Moulay Abdelmajid Kassem *Editor*

Soybean Seed Composition

Protein, Oil, Fatty Acids, Amino Acids, Sugars, Mineral Nutrients, Tocopherols, and Isoflavones

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Editor
Moulay Abdelmajid Kassem
Department of Biological and Forensic Sciences
Fayetteville State University
Fayetteville, NC, USA

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This book is dedicated to the souls of my lovely mother Ait Elhaj Ijja (1941–2018) and father My Abderrahmane Kassem (1937–1993), to my lovely wife Sanaa Rajaallah, and our children Zakariah, Safiah, and Sakinah.
Thank you. Without your support and patience, I would have never achieved my dream.

Foreword

The book *Soybean Seed Composition: Protein, Oil, Fatty Acids, Amino Acids, Sugars, Mineral Nutrients, Tocopherols, and Isoflavone* is a timely update for those interested in seed composition and the healthful effects of phytochemicals. Protein, oil, and Isoflavone can improve human and animal health and also behavior.

Chapter 1 covers seed protein, oil, fatty acids, and amino acids and the effects of genetic and environmental factors on them. Chapter 2 covers QTL that control seed protein, oil, and fatty acids contents, and Chap. 3 covers seed amino acids, macronutrients, micronutrients, sugars, and other compounds that are key to selection for crop improvement. Chapter 4 covers two decades of QTL mapping of mineral deficiencies in soybean, which sheds light on the importance of a balanced mineral nutrition in soybean and other crops. Chapter 5 covers 16 years of salt stress tolerance QTL mapping, which is another, challenge that faces soybean and other crop production worldwide. Chapters 6, 7, 8, 9, and 10 cover in great detail the important soybean seed Isoflavone from their biosynthesis and quantification methods, locations, and variations in seeds, roots, leaves to their QTL mapping for over two decades, and Chap. 11 covers lunasin, a bioactive anticancer peptide in soybean seeds, which will help farmers and breeders to develop soybean cultivars with improved seed Isoflavone and lunasin contents.

The book makes a great primer for those new to the field, including undergraduate and graduate students, and also serves as a great assistance to the alleged experts. It should help direct policy and funding agencies alike.

Carbondale, IL, USA — Khalid Meksem

Reviewers

I wholeheartedly thank the following colleagues who took time from their busy schedules to review one or more chapters of this book. All chapters are peer-reviewed by at least two of these experts:

Khalid Meksem, Department of Plant, Soil, and Agricultural Systems, Southern Illinois University at Carbondale, Carbondale, IL, USA
Istvan Rajcan, Plant Agriculture, University of Guelph, Guelph, ON, Canada
Cevdet Akbay, Department of Chemistry, Physics, and Materials Sciences, Fayetteville State University, Fayetteville, NC, USA
Shubo Han, Department of Chemistry, Physics, and Materials Sciences, Fayetteville State University, Fayetteville, NC, USA
Mohammad Atik Rahman, Center for Viticulture and Small Fruit Research, Biotechnology Unit, Florida A&M University, Tallahassee, FL, USA
Naoufal Lakhssassi, Department of Plant, Soil, and Agricultural Systems, College of Agriculture, Southern Illinois University, Carbondale, IL, USA
Tri Vuong, Molecular Genetics & Soybean Genomics Laboratory, Division of Plant Sciences, College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO, USA
Patil Gunvant, Department of Plant and Soil Science, Institute of Genomics for Crop Abiotic Stress Tolerance, Texas Tech University, Lubbock, TX, USA
Prakash Niraula, Department of Biological Sciences, Mississippi State University, Mississippi State, MS, USA
Xiaofang Xie, Fujia Agriculture and Forestry University, Fuzhou, China
Guanshui Chen, Fujia Agriculture and Forestry University, Fuzhou, China

Preface

Soybean [*Glycine max* (L.) Merr.] seeds are a great source of Isoflavone (mainly daidzein, genistein, and glycitein), protein, oil, fatty and amino acids, nutrients, and many other beneficial compounds for human and animal consumption.

The idea of writing this book started when I wrote what was intended to be a review paper entitled "Isoflavone Quantitative Trait Loci (QTL) Mapping" back in fall 2014. In this review paper, I decided to cover soybean seed Isoflavone QTL mapping for over one and a half decade and the paper was getting too large; therefore, I changed my mind to add a few chapters on soybean seed Isoflavone such as "Isoflavone Biosynthetic Pathways and Methods of Quantification," "Isoflavone Locations and Variations in Seeds, Roots, Leaves, and Other Plant Parts," "Isoflavone Positive and Negative Effects on Humans, Animals, and Plants," and "Environmental Factors Affecting Isoflavone Contents" and make it a book about seed Isoflavone only. However, with the passage of time, I decided to expand the book and change its title to "Soybean Seed Composition: Protein, Oil, Fatty Acids, Amino Acids, Sugars, Mineral Nutrients, Tocopherols, and Isoflavone," added the chapters "Seed Protein, Oil, Fatty Acids, and Amino Acids: Effect of Genetic and Environmental Factors," "QTL That Control Seed Protein, Oil, and Fatty Acids Contents," "Seed Amino Acids, Macronutrients, Micronutrients, Sugars, and Other Compounds," "Two Decades of QTL Mapping of Mineral Deficiencies in Soybean," "Sixteen Years (2004–2020) of Salt Stress Tolerance QTL Mapping in Soybean," and "Bioactive Anticancer Peptides in Soybean Seeds," and decided to cover literature up to December 2020 and end the book by this date.

I hope that this book will add to the knowledge of soybean seed composition, especially Isoflavone, their biosynthesis, effects on humans and animal health and food, and their genetic mapping but also protein, oil, fatty acids, amino acids, mineral nutrients and other compounds and that it will benefit undergraduate and graduate students, faculty, scientists, and other individuals interested in these subjects.

Fayetteville, NC, USA — Moulay Abdelmajid Kassem

Acknowledgments

I would like to thank Prof. Dr. Khalid Meksem of Southern Illinois University at Carbondale (SIUC) for reviewing the book and writing its foreword, Dr. Ali Siamaki of Fayetteville State University (FSU) for drawing several figures, and all reviewers who reviewed one or more chapters of this book.

Chapter 10 was written while I served as the Dean of the School of Arts and Sciences (SAS) at the American University of Ras Al Khaimah (AURAK) in 2014–2015; therefore, I thank the President Prof. Hassan Al Alkim and AURAK. I also thank Fayetteville State University (FSU) for providing a great academic atmosphere to compete the book by December 2020.

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About the Editor

Moulay Abdelmajid Kassem is currently working as a Professor of Botany and Chair of the Department of Biological and Forensic Sciences at Fayetteville State University, Fayetteville, NC, USA. He earned a Bachelor of Science in Plant Biology from Faculty of Sciences, University Mohamed V, Morocco, in 1992; a Master of Science in Enzymatic Engineering, Bioconversion, and Microbiology from University of Picardie Jules Verne, Amiens, France, in 1995; and a PhD in Plant Biology from Southern Illinois University, Carbondale, IL, USA, in 2003.

Dr. Kassem worked as a high school teacher with Chicago Public Schools, Chicago, IL, from 2001 to 2004 and as an Assistant Professor of Botany in the Department of Biology at Kean University, Union, NJ. He then moved to Fayetteville State University in fall 2006 as an Associate Professor of Botany. He was promoted to Full Professor in 2009 and to Department Chair in 2010.

Dr. Kassem has 28+ years of experience in plant genetics, genomics, and biotechnology. His main areas of research include genetic and quantitative trait loci (QTL) mapping of important agronomic traits in soybean with a focus on yield, yield components, and seed composition traits. Dr. Kassem published 60+ research articles in high-quality peer-reviewed international journals, 80+ abstracts in national and international conferences, and 4 book chapters and received over $1.4 million in grants, including federal grants from the Department of Defense and the Department of Education.

Dr. Kassem is the founder and CEO of Atlas Publishing, LLC (www.atlas-publishing.org). He serves as the Editor-in-Chief of *Atlas Journal of Biology*, a member of the Editorial Board of the *Journal of Biotech Research*, and a reviewer for several international journals and granting agencies. He also co-organizes the American Moroccan Agricultural Sciences Conference (AMAS Conference; www.amas-conference.org) and organizes a workshop entitled "Teaching Genetics, Genomics, Biotechnology, and Bioinformatics" at the prestigious International Plant and Animal Genome Conference (PAG Conference, www.intlpag.org).

Contributors

Nacer Bellaloui USDA, Agriculture Research Service, Crop Genetics Research Unit, Stoneville, MS, USA

Meriam Bousselham Plant Genomics and Biotechnology Lab, Department of Biological and Forensic Sciences, Fayetteville State University, Fayetteville, NC, USA

Moulay Abdelmajid Kassem Plant Genomics and Biotechnology Lab, Department of Biological and Forensic Sciences, Fayetteville State University, Fayetteville, NC, USA

Jiazheng Yuan Plant Genomics and Biotechnology Lab, Department of Biological and Forensic Sciences, Fayetteville State University, Fayetteville, NC, USA

Abbreviations

4CL	4-coumarate-CoA-ligase
AAP	American Academy of Pediatrics
Ab	Antibody
ACNA	Acyl-CoA n-acyltransferase
ACP	Acyl carrier protein
AFLP	Amplified fragment length polymorphism
Agq	Antigen
Al	Aluminum
ALA	Alanine
ANOVA	Analysis of variance
ARD	Average root diameter
ARG	Arginine
ARV	Average root volume
ASN	Asparagine
ASP	Aspartate
B	Boron
BBI	Bowman-Birk protease inhibitor
BC	Backcross
BMI	Body mass index
C	Carbon
C4H	Cinnamate-4-hydroxylase
Ca	Calcium
CHI	Chalcone isomerase
CHR	Chalcone reductase
CHS	Chalcone synthase
CIM	Composite interval mapping
Cl	Chlorine
CO_2	Carbon dioxide
CSPS	Conventional soybean planting system
Cu	Copper
Cys	Cysteine

DAG	Diacylglycerol
DMPBQ	2,3-dimethyl-6-phytyl-plastoquinol
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ESPS	Early soybean planting system
F6H	Flavonone-6-hydroxylase
FA	Fatty acid
FAD	Fatty acid desaturase
FatB	Fatty acyl-ACP thioesterase B
FDA	Food and Drug Administration
Fe	Iron
G3P	Glycerol-3-phosphate
GC	Gas chromatography
GC-MS	Gas chromatography mass-spectrometry
GLN	Glutamine
GLU	Glutamate
GLY	Glycine
GRAS	Generally Recognized as Safe
GT	Glucosyltransferase
GWAS	Genome-wide association study
H	Hydrogen
H_2O	Water
HDL	High-density lipoproteins
His	Histidine
HPLC	High-performance liquid chromatography
HPT	Phytyl transferase
IDC	Iron deficiency chlorosis
IFS	2-hydroxyisoflavanone synthase
ILE	Isoleucine
IM	Interval mapping
IMT	Isoflavone methyl-transferase
K	Potassium
lacZ	Operon lactose Z gene
LASH	Leaf ash
LCC	Leaf chloride content
LC-MS	Liquid chromatography mass-spectrometry
LCO	Lipo-chitooligosaccharides
LDL	Low-density lipoproteins
Leu	Leucine
LN	Leaf number
LPA	Lysophosphatidic acid
LSS	Leaf scorch score
Lys	Lysine
MEP	2-C-methyl-d-erythritol-4-phosphate (MEP) pathway
Met	Methionine

Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
MPBQ	Methyl-6-phytyl-1,4-benzoquinone
MPBQ-MT	MPBQ-methyltransferase
MPK	Mitogen-activated protein kinase
MRL	Maximum root length
MT	Malonyl-transferase
N	Nitrogen
Na	Sodium
Ni	Nickel
NIL	Near isogenic line
NIRS	Near-infrared reflectance spectroscopy
NPC	Nonpolar compounds (urinary)
O	Oxygen
OVX	Ovariectomized
OZR	Obese Zucker (rats)
P	Phosphorus
PAL	Phenylalanine ammonia-lyase
PBS	Phosphate-buffered saline
PC	Phosphatidylcholine
PDHK	Pyruvate dehydrogenase kinase
PDW	Plant dry weight
PE	Phosphorus efficiency
PH	Plant height
PHE	Phenylalanine
PHOs	Partially hydrogenated oils
PLC	Phospholipase C
PLD	Phospholipase D
PLE	Pressurized liquid extraction
PPAR	Peroxisome-proliferator activated receptor
PPS	Percentage of plant survival
PRO	Proline
PSE	Pressurized-solvent extraction
PYR	Pyruvate
QTL	Quantitative trait loci
QTN	Quantitative trait network
RDW	Root dry weight
RFLP	Restriction fragment length polymorphism
RFW	Root fresh weight
RIL	Recombinant inbred line
RL	Root length
RNA-Seq	Ribonucleic acid sequencing
ROD1	Reduced oleate desaturation 1
RRE	Relative root elongation

RRG	Root relative growth
RSA	Root surface area
RTE	Root tap extension
RTI	Root tolerance index
RT-PCR	Reverse-transcription polymerase chain reaction
RT-qPCR	Real-time quantitative reverse transcription polymerase chain reaction
RV	Root volume
S	Sulfur
SAD	Stearoyl-acyl-carrier-protein desaturase
SDW	Shoot dry weight
SER	Serine
SFE	Supercritical carbon dioxide fluid extraction
SFW	Shoot fresh weight
Si	Silicon
SNP	Single nucleotide polymorphism
SPAD	Leaf chlorophyll (SPAD value)
SSR	Simple sequence repeats
STR	Salt tolerance rating
TAG	Triacylglycerol
TC or TOC	Tocopherol (α–, β–, γ–, and δ–tocopherol)
THR	Threonine
TPO	Thyroid peroxidase
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
VIGS	Virus-induced gene silencing
WD	Water deficit
WDT	Water deficit tolerance
WUE	Water use efficiency
Zn	Zinc

Chapter 1
Seed Protein, Oil, Fatty Acids, and Amino Acids: Effects of Genetic and Environmental Factors

Nacer Bellaloui and Moulay Abdelmajid Kassem

1.1 Introduction

Soybean is a major crop in the world and a source of high-quality protein, oil, and other nutrients. Soybean seed nutrients (seed composition constituents) include protein (40–45%), oil (18–24%) (Medic et al. 2014), and fatty acids such as palmitic (C16:0, 8–12%), stearic (C18:0, 3–5%), oleic (C18:1, 18–24%), linoleic (C18:2, 48–58%), and linolenic (C18:3, 5–10%) acids. Soybean seed fatty acids biosynthetic pathway is shown in Fig. 1.1 (Fang et al. 2017). Soybean seed contains high-quality protein for human nutrition and livestock meal. Also, it contains amino acids, isoflavones, and minerals. Health benefit to human has been previously reported by various studies (Hu et al. 1997; Maestri et al. 1998; Federal Register 2003; Fehr 2007; Business Sphere 2007; Clemente and Cahoon 2009). Breeders goal for desirable seed composition constituents include high oleic and low linolenic acids, low phytic acid, high sucrose, and low raffinose and stachyose levels. It has been shown that soybeans with higher levels of mono-unsaturated fatty acids such as oleic acid or lower levels of polyunsaturated fatty acids such as linoleic or linolenic are more desirable for human consumption than saturated fatty acids such as palmitic and stearic acids (Haun et al. 2014). However, higher levels of monounsaturated fatty acids such as oleic acid and low levels of polyunsaturated fatty acids such as linoleic and linolenic acid are desirable by the industry as they contribute to oil oxidative stability, short shelf life, and less rancidity. This trait is desirable because it can minimize hydrogenation of the oil. Hydrogenation has been reported

N. Bellaloui
USDA, Agriculture Research Service, Crop Genetics Research Unit, Stoneville, MS, USA

M. A. Kassem (✉)
Plant Genomics and Biotechnology Lab, Department of Biological and Forensic Sciences, Fayetteville State University, Fayetteville, NC, USA
e-mail: mkassem@uncfsu.edu

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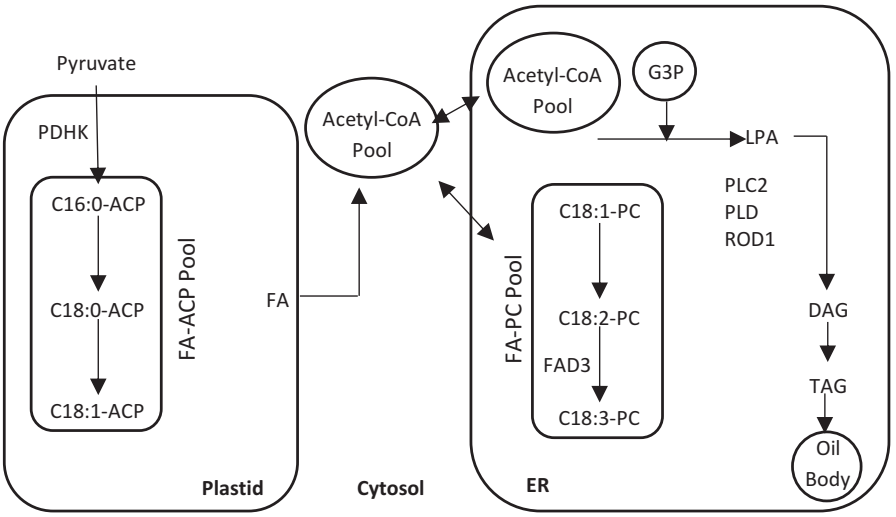

Fig. 1.1 Fatty acids biosynthetic pathways. ACP acyl carrier protein, DAG diacylglycerol, G3P glycerol-3-phosphate, FA fatty acid, LPA lysophosphatidic acid, PC phosphatidylcholine, PYR pyruvate, TAG triacylglycerol, ACNA acyl-CoA n-acyltransferase, FAD fatty acid desaturase, FatB fatty acyl-ACP thioesterase B, PDHK pyruvate dehydrogenase kinase, PLC phospholipase C, PLD phospholipase D, ROD1 reduced oleate desaturation 1, SAD stearoyl-acyl-carrier-protein desaturase, ER endoplasmic reticulum. (Adopted from Fang et al. 2017)

to have undesirable health effects by increasing the risk of coronary heart disease due to higher LDL-cholesterol and lower HDL-cholesterol (Federal Register 2003; Business Sphere 2007; Clemente and Cahoon 2009). The partial hydrogenation of polyunsaturated fatty acids such as linolenic acid results in the conversion of linolenic acid to oleic and stearic acids, thereby reducing polyunsaturated fatty acids to about 18% and linolenic acid to below 2% (Gerde et al. 2007; Clemente and Cahoon 2009; Haun et al. 2014).

Therefore, the main goal of breeders is to breed soybean with low linolenic acid and high oleic acid so as to reduce the hydrogenation process, thereby minimizing the level of trans-fatty acids in foods. In 2015, the Food and Drug Administration determined that partially hydrogenated oils (PHOs) are not generally recognized as safe (GRAS). This determination was based on research and input from stakeholders during the public comment. The PHOs are considered the primary dietary source of artificial transfat in processed foods. Removing PHOs from processed foods could prevent thousands of heart attacks and deaths each year (US Food and Drug Administration 2018: https://www.fda.gov/food/food-additives-petitions/final-determination-regarding-partially-hydrogenated-oils-removing-trans-fat) "GRAS: is an acronym for the phrase Generally Recognized As Safe. Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (the Act), any substance that is intentionally added to food is a food additive, that is subject to premarket review

and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excepted from the definition of a food additive" (US Food and Drug Administration 2019: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras)

Also, higher raffinose and stachyose are not desirable as these sugar fractions are indigestible and cause flatulence or diarrhea in human and monogastric animals such as swine and chicken (Liu 1997; Obendorf et al. 1998). High phytic acid is another antinutritional component in soybean. Phytic acid is the major storage form of phosphorous in soybean, known as a food inhibitor. Phytic acid chelates micronutrient such as Fe and Zn and prevents phosphorus from absorption and bioavailability for monogastric animals, including humans. This occurs due to lack of enzyme phytase in their digestive tract. Therefore, genetic improvement of soybean with low phytic acid is still among the goals of soybean breeders. Phytic acid pretreatment method such as enzymatic treatment of soybean seed with phytase enzyme is a regular method used in industries (Gupta et al. 2015). This chapter will focus on reviewing soybean seed protein, oil, and fatty acids and highlight the main research conducted on improving soybean seed nutritional traits from the perspectives of genetic, environmental, and agricultural practices.

1.2 Interactions of Seed Composition Constituents with Genetics and Environmental Factors

Protein, oil, and fatty acids (oleic, linoleic, linolenic, stearic, and palmitic acids) are among the top compounds available in soybean seeds, consumed by humans, and highly desirable traits to improve in breeding programs (Yazdi-Samadi et al. 1977; Burton 1987; Blackman et al. 1992). Their contents vary depending on the genotype, abiotic and biotic stresses, and environmental conditions (Table 1.1) (Badami et al. 1984; Hartwig and Kilen 1991; Bellaloui et al. 2014a; Rincker et al. 2014; Gulluoglu et al. 2018; Wijewardana et al. 2019).

Several studies have reported that crop rotation affects seed yield and seed composition including protein, oil, fatty acids, and nutrient contents (Temperly and Borges 2006; Bellaloui et al. 2010b). It was shown that soybean seed protein content decreased from 357 g kg^{-1} to 351 g kg^{-1} from the 1st to 5th year of soybean consecutive growth after five years of corn consecutive growth (Temperly and Borges 2006).

It is well known that elevated temperatures and CO_2 levels affect plant growth and development including seed composition (Thomas et al. 2003; Long et al. 2004; Prasad et al. 2005; Hay and Porter 2006; Taub et al. 2008; Bellaloui et al. 2016). In another study, authors investigated the effects of increased temperatures (28 °C, 32 °C, 36 °C, 40 °C, 44 °C) and CO_2 levels (350, 700 µmol mol^{-1}) on seed composition. They found that seed oil, linolenic acid, and carbohydrates contents decreased

Table 1.1 Soybean seed protein, oil, and fatty acids (oleic, linoleic, linolenic, stearic, and palmitic acids) contents

Trait	Content (seed dry weight basis or %)	Reference
Protein	36.3–41.9% 41% 380–420 g kg^{-1} 35.16–37.35%	Badami et al. (1984) Hartwig and Kilen (1991) Bellaloui et al. (2014a) Bolon et al. (2014)
Oil	15.6–25.8% 21% 190–230 g kg^{-1} 15.29–17.43%	Badami et al. (1984) Hartwig and Kilen (1991) Bellaloui et al. (2014a) Bolon et al. (2014)
Saturated fatty acids	*8.6–16.7%*	Badami et al. (1984)
Stearic acid	2.2–7.2% - 4%	Badami et al. (1984) Bellaloui et al. (2014a) Hartwig and Kilen (1991)
Palmitic acid	120–130 g kg^{-1} 10%	Bellaloui et al. (2014a) Hartwig and Kilen (1991)
Unsaturated fatty acids	*83.3–91.4%*	Badami et al. (1984)
Oleic acid	22% 30–40 g kg^{-1}	Hartwig and Kilen (1991) Bellaloui et al. (2014a)
Linoleic acid	54% 480–580 g kg^{-1}	Hartwig and Kilen (1991) Bellaloui et al. (2014a)
Linolenic acid	10% 50–80 g kg^{-1}	Hartwig and Kilen (1991) Bellaloui et al. (2014a)

when temperatures increased, and seed oleic acid, N, and P contents increased when temperatures increased. However, CO_2 increase had a minimal effect on seed composition (Thomas et al. 2003). On the other hand, other researchers have predicted that the changes in seed yield and composition were due to global climate changes including high CO_2, affecting stomatal conductance and photosynthetic rates (Long et al. 2004; Prasad et al. 2005; Hay and Porter 2006; Taub et al. 2008). A recent research on the effect of high temperatures [26 °C and 45 °C] and CO_2 [360 and 700 µmol mol^{-1}] on seed composition showed that seed protein, linolenic acid, and mineral nutrient (P, K, N, Fe, Zn, and B) contents decreased and seed oil and oleic acid contents increased with the increase of temperatures and CO_2 levels. They also found that seed fructose, glucose, and sucrose contents increased with increased temperature and CO_2 levels; however, seed raffinose and stachyose contents did not change (Bellaloui et al. 2016).

The effects of soybean–corn crop rotation on seed composition in Stoneville, MS, USA (Bellaloui et al. 2010c) were also studied. They showed that three-year rotation increased the level of fatty acids content from 61% to 68%, P content from 60% to 75%, Fe content from 70% to 71%, B content from 34% to 69%, and oleic acid content from 22.63% to 30.22%; however, a decrease in linoleic acid content was noticed (Bellaloui et al. 2010c).

Several studies showed the effect of row spacing and seeding rate (SDR) on soybean seed yield and composition (Al-Tawaha and Seguin 2006; Ragin et al. 2014;

Bellaloui et al. 2014a; Bellaloui et al. 2020). The effect of row spacing (RS) and seeding rate (SDR) on seed composition was investigated in four soybean cultivars (P 93M90, AG 3906, P 94B73, and V 52N3) over two years (2006–2007) in a field in Stoneville, MS (Bellaloui et al. 2014a). The results showed that SDR, cultivar, and year significantly affected seed Fe, P, B, sucrose, protein, and oil contents, but no stachyose and linolenic acid contents were observed. Similarly, RS significantly affected Fe, B, raffinose, and sucrose contents, but no protein and oil contents were recorded (Bellaloui et al. 2014a).

In an experiment, Gulluoglu et al. (2018) investigated the effects of two cropping systems (main and double cropping systems) on seed composition traits (fatty acids and oil contents) in several soybean varieties adapted to Europe (Turkey). The temperatures ranged from 19.5 °C to 28.6 °C and from 19.5 °C to 28.6 °C during the growing seasons. They found that soybean plants grown in the main cropping system showed an increase in seed oil contents from 18.45% to 19.99%, while those grown in the double cropping system from 17.11% to 19.37%. They also found that a positive correlation between high temperatures and high seed oil content was observed, in agreement with those reported by others (Belalloui et al. 2015a, b). Fatty acids such as oleic, linoleic, and linolenic acids were also affected in the following ways. The content of seed palmitic acid in double cropping system ranged from 10.76% to 12.23% – higher than its range of 10.59% to 12.04% in the main cropping system. The content of seed stearic acid in double cropping system ranged from 3.94% to 4.87% – higher than its range of 3.11% to 4.52% in the main cropping system. Similarly, for seed oleic acid, the content ranged from 22.69% to 29.51% – lower than its range of 27.02% to 34.09% in the main cropping system; For seed linoleic acid, the content ranged from 48.40% to 54.14% of total oil in double cropping system – higher than its range of 44.5% to 51.80% in the main cropping system. Similar pattern was also observed for seed linolenic acid content that ranged from 5.41% to 6.62% in double cropping system – higher than its range of 4.44% to 5.61% in the main cropping system (Gulluoglu et al. 2018).

The accumulation levels of protein, oil, and fatty acids in seeds are genetically controlled, although environmental conditions and agricultural practices can significantly alter the levels of these seed constituents in seeds. For example, Dardanelli et al. (2006) investigated the effects of maturity group (MG) and environment (E) on protein and oil in a 3-year experiment using six maturity groups (IIIII, IV, V, VI, VII, and VIII-IX) and in 14–24 environments each year. They found that the environment was the most important source of variation for protein and oil content, and the main effect of MG was higher than that of MG × E interaction for oil content and oil + protein content. They also found that oil content was higher in seeds from MGs II-III and IV. Protein content was higher in MG VI in some environments, whereas it was higher in MG II-III in some other environments. The high temperatures during seed-fill period could cause consistent higher oil across years and environments in early MGs (Dardanelli et al. 2006). Heat effects on seed protein, oil, and fatty acids were also evaluated by using a growth chamber where one soybean was grown at normal temperature (25/20 °C), higher temperature (36/30 °C), and at heat stress (40/36 °C). Light intensity was about 1000 μmol $m^{-2} \cdot s^{-1}$, which was supplied with

a combination of 10 high pressure sodium and metal halide lights, each of 400 W. Results showed that soybean grown under high temperature showed a lower content of protein and linolenic acid, but higher content of oil and oleic acid (Figs. 1.2a, b, 1.3, and 1.4a, b). Although high temperature may promote oil production, this pattern cannot be generalized as moderate high temperature can increase protein, but severe high temperature can decrease protein. The effect of temperature and maturity on seed protein and oil was also investigated by previous researchers. For example, in an experiment conducted by Piper and Boote (1999), they evaluated the effects of field experiments across 60 environments in 20 cultivars from 10 MGs. Temperatures across these environments ranged from 14.6 to 28.7 °C. Results obtained from these field experiments showed a quadratic relationship between protein and mean daily temperature during seed fill with higher concentrations of protein with temperatures below 20 °C. Other research conducted to investigate the effects of mean temperature during the developmental period of soybean on seed composition showed a negative correlation between oil content and mean temperature during seed maturation (Maestri et al. 1998), but no effect of temperature on protein or fatty acids was recorded. It was concluded that the variability of seed composition constituents was due to MG, genotype within MG, environment, and their interactions (Bellaloui et al. 2009c).

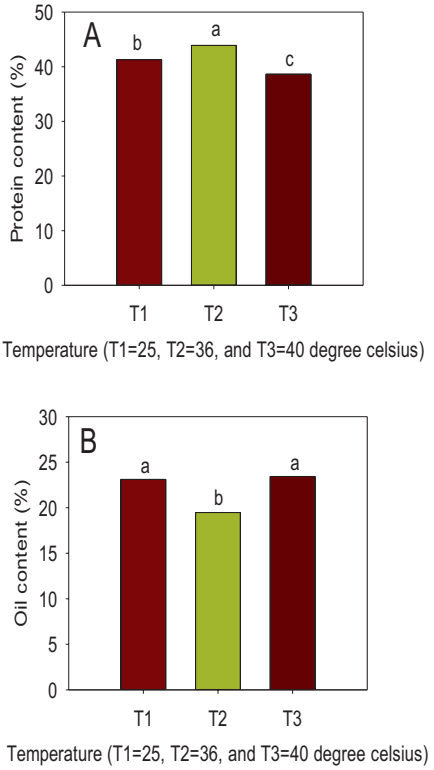

Fig. 1.2 Effect of temperature (normal, 25/20 °C; higher temperature, 36/30 °C; and heat stress, 40/36 °C) on seed protein (**a**) and oil (**b**) in Hutcheson cultivar. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$

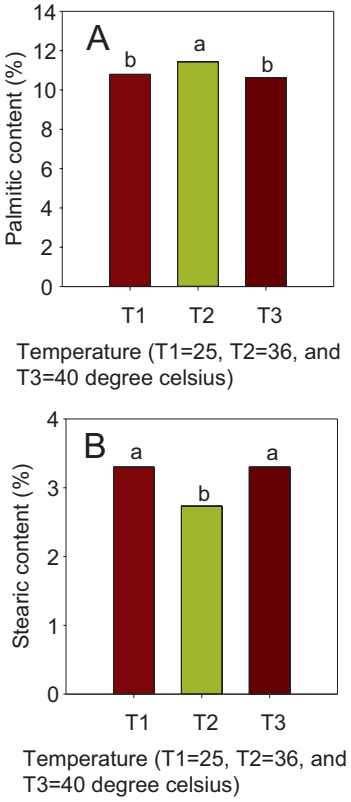

Fig. 1.3 Effect of temperature (normal, 25/20 °C; higher temperature, 36/30 °C; and heat stress, 40/36 °C) on seed palmitic acid (**a**) and stearic acid (**b**) in Hutcheson cultivar. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$

Rincker et al. (2014) studied MG II, MG III, and MG IV soybean cultivars over a period of 80 years and reported a decrease of 0.16–0.22 g kg^{-1} $year^{-1}$ in seed protein contents and a decrease of 0.05–0.14 g kg^{-1} $year^{-1}$ in seed oil contents due to the fact that protein and oil contents are negatively correlated. Other studies reported similar results of decrease in seed protein and oil contents over times depending on the genetic backgrounds (Voldeng et al. 1997; Wilcox 2001; Wilson 2004).

A recent study showed that the use of harvest aids such as paraquat, carfentrazone-ethyl (AIM), glyphosate, and sodium chlorate affect seed composition, especially seed protein, fructose, oleic acid, oil, and fructose contents; however, they had small effects on seed amino acids contents during development stages R6 and R7 depending on the year of growth (Bellaloui et al. 2020). For example, seed palmitic acid, stearic acid, and linolenic acid have not been affected by the addition of harvest aids at the R7 growth stage; however, the addition of paraquat plus AIM or paraquat caused a decrease in seed oleic acid content and a high increase in seed oil content. The addition of glyphosate increased the seed protein content, while the addition of $NaClO_3$ decreased it. The addition of both AIM and glyphosate decreased the seed fructose content; however, a small non-significant effect of the addition of these harvest aids was observed for seed amino acids contents (Bellaloui et al. 2020).

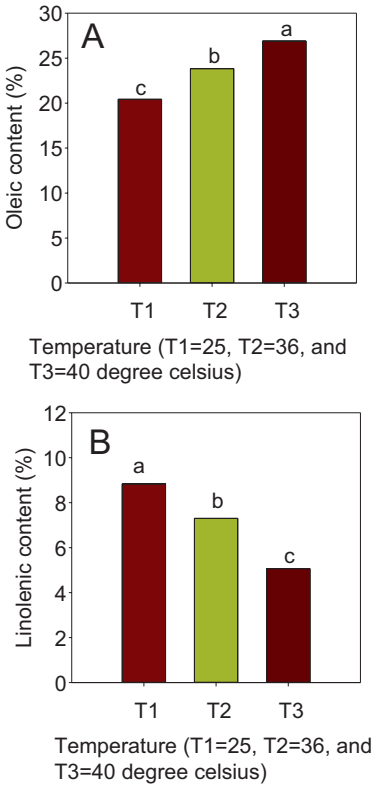

Fig. 1.4 Effect of temperature (normal, 25/20 °C; higher temperature, 36/30 °C; and heat stress, 40/36 °C) on seed oleic acid (**a**) and linoleic acid (**b**) in Hutcheson cultivar. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$

To separate the effects of genotype from environment and maturity effects, a 2-year experiment was conducted to study the effect of maturity on seed composition without the bias of the confounding factors of genotype and maturity (Bellaloui et al. 2009a, b, c). In this research, two sets of near-isogenic lines that developed with different maturities within a common genotypic background were used. One set with nine isolines was derived from "Clark" (Johnson 1958) and the other isoline set with seven lines was derived from "Harosoy" (Weiss and Stevenson 1955). The maturity in each set was different due to the combination of maturity genes (E1, E2, E3, E5) (the maturity of each line within a set varied, but all had a common genotypic background). This experiment allowed investigating the effects of maturity among and between the Clark and Harosoy isoline sets on seed composition in the Early Soybean Production System of the midsouth (Bellaloui et al. 2009a, b, c). They found that there were positive linear relationships between protein content and maturity among isolines of the Clark set in 2004 ($R^2 = 0.75$; $P \leq 0.001$) and 2005 ($R^2 = 0.63$; $P \leq 0.001$). However, in Harosoy isolines, there was no relationship between protein and maturity. On the other hand, there were negative linear relationship between oil content and maturity for Clark (in 2004, $R^2 = 0.82$, $P \leq 0.001$; in 2005, $R^2 = 0.91$, $P \leq 0.0001$) and Harosoy (in 2004, $R^2 = 0.19$, $P \leq 0.05$; in 2005, $R^2 = 0.36$, $P \leq 0.01$). Also, they found that maturity had a bigger influence on seed

composition than maximum temperature. They concluded that the relationship between seed composition and maturity was different between the Clark and Harosoy sets of isolines, depending on the range of temperature, as the range of maximum temperature during the last 20 days before maturity for the Harosoy isoline set (early isolines) was from 31.6 to 33.6 °C in 2004 and from 33.5 to 35.5 °C in 2005. However, the maximum temperature for the Clark isoline set was from 31.8 to 33.5 °C in 2004 and from 33.2 to 36 °C in 2005. The lowest protein concentrations were found when temperature ranges from 20 °C to 25 °C and the highest protein contents were found at temperatures lower than 20 °C or greater than 25 °C (Piper and Boote 1999; Dardanelli et al. 2006). Moreover, temperature during seed-fill was the main reason for maximal protein in MG II-III and MG VI in some environments where MG II-III generally matured under higher temperatures than MG VI. The total oil content increased as temperature increased to a certain point, then decreased as temperature increased (Gibson and Mullen 1996; Dornbos and Mullen 1992).

Effects of drought on seed protein, oil, and fatty acids were investigated in a greenhouse experiment. The experiment was conducted to evaluate the responses of slow-wilting trait to heat and drought using NC-Roy (fast wilting: Control1), Boggs (intermediate in wilting: Control2), and NTCPR94–5157 (slow-wilting: SW1) and N04–9646 (slow-wilting: SW2) genotypes. Plants were either well watered or drought stressed. Soil of watered plants was kept between −15 and −20 kPa and this was considered the field capacity and used as control. Drought-stressed plants were kept between −90 and −100 kPa (Bellaloui et al. 2010a) and were considered fully matured when they reached R8 (full maturity) according to Fehr and Caviness (1977). At full maturity, 95% of pods reached full maturity. Three replicates were used in each treatment. Greenhouse temperature conditions were kept at 34 °C ± 9 °C during the day and 28 °C ± 7 °C at night. Photosynthetic photon flux density (PPFD) during the day of about 800–2300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was measured by a quantum meter (Spectrum Technologies, Inc., Aurora. Illinois, USA). The wide range of light intensity reflects a bright, sunny, or cloudy day. The experiment was conducted during the normal growing season (from April to September) to simulate the growing season photoperiod of soybean production in the midsouth USA. The fully expanded leaves at seed-fill stages (R5-R6) were analyzed for water potential and mineral nutrition. Mature seeds at R8 were harvested for seed protein, oil, and fatty acids. The results from this experiment showed that protein and oleic acid were higher in slow-wilting soybeans than the controls (Figs. 1.5a,b, 1.6, 1.7, 1.8, 1.9, 1.10, and 1.11a, b) because of the inverse relationships between protein and oil and between oleic acid and linolenic acid. This was explained by the fact that slow-wilting soybean has the ability to maintain its cell water turgor and higher leaf water potential compared to controls.

The genetic modification of the fatty acid composition of soybean oil has been previously reported by Fehr (2007). For example, modified soybean oils have been sold commercially, including oils in which linolenic acid (18:3) content has been reduced from 8 to 1% and oleic acid (18:1) has been increased from 25 to >80%. This oil composition reduces or eliminates the need for hydrogenation to achieve

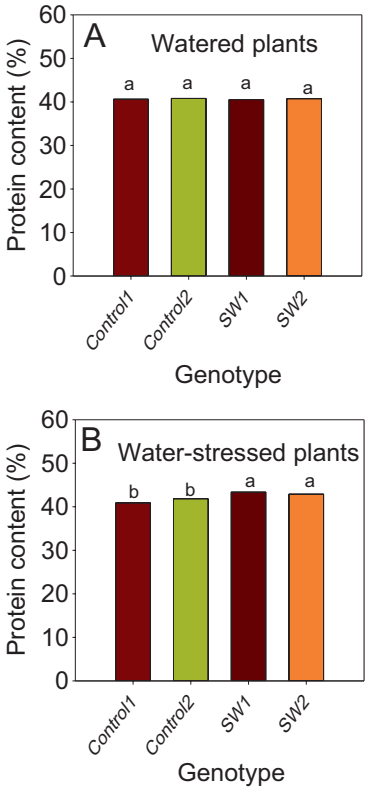

Fig. 1.5 Effect of water stress (drought) on seed protein in NC-Roy (fast wilting: Control1), Boggs (intermediate wilting: Control2), NTCPR94–5157 (slow wilting: SW1), and N04–9646 (slow wilting: SW2) genotypes. Plants were either well watered (**a**) or drought stressed (**b**). Soil of watered plants was kept between −15 and −20 kPa and this was considered the field capacity and used as control. Drought-stressed plants were kept between −90 and −100 kPa. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$

stability, minimize or eliminate transfats production, and prolong shelf life. Further, oil with palmitic acid (16:0) levels reduced from 11 to <4% has been achieved, resulting in oil low in saturated fatty acids content, a desirable oil trait for cardiovascular health. Genetic modification of oils has been achieved by genetic engineering and conventional breeding. It was reported that mutagenesis was the conventional breeding method used to develop the major genes contributing to reduced palmitic and linolenic acids, while genetic engineering was used to increase oleic acid to >80%. Leamy et al. (2017) reported that soybean (*Glycine max*) is a major crop in the world (Medic et al. 2014; Leamy et al. (2017). It was reported that decreased levels of saturated palmitic acid and increased levels of unsaturated oleic acid in soybean oil are beneficial for human cardiovascular health. Therefore, these oil traits became one of the major goals of soybean breeders.

In a recent study, Del Conte et al. (2020) found strong positive correlations between seed numbers, pod numbers, and plant node numbers and seed oil content, and these agronomic traits can be used for indirect selection for increased seed oil content.

Soybean [*Glycine max* (L.) Merr.] is grown worldwide due to the high protein and oil contents of its seed (Medic et al. 2014), and the characterization of soybean

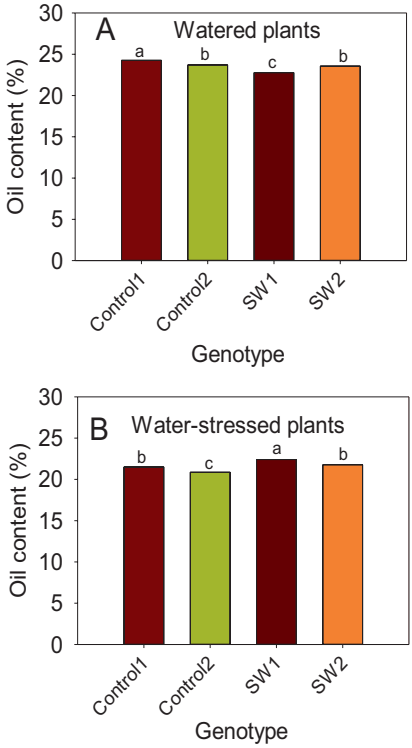

Fig. 1.6 Effect of water stress (drought) on seed oil in NC-Roy (fast wilting: Control1), Boggs (intermediate wilting: Control2), NTCPR94–5157 (slow wilting: SW1), and N04–9646 (slow wilting: SW2) genotypes. Plants were either well watered (**a**) or drought stressed (**b**). Soil of watered plants was kept between −15 and −20 kPa and this was considered the field capacity and used as control. Drought-stressed plants were kept between −90 and −100 kPa. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$

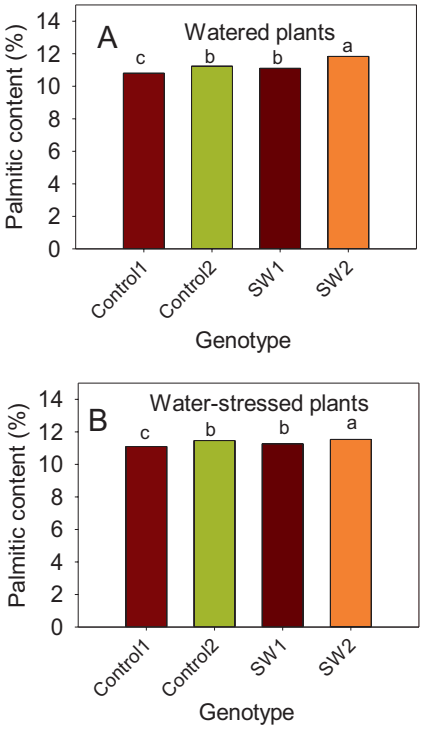

Fig. 1.7 Effect of water stress (drought) on seed palmitic acid in NC-Roy (fast wilting: Control1), Boggs (intermediate wilting: Control2), and NTCPR94–5157 (slow wilting: SW1), and N04–9646 (slow wilting: SW2) genotypes. Plants were either well watered (**a**) or drought stressed (**b**). Soil of watered plants was kept between −15 and −20 kPa and this was considered the field capacity and used as control. Drought-stressed plants were kept between −90 and −100 kPa. Bars are mean of three replicates. Different letters on the bars indicate significant differences at $P \leq 0.05$