher, M., 2020. Global-scale drought risk assessment for agricultural systems. Nat. Hazards Earth Syst. Sci. 20, 695–712. https://doi.org/10.5194/nhess-20-695-2020

Moellering, E.R., Muthan, B., Benning, C., 2010. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. Science (80-.). 330, 226–228. https://doi.org/10.1126/science.1191803

Mohammadi, S., Najafi, B., 2008. Prediction of Cetane Number of biodiesel fuel from fatty acid ethyl ester (FAEE) composition. Indian J. Sci. Technol. 22, 21–29. https://doi.org/10.17485/ijst/2015/v8i35/78272

Nakamura, Y., 2017. Plant Phospholipid Diversity: Emerging Functions in Metabolism and Protein–Lipid Interactions. Trends Plant Sci. 22, 1027–1040. https://doi.org/10.1016/j.tplants.2017.09.002

Naszradi, T., Badacsonyi, A., Keresztényi, I., Podar, D., Csintalan, Z., Tuba, Z., 2007. Comparison of two metal surveys by moss Tortula ruralis in Budapest, Hungary. Environ. Monit. Assess. 134, 279–285. https://doi.org/10.1007/s10661-007-9617-2

Nikiforidis, C. V., 2019. Structure and functions of oleosomes (oil bodies). Adv. Colloid Interface Sci. 274, 102039. https://doi.org/10.1016/j.cis.2019.102039

Norwood, M., Toldi, O., Richter, A., Scott, P., 2003. Investigation into the ability of roots of the poikilohydric plant Craterostigma plantagineum to survive dehydration stress. J. Exp. Bot. 54, 2313–2321. https://doi.org/10.1093/jxb/erg255

Ohsaki, Y., Kawai, T., Yoshikawa, Y., Cheng, J., Jokitalo, E., & Fujimoto, T. 2016. PML isoform II plays a critical role in nuclear lipid droplet formation. The Journal of cell biology, 212(1), 29–38. https://doi.org/10.1083/jcb.201507122

Oliver, M.J., Dowd, S.E., Zaragoza, J., Mauget, S.A., Payton, P.R., 2004. The rehydration transcriptome of the desiccation-tolerant bryophyte Tortula ruralis: Transcript classification and analysis. BMC Genomics 5, 1–19. https://doi.org/10.1186/1471-2164-5-89

Oliver, M.J., Murdock, A.G., Mishler, B.D., Kuehl, J. V., Boore, J.L., Mandoli, D.F., Everett, K.D.E., Wolf, P.G., Duffy, A.M., Karol, K.G., 2010. Chloroplast genome sequence of the moss Tortula ruralis: Gene content, polymorphism, and structural arrangement relative to other green plant chloroplast genomes. BMC Genomics 11, 143. https://doi.org/10.1186/1471-2164-11-143

Pacheco, C., Palla, C., Crapiste, G.H., Carrín, M.E., 2014. Simultaneous Quantitation of FFA, MAG, DAG, and TAG in Enzymatically Modified Vegetable Oils and Fats. Food Anal. Methods 7, 2013–2022. https://doi.org/10.1007/s12161-014-9830-x

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I. V., Lyons, E., Maher, C.A., Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M., Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Mehboob-Ur-Rahman, Ware, D., Westhoff, P., Mayer, K.F.X., Messing, J., Rokhsar, D.S., 2009. The Sorghum bicolor genome and the diversification of grasses. Nature 457, 551–556. https://doi.org/10.1038/nature07723

Pandey, V., Ranjan, S., Deeba, F., Pandey, A.K., Singh, R., Shirke, P.A., Pathre, U. V., 2010. Desiccation-induced physiological and biochemical changes in resurrection plant, Selaginella bryopteris. J. Plant Physiol. 167, 1351–1359. https://doi.org/10.1016/j.jplph.2010.05.001

Pappan, K., Zheng, S., Wang, X., 1997. Identification and characterization of a novel plant phospholipase D that requires polyphosphoinositides and submicromolar calcium for activity in Arabidopsis. J. Biol. Chem. 272, 7048–7054. https://doi.org/10.1074/jbc.272.11.7048

Pardo, J., Wai, C.M., Chay, H., Madden, C.F., Hilhorst, H.W.M., Farrant, J.M., VanBuren, R., 2020. Intertwined signatures of desiccation and drought tolerance in grasses. Proc. Natl. Acad. Sci. U. S. A. 117, 10079–10088. https://doi.org/10.1073/pnas.2001928117

Parthibane, V., Iyappan, R., Vijayakumar, A., Venkateshwari, V., Rajasekharan, R., 2012. Serine/threonine/tyrosine protein kinase phosphorylates oleosin, a regulator of lipid metabolic functions. Plant Physiol. 159, 95–104. https://doi.org/10.1104/pp.112.197194

Paswan, S.K., Gautam, A., Verma, P., Rao, C.V., Sidhu, O.P., Singh, A.P., Srivastava, S., 2017. The Indian magical Herb "Sanjeevni" (Selaginella bryopteris L.) - A promising anti-inflammatory phytomedicine for the treatment of patients with inflammatory skin diseases. J. Pharmacopuncture 20, 93–99. https://doi.org/10.3831/KPI.2017.20.012

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I. V., Lyons, E., Maher, C.A., Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M., Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Mehboob-Ur-Rahman, Ware, D., Westhoff, P., Mayer, K.F.X., Messing, J., Rokhsar, D.S., 2009. The Sorghum bicolor genome and the diversification of grasses. Nature 457, 551–556. https://doi.org/10.1038/nature07723

Pérez, S., Bertoft, E., 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. Starch/Staerke 62, 389–420. https://doi.org/10.1002/star.201000013

Phillips, J.R., Fischer, E., Baron, M., Van Den Dries, N., Facchinelli, F., Kutzer, M., Rahmanzadeh, R., Remus, D., Bartels, D., 2008. Lindernia brevidens: A novel desiccation-tolerant vascular plant, endemic to ancient tropical rainforests. Plant J. 54, 938–948. https://doi.org/10.1111/j.1365-313X.2008.03478.x

Pol, A., Gross, S.P., Parton, R.G., 2014. Biogenesis of the multifunctional lipid droplet: Lipids, proteins, and sites. J. Cell Biol. 204, 635–646. https://doi.org/10.1083/jcb.201311051

Pyc, M., Cai, Y., Greer, M.S., Yurchenko, O., Chapman, K.D., Dyer, J.M., Mullen, R.T., 2017. Turning Over a New Leaf in Lipid Droplet Biology. Trends Plant Sci. 22, 596–609. https://doi.org/10.1016/j.tplants.2017.03.012

Qi, B., Fraser, T., Mugford, S., Dobson, G., Sayanova, O., Butler, J., Napier, J.A., Stobart, A.K., Lazarus, C.M., 2004. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat. Biotechnol. 22, 739–745. https://doi.org/10.1038/nbt972

Qin, C., Wang, X., 2002. The Arabidopsis phospholipase D family. Characterization of a calcium-independent and phosphatidylcholine-selective PLDζ1 with distinct regulatory domains. Plant Physiol. 128, 1057–1068. https://doi.org/10.1104/pp.010928

Quettier, A.L., Eastmond, P.J., 2009. Storage oil hydrolysis during early seedling growth. Plant Physiol. Biochem. 47, 485–490. https://doi.org/10.1016/j.plaphy.2008.12.005

Review, A.M., Reserved, A.R., Reprint, N., Written, W., 2001. Monograph. Plant sterols and sterolins. Altern. Med. Rev. 6, 203–6

Reszczyńska E, Hanaka A. Lipids Composition in Plant Membranes. Cell Biochem Biophys. 2020 Dec;78(4):401-414. doi: 10.1007/s12013-020-00947-w

Rodriguez, M.C.S., Edsgärd, D., Hussain, S.S., Alquezar, D., Rasmussen, M., Gilbert, T., Nielsen, B.H., Bartels, D., Mundy, J., 2010. Transcriptomes of the desiccation-tolerant resurrection plant Craterostigma plantagineum. Plant J. 63, 212–228. https://doi.org/10.1111/j.1365-313X.2010.04243.x

Rosa, L., Chiarelli, D.D., Rulli, M.C., Dell'Angelo, J., D'Odorico, P., 2020. Global agricultural economic water scarcity. Sci. Adv. 6, 1–11. https://doi.org/10.1126/sciadv.aaz6031

Roston, R.L., Wang, K., Kuhn, L.A., Benning, C., 2014. Structural determinants allowing transferase activity in sensitive to freezing 2, classified as a family i lycosyl hydrolase. J. Biol. Chem. 289, 26089–26106. https://doi.org/10.1074/jbc.M114.576694

Roughan, P.G., Slack, C.R., Holland, R., 1978. Generation of phospholipid artefacts during extraction of developing soybean seeds with methanolic solvents. Lipids 13, 497–503. https://doi.org/10.1007/BF02533620

Singh, S., Singh R., 2015. A review on endemic Indian resurrecting herb Selaginella bryopteris (L.) Bak. “Sanjeevani.” International Journal of Pharmaceutical Sciences and Research. 6, 50–56. https://doi.org/10.13040/IJPSR.0975-8232.6(1).50-56

Sang, Y., Cui, D., Wang, X., 2001. Phospholipase D and phosphatidic acid-mediated generation of superoxide in arabidopsis. Plant Physiol. 126, 1449–1458. https://doi.org/10.1104/pp.126.4.1449

Sasser, M., 1990. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, MIDI Technical Note 101. Newark, DE, MIDI Inc 1–6.

Schaller, H., Grausem, B., Benveniste, P., Chye, M.L., Tan, C.T., Song, Y.H., Chua, N.H., 1995. Expression of the Hevea brasiliensis (H.B.K.) Müll. Arg. 3-hydroxy-3-methylglutaryl-coenzyme a reductase 1 in tobacco results in sterol overproduction. Plant Physiol. 109, 761–770. https://doi.org/10.1104/pp.109.3.761

Schaller, H., 2003. The role of sterols in plant growth and development. Progress in lipid research, 42(3), 163–175. https://doi.org/10.1016/s0163-7827(02)00047-4

Schaller, H., 2004. New aspects of sterol biosynthesis in growth and development of higher plants. Plant Physiol. Biochem. 42, 465–476. https://doi.org/10.1016/j.plaphy.2004.05.012

Schneitz, K., Sablowski, R., Lemieux, B., Grossniklaus, U., Dunwell, J., Metraux, J.-P., Palme, K., McAinsh, M.R., Berger, F., 2002. Plant biology, Current Opinion in Plant Biology. https://doi.org/10.1016/s1369-5266(02)00242-x

Shen, Y., Xie, J., Liu, R.D., Ni, X.F., Wang, X.H., Li, Z.X., Zhang, M., 2014. Genomic analysis and expression investigation of caleosin gene family in Arabidopsis. Biochem. Biophys. Res. Commun. 448, 365–371. https://doi.org/10.1016/j.bbrc.2014.04.115

Shimada, T.L., Hara-Nishimura, I., 2015a. Leaf oil bodies are subcellular factories producing antifungal oxylipins. Curr. Opin. Plant Biol. 25, 145–150. https://doi.org/10.1016/j.pbi.2015.05.019

Shimada, T.L., Hara-Nishimura, I., 2015b. Leaf oil bodies are subcellular factories producing antifungal oxylipins. Curr. Opin. Plant Biol. 25, 145–150. https://doi.org/10.1016/j.pbi.2015.05.019

Shimada, T.L., Takano, Y., Hara-Nishimura, I., 2015. Oil body-mediated defense against fungi: From tissues to ecology. Plant Signal. Behav. 10, 3–6. https://doi.org/10.4161/15592324.2014.989036

Singh, A.K., Jha, A., Bit, A., Kiassov, A.P., Rizvanov, A.A., Ojha, A., Bhoi, P., Patra, P.K., Kumar, A., Bissoyi, A., 2017. Selaginella bryopteris Aqueous Extract Improves Stability and Function of Cryopreserved Human Mesenchymal Stem Cells. Oxid. Med. Cell. Longev. 2017, 1–10. https://doi.org/10.1155/2017/8530656

Smith, A.M., 2001. The biosynthesis of starch granules. Biomacromolecules 2, 335–341. https://doi.org/10.1021/bm000133c

Smith, A.M., Denyer, K., Martin, C., 1997. The synthesis of the starch granule. Annu. Rev. Plant Biol. 48, 67–87. https://doi.org/10.1146/annurev.arplant.48.1.67

Tandang-Silvas, M.R.G., Tecson-Mendoza, E.M., Mikami, B., Utsumi, S., Maruyama, N., 2011. Molecular design of seed storage proteins for enhanced food physicochemical properties. Annu. Rev. Food Sci. Technol. 2, 59–73. https://doi.org/10.1146/annurev-food-022510-133718

Tzen, J.T.C., 2012. Integral Proteins in Plant Oil Bodies. ISRN Bot. 2012, 1–16. https://doi.org/10.5402/2012/173954

Tzen, J.T.C., Huang, A.H.C., 1992. Surface structure and properties of plant seed oil bodies. J. Cell Biol. 117, 327–335. https://doi.org/10.1083/jcb.117.2.327

Uzbekov, R., Roingeard, P. 2013. Nuclear lipid droplets identified by electron microscopy of serial sections. BMC Res Notes 6, 386. https://doi.org/10.1186/1756-0500-6-386

Valitova, J.N., Sulkarnayeva, A.G., Minibayeva, F. V., 2016. Plant sterols: Diversity, biosynthesis, and physiological functions. Biochem. 81, 819–834. https://doi.org/10.1134/S0006297916080046

Van Buren, R., Wai, C.M., Pardo, J., Giarola, V., Ambrosini, S., Song, X., Bartels, D., 2018. Desiccation tolerance evolved through gene duplication and network rewiring in Lindernia. Plant Cell 30, 2943–2958. https://doi.org/10.1105/tpc.18.00517

Vanburen, R., Bryant, D., Edger, P.P., Tang, H., Burgess, D., Challabathula, D., Spittle, K., Hall, R., Gu, J., Lyons, E., Freeling, M., Bartels, D., Ten Hallers, B., Hastie, A., Michael, T.P., Mockler, T.C., 2015. Single-molecule sequencing of the desiccation-tolerant grass Oropetium thomaeum. Nature 527, 508–511. https://doi.org/10.1038/nature15714

VanBuren, R., Wai, C.M., Keilwagen, J., Pardo, J., 2018. A chromosome-scale assembly of the model desiccation tolerant grass Oropetium thomaeum. Plant Direct 2, 1–9. https://doi.org/10.1002/pld3.96

Vogel, J.P., Garvin, D.F., Mockler, T.C., Schmutz, J., Rokhsar, D., Bevan, M.W., Barry, K., Lucas, S., Harmon-Smith, M., Lail, K., Tice, H., Grimwood, J., McKenzie, N., Huo, N., Gu, Y.Q., Lazo, G.R., Anderson, O.D., You, F.M., Luo, M.C., Dvorak, J., Wright, J., Febrer, M., Idziak, D., Hasterok, R., Lindquist, E., Wang, M., Fox, S.E., Priest, H.D., Filichkin, S.A., Givan, S.A., Bryant, D.W., Chang, J.H., Wu, H., Wu, W., Hsia, A.P., Schnable, P.S., Kalyanaraman, A., Barbazuk, B., Michael, T.P., Hazen, S.P., Bragg, J.N., Laudencia-Chingcuanco, D., Weng, Y., Haberer, G., Spannagl, M., Mayer, K., Rattei, T., Mitros, T., Lee, S.J., Rose, J.K.C., Mueller, L.A., York, T.L., Wicker, T., Buchmann, J.P., Tanskanen, J., Schulman, A.H., Gundlach, H., Beven, M., Costa De Oliveira, A., Da C. Maia, L., Belknap, W., Jiang, N., Lai, J., Zhu, L., Ma, J., Sun, C., Pritham, E., Salse, J., Murat, F., Abrouk, M., Bruggmann, R., Messing, J., Fahlgren, N., Sullivan, C.M., Carrington, J.C., Chapman, E.J., May, G.D., Zhai, J., Ganssmann, M., Gurazada, S.G.R., German, M., Meyers, B.C., Green, P.J., Tyler, L., Wu, J., Thomson, J., Chen, S., Scheller, H. V., Harholt, J., Ulvskov, P., Kimbrel, J.A., Bartley, L.E., Cao, P., Jung, K.H., Sharma, M.K., Vega-Sanchez, M., Ronald, P., Dardick, C.D., De Bodt, S., Verelst, W., Inzé, D., Heese, M., Schnittger, A., Yang, X., Kalluri, U.C., Tuskan, G.A., Hua, Z., Vierstra, R.D., Cui, Y., Ouyang, S., Sun, Q., Liu, Z., Yilmaz, A., Grotewold, E., Sibout, R., Hematy, K., Mouille, G., Höfte, H., Micheel, T., Pelloux, J., O'Connor, D., Schnable, J., Rowe, S., Harmon, F., Cass, C.L., Sedbrook, J.C., Byrne, M.E., Walsh, S., Higgins, J., Li, P., Brutnell, T., Unver, T., Budak, H., Belcram, H., Charles, M., Chalhoub, B., Baxter, I., 2010. Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature 463, 763–768. https://doi.org/10.1038/nature08747Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.E., Rajashekar, C.B., Williams, T.D., Wang, X., 2002. Profiling membrane lipids in plant stress responses: Role of phospholipase Dα in freezing-induced lipid changes in arabidopsis. J. Biol. Chem. 277, 31994–32002. https://doi.org/10.1074/jbc.M205375200

Wewer, V., Dombrink, I., Vom Dorp, K., Dörmann, P., 2011. Quantifi cation of sterol lipids in plants by quadrupole time-of-fl ight mass spectrometry. J. Lipid Res. 52, 1039–1054. https://doi.org/10.1194/jlr.D013987

Willigen, C. Vander, Pammenter, N.W., Mundree, S.G., Farrant, J.M., 2004. Mechanical stabilization of desiccated vegetative tissues of the resurrection grass Eragrostis nindensis: Does a TIP 3;1 and/or compartmentalization of subcellular components and metabolites play a role? J. Exp. Bot. 55, 651–661. https://doi.org/10.1093/jxb/erh089

Wilson, T.M., 1907. On the chemistry and staining properties of certain derivatives of the methylene blue group when combined with eosin. J. Exp. Med. 9, 645–670. https://doi.org/10.1084/jem.9.6.645

Wu, G., Truksa, M., Datla, N., Vrinten, P., Bauer, J., Zank, T., Cirpus, P., Heinz, E., Qiu, X., 2005. Stepwise engineering to produce high yields of very long-chain polyunsaturated fatty acids in plants. Nat. Biotechnol. 23, 1013–1017. https://doi.org/10.1038/nbt1107

Xue, H.W., Hosaka, K., Plesch, G., Mueller-Roeber, B., 2000. Cloning of Arabidopsis thaliana phosphatidylinositol synthase and functional expression in the yeast pis mutant. Plant Mol. Biol. 42, 757–764. https://doi.org/10.1023/A:1006308909105

Yobi, A., Schlauch, K.A., Tillett, R.L., Yim, W.C., Espinoza, C., Wone, B.W.M., Cushman, J.C., Oliver, M.J., 2017. Sporobolus stapfianus: Insights into desiccation tolerance in the resurrection grasses from linking transcriptomics to metabolomics. BMC Plant Biol. 17, 1–30. https://doi.org/10.1186/s12870-017-1013-7

Zhang, M., Fan, J., Taylor, D.C., Ohlrogge, J.B., 2009. DGAT1 and PDAT1 acyltransferases have overlapping functions in Arabidopsis triacylglycerol biosynthesis and are essential for normal pollen and seed development. Plant Cell 21, 3885–3901. https://doi.org/10.1105/tpc.109.071795

Zhou, X., Chen, X., Du, Z., Zhang, Y., Zhang, W., Kong, X., Thelen, J.J., Chen, C., Chen, M., 2019. Terpenoid esters are the major constituents from leaf lipid droplets of Camellia sinensis. Front. Plant Sci. 10, 1–12. https://doi.org/10.3389/fpls.2019.00179

Zou, J., Wei, Y., Jako, C., Kumar, A., Selvaraj, G., Taylor, D.C., 1999. The Arabidopsis thaliana TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene. Plant J. 19, 645–653. https://doi.org/10.1046/j.1365-313X.1999.00555