HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES (MATE)

GEORGIKON CAMPUS

FESTETICS DOCTORAL SCHOOL

Head of the doctoral School

Prof. habil Angéla Anda, DSc

Comparative analysis of expressed genes in male and female inflorescences of the common ragweed (*Ambrosia artemisiifolia* L.)

Doctoral (Ph.D.) dissertation

Name of Author (PhD candidate)

Kinga K Mátyás Petrovicsné

Supervisor

Dr. Eszter Virág, PhD

KESZTHELY
2021

Name of Doctoral School: Festetics Doctoral School

Discipline: Plant Production and Horticulture

Head of Doctoral School: **Dr. habil. Angéla Anda, D.Sc.**
professor
Hungarian University of Agriculture and Life Sciences,
Institute of Environmental Sciences,
Department of Irrigation and Land Improvement

Supervisor: **Dr. Eszter Virág, PhD**

University of Debrecen,
Faculty of Science and Technology
Institute of Biotechnology
Department of Molecular Biotechnology and Microbiology

....	....
Approval of Head of Doctoral School	Approval of Supervisor

TABLE OF CONTENT

List of abbrevations 5

Introduction 9

Objectives of the study 11

Chapter 1 - Background 12

1.1. Description of *Ambrosia artemisiifolia* L. 12
1.1.1. Taxonomy and morphology 12
1.1.2. Reproduction biology 17
1.1.3. Origin and Distribution 17
1.1.4. Economic and human impact 19
1.2. Regulation of flowering 20
1.2.1. Environmental and endogenous pathways that regulate flowering 21
1.2.2. Floral architecture formation –ABC(E) genes 23
1.2.3. Male and female candidate genes 25
1.3.1. New generation sequencing methods 25
1.3.2. RT - qPCR 26
1.3.3. Bioinformatics methods 26

Chapter 2 – Materials and Methods 27

2.1. Plant materials and cultivation conditions 27
2.2. RNA-seq library construction and sequencing 28
2.3. Construction of *A. artemisiifolia* transcript datasets, identification of differentially expressed (DE) sequences and filtering pistillate specific transcripts 29
2.4. Selection of genes for floral transcriptome characterization 30
2.5. Digital gene expression analysis 31
2.6. Gene expression analysis with RT-qPCR amplification 31
2.7. Annotation of DE transcripts 32

Chapter 3 – Results and Discussion 33

3.1. Generating RNA-seq libraries from common ragweed inflorescences 34
3.1.1. RNA sequencing and assembly of *A. artemisiifolia* floral libraries 34
3.1.2. Validation of the *A. artemisiifolia* floral transcriptome 35
3.2. Identify flower development genes and determination of gene expression with *in silico* technique 38
3.3. Determine floral organ, ABC(E) genes and gene expression with *in silico* technique 42
3.4. Identify the sequences and gene expression of the most expressed transcription factors that control flowering 44

3.5. Selection, validation and annotation and determine expression level of *A. artemisiifolia* unigenes 48
3.5.1. Selection of *A. artemisiifolia* unique and differentially expressed genes 48
3.5.2. Annotation and functional classification of *A. artemisiifolia* unigenes 52
3.5.3. Expression of female flower specific genes 52
3.5.4. Expression of male flower specific genes 55
3.6. Validation of tissue specific genes with RT-qPCR technique 58

Thesis Points 63
Summary 64
Összefoglalás 65
References 66
Publication List 85
Appendix 91
Acknowledgements 98

List of abbrevations

ABA	abscisic acid
ALS	Acetolactate Synthase
BLAST	Basic Local Alignment Search Tool
bp	base pair
CDS	Coding Sequence
DE	Differentially Expressed
DNA	Deoxyribonucleic acid
cDNA	complementary DNA
EPSP	5-EnolPyruvylShikimate-3-Phosphate
FOIG	Floral Organ Identity Gene
GO	Gene Ontology
MIG	Meristem Identity Gene
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
NWS	National Weed Survey
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PPO	Protoporphyrinogen Oxidase
PSII	Photosystem II
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
RPKM	Reads Per Kilobase Million
RSEM	RNA-seq by Expectation-Maximization
RT-qPCR	Quantitative Reverse Transcription PCR
SRA	Sequence Read Archive
TF	Transcription factor
TSA	Transcriptome Shotgun Assembly
UniProt	Universal Protein Resource

Names of genes

ACA7	*ALPHA CARBONIC ANHYDRASE 7*
AG	*AGAMOUS*
AGL 24	*AGAMOUS-LIKE 24*
AMS	*ABORTED MICROSPORES transcription factor*
ANT	*APETALA 2-like ethylene-responsive transcription factor, AINTEGUMENTA*
AP	*APETALA*
BEE1	*BRASSINOSTEROID ENHANCED EXPRESSION 1*
CAL	*CAULIFLOWER transcription factor*
CO	*Zinc finger protein CONSTANS*
COP 1	*CONSTITUTIVE PHOTOMORPHOGENIC 1*
CRY	*CRYPTOCHROME*
CSTF	*CLEAVAGE STIMULATORY FACTOR*
CUC	*CUP-SHAPED COTYLEDON*
CYP450	*CYTOCHROME P450*
EIN3	*ETHYLENE INSENSITIVE3*
ELF3	*EARLY FLOWERING 3*
EMBF2	*POLYCOMB EMBRYONIC FLOWER 2*
FD	*FLOWERING LOCUS D*
FLC	*FLOWERING LOCUS C*
FRI	*FRIGIDA*
FT	*FLOWERING LOCUS T*
FUL	*FRUITFULL=AGL8 AGAMOUS-LIKE 8 MADS-BOX PROTEIN*
GA2OXS	*2-BETA-HYDROXYLATION OF GA 2-OXIDASES*

GDSL2	*ESTERASE/LIPASE 2; GLYCINE (G), ASPARAGINE (A), SERINE (S), LEUCINE (L)*
GID	*GA INSENSITIVE DWARF*
IAA	*INDOLE-3-ACETIC ACID*
ILR3	*IAA-LEUCINE RESISTANT 3*
LAP	*LESS ADHERENT POLLEN*
LFY	*LEAFY*
LMI1	*LATE MERISTEM IDENTITY1*
MSE1	*MALE SPECIFIC EXPRESSION 1*
MYB	*MYELOBLASTOSIS VIRAL ONCOGENE HOMOLOG*
NIP	*NODULIN 26-LIKE INTRINSIC*
OAS	*O-ACETYLSERINE (THIOL) LYASE*
PBL9	*PROTEIN KINASE 1A*
PCC13-62	*DESICCATION-RELATED PROTEIN*
PG	*POLYGALACTURONASES*
PHYA	*PHYTOCHROME A*
PHYB	*PHYTOCHROME B*
PI	*PISTILLATA*
MADS	*MINICHROMOSOME MAINTENANCE FACTOR 1 (M), AGAMOUS (A) DEFICIENS (D), SERUM RESPONSE FACTOR (S)*
PME	*PECTIN METHYLESTERASE*
SEP	*SEPALLATA*
SOC1	*SUPRESSOR OF CONSTANS OVEREXPRESSION 1*
SPA	*SUPPRESSOR OF PHYA*
STIG1	*STIGMA SPECIFIC1*
SUP	*SUPERMAN transcriptional regulator gene*

SYN	*SYNTAXINS MEMBRANE PROTEINS*
TCP12	*BRANCHED 2, TEOSINTE BRANCHED 1 (T), CYC (C), PROLIFERATING CELL FACTORS 1 AND 2 (P)*
TET8	*TETRASPANIN 8*
TFL1	*TERMINAL FLOWER 1*
TSF	*TWIN SISTER OF FT PROTEINS*
VIN 3	*VERNALIZATION INSENSITIVE 3*
VIP	*VERNALIZATION INDEPENDENCE*
VRN	*VERNALIZATION*
WIP2	*ZINC FINGER PROTEIN 2, TRYPTOPHAN (W), ISOLEUCINE (I), PROLINE (P)*
YAB	*AXIAL REGULATOR YABBY*

Introduction

Common ragweed is native to North and Central America (Lorenzi and Jeffery 1987; Kovalev 1989), but now this species is present world-wide; Africa (CJB, 2016), Asia (Flora of China Editorial Committee, 2016), Australia (Council of Heads of Australasian Herbaria, 2016) and Europe (Euro + Med, 2016). The distribution of the common ragweed in Europe covers the area at medium latitude characterized by continental climate started its expansion from two centers southwestern France and southwestern Hungary and meaning a current problem from both agricultural and public health aspects (Makra et al. 2004).

Since in the last 20-25 years the number of ragweed pollen has dramatically increased, it would be important to get global attention and find the optimal control against the rapid spread of the plant caused by extraordinary plant-adaptability, a large proportion of derelict land areas, the absence of specific pest insects and diseases, and not least the development of high-level resistance against herbicides. Considering the invasion migratory pathways, there is a high risk for the introduction of herbicide-resistant genotypes from Eastern to Western Europe.

Understanding the genetic control of sex determination during the plant life cycle may contribute to finding an ecologically safer strategy in the common ragweed control. Flower development is a complex and accurately coordinated biological and morphological process consisting of spatial regulation of a considerable number of organ-specific genes during the life cycle of higher plants (Taiz et al. 2015).

Many genes affecting floral meristem and structure formation were studied extensively using the model organism, *Arabidopsis thaliana* (*A. thaliana*) (Komeda 2004), which is a monoecious long-day plant with unisexual flowers. Investigated genes discussed in this study were chosen based on the genetic knowledge of *Arabidopsis* flowering (Chandler 2011, Irish 2010). Based on *Arabidopsis* transcriptome analysis the majority of expressed transcripts were found in the reproductive part of flowers instead of the perianth, reflecting the more complex anatomy of tissue and cell types within stamens and carpels and major developmental events such as ovule and pollen formation (Chandler 2011).

Members of the *Asteraceae* family are characterized by a more complex and modified floral formula compared to *Arabidopsis thaliana. Asteraceae* are characterized by capitulum inflorescence and this flower head can be monogamous or heterogamous (Harris 1999).

The inflorescences are protected by bracts, and their calyx reduced to pappus, scales, or coronula, have a corolla, stamens are connate and their cypsela fruit type is achene (Katinas et al. 2016).
Therefore, we used the ABC(E) gene sequences of different species from the *Asteraceae* family (*Gerbera sp.; Helianthus sp.; Tagetes sp.; Chrysanthemum sp.*) to determine the flower organ identify genes (FOIGs).

Due to the adverse effects on human health and weed control technique - mentioned above - it is of paramount importance to identify genes and pathways that regulate the development of flower organs and are responsible for pollen production and seed mass.

Next generation sequencing (NGS) is an advantaged technology to capture the diversity of differentially expressed transcripts in male and female flowers. In the present work, we report Illumina RNA-sequencing of two developmental stages of female flower compared with male and leaf transcriptomic data. Differences between expression levels of the above-described flowering pathways and two gene categories such as MIGs and FOIGs (ABC(E) ortholog - MADS box genes) identified in each type of flowers are also discussed. This study reports firstly about the sex-specific floral development in the common ragweed and the underlying genetic expression pattern. The genetic events of staminate and pistillate inflorescence formation were investigated through expression analysis in wild-growing and *in vitro* cultivated plants.

Objectives of the study

The aim of the present work is to identify genes that control the common ragweed flowering and their specificity to male and female inflorescence formation.

The aims can be summarized as:

1. Create transcriptome libraries from *A. artemisiifolia* L., early and late developmental stages of male and female inflorescences.

2. Identify the coding sequences and the expression level of flowering related genes of *A. artemisiifolia* L. inflorescences: Meristem Identity Genes (MIGs), Floral Organ Identity Genes (FOIGs), and Transcription factors (TF). Validation of tissue specific genes with RT-qPCR technique.

3. Determine the coding sequences and the expression level of the specific floral organ, ABC(E) genes during floral development.

4. Screening and identify of gender specific genes in male and female inflorescences and determine their expression level.

5. Determine the biochemical pathways that regulate male and female flower formation.

Chapter 1
Background

1.1. Description of *Ambrosia artemisiifolia* L.

1.1.1. Taxonomy and morphology

Ambrosia artemisiifolia L. (common or short ragweed, *A. artemisiifolia*) is a monoecious, anemophilous plant (Fig. 1.A) from the *Asteraceae* family and tribe *Heliantheae* (Chase et al. 2016; Essl et al. 2015). Common ragweed is an annual herb, which grows up 20-150 cm high (Fig. 1.B). The plant is characterized by erect, branched hairy stem with various shaped leaves (Soó 1970, Béres et al. 2005). Cotyledons of the seedling plant are bald, elliptical with obtuse apex (Fig. 1.C). Leaves mostly opposite, alternate above, deltate to lanceolata or elliptic deeply pinnatifid, the upper leaves margins are often entire, the surface is finely hairy and gland-dotted (Soó 1970, Essl et al. 2015).

Figure 1. Common ragweed **A-** Inflorescence of common ragweed, **B**- Adult flowering common ragweed, **C-** Ragweed seedlings with cotyledons and two mature leaves **D-** Male inflorescences of common ragweed.

Common ragweed is cosexual plant with a highly modified flower structure. It produces separate male and female flower heads in the same individual (Lloyd 1984). This modification in *Asteraceae* family is due to wind pollination (anemophily) and observed in the genera *Iva, Dicoria, Euphrosyne, Hymenoclea,* and *Xanthium* (Payne 1963). This gender system shows 5 % occurrence in sexually reproducing flowering plants mainly in gymnosperm pines and plants with catkins indicating an ancestral angiosperm state (Renner and Ricklefs 1995). Staminate heads cluster racemelike and increase and maturing acropetally (Payne 1963). Several male flowers are located in one staminate head (Fig. 1.D). Different data on the number of male flowers in a male staminate head can be found in the literature, Hegi (1906) 10-15, Bassett and Crompton (1975) 10-100 and Essl (2015) referred 9-39 flowers. Orientations of male flowers tend to be downwards; thus, the pollen falls into the air (Payne 1963). The phyllaries are laterally connate together to form a saucer-shaped involucre (Fig. 2.A). Trichomes can be observed on the surface and margin of the phyllaries (Fig. 2.B). The diameter of the staminate head is usually 2 to 3 mm. The inflorescence is open, flowers are arranged in a spiral and developing from the margin to the central (Fig. 2.C).

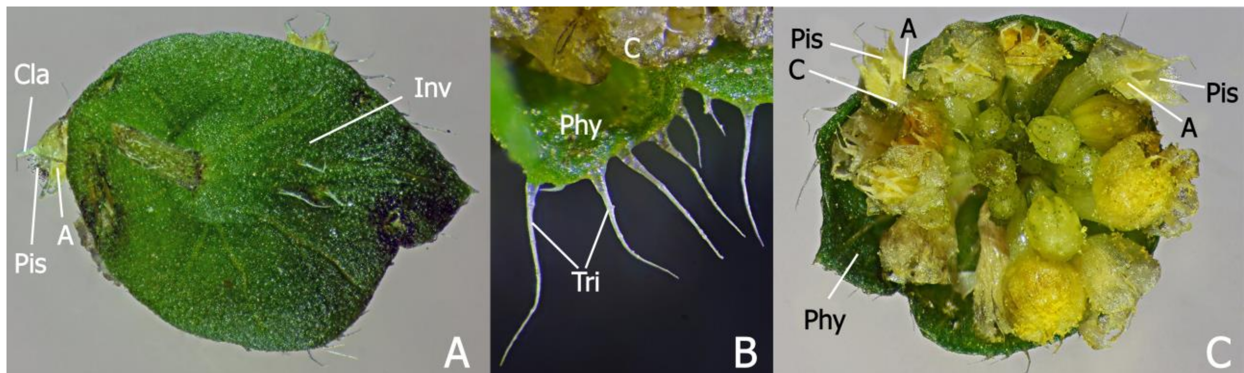


Figure 2. Male inflorescences of *Ambrosia artemisiifolia* L. **A**- Bottom-view of a campanulate involucre with connate phyllaries, M= 12x; **B**- Trichomes on involucre, M= 20x; **C**- Flowers in different developmental stages in the male inflorescence, M= 10x. M: magnification. Abbrevations: A – anther, C – corolla, Cla – claws, Inv – involucre, Phy – phyllaries, Pis – pistillodium, Tri – trichoma.

In case of optimal weather conditions in one staminate head, flowers can develop continuously. The number of flowers (10-25) observed at one time is not the same as the total number of flowers, as the buds are continuously formed, up to a hundred flowers can develop during a growing season. The size of the male flower is various, from about 1 to 2 mm long. The male flowers have a 5-lobed corolla, in which the fused petals are well visible (Fig. 3.B). On the surface of the corolla small glands, also known as colleters, can be observed. Colleters secrete a sticky resinous substance, which protects flowers from dehydration and infection (Fig. 3.A-D) (Darók 2011). After pulling off the corolla, the stamens become free and can be observed the flattened anthers (Fig. 3.D) (Mátyás et al. 2020). The claws at the top of the anthers (Fig. 3.C-E) weakly hold the anther ring together until the time of pollen discharge.

Figure 3. Parts of male flower of *Ambrosia artemisiifolia* L. **A**- Male flower bud with five-lobed corolla covered by colleters, M= 19x; **B-** Male flower bud from top view M= 45x; **C-** Blooming male flower, M= 17x; **D-** Stamens and pistillodium clearly visible after removal of corolla, M= 34x; **E-** Stamens with claws, M= 74x. M: magnification. Abbrevations: A – anther, C – corolla, Cla – claws, Col – colleter, Fil – stamen, Pis – pistillodium.

In male flowers can be observed a strongly reduced and modified pistil, called pistillodium (Fig. 4.A). The shape of the pistillodium is tubular, with brush-like hairs at the top (Fig. 4.B-C). After primary pollen discharge, the pistillodium begins to grow and pushes the remaining pollen out of the anther ring, thus providing a secondary pollen supply (Payne 1963).

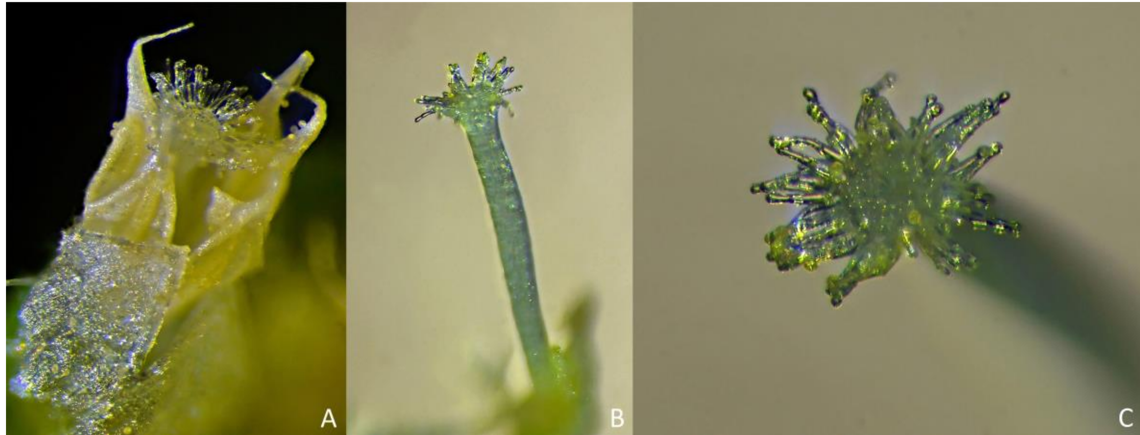


Figure 4. Pistillodium **A-** Pistillodium in a blooming male flower, M= 18x; **B-** Dissected pistillodium, M= 32x; C- Pistillodium from top view, M= 55x. M: magnification.

The female flowers are one or two flowered and are inconspicuously situated below the male ones, in the leaf axils (Bassett and Crompton 1975). Pistillate heads are often not solitary they develop continuously in the protection of bracts (Fig. 5.A). In the axils of one leaf, 3 to 6 female inflorescences can develop over several weeks. The female flowers range from 2 to 5 mm in length (Mátyás et al. 2020). The pistils are relatively long and the inner surface covered with papillose (Fig. 5.A-D). The pistil is surrounded by a vase-shaped organ, which is formed from the fusion of phyllaries. The surface of the phyllaries is covered with trichomes and spikes can be observed on the tips (Payne 1963). After maturation the inner part of the woody fruiting involucres, mononuclear achene is formed (Fig. 6.A-B).

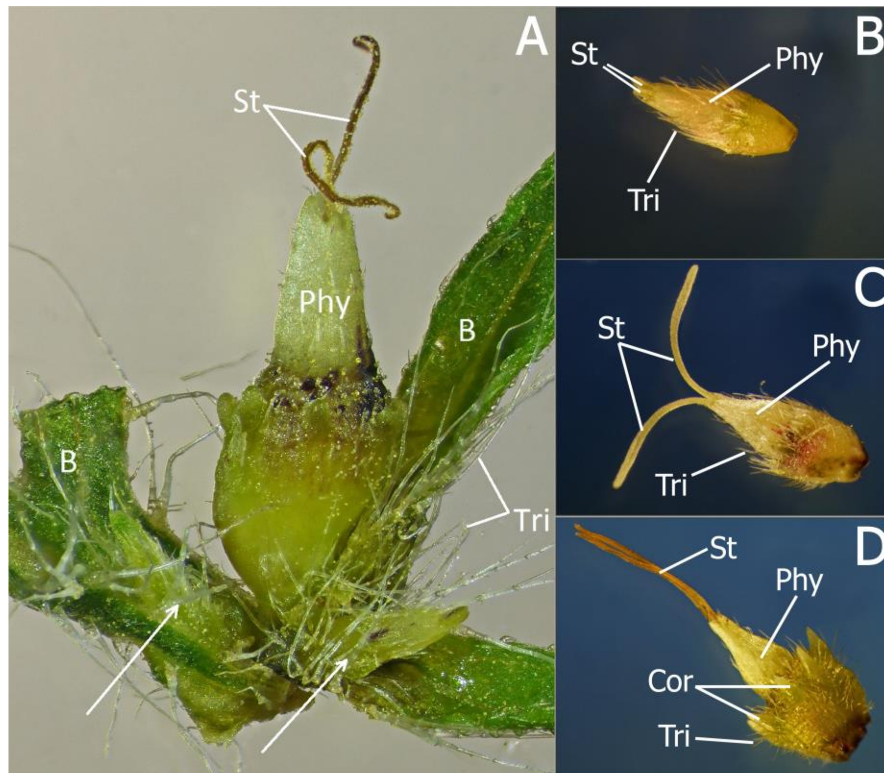


Figure 5. Female inflorescences of *Ambrosia artemisiifolia* L. **A-** Female inflorescences; a mature and fertilized inflorescence in the middle, and juvenile inflorescences visible to the right and left of it (indicated with arrows), M= 15x; **B-** Female flower in early developmental stage, M= 16x; **C-** Female flower with mature stigmas M= 9x; **D-** Fertilized female flower, with withered stigmas, swollen core and tubercles, M= 8x. M: magnification. Abbrevations: B – bract, Cor – coronula, Phy – phyllaries, St – pistil, Tri – trichoma.

Figure 6. **A**- One-seeded syconiums (image recorded with a digital microscope); **B**- Achenes recorded with a digital microscope (with sandpaper particles on their surface).

1.1.2. Reproduction biology

Freshly matured seeds have dormancy during the autumn and this can be eliminated by 6 to 12 weeks of stratification. Under the field conditions in continental climate the main germination period lasts from April to May, even after this period the germination remains until the first frosts, but less intense. Dry and warm weather can induce secondary dormancy (Bazzaz 1979). Seeds germinate in light and dark as well, but light stimulates the germination. Seeds may lose their germination capacity on the soil surface in four years, but in deeper layers, they remain to germinate for a long time (Béres et al. 2005). The germination time determines the biomass and the seed production of the individuals. Flowering time is influenced by germination time and average temperature (Béres 1993). Photoperiodic length determines the development of flowers, after the days have shortened, primordia of flowers appear, and therefore it is called a short-day plant (Allard 1943). The first male flowers appear between mid-July and early August, while the female flowers develop about two weeks after the male (Bassett and Crompton 1975, Brandes and Nitzsche 2006; Ziska et al. 2011). Under rising temperature and low humidity conditions anthers open at morning times (Martin et al. 2010). As a result, pollen grains desiccate and can easily disperse in the air. Pollen production varies between 0.1 and 3.8 billion (Fumanal et al. 2007) that depends on the plant size. The size of pollen grain diameter is 18 - 22 μm (Taramarcaz et al. 2005). During vegetation period active pollen spraying is from August to the end of October. Seed production of medium-sized and large individuals are 3.000 and up to 60.000, on average (Dickerson and Sweet 1971).

1.1.3. Origin and Distribution

Species of the *Ambrosia* genus come from North America and their gene centre is localized in the Sonora desert (Bohár 1996). The intensive spread of the common ragweed dates in Europe in the second half of the 19th century. The first record of its appearance was in 1893, Germany (Hegi 1906). According to some assumptions, the common ragweed seeds arrived in Europe with grain shipments at several stages after the I World War (Makra et al. 2005). The most infected areas are located in Northern Italy, in the Rhone Valley and in the Carpathian Basin (Jager 2001, Béres 2003).

Nowadays the proportions of infected areas are constantly increasing, and it is present in a large amount in the Alpine and Baltic countries (Kazinczi et al. 2008). Scalone and colleagues found that in Europe, from latitude 51° N to latitude 59° N, it was better adapted to light and temperature conditions than on the North American continent (Scalone et al. 2016). Storkey and colleagues predict the extent of the spread of the common ragweed in Europe to 2050 using the Sirius 2010 plant growth simulation model, considering climate change scenarios. Their results show that the species spread northwards, mainly to the UK and Denmark, while in the southern countries, coverage will not change due to the stress effects of drought (Storkey et al. 2014) (Fig. 7.).

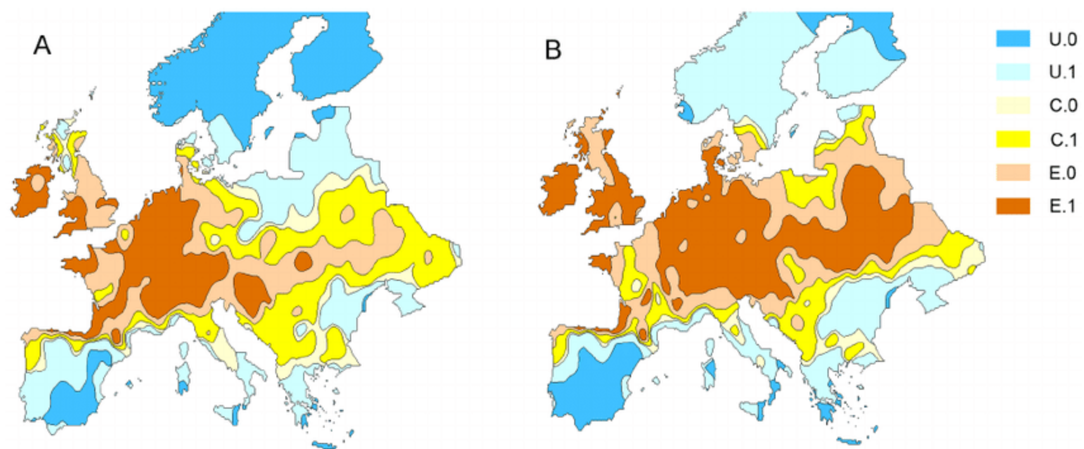


Figure 7. Distribution of *Ambrosia artemisiifolia* (common ragweed) in Europe under climate change as predicted by the process-based model. **A-** Using HadCM3 (A1B) scenarios for near future 2010–2030 and **B-** long-term future 2050–2070. The categories are: U.0 - highly unsuitable, U.1 - unsuitable, C.0 - casual (less likely), C.1 - casual, E.0 - established, E.1 - well established. doi: 10.1371/journal.pone. 0088156. g003

Recent research based on herbarium specimens from Orsova and Herculesfürdő suggests that the first appearance in the Carpathian-Pannonian region of this species dates at 1907 (Csontos et al. 2010). More individuals were detected in the 1920s in the southern part of Somogy county (Lengyel 1923). After adapting to climate conditions, it was spread to the northeast way in the whole country (Tímár 1955). The data of the National Weed Surveys (NWS, Hungary) also well reflect the intensity of the spread of this species.

At the time of the first NWS (1947-1953), it was ranked as 21st, with 0.39% coverage of the list of the most common weeds, and after fifty years it was ranked as first (4th NWS, 1996-1997) with 4.7% coverage (Novák et al. 2009) and its coverage was 5.33% in 2011 (Novák et al. 2011). Based on this data, its spread is constantly growing. In addition to its excellent adaptability, wrong cultivation practices can also contribute to its spread in Hungary (Pálmai 2009).
Mátyás et al. studied ragweed populations in the Carpathian Basin, and their results showed that common ragweed should be a metapopulation in which populations are not separated and there is a continuous gene flow between them due to random hybridization of individual plants (Mátyás et al. 2012). Domonkos et al. monitored the distribution of common ragweed in Csallóköz and Szigetköz using Geographic Information System (GIS) tools. They found that its spatial occurrence was not homogeneous, ragweed-free and heavily infested areas can also be found in each region (Domonkos et al. 2016).

1.1.4. Economic and human impact

Common ragweed has been in prominence of research over the past 50 years due to its rapid spread and allergenic effects. Lehoczky (2004) and Lehoczky et al. (2012) show that the adaptability of this species is high and it appears almost everywhere, except in extreme conditions (Lehoczky 2004), (Lehoczky et al. 2012). According to Skálová et al., this species is the dominant weed at train stations, in ports, next to stables, in agricultural and industrial areas (Skálová et al. 2017).

Lehoczky (2004) studied ragweed shoot and root mass ratios at different nitrogen levels. It produced the highest shoot and root mass on soils with a nitrogen supply of 100–300 mg/kg, and it was found that ragweed can tolerate a very high nitrogen content of 400 mg/kg soil and blooms even under unfavourable, nutrient-deficient conditions (Lehoczky 2004). This species is an effective competitor that may cause a high yield reduction in agricultural crops.
It was monitored that in maize culture 26 plants/m^2 density of common ragweed caused 70% (Varga et al. 2000), in sunflower culture 10 plants/m^2 caused 30% (Kazinczi et al.2009) and in sugar beet culture 5 plants/m^2 caused 50% (Bosak and Mod 2000) yield reduction. In addition, common ragweed problem becomes more difficult, because of the occurrence of new herbicide-resistant biotypes such as plants resistant to PSII, ALS, PPO, EPSP synthase inhibitors and several individuals with multiple resistance.

Currently, herbicide resistance in common ragweed has been described in the mentioned 4 herbicide groups, from the 25 inhibitor groups which are registered on the International Herbicide-Resistant Weed Database (Taylor et al. 2002, Tranel et al. 2004, Zheng et al. 2005, Saint-Louis et al. 2005, Brewer and Oliver 2009, Rousonelos et al. 2012, Heap 2020).

The highly allergenic pollen of the common ragweed causes serious problems in the health care. An average-sized plant can produce eight billion of pollen in 2-3 months from August to October. Pollen may spread long distances by the wind, pollen grains were found at 5000 meters and 160 kilometres from the mainland (Járainé 2003). Clinical studies show that 10, 7% of the European population can be sensitized with *Ambrosia* pollen (Burbach et al. 2009) that may cause allergic rhinitis, asthmatic symptoms, and dermatitis or phytophotodermatitis triggered by the direct contact of the plant itself (Epstein 1960, Hjorth et al. 1976). Twelve allergens have been described among which the major pollen allergen, Amb a 1 belongs to the pectatelyase protein family (Adolphson et al. 1978; Gadermaier et al. 2008). Pectate lyase enzymes are the most aggressive proteins from the surface of the pollen grains (Juhász 2012) these enzymes are responsible for degrading cell walls and are also found in other parts of the plants (Marín-Rodríguez et al. 2002). Due to cross-allergy, it is important to mention that the enzyme pectate lyase is found not only in pollen but also in the fruits of many species, such as tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*), mouse-ear grass (*Arabidopsis thaliana*), rice (*Oryza sativa*) and strawberry (*Fragaria ananassa*) (Egger et al. 2006). *In vitro* allergen study showed that 90% of patients responded to Amb a 1, while sensitivity to other ragweed allergen molecules ranged from 20 to 35% (Asero et al. 2006). An additional problem that global warming may amplify the common ragweed invasion such as average temperatures and higher CO_2 concentrations increase of ragweed biomass and higher pollen production (Ziska et al.2003).

1.2. Regulation of flowering

The main purpose of plant life is a reproduction. To achieve a reproductive state, they undergo several changes that are determined by both external and internal influences. In our studies, genes of the photoperiodic, vernalization, and hormonal (gibberellic acid) signaling pathways and genes associated with flower and flower organ development were examined.

1.2.1. Environmental and endogenous pathways that regulate flowering

The dynamic process of flowering in *A. thaliana* is induced from different pathways through the integration of environmental (photoperiodism and vernalization) and endogenous (hormonal regulation) signals. These signals trigger a complex system of interacting genes and proteins with repressor and activator roles influencing one another. Corbesier and colleagues identified a floral inducer *FLOWERING LOCUS T (FT)* playing an integral role between the different pathways (so-called mobile 'floral integrator'), that determine the floral development (Corbesier et al. 2007).

In angiosperms, the circadian clock regulates floral induction in response to seasonal changes. In most cases, the day-length as the main factor of photoperiodism may affect the flowering in plants. For example, *Cannabis sp*., *Gossypium sp*., *Xanthium sp*. respond to short day (SD) signal however *A. thaliana*, *Avena sp*., *Lolium sp*., *Trifolium sp*. respond to long day (LD) signals. In photoperiodic sensing there is a critical role of the *CO* gene. Its gene product the CONSTANS protein is stabilized by light and initiates a complex interaction with inducers and repressors and binds to the *FT* promoter. Such a way it may upregulate the floral inducer *FT* mRNA in long-day conditions, when the circadian clock-controlled expression peak of *CO* in the evening coincides with daylight (Putterill et al. 1995, Samach et al. 2000, Song et al. 2010, Wigge et al. 2005).

To induce flowering during long-day lighting conditions the CO protein activate the *FT*-mRNA in long-day plants, however in the same conditions there is a repression process in short-day plants regulated by a light-stabilized *CO* ortholog *Hd1*, that repress the *FT* ortholog *HEADING DATE 3 (Hd3a)* (Hayama et al. 2003). With the illumination of far red and blue (late afternoon lightening conditions, daylight >700nm) translation and stability of *CO* is regulated by *PHYTOCHROME A (PHYA)* and *CRYPTOCHROME (CRY)* via repressing degradation of CO by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF PHYA proteins (SPA) (Jang et al. 2008, Laubinger et al. 2006, Yu et al. 2008).

PHYTOCHROME B (PHYB) facilitates degradation of CO in the morning and delays flowering (Song et al. 2013). *FLOWERING LOCUS C (FLC)* is an epigenetically controlled suppressor to inhibit floral induction in the vernalization pathway during the autumn period (Amasino 2010. In this period the prolonged cold exposure (10°C to -1°C), called vernalization, is required for the plant meristem to be competent to respond to floral induction conditions (Simpson and Dean 2002).

This is important to prevent flower development during the winter months when low temperatures may cause serious damage in floral tissue and most pollinators are absent. High level of *FLC* mRNA is maintained by *FRIGIDA (FRI)* (Gazzani et al. 2003, Michaels and Amasino 2001) leading to transcriptional downregulation of the floral activators *SUPRESSOR OF CONSTANS OVEREXPRESSION1 (SOC1), FLOWERING LOCUS D (FD)* and *FT*.
FLC proteins bind directly to the promoters of *SOC1* and *FD* and a region in the first intron of *FT*, inhibiting the transcriptional activation of these genes and subsequent floral transition (Helliwell et al. 2006, Searle et al. 2006).

Vernalization mediates repression of *FLC* expression by transcriptional and epigenetic routes. *VERNALIZATION INSENSITIVE 3 (VIN3)* plays a central role in vernalization by mediating the initial transcriptional repression of *FLC* (Sung and Amasino 2004). However, due to its transient expression, it cannot maintain repression of *FLC*, which is then maintained by Polycomb Group complexes containing VRN1 and VRN2 throughout development. Stable repression of *FLC* requires specific methylation and deacetylation of histones around the *FLC* promoter. Upon receiving the above-described signals, under appropriate conditions, floral meristem identity genes (FMIGs) and the homeotic floral organ identity genes (FOIGs) are regulated by additional pathways. The upregulation of *LEAFY (LFY)* and *APETALA1 (AP1)* (FMIGs) evocates a cascade that regulates FOIGs expression (Mandel and Yanofsky 1995, Weigel and Nilsson 1995) toward floral whorl formation. *LFY* interacts with several homeotic genes to control their expression and therefore it is directly involved in the determination of floral architecture. Based on positive or negative regulation effect FMIGs can be divided into two distinct classes. The first class - such as *LEAFY, APETALA1*, and *CAULIFLOWER* - promotes flower meristem identity.
The second class such as TERMINAL FLOWER (TFL1) has the opposite effect and maintains the identity of inflorescence shoot meristems.

Hormonal regulation of reproductive development is highly dependent on the gibberellin signal and may vary among different species. Gibberellin involvement in floral initiation, therefore is more complex. While gibberellins (GAs) promote flowering in some LD and biennial species, their effects in other species are absent or may inhibit flowering of some perennials.

In the case of SD plants, the photoperiod flowering pathway is not decisive, therefore the GA pathway plays a major role and becomes significant. GID (GA INSENSITIVE DWARF) proteins - encoded by *GID1A, GID1B* - are soluble GA receptors and bind with high affinity the biologically active GAs, whose active state is catabolized by 2-beta-hydroxylation of GA 2-oxidases (GA2OXs). During flowering in response to GA, *GIDs* interact with specific DELLA proteins (e.g., GAI), known as repressors of GA-induced growth, and target them for degradation via proteosome (Griffiths et al. 2006). In case DELLA interacting with the *MYC3* transcription factor, which is an *FT* suppressor, preventing the LD plants from precocious flowering under SDs (Bao et al. 2019). In addition, DELLAs can directly interact with the flowering repressor *FLC* and promote its repressive function (Li et al. 2016). Gibberellin activates several *GAMYB* transcription factors that positively regulate the *LFY* expression, and stimulates *SOC1* through several pathways. It promotes the expression of genes that induce *SOC1* and at the same time represses the inhibitors of flowering, such as *SVP* (Li et al. 2008, Achard et al. 2004).
During the processes of floral transition, the cytokinins have an important role as part of hormonal regulation. It was demonstrated experimentally that the response to cytokinin treatment does not require *FT*, but activates its paralogue *TWIN SISTER OF FT (TSF)*, as well as *FD*, which encodes a partner protein of TSF, and the downstream gene *SOC1* (D'Aloia et al. 2011).

1.2.2. Floral architecture formation –ABC(E) genes

Floral organ identity determination is regulated by three classes of homeotic genes (FOIGs, encoding the A, B and C functions). The ABC(E) combinatory model (Coen and Meyerowitz 1991) predicts that these classes act alone or combinatorically to give rise to sepals, petals, stamens, and carpels. The activities of class A such as APETALA (AP1, AP2), class B such as APETALA3 (AP3), PISTILLATA (PI), and class C such as AGAMOUS (AG) are active in two adjacent whorls where their individual and combined activities specify the fate of organ primordia. A alone (whorl 1) specifies sepals, C alone (whorl 4) specifies carpels, and the combined activities of AB (whorl 2) and BC (whorl 3) specify petals and stamens, respectively. (The A and C activities are mutually antagonistic, such that A prevents the activity of C in the outer two whorls, and C prevents the activity of A in the inner two whorls.

The C activity is also required for floral determinacy: if the C activity is absent, an indeterminate number of floral whorls develop (Pelaz et al. 2000). *SEPALLATA (SEP1, SEP2, SEP3, SEP4)* genes form an integral part of models that outline the molecular basis of floral organ determination and are hypothesized to act as co-factors with ABC(E) floral homeotic genes in specifying different floral whorls (Fig. 8.).

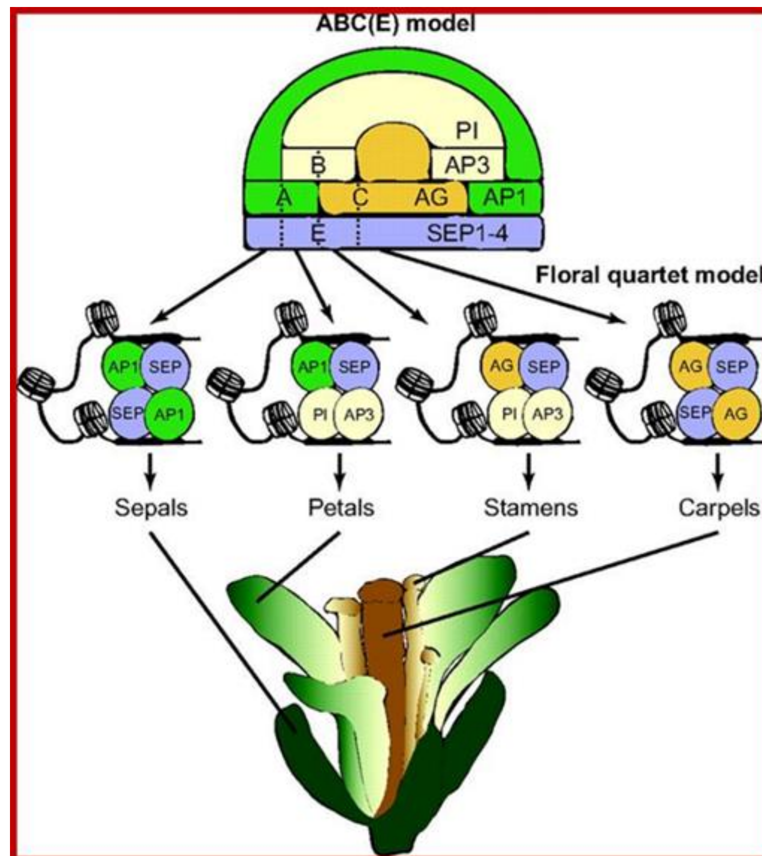


Figure 8. The ABC(E) model of flower development in *Arabidopsis thaliana*. The upper part of the figure indicates a schematic drawing of the flower meristem. The middle part indicates the single or combined effect of each transcriptional regulatory factor. The lower part indicates the mature flower. www.dev.biologists.org

1.2.3. Male and female candidate genes

To validate our transcriptome libraries, we used sex-specific genes described in the literature previously. The transcription factor *ABORTED MICROSPORES (AMS)* (Lou et al. 2014, Xu et al. 2010) and genes such as *LESS ADHERENT POLLEN3 (LAP3)* (Dobritsa et al. 2009), *LESS ADHESIVE POLLEN5,* and *LESS ADHESIVE POLLEN6 (LAP5, LAP6)* (Dobritsa et al. 2010) encode anther-specific proteins required for pollen exine development, so-called male candidate genes (Rocheta et al. 2014). *MALE SPECIFIC EXPRESSION 1 (MSE1)*, is specifically expressed in male flowers. It is essential to early anther development (Murase et al. 2017). Expression of *POLYGALACTURONASES (PG1* and *PG2)* and *PECTIN METHYLESTERASE (PME)* are characteristic in the tissues of mature pollen grains after microspore mitosis (Futamura et al. 2000). Female candidate genes such as filamentous floral proteins AXIAL REGULATOR YABBY1 and YABBY4 (YAB1, YAB4) (Meister et al. 2002, Siegfried et al. 1999, Villanueva et al. 1999) and transcription factor AINTEGUMENTA (ANT) (Elliott et al. 1996, Klucher et al. 1996, Long and Barton 1998) are relevant in pollen recognition, ovule formation and embriogenesis identifying pistillate tissues. STIGMA SPECIFIC1 (STIG1) protein was found also as a female candidate in *Quercus suber* described by Rocheta et al. (Rocheta et al. 2014). (Functional descriptions are summarized in Table 1-2).

1.3. Methods used to identify flower development genes and study for gene expression

1.3.1. New generation sequencing methods

Next-generation sequencing (NGS) technology allows all genome and transcriptome analysis of different species and individuals. This high throughput technology results in a large amount of data. In the case of RNA sequencing (RNA-seq) it provides comprehensive information on the expression of all genes at the time of sample collection (Wang et al. 2009). Using RNA-seq we can reconstruct the entire transcriptome based on a reference genome or *de novo* assembly (Garber et al. 2011). The degree of reading abundance may provide information on the gene expression at the transcriptome level.

The RNA-seq has already been used to identify flower development genes and mechanisms, not only in species with a model flower system but also in species with other floral gender systems, such as *Cucurbitaceae*, *Quercus spp*., *Zea mays*, *Salix sp.* (Ueno et al. 2010, Rocheta et al. 2014, Du et al. 2020, Forestan et al. 2020, Liu et al. 2013).

1.3.2. RT - qPCR

RT-qPCR is an excellent method to validate high-throughput results and quantify gene expression due to its sensitivity and specificity (Chuaqui et al. 2002; Czechowski et al. 2005; Die et al. 2010). Normalization of RT-qPCR is required, which can be done using a reference gene expressed at a constant level (Radonic et al. 2004; Huggett et al. 2005).
Several reports have shown that there are no generally applicable unchanged reference genes, for different species and RNA samples (Czechowski et al. 2005; Gutierrez et al. 2008; Bustin et al. 2015; Artico et al. 2010), so preliminary tests should be performed to select the reference gene before each experiment.

1.3.3. Bioinformatics methods

Contigs of transcripts generated during RNA-seq can be identified by the BLAST method based on homologous sequences in public databases (NCBI, UniProt).
Based on the results of genome sequencing, it can be stated that the biological function most of the genes is the same in eukaryotes. Therefore, the goal of the Gene Ontology Consortium was to create a dynamic, controlled vocabulary that will apply to most target organisms. Ontologies are (http://www.geneontology.org): biological process, molecular function, and cellular component. A biological process describes the biological role of a gene or gene product. This biological process can take place in several steps and undergo a chemical and physical transformation. Molecular function refers to the biochemical activity of a gene product and also shows what it potentially carries. The cellular component shows where the product of that gene is active (Ashburner et al. 2000).

Chapter 2
Materials and methods

2.1. Plant materials and cultivation conditions

The RNA-seq experiments were based on common ragweed samples from four bulked individuals. Seeds were collected from Cserszegtomaj, Zala county, Hungary (GPS: 46.79528, 17.26005) and were surface sterilized for 1 min in 70% (v/v) ethanol and washed with tap water for 23-30 min, followed by dipping in a 7% solution of calcium hypochlorite with TWEEN 20 for 20 min, and rinsing three times with sterile distilled water. The sterilized seeds were then germinated on MS medium (Murashige and Skoog, 1962) with 0.3 mg/l metatopolin hormon supplemented with 2% sucrose/litre and solidified with 0.8% phytoagar. The pH was adjusted to 5.8 prior to autoclaving at 120 °C for 20 min. All the cultures were maintained at 25 °C under a 16/8 light/dark photoperiodic regime with a light intensity of about 2400 to 2800 lux. Node segments (1.5 -2 cm long) were excised from 21- day-old plants and transferred to the above described MS medium.

Nine developmental stages of female flowers were collected from 12 *in vitro* cultivated individuals of common ragweed. The classification of the different phenological phases was based on visual observation (Fig. 9.). For RNA-seq two categories of nine phenological phases (1F and 2F) were established and combined such as early 1F (Fig. 9.A-C) and late 2F (Fig. 9.G-I) developmental stages. Male flower buds were collected from genotypes used in F, M, L transcriptomes (Virág et al. 2016). For gene expression comparisons we used 1M and 2M libraries as indicated in Fig. 9. (J-L, 1M; P-Q, 2M). To equal distribution, we determined the weight of the samples from each developmental stage (Appendix 1.).
Along with RT-qPCR analysis, we used four additionally wild-growing genotypes (collected in Keszthely, Zala county, Hungary, GPS: 46.7654716, 17.2479554).

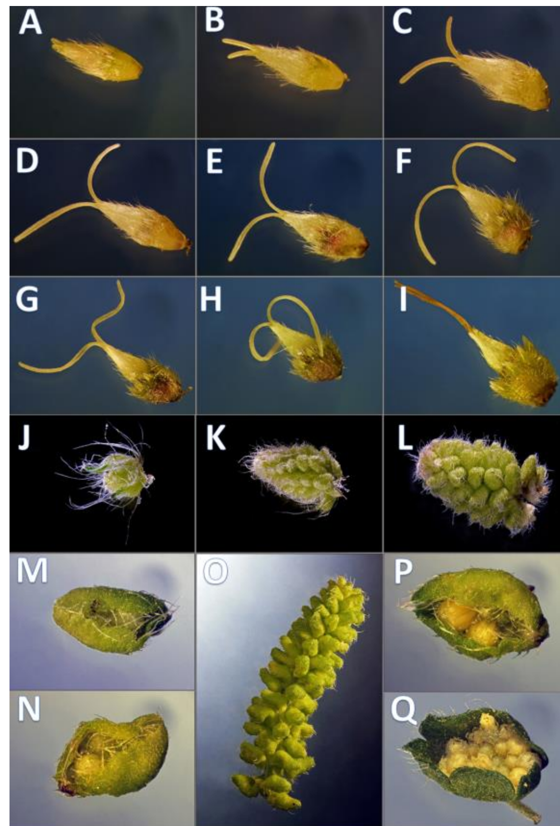


Figure 9. Developmental phenophases of female and male flowers used for RNA-seq. Symbols: Female flowers (F) and male flowers (M). For RNA extraction for *A. artemisiifolia* reference sequence the pool of F (A-I) and M (J-Q) of wild-growing samples were performed, separately. In order to obtain pistillate specific transcriptomes during floral organ formation, RNA extractions were performed from *in vitro* samples from F (A-C) and F (G-I), wild-growing samples from M (J-L) and M (P-Q) such as early and late developmental stages. Photo: Csaba Pintér (2013)

2.2. RNA-seq library construction and sequencing

Samples were frozen in liquid nitrogen immediately after collection. RNA extraction and on-column DNase digestion were performed from each category using TaKaRa Plant RNA Extraction Kit (Takara Bio Inc; Japan) following the manufacturer's instruction. RNA integrity and quantity were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies; USA).
2.0 µg of total RNA from 1F, 1M, and 2F, 2M samples were used as starting material for cDNA synthesis and library construction.

Enrichment of mRNA, cDNA synthesis, and library preparation for Illumina NextSeq paired-end sequencing were carried out using TruSeq™ RNA sample preparation kit (Low-Throughput protocol) using an oligo$(dT)_{18}$ as described by Virág et al. 2016.

The RNA-seq was performed using the Illumina NextSeq5000 system. Raw sequence data in FASTQ format were deposited in the NCBI SRA database under the accession SRR5965731 (1F) and SRR5965732 (2F). We used 1M and 2M for gene expression comparison in the case of investigated genes. 1M and 2M transcriptome datasets have not yet been uploaded to the NCBI database, and their analysis will be published elsewhere. Sequence reads of male inflorescence (M), leaves (L), and female (F) inflorescence containing all nine developmental stages were used from earlier reported and deposited data in National Centre for Biotechnology Information's (NCBI) Sequence Read Archive (SRA) under the accession SRP08007 (Virág et al. 2016).

2.3. Construction of *A. artemisiifolia* transcript datasets, identification of differentially expressed (DE) sequences and filtering female, and male specific transcripts

During preprocessing, raw reads quality was examined using the FastQC quality control software (Andrews 2010). Based on FastQC report, sequences found to be represented more than 0.1% of the total and low-quality bases (corresponding to a 0.1% sequencing error rate) were removed and trimmed using a self-developed application 'GenoUtils' written in Visual Studio integrated development environment in C#. Conversion of .fastq to .fasta files was also performed using this in-house application.

A sample-specific multiple assemblies of cleaned reads from seven libraries (M, L, F, 1F, 2F, 1M, and 2M) were performed. *De novo* assembly of each sample type was performed by using Trinity (Haas et al. 2013). For reference, guided alignments Bowtie2 short read aligner (Langmead et al. 2009) was applied. The assembly strategy was performed in a way with taking into account the refinement of female and male tissue-specific sequences in both developmental classes as the final outcomes.

First, a combined read set was assembled from the three sample libraries (M, F, and L) to generate a ‘reference’ *de novo* transcriptome assembly that was deposited in the NCBI TSA database under the accession GEZL00000000 and reported in our previous study (Virág et al. 2016). During this process, reads with a certain length of overlap were combined to form contigs (kmer size, K, = 25). This combined dataset was used as a reference shotgun assembly in the further alignments. In order to perform sample-specific expression analysis, we screened the differentially expressed sequences by aligning the original sample reads to the reference followed by abundance estimation using RSEM (Li and Dewey 2011).

The resulting differentially expressed transcripts were further clustered according to their expression patterns by applying Microsoft SQL Server Management Studio. Protein coding regions were extracted from the reference assembly using TransDecoder and further characterized according to likely functions based on sequence homology or domain content using BLAST+ (Altschul et al. 1990). In this way coding sequences (CDS) as unique transcripts of each seven samples were screened and compared. Separately, *de novo* assemblies of 1F, 2F, 1M, and 2M libraries were performed using Trinity and deposited in the NCBI TSA database under the accession GFWB00000000 (1F) and GFWS00000000 (2F). Using these reference contig sets 1F and 2F unique transcripts were realigned applying BLASTn with E value less than 10^{-5}. The resulting narrowed and specified sequences were annotated using NCBI nr protein database.

2.4. Selection of genes for floral transcriptome characterization

Genes representing different floral regulatory pathways - stimulated by environmental and endogenous signals - were selected to characterize genetic events during male and female floral development. Architecture formation wasanalized by determining ABC(E) genes expression pattern at generative growth (differences in FOIGs). Genes that enable and promote floral meristem initiation during vegetative growth (FMIGs) such as photoperiodic, vernalisation, gibberellin pathway and flower development genes (Fig. 10., Fig. 11.) were also investigated in both wild-growing and *in vitro* samples. Normalized expression of selected genes was performed digitally and expression values were compared.

2.5. Digital gene expression analysis

The obtained reads from each library were mapped to the selected gene CDSs by using Bowtie2. The mapped reads were used to estimate the transcriptome level by the reads per kilobase per million mapped reads (RPKM) method (Mortazavi et al. 2008):

$$RPKM = \frac{\text{CDS read count} * 10^9}{\text{CDS length} * \text{total mapped read count}}$$

In this equation, CDS represent the coding sequence of the investigated gene. Read count means the mapped reads from the total. CDS length is the nucleotide sequence length of the coding region of the investigated gene and total mapped reads are the total raw reads of a given cleaned library. For automatic calculation of RPKM values of genes, a self-developed pipeline as module of GenoUtils were applied.

2.6. Gene expression analysis with RT-qPCR amplification

Reverse transcription was performed starting from 1 µg total RNA using Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase (Thermo Scientific) according to the manufacturer's protocol, using an oligo$(dT)_{18}$ and random hexamer primers (Thermo Scientific), the final volume was 20 µl. cDNA (1 µl) was used for real-time PCR amplification on a Bio-Rad CFX96 System. qPCR analysis (and efficiency) was performed with 1µl of cDNA on a Bio-Rad CFX96 System using Xceed qPCR SG Mix (Institute of Applied Biotechnologies). The relative gene expression was calculated with ΔΔCt method using Bio-Rad CFX Manager™ Software v3.1. Primer efficiency was analyzed with CFX Manager™ Software v3.1 (Bio-Rad). PCR was performed as follows: an initial activation of the polymerase enzyme at 95 °C for 2 min was followed by 46 cycles at 95 °C 5 sec, 59 °C for 30 sec, ended with a 5 sec melt analysis ranging between 65-95 °C with 0.5 increments.

Primers (Appendix 2) were designed with Primer3 (Koressaar and Remm 2007, Untergrasser et al. 2012) based on *in silico* sequence prediction of CDS.

Gene expression analysis was established based on three technical and biological replicatesand normalized with the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Hodgins et al. 2013). In a pilot experiment, *GAPDH* was selected as a reference gene. In this investigation based on the literature CDS of fifteen housekeeping genes were determined. Subsequently, the relative expression levels were determined in three sample types (L, M, F) where six reference genes showed the same level, respectively. Among the equally expressed genes, three reference genes (*GAPDH, TUA*, *TUB*) were selected for RPKM value calculation (Appendix 3.) and PCR experiments.*GAPDH* was the most characteristic along with these investigations. Based on RPKM values *GAPDH* expression referred to an equivalent level order of magnitude level in each library ranging from 2480-4914.

2.7. Annotation of DE transcripts

Whereas *A. artemisiifolia* floral transcriptome was a newly targeted transcriptome the annotation procedure was performed as described by Haas and colleagues (Haas et al. 2013). Potential coding regions within reconstructed transcripts that were insufficiently represented by detectable homologies to known proteins were predicted based on metrics tied to sequence composition by applying TransDecoder include with Trinity.

Running this application on the Trinity-reconstructed transcripts the candidate protein-coding regions could be identified based on nucleotide composition, open reading frame (ORF) length, and (optional) Pfam domain content. To predict their functions the latest non-redundant (Nr) protein database 10/03/2016 (ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz) were used.
For further annotation of unigenes using various bioinformatics approaches, the unigenes were firstly searched against the non-redundant database and the Swiss-Prot protein database using local BLASTx (Gish and States 1993) with an E value cutoff of 10^{-5}. With Nr annotation, Blast2GO (Götz et al. 2008) was used to get GO annotation according to molecular function, biological process, and cellular component ontologies http://www.geneontology.org.

Chapter 3
Results and Discussion

Genetic control of floral morphogenesis is a well investigated field in the model plant *A. thaliana*. Using *Arabidopsis* hermaphrodite flowers and their mutants some genetic models were established for the structural development of floral organs in which a series of sequential steps were defined, for review see Krizek and Fletcher (Krizek and Fletcher 2005). As the initial destination, the floral meristem is regulated through the activation of FMIGs by flowering pathways. Subsequently, the floral meristem is patterned into the whorls of organ primordia through the activity of FOIGs. Then the FOIGs activate downstream effectors that specify the various tissues and cell types that constitute the different floral-organ types. Each of these steps are under strict genetic network control of positive and negative factors and regulatory elements interacting at various levels to regulate floral morphogenesis. Using NGS RNA-seq there is a great possibility to investigate the expression profile of these pathways in non-model organisms like *A. artemisiifolia*, where the male and female floral organs grow as separate entities in the same individual plant. Rocheta and colleagues report on a comparative study revealed a transcript dataset to be involved in flower and plant development in the monoecious tree *Quercus suber* with a similar disjunct gender system where the male flowers form catkins and female inflorescence grow on young leaf axils separately. This floral transcriptome analysis revealed a group of genes expressed exclusively in each type of flower gender that may have a functional role in the sexually different floral organ development and sex specification (Rocheta et al. 2014).

In this study, we present an RNA-seq approach to get closer to the mechanism of floral development in *A. artemisiifolia* using Illumina sequencing. In order to investigate the expression distribution of genes taking part in floral morphogenesis, we categorized transcript datasets of female and male flowers involving whole investigated phenophases (F, M), leaves (L), and female and male flowers in early and late developmental stages (1F, 2F, 1M, 2M). For normalized F, M, and L transcript libraries, we used the SRA dataset reported in our prior study (Virág et al. 2016) provided the first step in the common ragweed floral genomics.

For 1F, 2F, 1M, 2M libraries early and late categories of phenophases were collected and sequenced *de novo* from *in vitro* and wild-growing plants. This work and related NGS project are deposited in the NCBI Bioproject under the ID PRJNA335689.

3.1. Generating RNA-seq libraries from common ragweed inflorescences

3.1.1. RNA sequencing and assembly of *A. artemisiifolia* floral libraries

The M, F, and L libraries were obtained through HiSeq2000 platform. Library construction and assembly were described in detailed in our prior study, Virág and colleagues in 2016. In brief, after cleaning we obtained 18,472,374 (M); 15,290,200 (F) and 17,435,976 (L) from total raw reads of 24,110,256; 23,264,636 and 24,330,693 (2*100 bp). Assembly of combined read sets resulted in 229,116 transcripts and 162,494 trinity genes (unigenes) and used as a reference (Appendix 4.).

A total of 39,664,366 (1F) and 37,127,852 (2F) 2*80 bp reads were generated by the Illumina NextSeq500 system. High quality (>Q20) bases were more than 91 % in (paired-end) reads of both samples. The percentage of unresolved bases (Ns) and overrepresented sequences was observed to be minimal 0.197% and 0.0004% on average. In total of 92% were high quality sequences from raw data: numerically 36,491,216 (1F) and 34,157,623 (2F). These high quality, processed paired-end reads were used to assemble into contigs and transcripts. Their *de novo* assembly resulted in 109,452 (1F) and 97,239 (2F) contigs.

In comparision, the reference guided assembly led to 40% more contigs 147,457 (1F) and 141,58 (2F), respectively. However, N50 length showed a minor 5-11% dispersion between the ranges 626.9 – 697.5 in both methods. Sequencing statistics are summarized in Appendix 4. The identification of differences between the male and female floral transcriptomes was based on *in silico* analysis. We performed a local blast alignment by using the determined 80 CDS fasta sequences (MK098047- MK088126) against male (M) and female (F) transcriptomes. No qualitative differences were found among the sequences of commonly expressed genes in two floral organs.

3.1.2. Validation of the *A. artemisiifolia* floral transcriptome

The observed expression differences were validated by investigating the transcript expression levels of homolog genes responsible for pollen and embryo formation in model organism *A. thaliana*. The reliability of the male transcriptome was characterized by overexpression of the homologs of *AMS, LAP3, LAP5, LAP6, MSE1, PME, PG1, PG2*.
The female transcriptome validity was justified by overexpression of *YAB1, YAB4,* and *ANT* transcription factors. Additionally, we introduced *SUPERMAN (SUP, MK098096, GO:0003676)*, *Transcription repressor MYB5* (*MYB5, MK098099, GO:0003677*), *Transcription repressor MYB61 (MYB61, MK098101, GO:0003677)* and *Protein BRANCHED2* (*TCP12, MK098098, GO:0003700)* as female candidate genes based on our filtering by Microsoft SQL Server Management Studio. These regulators are involved in plant and floral tissue development, but their role during sex determination is still unclear. Expression differences and function of organ-specific genes are summarized in Table 1-2. Based on our results, it can be identified that our libraries are tissue-specific. Ragweed genes homologous to the sequences of the candidate genes showed the expected expression values in both male and female flowers.

The investigated genes showed differential expression ratios with meaningful results according to that male tissues were characterized with overexpression of genes taking part in pollen structural development, fatty acid, and exine constituent biosynthesis during the anther and pollen differentiation. In contrast, expression of these genes was completely absent in the pistillate tissues in which ovule polarity, initiation, structural development and embryogenesis influencing genetic elements (*YAB* class transcription factors) showed about 67% higher presence than in staminate samples.

The non-exclusive occurrence of these sequences in pistillate flowers may be explained by the contribution to setting up the polarity in stamen may be through their action in supporting the development of the floral meristem or/and regulating margin growth (Eshed et al., 2004) that was observed also in all lateral organs (Siegfried et al. 1999, Chen et al. 1999). Additionally, the validation method was confirmed through the very low incidence of candidate sequences in leaf transcriptomes.

Table 1. Function and expression values of male candidate genes selected to validate floral transcriptomes.

Gene homolog	Encoded protein	Function (UniProt description)	References	Normalized expression		
				M	L	F
Male candidate genes						
AMS	Transcription factor EN 48	Regulates male fertility and pollen differentiation.	Xu et al, 2010; Lou et al, 2014	**8,91**	0,44	0,52
LAP3	Protein strictosidine synthase-like 13	Required for proper exine formation during pollen development	Dobritsa et al, 2009	**9,61**	0,21	0
LAP5	Type III polyketide synthase B	Required for pollen development and biosynthesis of pollen fatty acids	Dobritsa et al, 2010; Rocheta et al, 2014	**6,65**	0	0
LAP6	Type III polyketide synthase A			**8,39**	0	0
STIG1	Stigma-specific protein	Involved in the temporal regulation of the exudate secretion onto the stigma	Rocheta et al, 2014	**3,12**	0	0
PG1	Polygalacturonase 1 beta-like protein 2	Involved in cell size determination.	Futamura et al, 2000	**3,42**	0	0
PG2	Polygalacturonase 1 beta-like protein 3	Involved in cell size determination. May serve as a chaperone for expansins through the secretory pathway.	Futamura et al, 2000	**4,71**	0,43	0
PME	Pectinesterase	Plays an important role in growth of pollen tubes. Involved in anther development and play role in tapetum and pollen development.	Futamura et al, 2000	**6,33**	0,42	0
MSE1	Putative duplicated homeodomain-like superfamily protein	Exhibits tight linkage with the Y chromosome, specific expression in early anther development and loss of function on the X chromosome.	Murase et al, 2017	**7,12**	0,41	0

STIG1 were found as male candidate instead of female as described in other species.

Table 2. Function and expression values of female candidate genes selected to validate floral transcriptomes.

Gene homolog	Encoded protein	Function (UniProt description)	References	Normalized expression		
				M	L	F
Female candidate genes						
YAB1	Axial regulator YABBY 1	In gynoecium, expressed in the abaxial cell layers differentiating into the valves meristem development.	Siegfried et al, 1999	1,78	0,51	**6,51**
YAB4	Protein INNER NO OUTER	Essential for the formation and the abaxial-adaxial asymmetric growth of the ovule outer integument.	Villanueva et al, 1999; Meister et al, 2002	2,61	2,73	**7,05**
ANT	AP2-like ethylene-responsive transcription factor ANT	Transcription activator. Required for the initiation and growth of ovules integumenta, and for the development of female gametophyte.	Klucher et al, 1996;Elliot et al, 1996;Long and Barton, 1998	2,04	0,35	**7,91**
*SUP**	Transcriptional regulator SUPERMAN	Transcriptional regulator considered as cadastral protein that acts indirectly to prevent the B class homeotic proteins APETALA3 and perhaps PISTILLATA from acting in the gynoecial whorl.	Meister et al, 2002	0	0	**3,4**
*TCP12**	Protein BRANCHED 2	Transcription factor that prevents axillary bud outgrowth.	Bush et al, 2011; Zhu et al, 2013	0	0	**3,45**
*MYB61**	Transcription factor MYB61	Transcription factor that coordinates a small network of downstream target genes required for several aspects of plant growth and development.	Asrovski et al,2009;Liang et al, 2005	0,96	0,43	**4,16**
*MYB5**	Transcription repressor MYB5	It is expressed in trichomes, seed coat and siliques.	Romano et al, 2012;Yang et al, 2012;	0,88	0,14	**5,37**

Genes marked with * were selected based on our filtering by using SQL Server Manager Studio (see Materials and methods).

3.2. Identify flower development genes and determination of gene expression with *in silico* technique

Plant development and architecture are regulated by meristems that initiate lateral organs on their flanks. Gene regulatory networks that control the transition of a vegetative shoot apical meristem into an inflorescence meristem (FMIGs), together with those necessary to specify floral meristem identity (FOIGs) have been explored in *A. thaliana* and are highly complex and redundant. We have evaluated and reported the most prominent cases in this study. Since the morphogenesis of *in vitro* cultivated female inflorescence of common ragweed is more intensive than in wild-growing plants, therefore genetic regulation of the pistillate inflorescence can be more effectively investigated in these samples. The investigated pathways and related genes showed the following correlations. Functional and structural analysis of *A. thaliana* gene homologs were investigated to elucidate floral regulation in the common ragweed. Expression patterns of photoperiodic, vernalization, gibberellin, and flower development pathways comparing with floral architecture formation are summarized in Fig. 10. and Fig. 11.

No interaction was found between *LFY, FRIGIDA (FRI)*, and *FLC* transcription factors that determine the initiating steps during vegetative growth. Homologous transcripts of these genes were not found in any of the investigated libraries and were not expressed in the examined samples. The absence of *LFY, FLC,* and *FRI* genes could indicate, that the blossom phase has exceeded the initial signals in the bio-rhythm of collected samples. Overexpression of photoperiodic pathway related transcripts of *PHYB (MK098056, GO:0000155)* in 1F, 2F, 1M, and 2M libraries and *COP1 (MK098059, GO:0009785), CO (MK098060, GO:0005634), and FT (MK098073, GO:0008429)* in the L library were found. In Arabidopsis, the expression of *PHYB* affects indirectly *FT* and is limited to the leaf, so for common ragweed, the expression of *PHYB* in flowers is interesting and unusual data. This may suggest that *PHYB* could also regulate flower development steps that occur after the FT switch. The transcription of *FT* is activated by *CO* in leaf, and the FT protein moves to the shoot apex to induce flowering, therefore we found these transcripts overexpressed only in leaf tissues in wild-growing samples.

The vernalization pathway gene homolog *VRN1 (MK098069, GO:0003677)*, showed high values in all developmental stages (1F, 2F, 1M, 2M), which function is to inhibit the flowering repressor *FLC* (Sung and Amasino 2004). Expression of the *VRN1* gene in both developmental stages of each flower maintains the generative morphogenesis of the flowering meristem. Expression of *VRN1* was increased during female flower maturation.

	Pathway	Gene	F	L	M
	Gibberellin	*GID1A*	83,32	353,76	160,19
		GID1B	15,85	349,98	31,58
		GID2	40,18	187,02	73,88
		GAI	36,01	540,92	73,89
		Ga2ox8	22,63	25,41	8,01
		Ga2ox1	8,44	86,51	39,89
		Ga2ox3	0,44	28,07	16,49
		Ga3ox1	0	67,16	9,65
		Cyp450	108,23	995,33	170,72
Floral meristem initiation (vegetative growth)	**Photoperiodic**	*PHYB*	22,10	150,76	53,89
		PHYA	2,36	5,76	8,03
		CRY1	14,37	533,24	38,92
		COP1	8,16	236,62	33,68
		CO	9,52	2168,15	98,54
		SPA	2,20	68,42	5,11
	Vernalization	*CSTF77*	5,48	84,59	19,67
		CSTF64	3,72	81,72	17,07
		VIP6	5,56	94,84	22,94
		VIP5	11,96	181,12	41,91
		VIP4	13,34	189,45	61,25
		VIP3	41,96	98,16	61,81
		VIN3	0,00	313,45	10,81
		VRN1	9,09	41,66	27,08
	Flower development	*EMBF2*	18,25	80,38	47,20
		CAL	114,80	0,00	1,94
		SOC1	0,00	0,00	0,00
		FT	0,00	1487,94	56,17
		FD	0,00	10,40	16,05
		LMI2	6,75	7,12	41,52
		FUL	6,17	103,31	75,64
		SVP	12,51	31,71	7,55
		TFL1	0,00	1461,09	56,95
		SPL3	122,02	0,00	293,90
		SPL1	80,85	29,85	96,28
Floral architecture formation (flowering) ABC	**A**	*AP2*	0,00	110,81	1,98
	A	*AP1*	162,27	1,93	6,11
	B	*PI*	0,00	0,00	385,99
	B	*AP3 3*	20,34	7,84	150,85
	B	*AP3 2*	0,00	0,00	187,24
	B	*AP3 1*	5,31	0,00	80,51
	C	*AG*	45,64	1,10	187,59
	E	*SEP4*	111,34	1,10	43,86
	E	*SEP3 2*	175,89	98,35	271,40
	E	*SEP3 1*	78,92	7,52	59,01
	E	*SEP2 2*	84,95	98,55	66,94
	E	*SEP2*	105,90	1,90	32,51
	E	*SEP1*	30,22	2,50	72,73

Figure 10. Investigated genes representing different floral regulatory pathways in F, M, L libraries. These genes were selected to characterizing genetic events during male and female floral architecture formation and floral meristem initiation during vegetative growth such as photoperiodic, vernalisation, and meristem identity genes. Normalized expression of selected genes was performed digitally and expression values were compared with each libraries F, M, L with HiSeq2000 technology. The color scale refers to expression values: blue, white, and red represent low, intermediate and high expression levels, respectively.

	Pathway	Gene	1F	2F	1M	2M
	Gibberellin	*GID1A*	718,72	818,45	371,26	323,73
		GID1B	60,25	188,07	77,34	64,89
		GID2	520,22	462,20	228,17	236,33
		GAI	306,10	295,90	407,41	420,34
		Ga2ox8	207,07	220,68	37,58	33,71
		Ga2ox1	83,26	48,55	147,25	157,31
		Ga2ox3	18,87	15,10	41,42	69,05
		Ga3ox1	0	0	162,01	177,76
		Cyp450	139,44	270,7	1252,2	3499,78
Floral meristem initiation (vegetative growth)	***Photoperiodic***	*PHYB*	298,71	244,20	224,23	209,08
		PHYA	57,85	41,89	77,83	80,84
		CRY1	230,51	424,48	95,38	102,69
		COP1	74,27	64,88	102,09	96,59
		CO	113,99	118,43	443,84	451,84
		SPA	20,27	15,92	20,98	19,29
	Vernalization	*CSTF77*	126,07	114,60	140,87	130,34
		CSTF64	91,56	67,24	55,48	55,49
		VIP6	169,16	168,28	106,73	109,52
		VIP5	153,24	177,16	144,57	151,45
		VIP4	426,24	492,56	388,79	392,59
		VIP3	218,86	156,07	137,23	128,29
		VIN3	8,61	14,47	48,01	49,08
		VRN1	177,92	273,02	100,43	103,99
	Flower development	*EMBF2*	143,32	128,64	114,59	107,31
		CAL	2076,87	3059,01	15,11	13,49
		SOC1	44,62	19,54	0	0
		FT	7,21	0,00	295,35	258,03
		FD	12,45	10,82	20,15	14,16
		LMI2	73,47	29,53	188,22	208,34
		FUL	81,42	70,65	316,24	298,81
		SVP	56,48	49,01	24,26	15,75
		TFL1	7,69	0,00	286,143	269,34
		SPL3	364,35	602,39	635,25	686,31
		SPL1	6,64	17,96	499,06	513,67
Floral architecture formation (flowering) ABC	***A***	*AP2*	0,00	0,00	4,97	5,59
	A	*AP1*	2435,41	3532,78	11,15	10,49
	B	*PI*	4,29	3,19	2370,99	2571,13
	B	*AP3 3*	229,98	247,66	994,37	1106,86
	B	*AP3 2*	163,94	223,67	1724,01	1757,56
	B	*AP3 1*	285,63	293,35	263,7	238,34
	C	*AG*	869,62	738,51	967,73	919,1
	E	*SEP4*	1285,10	1166,06	209,46	237,09
	E	*SEP3 2*	3073,76	1974,46	1031,59	1097,51
	E	*SEP3 1*	661,21	499,63	215,78	217,48
	E	*SEP2 2*	1229,70	1530,87	278,9	276,62
	E	*SEP2*	2060,41	1628,81	222,62	238,75
	E	*SEP1*	732,25	534,94	915,63	964,9

Figure 11. Investigated genes representing different floral regulatory pathways in 1F, 2F and 1M 2M libraries. The normalized expression values of flower development genes has also been studied in libraries 1F, 2F, 1M, 2M with NextSeq 500 technology derived from *in vitro* and wild growing plant materials. The color scale means blue low, white intermediate, and red are high expression values.

We found *SOC1 (MK098072, GO:0000957)* homolog only in 1F and 2F libraries. *SOC1* can integrate flowering signals from different pathways and activat the *APETALA1 (AP1)* and *LEAFY (LFY)* flower meristem identity genes. High expression of the *TFL1 (MK098078, GO:0005737*) gene, which maintains indeterminacy of the floral meristem, was observed in leaf samples in wild plants, and higher expression was detected in in vitro plants in male samples compared to female flowers. *SOC1* association with *AGL24 (MK098093, GO:0000977)* mediates the effect of gibberellins on flowering under short-day conditions and regulates the expression of *LFY*, which links floral induction and floral development (Liu et al. 2008). The lack of *SOC1, LFY* (RPKM=0), and overexpression of *FT* (RPKM=1487) in wild-growing samples indicated that flowering transition was regulated more intensively by the photoperiodic pathway in these samples. In contrast, hormonal pathway related genes *(SOC1, GID1A, GAI, Ga2ox8, CAL)* were more expressed in *in vitro* plants. Therefore we concluded, that both cultivation conditions reached the *AP1* expression that is the "first pass" of floral architecture formation, however, the vegetative pathway was altered under *in vitro* conditions. This phenomena has led to altered morphology and genesis of floral genders. The female inflorescences grow faster and in greater abundance in the case of *in vitro* individuals than in the wild-growing plants (see Fig. 12.). Male inflorescences, however, were barely appeared *in vitro*. Under wild-growing conditions, male flowers dominate probably because of the intense light mediated signals.

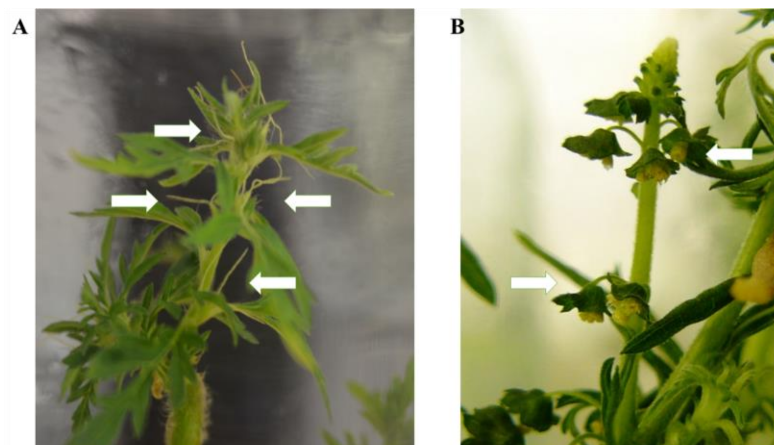


Figure 12. *In vitro* cultivated *A. artemisiifolia* individuals. During the life cycle of the plant under *in vitro* conditions, the female flowers were appeared earlier and in greater amounts (A). Appearance of male inflorescences were minimal (B) which is the opposite phenomena that may be observed under wild-growing conditions. Arrows show the appearance of dense pistillate and spars staminate flowers *in vitro*.

Among the genes belonging to the hormonal pathway, *GID1A (MK098047, GO:0016787), GAI (MK098050, GO:0003712), Ga2ox8 (MK098051, GO:0016491), CAL (MK098071, GO:0000977)* were more expressed in the 1F and 2F samples compared to male flowers, in contrast to those observed in wild-grown individuals. Confirmation of this finding and examination of whether cytokinin treatment by *in vitro* plants actually has an effect on altering the expression of these genes or caused by another effect requires further experimentation.

3.3. Determine floral organ, ABC(E) genes and gene expression with *in silico* technique

The ABC(E) genes responsible for the formation of flower organs were determined based on *Compositae* species (*Gerbera sp, Helianthus sp, Tagetes sp, Chrysanthemum sp*) found in public databases (NCBI, UniProt). The coding sequences and their expression patterns of the following thirteen gene homologs were determined: *AP1(MK098082), AP2 (MK098081), AP3 1 (MK098086), AP3 2 (MK098085), AP3 3 (MK098084), PI, AG (MK098087), SEP1 (MK098094), SEP2 1 (MK098092), SEP2 2 (MK098091), SEP3 1 (MK098090), SEP3 2 (MK098089) and SEP4* (*MK098088*) (Fig. 10., Fig. 11.). All of the investigated floral organ genes *except AP2 (GO:0003677)* have the same GO ID: *GO:0000977.* The *AP2*, *AP3 2,* and *PI* genes were not or very low expressed in the female samples, therefore we considered them as male specific genes of the common ragweed. *PI* with *AG* determine the stamen and *AP2* with *AP3* determine the petal formation that are male flower elements in the common ragweed (Fig. 13. A). The expression of the *AP1* gene showed a significantly higher value in female flowers than in male inflorescence (Fig. 13. B-C), suggesting that the initiation of petals and stamens is stronger in the pistillate flowers. The overexpression of *AP1* gene may be traced back to the hormonal pathway domination and through *SOC1* during the vegetative phase in female flowers (Kaufmann et al. 2010). In the early stage of development of female inflorescences (1F) a high expression rate was found for *SEP1, SEP2, SEP,* and *SEP4*. Supposedly these genes are responsible for the development of petal and carpel primordias. However, *SEP3 2* and *SEP2 2* showed high expression in the late phase (2F) that may be involved in the pistil prolongation (Fig. 13. B).

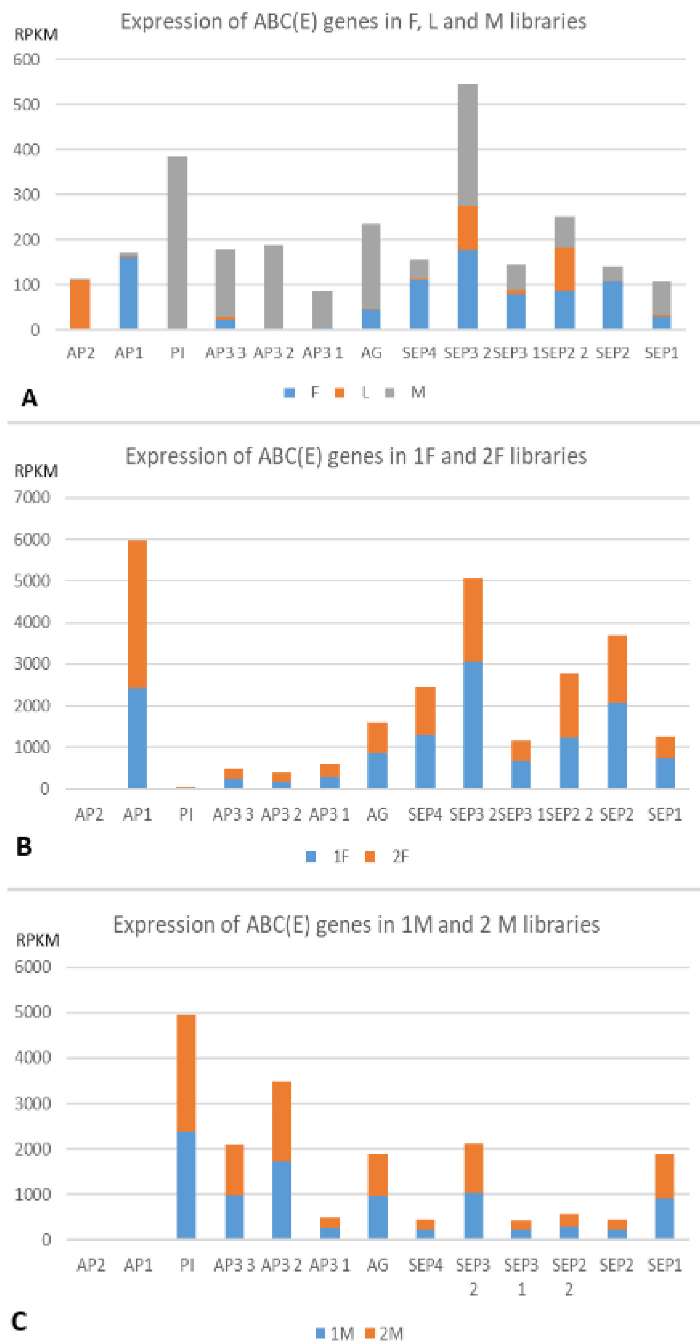


Figure 13. Expression differences between ABC(E) genes. Normalized expression rates were determined by calculating RPKM. **A**-Expression of ABC(E) in F, L, and M libraries **B-** Expression of ABC(E) in 1F and 2F libraries. **C-** Expression of ABC(E) in 1M and 2M libraries.

3.4. Identify the sequences and gene expression of the most expressed transcription factors that control flowering

Based on analysis of the different TF groups that are sex-specific and differentially expressed in each library the following factors were identified in our samples according to Rocheta and colleagues (Rocheta et al. 2014). Gene expression values of all investigated libraries and functional descriptions are summarized in Table 3 and Table 4.

Table 3. Gene expression values of transcription factors in F, M and L libraries

Gene homolog	RPKM value (L)	RPKM value (M)	RPKM value (F)	Function	References
MYB33	13.12	51.78	0.00	Proved to facilitate anther development redundantly	Millar and Gubler 2005
ILR3	333.63	106.07	33.06	Required to maintain Fe homeostasis in correlation with a large amount of Fe3+ in sepals	Sudre et al, 2013
CUC1	8.08	2.65	0.00	Responsible to mechanisms to separate organs developing at adjacent positions during early flower development.	Aida et al, 1997
CUC2	17.94	17.66	7.68		
IAA9	26.10	76.06	33.05	Repressors of early auxin response genes at low auxin concentrations.	Wang et al, 2009; Liscum and Reed, 2002
IAA27	173.21	23.97	15.68		
PIN1	52.44	17.25	43.85	Expression of PIN1 and essential for correct auxin efflux into the early stages of female gametophyte development	Wang et al, 2009
EIN3	161.47	45.70	13.69	Delays flowering via repression of the *LFY* and *SOC1* genes	Ceccato et al, 2011
COL4	646.84	192.96	155.56	Transcription factor involved in the light input to the circadian clock.	Lee et al, 2010
COL5	1099.61	51.63	18.13	Induce flowering in short-day grown Arabidopsis	Hassidim et al, 2009
COL9	8.19	2.70	0.00	Delays flowering by reducing expression of CO and FT.	Cheng and Wang, 2005

Table 4. Gene expression values of transcription factors in 1F,2F, 1M and 2M libraries

Gene homolog	RPKM value (1F)	RPKM value (2F)	RPKM value (1M)	RPKM value (2M)	Function	References
MYB33	17.98	23.39	233,20	228,81	Proved to facilitate anther development redundantly	Millar and Gubler 2005
ILR3	395.67	321.32	312,86	318,10	Required to maintain Fe homeostais in correlation with a large amount of Fe^{3+} in sepals	Sudre et al, 2013
CUC1	29.43	22.25	37,56	40,18	Responsible to mechanisms to separate organs developing at adjacent positions during early flower development.	Aida et al, 1997
CUC2	6.13	0.00	10,77	5,49		
IAA9	263.80	139.43	351,46	371,83	Repressors of early auxin response genes at low auxin concentrations.	Wang et al, 2009; Liscum and Reed, 2002
IAA27	204.66	546.24	68,02	59,06		
PIN1	155.54	29.07	43,34	42,69	Expression of PIN1 and essential for correct auxin efflux into the early stages of female gametophyte development	Wang et al, 2009
EIN3	97.18	134.97	154,33	130,72	Delays flowering via repression of the *LFY* and *SOC1* genes	Ceccato et al, 2011
COL4	1478.03	1564.22	552,40	509,76	Transcription factor involved in the light input to the circadian clock.	Lee et al, 2010
COL5	478.01	549.48	257,51	261,93	Induce flowering in short-day grown Arabidopsis	Hassidim et al, 2009
COL9	53.41	38.76	15,38	15,62	Delays flowering by reducing expression of CO and FT.	Cheng et al, 2005

Transcription factors (TFs) play important roles in plant development and flower morphogenesis responding to the environment. These factors interact specifically with sequences located in the promoter regions of the genes they regulate. Transcription factors are classified in families according to the structure of their DNA-binding domain.

Based on RPKM values, homologs of the *CONSTANS-LIKE* zink-finger TF family were found to be characteristic in both female and male samples (*COL4, MK098124, GO:0005634, COL5, MK098125, GO:0005634*). Upregulation of *COL4* was found in 1F and 2F libraries indicating a stronger interaction of female tissues with the circadian clock and light signals than in male flowers. *col4* null mutants flowered early under short or long days. In contrast, *oscol4* activation-tagging mutants *(oscol4-d)* flowered late in either environment (Lee et al. 2010). Of these, the late flower showed a higher RPKM value in the correspondence of *CO* transcript level. Downregulation of *COL9 (MK098126, GO:0005634)* was observed in all wild-growing samples. Over-expression of *COL5* can induce flowering in short-day grown *Arabidopsis* (Hassidim et al. 2009). We observed the higher *COL5* expression in female flowers, mainly in the case of *in vitro* plants where pistillate flowering showed intensive growth under short day conditions.

The next most characteristic transcript was an *ILR3 (MK098117, GO:0046983)* homolog of *IAA-LEUCINE RESISTANT 3* protein representing the helix-loop-helix protein family. *ILR3* is required to maintain Fe homeostasis in correlation with a large amount of Fe^{3+} observed in the sepals in *A. thailana* (Zhang et al. 2015). Since this transcript was found to be expressed roughly equally in all investigated samples (1F, 2F, 1M, 2M, M, and L), we assumed that to keep at appropriate level the iron homeostasis is essential to all flower types, in fact, to maintain hormonal and redox balance for fertility (Sudre et al. 2013).

Among the hormone related factors, the auxin responsive AUX/IAA protein family genes such as *IAA27 (MK098121, GO:0005634)* and *IAA9 (MK098120, GO:0005634)* were found to be characteristic in both reproductive tissues. These transcriptional factors are repressors of early auxin response genes at low auxin concentrations (Liscum and Reed 2002). RT-qPCR and *in situ* hybridization have shown that tissue-specific gradient of *IAA9* expression was established during flower development, and the release of which, upon pollination and fertilization, triggers the initiation of fruit development (Wang et al. 2009). These results explain the higher expression rate in female tissues in both early and late phenophases in *Ambrosia*.

The auxin efflux carrier homolog, *PIN-FORMED 1 (PIN1, MK098122, GO:0009734)* was also found to be upregulated in early female flowers (1F) indicating the female tissue determination might be under strong control of auxin. These results correspond to the study of Ceccato and colleagues where *PIN1* expression and cellular localization in the ovule were essential for correct auxin efflux in the early stages of female gametophyte development (Ceccato et al. 2011). In the ethylene response pathway, the protein *ETHYLENE INSENSITIVE3 (EIN3, MK098123, GO:0003677)* regulates ethylene-responsive genes as a positive regulator and results in flowering delay via repression of the floral meristem-identity genes *LFY* and *SOC1* (Achard et al. 2007). In our flowering system, it was upregulated in M, 1M, 2M libraries resulting no expression of *SOC1* and *LFY* in male tissues (see Fig. 10. and Fig. 11.). No *LFY* homolog was identified in any of the floral libraries in correlation of higher expression of this transcription factor. Wild-growing *Ambrosia* individuals grow male flowers intensively in late summer, which is a delayed state compared to the female blossom. However, *SOC1* transcript was found only in the *in vitro* samples referring to the altered (earlier) floral regulation we assumed that this phenomena is based on changes in hormonal pathways.

NAC-domain containing transcription factors are essential for normal plant morphogenesis representatives of which are the *CUP-SHAPED COTYLEDON* proteins encoded by *CUC1 (MK098118, GO:0003677)* and *CUC2 (MK098119, GO:0003677)* genes less expressed in our flower samples. Mutations in *Arabidopsis CUCl* and *CUC2* caused fusion of cotyledons, sepals, and stamens suggesting that these factors are responsible for mechanisms that separate organ developing at adjacent positions (Aida et al. 1997). No expression of *CUC1* was detected in wild-growing pistillate tissues and its expression was lowest among the investigated transcription factors in staminate samples. In *in vitro* samples, *CUC2* was expressed only in early phenophases (1F) in female flowers with a minimal expression level. Since these factors are involved mainly in the early development of cotyledonary primordia (Aida et al. 1997), the phenomena of downregulation of these genes may be explained by the flowering separation that has overtaken this critical state in our samples.

The homeodomain like *MYB* transcription factors are involved in the control of the cell cycle of plants. The majority of them are miRNA regulated and are involved in numerous cell biochemical pathways like hormonal regulation.

The gibberellin pathway related *MYB33 (MK098116, GO:0003677)* homolog was expressed only in male flower in wild-growing *Ambrosia* samples in agreement with Rocheta (2014), and Millar (2005) in which studies the *GAMYB-like MYB33* was proved to facilitate anther development redundantly (Millar and Gubler 2005, Rocheta et al. 2014). In *in vitro* originated 1F and 2F libraries homolog transcripts of *MYB33* were also found indicating repeatedly altered hormonal regulation of this system.

3.5. Selection, validation and annotation and determination of expression level of *A. artemisiifolia* unigenes

3.5.1. Selection of *A. artemisiifolia* unique and differentially expressed genes

In order to identify exclusively expressed transcripts in the sex-specific floral tissues, we used Bowtie2 alignments of F, M, L, 1F, 2F, 1M and 2M libraries for the *Ambrosia* reference transcriptome GEZL00000000. In order to prove the 1F and 2F exclusive transcripts in the female sample (F), and 1M, 2M exclusive transcripts in the male sample (M), contigs were realigned using BLASTn algorithm to the 1F, 2F, 1M and 2M *de novo* Trinity transcripts (GFWB00000000, GFWS00000000). The transcript datasets were extracted from Trinotate heat map and post-processed using Microsoft SQL Server Management Studio. The queries for unique organ-specific sequences resulted in 5659 (M), 1691 (F), and 4267 (L). The number of exclusive and shared transcripts in wild-growing male and female flowers and leaf are visualized in Venn diagram. For visualiszation we used FunRich (V3) software (Pathan et al. 2015) (Fig. 14). The number of annotable unique transcripts was 10507 in M, F and L libraries. Among which the most important organ-specific genes are summarized in Table 5-6. The presented genes were within the upper limit (>80%) of the total expression level value.

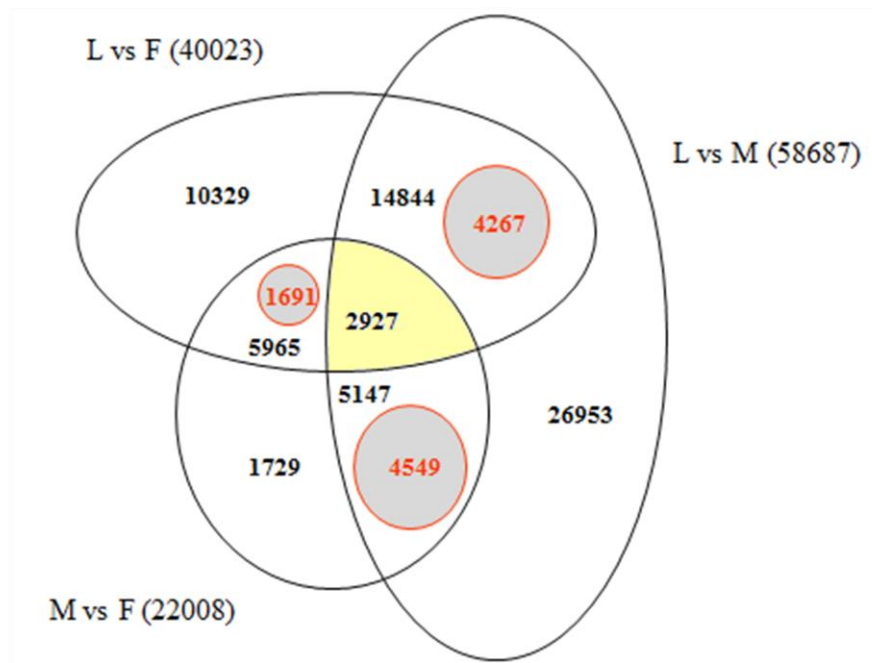


Figure 14. Venn diagram indicating the number of exclusive and shared transcripts in wild-growing male (M), female (F) flowers, and leaf (L) samples. The datasets were extracted from Trinotate heat map and visualized with FunRich. The comparison and distribution of transcripts were specified according to the expressed sequences in groups of leaf versus (vs) female, male vs female and leaf vs male transcriptomes of *A. artemisiifolia* reference GEZL00000000. A total of 40,023 transcripts were detected in the leaf (L) and female (F) sample pair, and 10,329 transcripts showed a significant difference. In the leaf (L) and male (M) sample pair, were 58687 transcripts, of which 26953 were differentially expressed. The male (M) and female (F) sample pair had 22008 transcripts, of which 1729 showed different expression values. In all samples, 2927 common transcripts were found, which expressed differently.The SQL queries for organ specific unique transcripts resulted in 4549 (unique M), 1691 (unique F), and 4267 (unique L).

Table 5. Genes expressed exclusively in female transcriptomes (F, 1F and 2F).

Gene homolog	Encoding protein	RPKM value (F)	RPKM value (1F)	RPKM value (2F)	Function	References
PBL9	serine/threonine protein kinase	88.97	640.35	57.32	Exhibits serine/threonine activity.	Hirayama and Oka, 1992; Ito et al, 1997
SUP	Transcriptional regulator SUPERMAN	7.52	123.31	56.55	Acts indirectly to prevent AP3 and perhaps PI proteins from acting in the gynoecial whorl.	Kazama et al, 2009; Bowman et al, 1992
ACA7	calcium-transporting ATPase 7, plasma membrane-type	22.57	0	4.92	Carbonic anhydrase that catalyses the reaction of reversible hydration of CO_2.	Henry, 1996; Smith and Ferry, 2000
TCP12	Transcription factor TCP12	12.6	239.69	134.51	Prevents axillary bud outgrowth, delay early axillary bud development.	Bush et al, 2011; Zhu et al, 2013
MYB5	Transcription repressor MYB5	19.01	122.98	75.4	Involved in seed coat formation, trichome morphogenesis and mucilage secretion.	Romano et al, 2012; Yang et al, 2012; Li et al, 2009; Liu et al, 2014
WIP2	Zinc finger protein WIP2	7.29	131.11	114.05	Required for normal differentiation of the ovary transmitting tract cells.	Crawford and Yanofsky, 2008; Marsch-Martínez et al, 2014
MYB61	Transcription repressor MYB61	7.59	205.63	349.61	Required for seed coat mucilage deposition.	Asrovski et al, 2009; Liang et al, 2005
LAC2	Laccase-2	30.84	0	3.93	LAC2 deficient lead to early flowering	Tashian 1989
BEE	Transcription factor BEE1	5.29	0	59.63	Positive regulator of brassinosteroid signaling (BR).	Domaglaska et al, 2007

Table 6. Genes expressed exclusively in male transcriptomes (M, 1M, 2M).

Gene homolog	Encoding protein	RPKM value (M) HiSeq2000	RPKM value (1M) NextSeq500	RPKM value (2M) Nextseq500	Function	References
pcC13-62	Desiccation-related protein	1126.37	4335.81	5839.66	Expressed exclusively in the stylopodium.	Zha et al, 2013
CYP450 86B1	Cytochrome P450	906.19	4304.38	3499.78	Effect the suberin and cutin biosynthesis.	Duan and Schuler, 2005, Compagnon et al, 2009
GDSL2	Lipase/Acylhydrolase superfamily protein	804.54	2230.36	1603.26	Expressed during petal differentiation.	Ji et al, 2017
OAS	3-oxoacyl-[acyl-carrier-protein] synthase	737.44	326.14	303.02	Catalyses the condensation reaction of fatty acid synthesis.	Dayan and Duke, 2014; Hakozaki et al, 2008
PMADS 2	PMADS 2	309.49	1968.72	2038.71	Pi (Pistillata) homologue, predominantly expressed in petals and stamens.	van der Krol, 1993
MYB80	Transcription factor MYB80	170.82	440.74	381.95	Play an essential role during anther development.	Higginson et al, 2003; Phan et al, 2011
MYB35	Transcription factor MYB35	144.81	426.79	398.56	Required for anther development and early tapetal function during microspore maturation.	Zhu et al, 2011;
TET8	Tetraspanin 8	140.82	236.92	250.22	Expression was observed during the pollen development and in mature pollen.	Reimann et al, 2017; Honys and Twell, 2004; Pina et al, 2005; Boavida et al, 2013
MYB44	Transcription factor MYB44	99.18	137.58	135.46	Represses the expression of protein phosphatases 2C in response to abscisic acid.	Jung et al, 2008
NIP	Aquaporin	49.68	51.78	25.27	Play role in pollen germination and pollination	Di Giorgio et al, 2016
SYN	Syntaxin	45.70	80.96	76.05	Syntaxins mediate membrane fusion.	Sanderfoot et al, 2001
MYB26	Transcription factor MYB26	15.57	183,91	212,97	Regulates lignified secondary cell wall thickening of the anther endothecium.	Mitsuda et al, 2006; Yang et al, 2007

3.5.2. Annotation and functional classification of *A. artemisiifolia* unigenes

Functional annotation of the transcriptome sequences and analysis of the annotation of three-specific RNA pools (M, F, L) were performed using BLASTx search (Gish and States 1993)and Blast2GO (Götz et al. 2008, Conesa et al. 2005). This process resulted in 81.56 % translated contigs in total. Based on GO annotation the cell part, organic cyclic compound binding, and organic substance metabolic process were the most abundant GO slims in the categories of cellular component, molecular function, and biological process, respectively (Appendix 5.). The high physiological activity of *Ambrosia* reproductive tissues was suspected from the high representation of the primary metabolic process and cellular metabolic process groups within the biological process category. Representation of the significant sub-ontologies of cellular component, molecular function, and biological process are summarized in Appendix 5. GO enrichment analysis were performed with the investigated floral genes wich results are summarized in Appendix 6 -8.

3.5.3. Expression of female flower specific genes

In order to identify exclusively expressed genes in female flowers, the combined assembly of five (M, L, F, 1F, and 2F) non-normalized libraries were analysed. In the analysis, 1691 unique transcripts were found in the wild growing F library, representing the nine developmental stages. Transcripts longer than 700 bp were further investigated. After this filtering, we found 60 transcripts showing evaluable ORF, among which nine genes were well annotable using NCBI nr and Swiss-Prot databases. Based on this, the following protein coding and transcription factors were identified: *Protein kinase 1A (PBL9, MK098095, GO:0004674), SUP, Alpha carbonic anhydrase-7 (ACA7, MK098097, GO:0004089), Laccase-2 (LAC2, MK098102, GO:0005507), TCP12, MYB5, MIB61, Zinc finger protein WIP2 (MK098100, GO:0003677),* and *Protein Brassinosteroid enhanced expression 1 (BEE1, MK098103, GO:0006355)* (see Table 5.). In order to find genes that might correspond to early and late flower development, exact CDSs of these genes were determined *in silico* and validated with sanger sequencing from *in vitro* plant materials.

RPKM values were calculated in F, L, M, 1F, 2F, 1M, and 2M libraries using validated CDSs. As expected, all of these investigated genes are not expressed in M, L, 1M, and 2M libraries (RPKM=0) except *BEE1* in leaf samples with a very low value.

In early female flower development, the serine/threonine protein kinase *PBL9 (MK098095, GO: 0004674)* showed an exceptionally high value. *SUP, TCP12,* and *MYB5* were expressed twice as high as in matured flowers before the ovule formation. Two catalytic protein genes *LAC2* and *ACA7* were also found as unique genes in late 2F samples with a low expression suggesting a not characteristic role, but taking part in necessary metabolic processes during floral maturation**.** *PBL9 (alternatively APK1)* is very weakly expressed in flower tissues. It was described as *A. thaliana* serine/threonine protein kinase that phosphorylates tyrosine, serine, and threonine *(APK1)* during signal transduction (Hirayama and Oka 1992). The *APK* family is negatively regulated by the floral homeotic protein AG (AGAMOUS) (Ito et al. 1997) involved in the control of organ identity, whose expression was equal in early and late female libraries, respectively. The unique expression of *PBL9* in female flowers indicated that the developments of staminate and pistillate tissues are regulated by different kinases at protein level during signal transduction mechanisms. Similarly, a receptor-like protein kinase was also reported as female specific gene in the monoecious *Quercus suber* (Rocheta et al. 2014). Additionally, a different expression of these genes suggested altered protein phosphorylation during female flower formation; accurately it is more significant at the beginning of the pistillate flower development.

Despite *SUP* is not a sex determination gene (not located on the sex – Y – chromosome) it was considered as female specific gene. Their function in the female flower differentiation pathway was proved in the diecious plant *S. latifolia*. During the development of the female flower in *S. latifolia*, the expression of *SUP* is firstly detectable in whorls 2 and 3 when the normal expression pattern of the B-class flowering genes was already established and persisted in the stamen primordia until the ovule had matured (Kazama et al. 2009, Bowman et al. 1992). It is probably controlled by sex determination genes on the Y chromosome in dioecious plants.

The role of the MYB transcription factor superfamily in plant reproductive development is unequivocal. MYB proteins include a conserved domain, the MYB DNA-binding domain regulating a variety of plant-specific processes and they may play roles in different plant species (Ambawat et al. 2013). For example, *R2R3 MYB* and *MYB88* regulates female flower reproduction in *A. thaliana* (Makkena et al. 2012), *MYB35 (MSE)* regulates male specific expression in *Asparagus officinalis* (Murase et al. 2017).

MYB1 and *MYB16* regulate petal development in *Petunia hybrida* and *A. thaliana* (Noda et al. 1994, Baumann et al. 2007). Overexpression of *MYB61* was demonstrated in several aspects of plant growth and development, such as xylem formation and xylem cell differentiation, and lateral root formation (Liang et al. 2005, Arsovski et al. 2009, Romano et al. 2012, Matías-Hernández et al. 2017). Importance of *MYB5* and *MYB61* in seed coat formation, trichome morphogenesis, and mucilage secretion was also described by several studies (Arsovski et al. 2009, Gonzalez et al. 2009, Li et al. 2009, Liu et al. 2014) consistent with overexpression in seed coat and siliques. In our investigations *MYB5* and *MYB61* showed unique expression in female flowers, however, *MYB5 (MK098099) MYB61 (MK098101)* was found to be more characteristic in early and late phenophases respectively.

TCP12 belongs to the plant-specific transcription factor family TCPs. The founding members of this family have been characterized to be involved in growth, cell proliferation, and organ identity in plants (Yang et al. 2012). *TCP1-like* genes are involved in floral development with different regulatory and evolutionary mechanisms underlying the diverse forms of floral symmetry, such as dorsal/ventral dosage effect (Busch et al. 2012). We found that the female specific *TCP12* was stronger expressed in earlier phenophases suggesting a *TCP12*-related zygomorphic regulation at the beginning of floral morphogenesis in this species.

The transcription factor *BEE1* is a positive regulator of brassinosteroid signaling (BR) regulating plant development and physiology. The BR signal is transduced by a receptor kinase-mediated signal transduction pathway which ultimately results in altered expression of numerous genes. During flowering brassinosteroids stimulate floral transition through positively regulating the circadian clock pathway genes and inhibiting *FLC* repressor (Zhu et al. 2013, Domagalska et al. 2007). The deficiency of these phytohormones leads to the delay of flowering and because of the disruption of the pollen tube, BR deficient plants are practically male-sterile (Clouse 1996). Characteristic overexpression of the BR enhancer *BEE1* uniquely in late-female flowers suggests on one hand an altered regulation of BR signal transduction compared with male flower development.

Transcription factor *WIP2* is a zinc finger protein and was found to be responsible for transmitting tract formation in the gynoecium, which is important for pollen tube growth (Crawford and Yanofsky 2008). *WIP2* plays role in replum development and regulation of *AGL8/FUL (MK098076, GO:0000957)*, which is required for the normal pattern of cell division, expansion, and differentiation during morphogenesis of the silique (Marsch-Martínez et al. 2014).

Expression of this gene was equal in early and late developmental flowers indicating an intensive cell division in the gynoecial tissues during fruit development.

LAC2 is a multicopper-containing glycoprotein belonging to the laccase family. The exact function of *LAC* genes is not yet cleared. Laccase genes were highly expressed in lignifying tissues and are known to be associated with lignin synthesis (Sato et al. 2001, Gavnholt and Larsen 2002). In *Arabidopsis* array experiments the majority of investigated laccase members were found primarily in root tissues and in flower and stem samples secondly. In a mutant screening experiment, *lac8* and *lac2* deficient mutants lead to early flowering and reduced root elongation phenotypes. *LAC2* expression was also detected in flower tissues in the *Arabidopsis* array (TAIR database) (Gavnholt and Larsen 2002). In our *Ambrosia* flowering system, *LAC2* was found expressed uniquely in late, however, 2F flowers were represented at the level an order of magnitude lower compared to the other female specific genes.

The role of *ACA7* in flowering pathways is not yet reported. It belongs to the Zn metalloenzymes carbonic anhydrases (CAs) that catalyse the reaction of reversible hydration of CO2 with exceptionally high efficiency. CAs have been involved in a broad range of biochemical processes that involve carboxylation or decarboxylation reactions including photosynthesis and respiration. CAs also participate in pH regulation, inorganic carbon transport, ion transport, water, and electrolyte balance (Tashian 1989, Henry 1996, Smith and Ferry 2000). However, an interesting correlation revealed, that *ACA7* location was mapped on the same chromosome region as *EARLY FLOWERING 3 (ELF3)* including the earliness *per se* locus Eps-Am1 in diploid wheat (*Triticum monococcum*). This locus affects the duration of early developmental phases and it is responsible for the optimal photoperiod sensing and vernalization during the flowering time (Gavnholt and Larsen 2002). These results suggest that female flower differentiation may differ mainly in regulatory elements of the flowering pathway genes such as altered signal transduction and transcription factor activity.

3.5.4. Expression of male flower specific genes

The uniquely expressed transcript number in male tissues was 4549 (Fig. 14.) of which sequences longer than 700 bp were filtered out. In this way, total coding sequences of 41 genes were identified based on NCBI nt Blast and NCBI ORF finder databases. The 12 most characteristic male specific genes, RPKM values and functional properties are summarized in Table 6. Of the annotable genes, the most expressed sequences showed homology with the followings.

The homolog of desiccation-related protein, *PCC13-62 (MK098104, GO: no terms)* showed the highest RPKM value, 1126.37, which is activated during the drought stress in the pollen, seeds, and vegetative organs in succulent plants (Giarola et al. 2018). It was expressed exclusively in the stylopodium of the hermaphrodite flower in *Mucona sempervivens*, where the nectary is located (Zha et al. 2013).

Since the male flower pistillodium is a modified pistil with a function to help to dispensing the pollen from staminate flowers in *A. artemisiifolia* (Payne 1963), the anatomical presence of this transcript may be explained. Functionally, however, this protein may serve to protect the cytoplasmic contents in the pollen, since the pollen grain is an extremely desiccated structure at maturity. Desiccation-related proteins were also reported in the *A. thailana* columbia ecotype pollen (Sheoran et al. 2006).

The second most significantly expressed sequence was the *CYP450 86B1 (MK098105, GO: 0004497)* homolog. This gene was found to be expressed in many parts of the flower, including the epidermis, anther, and pistil. In young inflorescences this gene effects the suberin and cutin biosynthesis playing an important role in defensive mechanisms during biotic and abiotic stresses and supports the maintenance of reproduction (Duan and Schuler 2005, Compagnon et al. 2009).

The third most significantly expressed gene homolog was found from the lipolytic enzyme family, *GDSL2 (MK098106, GO:0016021)*, which was expressed during petal differentiation and is responsible for lipid catabolic processes (Ji et al. 2017). Ji and colleagues (2017) identified a *GDSL* lipase gene in *Brassica rapa* playing an important role in the anther and pollen development. *Arabidopsis* GDSL lipase 2 plays role in pathogen defense via negative regulation of auxin signaling and a positive correlation between late flowering and resistance to *F. oxysporum* in *A. thaliana* natural ecotypes was observed related to GDSL lipases (Lyons et al. 2015). The above-described male characteristic genes with outstanding high RPKM values refer to the most intensive physiological processes that are related to pollen exposure and stability and biotic and abiotic defense during anther formation.

Because all of the most male characteristic genes are related to flowering, secondary metabolism, plant cell structure, biotic and abiotic stresses, these may be considered to be essential to male specificity in the common ragweed. Gene homologs discussed below were found to be important for male flower physiology based on RPKM values.

TET8 (MK098111, GO: 0016021), which encodes a transmembrane protein tetraspanin 8, a member of tetraspanin family. TET8 play important role in plant development, reproduction and stress responses (Reimann et al. 2017). Expression of tetraspanins including tetraspanin 8 was described during the pollen development and in mature pollen in *A. thaliana* (Honys and Twell 2004, Pina et al. 2005, Boavida et al. 2013). *pMADS2* was found expressed in flowers in the second whorl, however, in vegetative organs were not found in *Petunia* (van der Krol et al. 1993). In *Ambrosia,* this gene was found to be homologous to *PI* which is predominantly expressed in petals and stamens, and less in carpels and sepals.

The 3-oxoacyl-[acyl-carrier-protein] synthase coding *OAS (MK098107, GO:0006633)* is responsible for the condensation reaction of fatty acid synthesis and confers resistance to low temperatures by maintaining chloroplast membrane integrity (Dayan and Duke 2014). This protein is also involved in the regulation of fatty acid ratio during seed metabolism. It is required for embryo development, especially at the transition from globular to heart stage (Hakozaki et al. 2008).

Another gene family responsible for membrane structure, the syntaxins (*SYN*) showed also unique expression in male tissues. These proteins play role in the fusion of transport vesicles to target membranes. Inactivation of genes from two syntaxin families has led to the lethality of the male gametophyte (Sanderfoot et al. 2001). *NIP 4 1* and *NIP 4 2* are pollen specific aquaporins playing role in pollen germination and pollination in *A. thailana*. These genes are essential to the growth of pollen tubes and were considered to be exclusive components of the reproductive apparatus of angiosperms with partially redundant roles in pollen development and pollination (Di Giorgio et al. 2016).

MYB proteins are a superfamily of transcription factors that play regulatory roles in gene expression controlling direct organ development and defense responses in plants. We found uniquely expressed MYB transcription factors related to flower development in male tissues. *MYB80 (MK098109, GO:0003677)* showed the highest expression in male tissues playing an essential role during anther development and production of tapetum and microspores (Higginson et al. 2003, Phan et al. 2011). *MYB26 (MK098115, GO: 0003677)* regulates lignified secondary cell wall thickening of the anther endothecium, which is necessary for anther dehiscence. It may play role in specifying early endothecial cell development by regulating several genes linked to secondary thickening (Mitsuda et al. 2006, Yang et al. 2007). *MYB35 (MK098110, GO: 0003677)* is required for anther development and early tapetal function during microspore maturation.

It regulates callose dissolution and is required for microspores release from the tetrads (Zhu et al. 2011). The *MYB44 (MK098112, GO: 0003677)* represses the expression of protein phosphatases 2C in response to abscisic acid (ABA).

It confers resistance to abiotic stresses dependent of ABA. The overexpression of *AtMYB44* enhanced stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis* (Jung et al. 2008).

3.6. Validation of tissue specific genes with RT-qPCR technique

For the RT-qPCR validation, we selected the genes showing the largest differences (Table 1-2) and most interesting from functional aspects. *TCP12, SUP,* and *LAP6* were selected to justify their exclusive expression in female and male flowers. *STIG1* was chosen according to its opposite expression in male flowers. Additionally, we selected also *PI (MK098083, GO:0000977)* as ABC(E) transcript exclusively present in staminate tissues. The relative expression value of *PI* gene in male flowers was 8170.66, in leaf 1.59, and in female flowers 3077.59. For the *LAP6* gene, the highest expression value was 11599.13 in male flowers, 0.16 value was in leaf, and 486.28 value was in female flowers. The expression values of the *STIG1* gene were in male 1000.38, in leaf 0.38, and in female tissues 40.9. For the *SUP* gene, a relative expression value of 0.83 was detected in male samples, 0.63 in leaf samples, and 540.53 in female flowers. The *TCP12* gene was expressed with a relative value of 12.97 in male samples, 3.95 in leaf samples, and 146.44 in female samples. To evaluate the results, genes were grouped according to their specificity. According to this, the female transcriptome was validated based on *TCP12* and *SUP* expressional patterns. Female specificity of *SUP* and *TCP12* was confirmed (Fig. 15. B). The expression of *TCP12* was three times higher than of the *SUP* in female flowers confirming the *in silico* RPKM results. The male transcriptome was validated according to the expression pattern of *LAP6*, *STIG1*, and *PI*. The non-exclusive expression of *PI* was observed indicating the ABC(E) function and B class activity in both flower types.

Since in female flower, the petal differentiation is vestigial and this whorl is united into a single tubular flower, therefore *PI* homolog transcript is less present than in male flowers where petals are united into short anthers with long stigma and stamen structures represents a large amount.

Expression of *STIG1* homolog in male flowers (Table 1.) was confirmed by RT-qPCR, indicating the modified pistil origin in the pistillodium structure. However this transcript showed a minimal expression also in female flowers (Fig. 15. A). This phenomena reveals the common origin of these flower types which were separated during evolution.

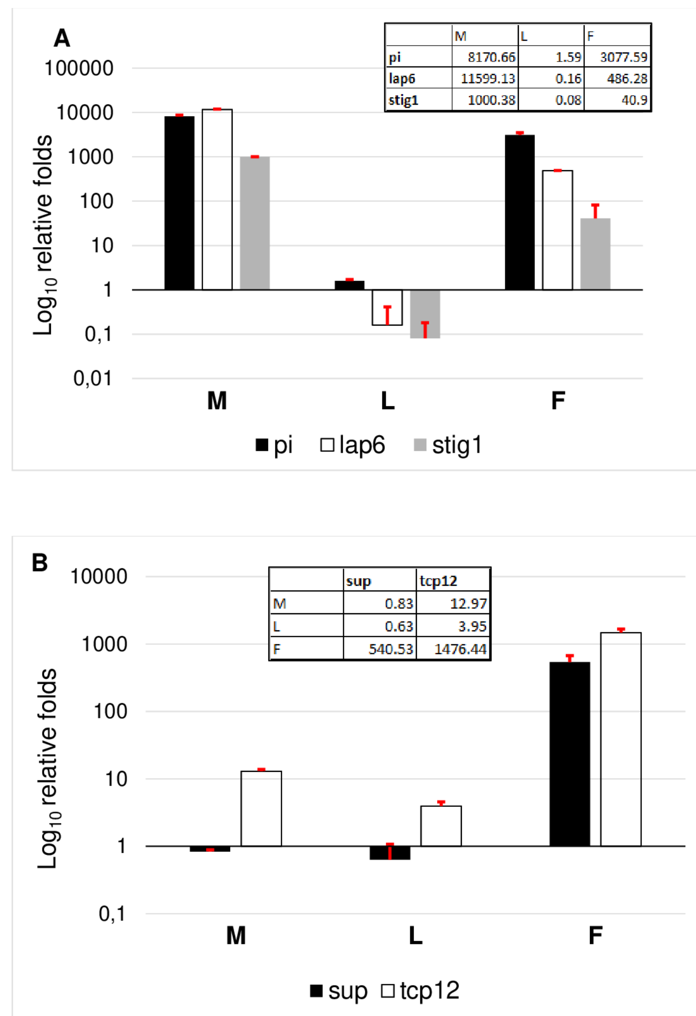


Figure 15. Comparative analysis of male specific (A) and female specific (B) gene expression. RT-qPCR analysis was determined as $\log_{10}$ relative fold. The tables inserted in the diagram show the real values of expression measured by RT-qPCR. In order to plot the results on one diagram, which ranged from 0.08 to 11599.13, $\log_{10}$ was used.

Conclusions

The genetic program of the formation of separated male and female flower development in monoecious plants is a less understood mechanism in flowering biology. Available genomic data of the majority of these species are insufficient or inexistent to date. It makes it more difficult, that the reproduction biology of *A. artemisiifolia* is less studied and the majority of information has been revealed exclusively in the pollen formation as part of male flowering biology. Today, large DNA or RNA data sets of the non-model organisms may be attainable using NGS technology; therefore, we sequenced and compared transcriptome libraries of wild-growing male, female, leaf, and *in vitro* female flowers representing early and late developmental phases. Comparative studies revealed a subset of transcripts that were differentially expressed in the different libraries known in flower or plant development in *A. thaliana*. However, genes showing differential expression previously were not characterized in *A. artemisiifolia* during flowering.

Genome-wide transcriptional profiling in five libraries revealed a high number of transcripts that were differentially expressed playing role - non-exclusively - in plant or flower development, but also in signal transduction, redox, and abiotic stress mechanisms. Induction of floral meristem initiation is preceded by a complex regulation network just in the vegetative phase which regulatory elements are the so-called flowering pathway genes. The expression pattern of components of these pathways is consistent with the short day-induced flowering of common ragweed and suggests that the initiation of flowering depends on PHYB-related light signals. Because of the small amount of collectable pistillate flowers, plants were also *in vitro* cultivated on meta-topolin supplemented media. In this way, influences of hormonal and photoperiodic pathways were modelled on the flowering pathway genes. Based on gene expression differences of flowering pathways in male and female samples and the shifts to intensive female morphogenesis under *in vitro* cultivation conditions, we concluded that before the generative transition, the determination of floral gender takes place just in the vegetative phase during the vegetative pathway dominancy that defines the subsequent floral organ morphogenesis. Transition to *AP1* expression and FOIGs induction is led by hormonal and photoperiodic pathways, and depending on the relative dominancy of these two routes, initiation of the female or the male flower development is facilitated.

Thus, flower identity is decided just in vegetative growth. Investigation of male flower indicated that photoperiodic pathway may induce the generative transition through FT / FD complex; however, gibberellin-related *SOC1* expression was not observed indicating it has no role in male gender formation.

On the other hand, expression of FT / FD was lacking in *in vitro* female samples that was probably due to the action of *SVP (MK098077, GO:* 0000977) or *AGL24*. Transcripts of *LFY* homolog were not found in any of the libraries, therefore it appears likely that floral organ development is induced through *LMI* - *CAL* regulation in female flowers. However, *FUL* and directly *(AP1)* - *AP2* regulation in male flowers in only induced when the *AP1* repression by *TFL1* was also in effect (Fig. 16.). Expression analysis of transcription factors indicated that similarly to other species investigated so far, the circadian clock takes a significant part in timing flowering initiation.

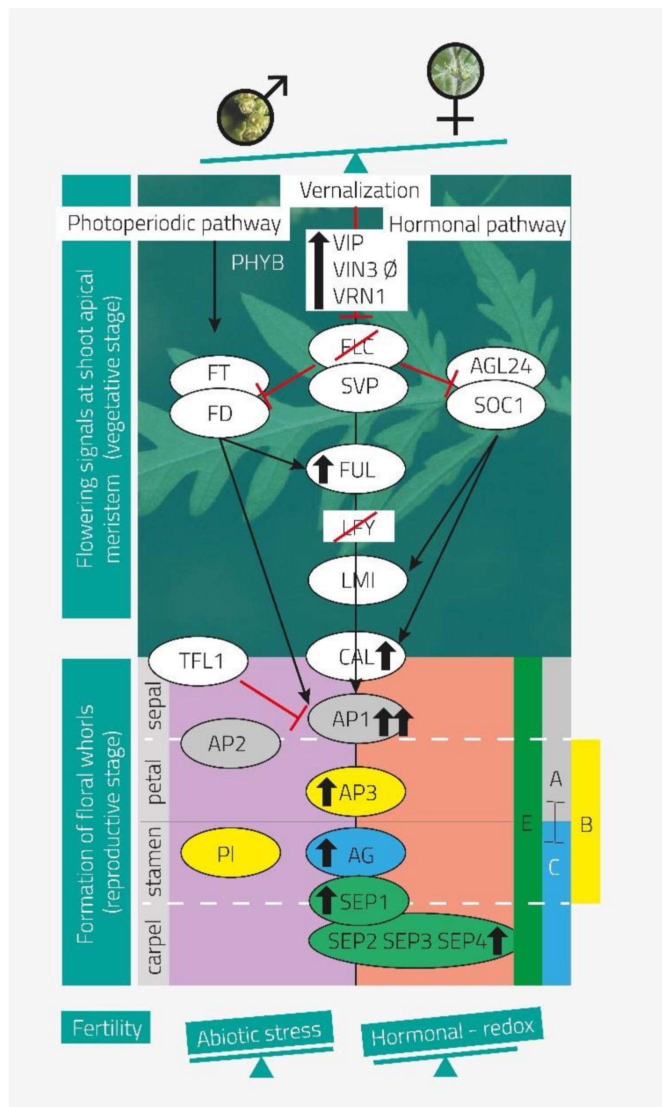


Figure 16. The development of the male and female floral morphogenesis indicated two different regulatory pathways during meristem initiation. Photoperiodic signal regulation was under FT / FD complex induction in male flowers stimulated by the photoreceptor *PhyB* leading to the generative „first Step" *AP2* upregulation via the MADS-box gene *FUL* (*AP1* was repressed by *TFL1* in male lines). Consequently, *PI* expression with *AG* upregulation was occurred for stamen formation and uniquely expressed in male tissues. Female flower morphogenesis was under a strong regulation of endogenous (hormonal) pathway signaling during the vegetative phase. *AP1* upregulation was controlled through *CAL* activated by AGL24 / SOC1 complex distinctively in female transcriptomes leading to the carpel formation that was regulated by the overexpression of *SEP* genes.

Thesis Points

1. Identification of male and female flower organs of *Ambrosia artemisiifolia* L. and determination of their developmental stages. Creation of transcriptome libraries of female and male inflorescences from early and late developmental stages and their biological validation.

2. Determination of the coding sequence of 80 flowering regulatory genes in *Ambrosia artemisiifolia* L. that were published in the NCBI (international) database. Identification their expression level with *in silico* and experimental method.

3. Uniquely expressed gender-specific genes were determined comparing all of the libraries. 9 female and 14 male specific genes were identified and validated.

4. Thirteen sp-called ABC(E) genes, such as floral architecture formation genes, in *A. artemisiifolia* L. monoecious flowering system were identified. These results showed that the *APETALA 2*, *APETALA3 2*, and *PISTILLATA* genes were no or slightly expressed in the female inflorescence, oppositely to male inflorescence, therefore so they can be determined male-specific in this species.

5. The development of male flowers occurs through the photoperiodic pathway which is regulated by the FT / FD complex; however, regulation of female flowers depends on the hormonal pathway affecting AGL 24 / SOC1 complex regulation.

Summary

The highly allergenic and invasive weed *Ambrosia artemisiifolia* L. (common ragweed, *A. artemisiifolia*) is a monoecious plant with separated male and female flowers.

The genetic regulation of floral morphogenesis is a less understood field in the reproduction biology of the common ragweed. Therefore, the objective of this work was to investigate the genetic control of sex determination during floral organogenesis. To this end, we performed a genome-wide transcriptional profiling of vegetative and generative tissues during the plant development comparing wild-growing and *in vitro* cultivated plants.

Prior to genetic studies, the male and female inflorescences of the common ragweed were have dissected out, examined by digital microscopy using the stereomicroscopic focus stacking technique, and individual flower organs were identified.

RNA-seq on the Illumina NextSeq 500 and HiSeq 2000 platforms with an integrative bioinformatics analysis indicated differences in 80 floral gene expression profiles depending on environmental and endogenous initial signals. Sex specificity of select genes was validated based on RT-qPCR experiments and the sequences of candidate genes described in other species in NCBI and UNIPROT databases. We found 9 and 14 uniquely expressed genes in female and male transcriptomes, respectively, potentially associated with fertility and abiotic stress responses. Increased levels of gene expression for homologues in each tissue type of *FD, FT, TFL1* and *CAL, AP1* were characteristic to male and female floral meristems during organogenesis. Homologue transcripts of *LFY* and *FLC* were not found in the investigated generative and vegetative tissues. We observed that morphogenesis of each flower type occurs by two different regulatory pathways, the development of male flowers through the photoperiodic pathway by the FT / FD complex, while that of female flowers under the hormonal pathway is regulated by the AGL24 / SOC1 complex. The nucleotide sequences of the 13 ABC(E) genes, which responsible for flower organ development were determined based on the sequences of various species belonging to the *Asteraceae* family.

Alterations of male and female floral meristem differentiation were demonstrated under photoperiodic and hormonal condition changes by applying *in vitro* treatments.

Összefoglalás

Az ürömlevelű parlagfű (*Ambrosia artemisiifolia* L.) hím és nő virágzatában kifejeződő gének összehasonlító elemzése

Az erősen allergén és invazív ürömlevelű parlagfű (common ragweed, *Ambrosia artemisiifolia* L) egylaki gyomnövény, hím és nő virágai elkülönülten fészekvirágzatokba rendeződve fejlődnek. A faj szaporodásbiológiájában a virágmorfogenezis genetikai szabályozása még nem ismert, ezért munkánk során célul tűztük ki a parlagfű esetében a hím és nő virágzatokra jellemző, valamint a virág szervek kialakulásáért felelős gének izolálását. Ennek érdekében az ürömlevelű parlagfű vad és *in vitro* példányainak vegetatív és generatív szöveteiből mRNS szekvenálással transzkriptom adatbázisokat hoztunk létre.

A genetikai vizsgálatok előtt azonosítottuk a parlagfű virágszerveit sztereomikroszkópos rétegfotózás segítségével, melyről elsőként készítettünk magyar nyelvű leírást.

Az Illumina NextSeq 500 és HiSeq 2000 platformokon végzett RNS szekvenálást követő bioinformatikai elemzések során 80 virággén szekvenciáját határoztunk meg. A meghatározott gének nemhez kötött specifikusságát RT-qPCR technikával, valamint és az NCBI és az UNIPROT adatbázisokban már más fajokban leírt gének szekvenciái alapján validáltuk. 9 és 14 egyedileg expresszált gént találtunk a nő és a hímvirág transzkriptomokban, amelyek elsősorban a szaporodásért és az abiotikus stresszre adott válaszokért lehetnek felelősek.

Az *FD, FT, TFL1, CAL, SOC1* és *AP1* gén homológok expressziója a hím és a női virágzati merisztémákban is megfigyelhető volt az organogenezis során. Az *LFY* és az *FLC* homológ gének szekvenciáit nem tudtuk azonosítani a vizsgált generatív és vegetatív szövetekben sem.

Megfigyeltük, hogy az egyes virágtípusok morfogenezise két különböző szabályozási útvonalon történik, a hím virágok fejlődésének szabályozása a fotoperiodikus útvonalhoz kötött és az FT / FD komplex kezdi a szabályozást, míg a nő virágok szabályozása a hormonális útvonalhoz kötött az AGL24 / SOC1 útvonalon keresztül. A virágszervek fejlődéséért felelős 13 ABC(E) gén szekvenciáját az *Asteraceae* családba tartozó fajok szekvenciái alapján határoztuk meg.

A hím és a női virágzati merisztéma differenciálódásának fotoperiodikus és hormonális szabályozását *in vitro* kísérletekkel is alátámasztottuk.

References

ACHARD, P., HERR, A., BAULCOMBE, D.C., HARBERD, N.P. 2004. Modulation of floral development by a gibberellin- regulated microRNA. *Development*, 131, 3357-3365.

ACHARD, P., BAGHOUR, M., CHAPPLE, A., HEDDEN, P., VAN DER STRAETEN, D., GENSCHIK, P., MORITZ, T. & HARBERD, N. P. 2007. The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proceedings of the National Academy of Sciences,* 104**,** 6484-6489.

ADOLPHSON, C., GOODFRIEND, L. & GLEICH, G. J. 1978. Reactivity of ragweed allergens with IgE antibodies: analyses by leukocyte histamine release and the radioallergosorbent test and determination of cross-reactivity. *Journal of Allergy and Clinical Immunology,* 62**,** 197-210.

AIDA, M., ISHIDA, T., FUKAKI, H., FUJISAWA, H. & TASAKA, M. 1997. Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. *The plant cell,* 9**,** 841-857.

ALLARD, H. 1943. The North American ragweeds and their occurrence in other parts of the world. *Science,* 98**,** 292-294.

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMAN, D. J. 1990. Basic local alignment search tool. *Journal of molecular biology,* 215**,** 403-410.

AMASINO, R. 2010. Seasonal and developmental timing of flowering. *The Plant Journal,* 61**,** 1001-1013.

AMBAWAT, S., SHARMA, P., YADAV, N. R. & YADAV, R. C. 2013. MYB transcription factor genes as regulators for plant responses: an overview. *Physiology and Molecular Biology of Plants,* 19**,** 307-321.

ANDREWS, S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.

ARSOVSKI, A. A., VILLOTA, M. M., ROWLAND, O., SUBRAMANIAM, R. & WESTERN, T. L. 2009. MUM ENHANCERS are important for seed coat mucilage production and mucilage secretory cell differentiation in Arabidopsis thaliana. *Journal of experimental botany,* 60**,** 2601-2612.

ARTICO, S., NARDELI, S. M., BRILHANTE, O., GROSSI-DE-SA, M. F. & ALVES-FERREIRA, M. 2010. Identification and evaluation of new reference genes in Gossypium hirsutumfor accurate normalization of real-time quantitative RT-PCR data. *BMC Plant Biology,* 10**,** 49.

ASERO, R., WOPFNER, N., GRUBER, P., GADERMAIER, G. & FERREIRA, F. 2006. Artemisia and Ambrosia hypersensitivity: co-sensitization or co-recognition? *Clinical & Experimental Allergy,* 36**,** 658-665.

ASHBURNER, M., BALL, C. A., BLAKE, J. A., BOTSTEIN, D., BUTLER, H., CHERRY, J. M., DAVIS, A. P., DOLINSKI, K., DWIGHT, S. S. & EPPIG, J. T. 2000. Gene ontology: tool for the unification of biology. *Nature genetics,* 25**,** 25-29.

BAO, S., HUA, C., HUANG, G., CHENG, P., GONG, X., SHEN, L., YU, H. 2019. Molecular basis of natural variation in photoperiodic flowering responses. *Developmental Cell,* 50, 90– 101

BASSETT, I. J. & CROMPTON, C. W. 1975. THE BIOLOGY OF CANADIAN WEEDS.: 11. Ambrosia artemisiifolia L. and A. psilostachya DC. *Canadian Journal of Plant Science,* 55**,** 463-476.

BAUMANN, K., PEREZ-RODRIGUEZ, M., BRADLEY, D., VENAIL, J., BAILEY, P., JIN, H., KOES, R., ROBERTS, K. & MARTIN, C. 2007. Control of cell and petal morphogenesis by R2R3 MYB transcription factors. *Development,* 134**,** 1691-1701.

BAZZAZ, F. 1979. The physiological ecology of plant succession. *Annual review of Ecology and Systematics,* 10**,** 351-371.

BÉRES, I. 1994. [Recent investigations into the biology of Ambrosia artemisiifolia L., the common ragweed].[German]. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*.

BÉRES, I. 2003. Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) elterjedése, jelentősége és biológiája. *Novenyvedelem,* 39**,** 293-302.

BÉRES, I., NOVÁK, R., HOFFMANNÉ PATHY, Z. & KAZINCZI, G. 2005. Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) elterjedése, morfológiája, biológiája, jelentősége és a védekezés lehetőségei.[Distribution, morphology, biology and importance of common ragweed (Ambrosia artemisiifolia L.) and protection facilities.]. *Gyomnövények, gyomirtás,* 6**,** 1-48.

BOAVIDA, L. C., QIN, P., BROZ, M., BECKER, J. D. & MCCORMICK, S. 2013. Arabidopsis tetraspanins are confined to discrete expression domains and cell types in reproductive tissues and form homo-and heterodimers when expressed in yeast. *Plant physiology,* 163**,** 696-712.

BOHÁR, G. & VAJNA, L. 1996. Occurrence of some pathogenic fungi of common ragweed (Ambrosia artemisiifolia var. elatior) in Hungary. *Novenyvedelem,* 32**,** 527-528.

BOSAK, P. & MOD, S. 2000. Influence of different weed species on sugar beet yield. *Növénytermelés,* 49**,** 571-580.

BOWMAN, J. L., SAKAI, H., JACK, T., WEIGEL, D., MAYER, U. & MEYEROWITZ, E. M. 1992. SUPERMAN, a regulator of floral homeotic genes in Arabidopsis. *Development,* 114**,** 599-615.

BRANDES, D. & NITZSCHE, J. 2006. Biology, introduction, dispersal, and distribution of common ragweed (Ambrosia artemisiifolia L.) with special regard to Germany. *Nachrichtenblatt-Deutschen Pflanzenschutzdienstes Braunschweig,* 58**,** 286.

BREWER, C. E. & OLIVER, L. R. 2009. Confirmation and resistance mechanisms in glyphosate-resistant common ragweed (Ambrosia artemisiifolia) in Arkansas. *Weed Science,* 57**,** 567-573.

BURBACH, G. J., HEINZERLING, L. M., EDENHARTER, G., BACHERT, C., BINDSLEV-JENSEN, C., BONINI, S., BOUSQUET, J., BOUSQUET-ROUANET, L., BOUSQUET, P. & BRESCIANI, M. 2009. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. *Allergy,* 64**,** 1507-1515.

BUSCH, A., HORN, S., MÜHLHAUSEN, A., MUMMENHOFF, K. & ZACHGO, S. 2012. Corolla monosymmetry: evolution of a morphological novelty in the Brassicaceae family. *Molecular Biology and Evolution,* 29**,** 1241-1254.

BUSTIN, S., BENES, V., GARSON, J., HELLEMANS, J., HUGGETT, J., KUBISTA, M., MUELLER, R., NOLAN, T., PFAFFL, M. & SHIPLEY, G. 2015. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *BMC Bioinformatics,* 16**,** 197.

CECCATO, L., MASIERO, S., ROY, D.S., BENCIVENGA, S., DITENGOU, F., PALME, K., SIMON, R., COLOMBO, L. (2011) The role of PIN1 in female gametophyte development. Hormonal Network Controlling Ovule Development in Arabidopsis thaliana:21

CHANDLER, J. (2011) The hormonal regulation of flower development. Journal of Plant Growth Regulation 30:242-254

CHASE, M. W., CHRISTENHUSZ, M., FAY, M., BYNG, J., JUDD, W. S., SOLTIS, D., MABBERLEY, D., SENNIKOV, A., SOLTIS, P. S. & STEVENS, P. F. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society,* 181**,** 1-20.

CHEN, Q., ATKINSON, A., OTSUGA, D., CHRISTENSEN, T., REYNOLDS, L. & DREWS, G. N. 1999. The Arabidopsis FILAMENTOUS FLOWER gene is required for flower formation. *Development,* 126**,** 2715-2726.

CHENG, X. F. & WANG, Z. Y. 2005. Overexpression of COL9, a CONSTANS-LIKE gene, delays flowering by reducing expression of CO and FT in Arabidopsis thaliana. *The Plant Journal,* 43**,** 758-768.

CHUAQUI, R. F., BONNER, R. F., BEST, C. J., GILLESPIE, J. W., FLAIG, M. J., HEWITT, S. M., PHILLIPS, J. L., KRIZMAN, D. B., TANGREA, M. A. & AHRAM, M. 2002. Post-analysis follow-up and validation of microarray experiments. *Nature genetics,* 32**,** 509-514.

CJB (2016). African Plant Database. Conservatoire et Jardin Botaniques de la Ville de Geneve, Geneva, Switzerland, and South African National Biodiversity Institute, Pretoria, South Africa. Geneva, Switzerland: CJB/SANBI. http://www.ville-ge.ch/musinfo/bd/cjb/africa/

CLOUSE, S. D. 1996. Molecular genetic studies confirm the role of brassinosteroids in plant growth and development. *Plant Journal (United Kingdom).*

COEN, E. S. & MEYEROWITZ, E. M. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature,* 353**,** 31-37.

COMPAGNON, V., DIEHL, P., BENVENISTE, I., MEYER, D., SCHALLER, H., SCHREIBER, L., FRANKE, R. & PINOT, F. 2009. CYP86B1 is required for very long chain ω-hydroxyacid and α, ω-dicarboxylic acid synthesis in root and seed suberin polyester. *Plant physiology,* 150**,** 1831-1843.

CONESA, A., GÖTZ, S., GARCÍA-GÓMEZ, J. M., TEROL, J., TALÓN, M. & ROBLES, M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics,* 21**,** 3674-3676.

COUNCIL OF HEADS OF AUSTRALASIAN HERBARIA 2016. Australia's Virtual Herbarium., Australia: Council of Heads of Australasian Herbaria. http://avh.ala.org.au

CORBESIER, L., VINCENT, C., JANG, S., FORNARA, F., FAN, Q., SEARLE, I., GIAKOUNTIS, A., FARRONA, S., GISSOT, L. & TURNBULL, C. 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *science,* 316**,** 1030-1033.

CRAWFORD, B. C. & YANOFSKY, M. F. 2008. The formation and function of the female reproductive tract in flowering plants. *Current Biology,* 18**,** R972-R978.

CZECHOWSKI, T., STITT, M., ALTMANN, T., UDVARDI, M. K. & SCHEIBLE, W.-R. 2005. Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiology,* 139**,** 5-17.

CSONTOS, P., VITALOS, M., BARINA, Z. & KISS, L. 2010. Eddig feldolgozatlan herbáriumi adatok újraértelmezik a parlagfű felbukkanását és korai terjedését a Kárpát-Pannon térségben. *BOTANIKAI KÖZLEMÉNYEK,* 97**,** 69-77.

D'ALOIA, M., BONHOMME, D., BOUCHÉ, F., TAMSEDDAK, K., ORMENESE, S., TORTI, S., COUPLAND, G. & PÉRILLEUX, C. 2011. Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the FT paralogue TSF. *The Plant Journal,* 65**,** 972-979.

DARÓK, J. 2011. *Növényanatómiai-botanikai terminológiai szótár*, Akadémiai Kiadó, Budapest.

DAYAN, F. E. & DUKE, S. O. 2014. Natural compounds as next-generation herbicides. *Plant physiology,* 166**,** 1090-1105.

DEEN, W., SWANTON, C. J. & HUNT, L. A. 2001. A mechanistic growth and development model of common ragweed. *Weed Science***,** 723-731.

DI GIORGIO, J. A. P., BIENERT, G. P., AYUB, N. D., YANEFF, A., BARBERINI, M. L., MECCHIA, M. A., AMODEO, G., SOTO, G. C. & MUSCHIETTI, J. P. 2016. Pollen-specific aquaporins NIP4; 1 and NIP4; 2 are required for pollen development and pollination in Arabidopsis thaliana. *The plant cell,* 28**,** 1053-1077.

DICKERSON JR, C. & SWEET, R. 1971. Common ragweed ecotypes. *Weed Science***,** 64-66.

DIE, J. V., ROMÁN, B., NADAL, S. & GONZÁLEZ-VERDEJO, C. I. 2010. Evaluation of candidate reference genes for expression studies in Pisumsativum under different experimental conditions. *Planta,* 232**,** 145-153.

DOBRITSA, A. A., LEI, Z., NISHIKAWA, S.-I., URBANCZYK-WOCHNIAK, E., HUHMAN, D. V., PREUSS, D. & SUMNER, L. W. 2010. LAP5 and LAP6 encode anther-specific proteins with similarity to chalcone synthase essential for pollen exine development in Arabidopsis. *Plant physiology,* 153**,** 937-955.

DOBRITSA, A. A., NISHIKAWA, S.-I., PREUSS, D., URBANCZYK-WOCHNIAK, E., SUMNER, L. W., HAMMOND, A., CARLSON, A. L. & SWANSON, R. J. 2009. LAP3, a novel plant protein required for pollen development, is essential for proper exine formation. *Sexual plant reproduction,* 22**,** 167.

DOMAGALSKA, M. A., SCHOMBURG, F. M., AMASINO, R. M., VIERSTRA, R. D., NAGY, F. & DAVIS, S. J. 2007. Attenuation of brassinosteroid signaling enhances FLC expression and delays flowering. *Development,* 134**,** 2841-2850.

DOMONKOS, Z., SZABÓ-SZIGETI, V., FARKAS, A., PINKE, G., REISINGER, P., ENZSÖL, E. & PETER, T. 2016. Study of common ragweed (Ambrosia artemisiifolia L.) in Žitný ostrov (Slovakia) and Szigetköz (Hungary) in 2015. *Magyar Gyomkutatás és Technológia*, *17*(1), pp.17-27.

DU, S. S., LI, L., LI, L., WEI, X., XU, F., XU, P., WANG, W., XU, P., CAO, X. & MIAO, L. 2020. Photoexcited cryptochrome2 interacts directly with TOE1 and TOE2 in flowering regulation. *Plant Physiology,* 184**,** 487-505.

DUAN, H. & SCHULER, M. A. 2005. Differential expression and evolution of the Arabidopsis CYP86A subfamily. *Plant physiology,* 137**,** 1067-1081.

EGGER, M., MUTSCHLECHNER, S., WOPFNER, N., GADERMAIER, G., BRIZA, P. & FERREIRA, F. 2006. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy,* 61**,** 461-476.

ELLIOTT, R. C., BETZNER, A. S., HUTTNER, E., OAKES, M. P., TUCKER, W., GERENTES, D., PEREZ, P. & SMYTH, D. R. 1996. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *The Plant Cell,* 8**,** 155-168.

EPSTEIN, S. 1960. Role of dermal sensitivity in ragweed contact dermatitis. *Archives of dermatology,* 82**,** 48-55.

ESHED, Y., IZHAKI, A., BAUM, S. F., FLOYD, S. K. & BOWMAN, J. L. 2004. Asymmetric leaf development and blade expansion in Arabidopsis are mediated by KANADI and YABBY activities. *Development,* 131**,** 2997-3006.

ESSL, F., BIRÓ, K., BRANDES, D., BROENNIMANN, O., BULLOCK, J. M., CHAPMAN, D. S., CHAUVEL, B., DULLINGER, S., FUMANAL, B. & GUISAN, A. 2015. Biological flora of the British Isles: Ambrosia artemisiifolia. *Journal of Ecology,* 103**,** 1069-1098.

EURO+MED 2016. Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. http://www.emplantbase.org/home.html

FLORA OF CHINA EDITORIAL COMMITTEE 2016 Flora of China. St. Louis, Missouri and Cambridge, Massachusetts, USA: Missouri Botanical Garden and Harvard University Herbaria. http://www.efloras.org/flora_page.aspx?flora_id=2

FORESTAN, C., FARINATI, S., ZAMBELLI, F., PAVESI, G., ROSSI, V. & VAROTTO, S. 2020. Epigenetic signatures of stress adaptation and flowering regulation in response to extended drought and recovery in Zea mays. *Plant, Cell & Environment,* 43**,** 55-75.

FUMANAL, B. (2007) Biological traits and evolutive processes of an invasiveplant species in France: Ambrosia artemisiifolia L. PhD Thesis, Universityof Burgundy, Dijon, France.

FUTAMURA, N., MORI, H., KOUCHI, H. & SHINOHARA, K. 2000. Male flower-specific expression of genes for polygalacturonase, pectin methylesterase and β-1, 3-glucanase in a dioecious willow (Salix gilgiana Seemen). *Plant and Cell Physiology,* 41**,** 16-26.

GADERMAIER, G., WOPFNER, N., WALLNER, M., EGGER, M., DIDIERLAURENT, A., REGL, G., ABERGER, F., LANG, R., FERREIRA, F. & HAWRANEK, T. 2008. Array-based profiling of ragweed and mugwort pollen allergens. *Allergy,* 63**,** 1543-1549.

GAN, Y., LIU, C., YU, H. & BROUN, P. 2007. Integration of cytokinin and gibberellin signalling by Arabidopsis transcription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate. *Development,* 134**,** 2073-2081.

GARBER, M., GRABHERR, M. G., GUTTMAN, M. & TRAPNELL, C. 2011. Computational methods for transcriptome annotation and quantification using RNA-seq. *Nature methods,* 8**,** 469-477.

GAVNHOLT, B. & LARSEN, K. 2002. Molecular biology of plant laccases in relation to lignin formation. *Physiologia plantarum,* 116**,** 273-280.

GAZZANI, S., GENDALL, A. R., LISTER, C. & DEAN, C. 2003. Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. *Plant physiology,* 132**,** 1107-1114.

GIAROLA, V., JUNG, N. U., SINGH, A., SATPATHY, P. & BARTELS, D. 2018. Analysis of pcC13-62 promoters predicts a link between cis-element variations and desiccation tolerance in Linderniaceae. *Journal of experimental botany,* 69**,** 3773-3784.

GISH, W. & STATES, D. J. 1993. Identification of protein coding regions by database similarity search. *Nature genetics,* 3**,** 266-272.

GONZALEZ, A., MENDENHALL, J., HUO, Y. & LLOYD, A. 2009. TTG1 complex MYBs, MYB5 and TT2, control outer seed coat differentiation. *Developmental biology,* 325**,** 412-421.

GÖTZ, S., GARCÍA-GÓMEZ, J. M., TEROL, J., WILLIAMS, T. D., NAGARAJ, S. H., NUEDA, M. J., ROBLES, M., TALÓN, M., DOPAZO, J. & CONESA, A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic acids research,* 36**,** 3420-3435.

GRIFFITHS, J., MURASE, K., RIEU, I., ZENTELLA, R., ZHANG, Z.-L., POWERS, S. J., GONG, F., PHILLIPS, A. L., HEDDEN, P. & SUN, T.-P. 2006. Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *The plant cell,* 18**,** 3399-3414.

GUTIERREZ, L., MAURIAT, M., GUÉNIN, S., PELLOUX, J., LEFEBVRE, J. F., LOUVET, R., RUSTERUCCI, C., MORITZ, T., GUERINEAU, F. & BELLINI, C. 2008. The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants. *Plant biotechnology journal,* 6**,** 609-618.

HAAS, B. J., PAPANICOLAOU, A., YASSOUR, M., GRABHERR, M., BLOOD, P. D., BOWDEN, J., COUGER, M. B., ECCLES, D., LI, B. & LIEBER, M. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature protocols,* 8**,** 1494-1512.

HAKOZAKI, H., PARK, J.-I., ENDO, M., TAKADA, Y., KAZAMA, T., TAKEDA, Y., SUZUKI, G., KAWAGISHI-KOBAYASHI, M. & WATANABE, M. 2008. Expression and developmental function of the 3-ketoacyl-ACP synthase2 gene in Arabidopsis thaliana. *Genes & genetic systems,* 83**,** 143-152.

HARRIS, E. M. 1999. Capitula in the Asteridae: a widespread and varied phenomenon. *The Botanical Review,* 65**,** 348.

HASSIDIM, M., HARIR, Y., YAKIR, E., KRON, I. & GREEN, R. M. 2009. Over-expression of CONSTANS-LIKE 5 can induce flowering in short-day grown Arabidopsis. *Planta,* 230**,** 481-491.

HAYAMA, R., YOKOI, S., TAMAKI, S., YANO, M. & SHIMAMOTO, K. 2003. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature,* 422**,** 719-722.

HEAP, I. 2020. The international herbicide-resistant weed database. *Available at: www.weedscience.org(accessed 4 October 2019).*

HEGI G. 1906: Illustrierte Flora von Mitteleuropa. Band VI. J. F. Lehmanns Verlag, München, 544 pp.

HELLIWELL, C. A., WOOD, C. C., ROBERTSON, M., JAMES PEACOCK, W. & DENNIS, E. S. 2006. The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *The Plant Journal,* 46**,** 183-192.

HENRY, R. P. 1996. Multiple roles of carbonic anhydrase in cellular transport and metabolism. *Annual review of physiology,* 58**,** 523-538.

HIGGINSON, T., LI, S. F. & PARISH, R. W. 2003. AtMYB103 regulates tapetum and trichome development in Arabidopsis thaliana. *The Plant Journal,* 35**,** 177-192.

HIRAYAMA, T. & OKA, A. 1992. Novel protein kinase of Arabidopsis thaliana (APK1) that phosphorylates tyrosine, serine and threonine. *Plant molecular biology,* 20**,** 653-662.

HJORTH, N., ROED-PETERSEN, J. & THOMSEN, K. 1976. Airborne contact dermatitis from Compositae oleoresins simulating photodermatitis. *British Journal of Dermatology,* 95**,** 613-620.

HODGINS, K. A., LAI, Z., NURKOWSKI, K., HUANG, J. & RIESEBERG, L. H. 2013. The molecular basis of invasiveness: differences in gene expression of native and introduced common ragweed (A mbrosia artemisiifolia) in stressful and benign environments. *Molecular ecology,* 22**,** 2496-2510.

HONYS, D. & TWELL, D. 2004. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. *Genome biology,* 5**,** R85.

HUGGETT, J., DHEDA, K., BUSTIN, S. & ZUMLA, A. 2005. Real-time RT-PCR normalisation; strategies and considerations. *Genes & Immunity,* 6**,** 279-284.

IRISH, V. F. 2010. The flowering of Arabidopsis flower development. *The Plant Journal,* 61**,** 1014-1028.

ITO, T., TAKAHASHI, N., SHIMURA, Y. & OKADA, K. 1997. A serine/threonine protein kinase gene isolated by an in vivo binding procedure using the Arabidopsis floral homeotic gene product, AGAMOUS. *Plant and Cell Physiology,* 38**,** 248-258.

JAGER, O. R. S. 2001. Ambrosia (ragweed) in Europe. *Allergy Clin Immunol Int,* 13**,** 60-66.

JANG, S., MARCHAL, V., PANIGRAHI, K. C., WENKEL, S., SOPPE, W., DENG, X. W., VALVERDE, F. & COUPLAND, G. 2008. Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *The EMBO journal,* 27**,** 1277-1288.

JÁRAINÉ, K. M. 2003: Pannon Enciklopédia Magyarország Növényvilága. Ubris Könyvkiadó. Budapest. 284–286.

JI, R., WANG, H., XIN, X., PENG, S., HUR, Y., LI, Z. & FENG, H. 2017. BrEXL6, a GDSL lipase gene of Brassica rapa, functions in pollen development. *Biologia plantarum,* 61**,** 685-692.

JUHÁSZ, M. 2012. Mitől allergénebb egyik pollen, mint a másik. *Elméleti allergológia,* 19**,** 17-19.

JUNG, C., SEO, J. S., HAN, S. W., KOO, Y. J., KIM, C. H., SONG, S. I., NAHM, B. H., DO CHOI, Y. & CHEONG, J.-J. 2008. Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. *Plant physiology,* 146**,** 623-635.

KATINAS, L., HERNÁNDEZ, M. P., ARAMBARRI, A. M. & FUNK, V. A. 2016. The origin of the bifurcating style in Asteraceae (Compositae). *Annals of botany,* 117**,** 1009-1021.

KAUFMANN, K., WELLMER, F., MUIÑO, J. M., FERRIER, T., WUEST, S. E., KUMAR, V., SERRANO-MISLATA, A., MADUENO, F., KRAJEWSKI, P. & MEYEROWITZ, E. M. 2010. Orchestration of floral initiation by APETALA1. *Science,* 328**,** 85-89.

KAZAMA, Y., FUJIWARA, M. T., KOIZUMI, A., NISHIHARA, K., NISHIYAMA, R., KIFUNE, E., ABE, T. & KAWANO, S. 2009. A SUPERMAN-like gene is exclusively expressed in female flowers of the dioecious plant Silene latifolia. *Plant and Cell Physiology,* 50**,** 1127-1141.

KAZINCZI, G., BÉRES, I., NOVÁK, R., BÍRÓ, K. & PATHY, Z. 2008. Common ragweed (Ambrosia artemisiifolia): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. *Herbologia,* 9**,** 55-91.

KAZINCZI, G., BÉRES, I., NOVÁK, R. & KARAMÁN, J. 2009. Újra fókuszban az ürömlevelű parlagfű. *Ambrosia artemisiifolia***,** 389-403.

KLUCHER, K. M., CHOW, H., REISER, L. & FISCHER, R. L. 1996. The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. *The Plant Cell,* 8**,** 137-153.

KOMEDA, Y. 2004. Genetic regulation of time to flower in Arabidopsis thaliana. *Annu. Rev. Plant Biol.,* 55**,** 521-535.

KORESSAAR, T. & REMM, M. 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics,* 23**,** 1289-1291.

KOVALEV, O. 1989. Spread of adventitious plants of the tribe Ambrosia in Eurasia and methods of biological control of weeds of the genus Ambrosia L.(Ambrosieae, Asteraceae). *Trudy Zoologicheskii, Institut Akademii Nauk SSSR,* 189**,** 7-23.

KRIZEK, B. A. & FLETCHER, J. C. 2005. Molecular mechanisms of flower development: an armchair guide. *Nature Reviews Genetics,* 6**,** 688-698.

LANGMEAD, B., TRAPNELL, C., POP, M. & SALZBERG, S. L. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome biology,* 10**,** R25.

LAUBINGER, S., MARCHAL, V., GENTILHOMME, J., WENKEL, S., ADRIAN, J., JANG, S., KULAJTA, C., BRAUN, H., COUPLAND, G. & HOECKER, U. 2006. Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development,* 133**,** 3213-3222.

LEE, Y. S., JEONG, D. H., LEE, D. Y., YI, J., RYU, C. H., KIM, S. L., JEONG, H. J., CHOI, S. C., JIN, P. & YANG, J. 2010. OsCOL4 is a constitutive flowering repressor upstream of Ehd1 and downstream of OsphyB. *The Plant Journal,* 63**,** 18-30.

LEHOCZKY, É. 2004. A növekvő adagú nitrogén ellátás hatása a parlagfű (Ambrosia artemisiifolia L.) növekedésére. *Magyar Gyomkutatás és technológia,* 5**,** 32-41.

LEHOCZKY, É., BUSZNYÁK, J., GÓLYA, G. & PÁLMAI, O. 2012. Green water-Ambrosia artemisiifolia L. on winter wheat stubble. *Növénytermelés,* 61**,** 259-262.

LENGYEL, G. 1923. Az Ambrosia artemisiifolia előfordulása Magyarországon. *BOTANIKAI KÖZLEMÉNYEK,* 21**,** 100.

LI, B. & DEWEY, C. N. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics,* 12**,** 323.

LI, D., LIU, C., SHEN, L., WU, Y., CHEN, H., ROBERTSON, M., HELLIWELL, C.A., ITO, T., MEYEROWITZ, E., YU, H. 2008. A repressor complex governs the integration of flowering signals in *Arabidopsis. Developmental Cell* 15, 110-120.

LI, J., LI, C. & SMITH, S. M. 2017. *Hormone metabolism and signaling in plants*, Academic Press.

LI, M., AN, F., LI, W., MA, M., FENG, Y., ZHANG, X., GUO, H. 2016 DELLA proteins interact with FLC to repress flowering transition. *Journal of Integrative Plant Biology,* 58, 642–655

LI, S. F., MILLIKEN, O. N., PHAM, H., SEYIT, R., NAPOLI, R., PRESTON, J., KOLTUNOW, A. M. & PARISH, R. W. 2009. The Arabidopsis MYB5 transcription factor regulates mucilage synthesis, seed coat development, and trichome morphogenesis. *The Plant Cell,* 21**,** 72-89.

LIANG, Y.-K., DUBOS, C., DODD, I. C., HOLROYD, G. H., HETHERINGTON, A. M. & CAMPBELL, M. M. 2005. AtMYB61, an R2R3-MYB transcription factor controlling stomatal aperture in Arabidopsis thaliana. *Current Biology,* 15**,** 1201-1206.

LISCUM, E. & REED, J. 2002. Genetics of Aux/IAA and ARF action in plant growth and development. *Plant molecular biology,* 49**,** 387-400.

LIU, C., CHEN, H., ER, H. L., SOO, H. M., KUMAR, P. P., HAN, J.-H., LIOU, Y. C. & YU, H. 2008. Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. *Development,* 135**,** 1481-1491.

LIU, C., JUN, J. H. & DIXON, R. A. 2014. MYB5 and MYB14 play pivotal roles in seed coat polymer biosynthesis in Medicago truncatula. *Plant physiology,* 165**,** 1424-1439.

LIU, J., YIN, T., YE, N., CHEN, Y., YIN, T., LIU, M. & HASSANI, D. 2013. Transcriptome analysis of the differentially expressed genes in the male and female shrub willows (Salix suchowensis). *PLoS One,* 8**,** e60181.

LLOYD, D. 1984. Gender allocations in outcrossing cosexual plants. *Plant population ecology*.

LONG, J. A. & BARTON, M. K. 1998. The development of apical embryonic pattern in Arabidopsis. *Development,* 125**,** 3027-3035.

LORENZI, H. J. & JEFFERY, L. S. 1987. Weeds of the United States and their control. AVI Book, New York, N.Y. 355 pp.

LOU, Y., XU, X.-F., ZHU, J., GU, J.-N., BLACKMORE, S. & YANG, Z.-N. 2014. The tapetal AHL family protein TEK determines nexine formation in the pollen wall. *Nature communications,* 5**,** 1-9.

LYONS, R., RUSU, A., STILLER, J., POWELL, J., MANNERS, J. M. & KAZAN, K. 2015. Investigating the association between flowering time and defense in the Arabidopsis thaliana-Fusarium oxysporum interaction. *PloS one,* 10**,** e0127699.

MAKKENA, S., LEE, E., SACK, F. D. & LAMB, R. S. 2012. The R2R3 MYB transcription factors FOUR LIPS and MYB88 regulate female reproductive development. *Journal of experimental botany,* 63**,** 5545-5558.

MAKRA, L., JUHÁSZ, M., BÉCZI, R. & BORSOS, E. K. 2005. The history and impacts of airborne Ambrosia (Asteraceae) pollen in Hungary. *Grana,* 44**,** 57-64.

MAKRA, L., JUHÁSZ, M., BORSOS, E. & BÉCZI, R. 2004. Meteorological variables connected with airborne ragweed pollen in Southern Hungary. *International Journal of Biometeorology,* 49**,** 37-47.

MANDEL, M. A. & YANOFSKY, M. F. 1995. A gene triggering flower formation in Arabidopsis. *Nature,* 377**,** 522-524.

MARÍN-RODRÍGUEZ, M. C., ORCHARD, J. & SEYMOUR, G. B. 2002. Pectate lyases, cell wall degradation and fruit softening. *Journal of experimental botany,* 53**,** 2115-2119.

MARSCH-MARTÍNEZ, N., ZÚÑIGA-MAYO, V. M., HERRERA-UBALDO, H., OUWERKERK, P. B., PABLO-VILLA, J., LOZANO-SOTOMAYOR, P., GRECO, R., BALLESTER, P., BALANZÁ, V. & KUIJT, S. J. 2014. The NTT transcription factor promotes replum development in A rabidopsis fruits. *The Plant Journal,* 80**,** 69-81.

MARTIN, M. D., CHAMECKI, M. & BRUSH, G. S. 2010. Anthesis synchronization and floral morphology determine diurnal patterns of ragweed pollen dispersal. *Agricultural and Forest Meteorology,* 150**,** 1307-1317.

MATÍAS-HERNÁNDEZ, L., JIANG, W., YANG, K., TANG, K., BRODELIUS, P. E. & PELAZ, S. 2017. Aa MYB 1 and its orthologue At MYB 61 affect terpene metabolism and trichome development in Artemisia annua and Arabidopsis thaliana. *The Plant Journal,* 90**,** 520-534.

MÁTYÁS, K.K., VIGNESH, M., TALLER, J. 2012 Előzetes eredmények az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) Kárpát-medencei populációgenetikai vizsgálatáról. *Magyar Gyomkutatás és Technológia* 13 21-36.

MÁTYÁS, K. K., BÓDIS, J., VIRÁG, E., TALLER, J. & PINTÉR, C. 2020. Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) virágzatának részletes leírása sztereomikroszkópos rétegfotózás használatával. *BOTANIKAI KÖZLEMÉNYEK,* 107**,** 103-109.

MEISTER, R. J., KOTOW, L. M. & GASSER, C. S. 2002. SUPERMAN attenuates positive INNER NO OUTER autoregulation to maintain polar development of Arabidopsis ovule outer integuments. *Development,* 129**,** 4281-4289.

MICHAELS, S. D. & AMASINO, R. M. 2001. Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *The plant cell,* 13**,** 935-941.

MILLAR, A. A. & GUBLER, F. 2005. The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *The plant cell,* 17**,** 705-721.

MITSUDA, N., HIRATSU, K., TODAKA, D., NAKASHIMA, K., YAMAGUCHI-SHINOZAKI, K. & OHME-TAKAGI, M. 2006. Efficient production of male and female sterile plants by expression of a chimeric repressor in Arabidopsis and rice. *Plant Biotechnology Journal,* 4**,** 325-332.

MORTAZAVI, A., WILLIAMS, B. A., MCCUE, K., SCHAEFFER, L. & WOLD, B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature methods,* 5**,** 621-628.

MURASE, K., SHIGENOBU, S., FUJII, S., UEDA, K., MURATA, T., SAKAMOTO, A., WADA, Y., YAMAGUCHI, K., OSAKABE, Y. & OSAKABE, K. 2017. MYB transcription factor gene involved in sex determination in Asparagus officinalis. *Genes to Cells,* 22**,** 115-123.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum,* 15**,** 473-497.

NODA, K.-I., GLOVER, B. J., LINSTEAD, P. & MARTIN, C. 1994. Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. *Nature,* 369**,** 661-664.

NOVÁK, R., DANCZA, I., SZENTEY, L. & KARAMÁN, J. 2009. Magyarország szántóföldjeinek gyomnövényzete. *Ötödik Országos Szántóföldi Gyomfelvételezés. Témadokumentáció, Budapest*.

NOVÁK, R., DANCZA, I., SZENTEY, L. & KARAMÁN, J. 2011. Az ötödik országos gyomfelvételezés Magyarország szántóföldjein. *Vidékfejlesztési Minisztérium Élelmiszerlánc-felügyeleti Főosztály, Növény-és Talajvédelmi Osztály, Budapest*.

PÁLMAI, O. 2009. A parlagfű elleni védekezés hazai ellentmondásai, Növényvédelem 5(8), 385-388.

PATHAN, M., KEERTHIKUMAR, S., ANG, C. S., GANGODA, L., QUEK, C. Y., WILLIAMSON, N. A., MOURADOV, D., SIEBER, O. M., SIMPSON, R. J. & SALIM, A. 2015. FunRich: An open access standalone functional enrichment and interaction network analysis tool. *Proteomics,* 15**,** 2597-2601.

PAYNE, W. W. 1963. The morphology of the inflorescence of ragweeds (Ambrosia-Franseria: Compositae). *American Journal of Botany,* 50**,** 872-880.

PELAZ, S., DITTA, G. S., BAUMANN, E., WISMAN, E. & YANOFSKY, M. F. 2000. B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature,* 405**,** 200-203.

PHAN, H. A., IACUONE, S., LI, S. F. & PARISH, R. W. 2011. The MYB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in Arabidopsis thaliana. *The plant cell,* 23**,** 2209-2224.

PINA, C., PINTO, F., FEIJÓ, J. A. & BECKER, J. D. 2005. Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. *Plant physiology,* 138**,** 744-756.

PUTTERILL, J., ROBSON, F., LEE, K., SIMON, R. & COUPLAND, G. 1995. The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell,* 80**,** 847-857.

RADONIĆ, A., THULKE, S., MACKAY, I. M., LANDT, O., SIEGERT, W. & NITSCHE, A. 2004. Guideline to reference gene selection for quantitative real-time PCR. *Biochemical and biophysical research communications,* 313**,** 856-862.

REIMANN, R., KOST, B. & DETTMER, J. 2017. Tetraspanins in plants. *Frontiers in plant science,* 8**,** 545.

RENNER, S. S. & RICKLEFS, R. E. 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany,* 82**,** 596-606.

ROCHETA, M., SOBRAL, R., MAGALHÃES, J., AMORIM, M. I., RIBEIRO, T., PINHEIRO, M., EGAS, C., MORAIS-CECÍLIO, L. & COSTA, M. M. 2014. Comparative transcriptomic analysis of male and female flowers of monoecious Quercus suber. *Frontiers in plant science,* 5**,** 599.

ROMANO, J. M., DUBOS, C., PROUSE, M. B., WILKINS, O., HONG, H., POOLE, M., KANG, K. Y., LI, E., DOUGLAS, C. J. & WESTERN, T. L. 2012. AtMYB61, an R2R3-MYB transcription factor, functions as a pleiotropic regulator via a small gene network. *New Phytologist,* 195**,** 774-786.

ROUSONELOS, S. L., LEE, R. M., MOREIRA, M. S., VANGESSEL, M. J. & TRANEL, P. J. 2012. Characterization of a common ragweed (Ambrosia artemisiifolia) population resistant to ALS-and PPO-inhibiting herbicides. *Weed Science,* 60**,** 335-344.

SAINT-LOUIS, S., DITOMMASO, A. & WATSON, A. K. 2005. A common ragweed (Ambrosia artemisiifolia) biotype in southwestern Québec resistant to linuron. *Weed Technology,* 19**,** 737-743.

SAMACH, A., ONOUCHI, H., GOLD, S. E., DITTA, G. S., SCHWARZ-SOMMER, Z., YANOFSKY, M. F. & COUPLAND, G. 2000. Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *science,* 288**,** 1613-1616.

SANDERFOOT, A. A., KOVALEVA, V., BASSHAM, D. C. & RAIKHEL, N. V. 2001. Interactions between syntaxins identify at least five SNARE complexes within the Golgi/prevacuolar system of the Arabidopsis cell. *Molecular Biology of the Cell,* 12**,** 3733-3743.

SATO, Y., WULI, B., SEDEROFF, R. & WHETTEN, R. 2001. Molecular cloning and expression of eight laccase cDNAs in loblolly pine (Pinus taeda). *Journal of Plant Research,* 114**,** 147-155.

SCALONE, R., LEMKE, A., ŠTEFANIĆ, E., KOLSETH, A.-K., RAŠIĆ, S. & ANDERSSON, L. 2016. Phenological variation in Ambrosia artemisiifolia L. facilitates near future establishment at northern latitudes. *PLoS One,* 11**,** e0166510.

SEARLE, I., HE, Y., TURCK, F., VINCENT, C., FORNARA, F., KRÖBER, S., AMASINO, R. A. & COUPLAND, G. 2006. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes & development,* 20**,** 898-912.

SHEORAN, I. S., SPROULE, K. A., OLSON, D. J., ROSS, A. R. & SAWHNEY, V. K. 2006. Proteome profile and functional classification of proteins in Arabidopsis thaliana (Landsberg erecta) mature pollen. *Sexual plant reproduction,* 19**,** 185-196.

SIEGFRIED, K. R., ESHED, Y., BAUM, S. F., OTSUGA, D., DREWS, G. N. & BOWMAN, J. L. 1999. Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. *Development,* 126**,** 4117-4128.

SIMPSON, G. G. & DEAN, C. 2002. Arabidopsis, the Rosetta stone of flowering time? *science,* 296**,** 285-289.

SKÁLOVÁ, H., GUO, W., WILD, J. & PYŠEK, P. 2017. Ambrosia artemisiifolia in the Czech Republic: history of invasion, current distribution and prediction of future spread. *Preslia,* 89**,** 1-16.

SMITH, K. S. & FERRY, J. G. 2000. Prokaryotic carbonic anhydrases. *FEMS microbiology reviews,* 24**,** 335-366.

SONG, Y. H., ITO, S. & IMAIZUMI, T. 2010. Similarities in the circadian clock and photoperiodism in plants. *Current opinion in plant biology,* 13**,** 594-603.

SONG, Y. H., ITO, S. & IMAIZUMI, T. 2013. Flowering time regulation: photoperiod-and temperature-sensing in leaves. *Trends in plant science,* 18**,** 575-583.

SOÓ, R. 1970. Taxonomical and Plant Geographical Handbook of Hungarian Flora and Vegetation. IV.(A magyar flóra és vegetáció rendszertani növényföldrajzi kézikönyve. IV.) Budapest. Akadémiai Kiadó.

STORKEY, J., STRATONOVITCH, P., CHAPMAN, D. S., VIDOTTO, F. & SEMENOV, M. A. 2014. A process-based approach to predicting the effect of climate change on the distribution of an invasive allergenic plant in Europe. *PLoS One,* 9**,** e88156.

SUDRE, D., GUTIERREZ-CARBONELL, E., LATTANZIO, G., RELLÁN-ÁLVAREZ, R., GAYMARD, F., WOHLGEMUTH, G., FIEHN, O., ÁLVAREZ-FERNÁNDEZ, A., ZAMARREÑO, A. M. & BACAICOA, E. 2013. Iron-dependent modifications of the flower transcriptome, proteome, metabolome, and hormonal content in an Arabidopsis ferritin mutant. *Journal of experimental botany,* 64**,** 2665-2688.

SUNG, S. & AMASINO, R. M. 2004. Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature,* 427**,** 159-164.

TAIZ, L., ZEIGER, E., MØLLER, I. & MURPHY, A. 2015. Plant physiology and development. Sinauer Associates. *Inc., Sunderland.*

TARAMARCAZ, P., LAMBELET, C., CLOT, B., KEIMER, C., HAUSER, C. (2005) Ragweed (Ambrosia) progression and its health risks: will Switzerland resist this invasion? Swiss Medical Weekly, 135, 538–548.

TASHIAN, R. E. 1989. The carbonic anhydrases: widening perspectives on their evolution, expression and function. *Bioessays,* 10**,** 186-192.

TAYLOR, J. B., LOUX, M. M., HARRISON, S. K. & REGNIER, E. 2002. Response of ALS-resistant common ragweed (Ambrosia artemisiifolia) and giant ragweed (Ambrosia trifida) to ALS-inhibiting and alternative herbicides. *Weed Technology***,** 815-825

TÍMÁR, L. 1955. Egy veszedelmes gyomkártevő előőrsei Szegeden. *Délmagyarország, Szeged,* 18.

TRANEL, P. J., JIANG, W., PATZOLDT, W. L. & WRIGHT, T. R. 2004. Intraspecific variability of the acetolactate synthase gene. *Weed Science***,** 236-241.

UENO, S., LE PROVOST, G., LÉGER, V., KLOPP, C., NOIROT, C., FRIGERIO, J.-M., SALIN, F., SALSE, J., ABROUK, M. & MURAT, F. 2010. Bioinformatic analysis of ESTs collected by Sanger and pyrosequencing methods for a keystone forest tree species: oak. *BMC genomics,* 11**,** 650.

UNTERGRASSER, A., CUTCUTACHE, I., KORESSAAR, T., YE, J., FAIRCLOTH, B., REMM, M. & ROZEN, S. 2012. Primer3–new capabilities and interfaces. *Nucleic Acids Research*, 40(15):e115

VAN DER KROL, A. R., BRUNELLE, A., TSUCHIMOTO, S. & CHUA, N.-H. 1993. Functional analysis of petunia floral homeotic MADS box gene pMADS1. *Genes & development,* 7**,** 1214-1228.

VARGA, P., BÉRES, I. & REISINGER, P. 2000. The effect of weeds on yield and leaf-area changes of maize in field trials. *Novenyvedelem,* 36**,** 625-631.

VILLANUEVA, J. M., BROADHVEST, J., HAUSER, B. A., MEISTER, R. J., SCHNEITZ, K. & GASSER, C. S. 1999. INNER NO OUTER regulates abaxial–adaxial patterning in Arabidopsis ovules. *Genes & development,* 13**,** 3160-3169.

VIRÁG, E., HEGEDŰS, G., BARTA, E., NAGY, E., MÁTYÁS, K., KOLICS, B. & TALLER, J. 2016. Illumina sequencing of common (short) ragweed (Ambrosia artemisiifolia L.) reproductive organs and leaves. *Frontiers in plant science,* 7**,** 1506.

WANG, H., SCHAUER, N., USADEL, B., FRASSE, P., ZOUINE, M., HERNOULD, M., LATCHÉ, A., PECH, J.-C., FERNIE, A. R. & BOUZAYEN, M. 2009a. Regulatory features underlying pollination-dependent and-independent tomato fruit set revealed by transcript and primary metabolite profiling. *The plant cell,* 21**,** 1428-1452.

WANG, Z., GERSTEIN, M. & SNYDER, M. 2009b. RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics,* 10**,** 57-63.

WEIGEL, D., NILSSON, O., 1995 A developmental switch sufficient for flower initiation in diverse plants. Nature 377:495

WIGGE, P. A., KIM, M. C., JAEGER, K. E., BUSCH, W., SCHMID, M., LOHMANN, J. U. & WEIGEL, D. 2005. Integration of spatial and temporal information during floral induction in Arabidopsis. *science,* 309**,** 1056-1059.

XU, J., YANG, C., YUAN, Z., ZHANG, D., GONDWE, M. Y., DING, Z., LIANG, W., ZHANG, D. & WILSON, Z. A. 2010. The ABORTED MICROSPORES regulatory network is required for postmeiotic male reproductive development in Arabidopsis thaliana. *The plant cell,* 22**,** 91-107.

YANG, C., XU, Z., SONG, J., CONNER, K., BARRENA, G. V. & WILSON, Z. A. 2007. Arabidopsis MYB26/MALE STERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. *The plant cell,* 19**,** 534-548.

YANG, X., PANG, H.-B., LIU, B.-L., QIU, Z.-J., GAO, Q., WEI, L., DONG, Y. & WANG, Y.-Z. 2012. Evolution of double positive autoregulatory feedback loops in CYCLOIDEA2 clade genes is associated with the origin of floral zygomorphy. *The plant cell,* 24**,** 1834-1847.

YU, J.-W., RUBIO, V., LEE, N.-Y., BAI, S., LEE, S.-Y., KIM, S.-S., LIU, L., ZHANG, Y., IRIGOYEN, M. L. & SULLIVAN, J. A. 2008. COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. *Molecular cell,* 32**,** 617-630.

ZHA, H.-G., LIU, T., ZHOU, J.-J. & SUN, H. 2013. MS-desi, a desiccation-related protein in the floral nectar of the evergreen velvet bean (Mucuna sempervirens Hemsl): molecular identification and characterization. *Planta,* 238**,** 77-89.

ZHANG, J., LIU, B., LI, M., FENG, D., JIN, H., WANG, P., LIU, J., XIONG, F., WANG, J. & WANG, H.-B. 2015. The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis. *The plant cell,* 27**,** 787-805.

ZHENG, D., PATZOLDT, W. L. & TRANEL, P. J. 2005. Association of the W574L ALS substitution with resistance to cloransulam and imazamox in common ragweed (Ambrosia artemisiifolia). *Weed Science*, 424-430.

ZHU, J.-Y., SAE-SEAW, J. & WANG, Z.-Y. 2013. Brassinosteroid signalling. *Development,* 140, 1615-1620.

ZHU, J., LOU, Y., XU, X. & YANG, Z. N. 2011. A genetic pathway for tapetum development and function in Arabidopsis. *Journal of Integrative Plant Biology,* 53, 892-900.

ZISKA, L., KNOWLTON, K., ROGERS, C., DALAN, D., TIERNEY, N., ELDER, M. A., FILLEY, W., SHROPSHIRE, J., FORD, L. B. & HEDBERG, C. 2011. Recent warming by latitude associated with increased length of ragweed pollen season in central North America. *Proceedings of the National Academy of Sciences,* 108, 4248-4251.

ZISKA, L. H., GEBHARD, D. E., FRENZ, D. A., FAULKNER, S., SINGER, B. D. & STRAKA, J. G. 2003. Cities as harbingers of climate change: common ragweed, urbanization, and public health. *Journal of Allergy and Clinical Immunology,* 111, 290-295.

Publication List

Publications related to the PhD thesis

Refereed International Journals

Mátyás KK, Hegedűs G, Taller J, Farkas E, Decsi K Kutasy B, Kálmán N, Nagy E, Kolics B, Virág E *Different expression pattern of flowering pathway genes contribute to male or female organ development during floral transition in the monoecious weed Ambrosia artemisiifolia L. (Asteraceae).* PEERJ 7 (2019) doi.org/10.7717/peerj.7421. **IF.2.38**

Virág E, Hegedűs G, Barta E, Nagy E, **Mátyás K**, Kolics B, Taller J *Illumina sequencing of common (short) ragweed (Ambrosia artemisiifolia L.) reproductive organs and leaves*. FRONTIERS IN PLANT SCIENCES data report (2016) doi:10.3389/fpls.2016.01506. **IF: 4.298**

National Publications (In Hungarian)

Mátyás KK, Bódis J, Virág E, Taller J, Pintér Cs *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) virágzatának részletes leírása sztereomikroszkópos rétegfotózás használatával.* BOTANIKAI KÖZLEMÉNYEK 107: 1 pp. 103-109., 7 p. (2020) **IF: 0.52**

International Conferences

Mátyás KK, Taller J, Hegedűs G, Kolics B, Farkas E, Nagy E, Parrag T, Farkas Z, Virág E *Identification of floral organ identity genes in the common ragweed (Ambrosia artemisiifolia L).* Fiatal Biotechnológusok III. Országos Konferenciája (2018), Budapest, Magyarország, 2018. március 28-29., 31p.

Virág E, Nagy E, **Mátyás K**, Kolics B, Kutasy B, Decsi K, Taller J . *Analysis of molecular background of flowering in the common ragweed (Ambrosia artemisiifolia L.).* In: Pannonian Plant Biotechnology Workshop "Integration fundamental research into the practical agriculture".(2015) Ljubljana, Szlovénia, pp. 33-34.

National Conferences (In Hungarian)

Mátyás KK, Pintér Cs, Bódis J, Virág E, Taller J *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) hím és nő virágzatának morfológiai jellemzése rétegfotózással.* MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA (2019) 20. évf. 1. sz, 84 p.

Virág E, Hegedűs G, **Mátyás K**, Nagy E, Taller J *Az ürömlevelű parlagfű váltivarú virágzatának kialakulásért felelős gének NGS alapú vizsgálata*. „GENETIKAI MŰHELYEK MAGYARORSZÁGON" XVI. Minikonferencia (2017), Szeged, Magyarország, 2017. szeptember 8., Elektronikus kiadvány, 1 p.

Conference articles (In Hungarian)

Mátyás KK, Taller J, Hegedűs G, Farkas E, Nagy E, Parrag T, Farkas Z, Virág E *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) hím és nő virágzatában kifejeződő gének összehasonlító elemzése.* XXIV. Ifjúsági Tudományos Fórum (2018), 2018. május 24, Keszthely, Elektronikus kiadvány, 10 p.

Virág E**, Mátyás KK**, Taller J, Farkas Z, Parrag T, Hegedűs G *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) váltivarú virágzatáért felelős genetikai háttér felderítése bioinformatikai elemzésekkel.* XXIV. Ifjúsági Tudományos Fórum (2018), 2018. május 24, Keszthely, Elektronikus kiadvány, 12 p.

Publications not related to the PhD thesis

Refereed International Journals

Kolics É, Specziár A, Taller J, Mátyás KK, Kolics B *Lithium chloride outperformed oxalic acid sublimation in a preliminary experiment for Varroa mite control in pre-wintering honey bee colonies*, ACTA VETERINARIA HUNGARICA 68 (4). 370-373. (2021) **IF: 1,019** (2019)

Kolics É, Parrag T, Házi F, Szepesi K, Heltai B, **Mátyás K** Kutasy B; Virág E; Taller J; Orbán L, Kolics B *An Alternative, High Throughput Method to Identify Csd Alleles of the Honey Bee*. INSECTS 11: 8 Paper: 483 (2020) **IF: 2,220** (2019)

Kolics É, **Mátyás K**, Taller J, Specziár A, Kolics B *Contact Effect Contribution to the High Efficiency of Lithium Chloride Against the Mite Parasite of the Honey Bee* INSECTS 11: 6 Paper: 333 (2020) **IF: 2,220** (2019)

Farkas E, Farkas Z, Hegedűs G, Kutasy B, Kolics B, **Mátyás KK**, Parrag T, Solti I, Virág E, Taller J *Identification and expression of pollen allergen transcripts in different organs of the common ragweed (Ambrosia artemisiifolia L.)*. GEORGOKIN FOR AGRICULTURE 23 (2) (2019) 116-130.

Taller J, Decsi K, Farkas E, Nagy E**, Mátyás KK,** Kolics B, Kutasy B, Virág E *De novo Transcriptome Sequencing Based Identification of Amb a 3-like Pollen Allergen in Common Ragweed (Ambrosia artemisiifolia L.).* JOURNAL OF BOTANICAL SCIENCES (2015) 5: 2 pp. 12-16.

Taksonyi P, Kocsis L, **Mátyás K**, Taller J *Investigation into effective control of powdery mildew with strobilurins in viticulture – a case study.* MITTELUNGEN KLOSTERNEUBURG (2013) 63: 1 pp. 30-37. **IF: 0,031**

Taksonyi P, Kocsis L, **Mátyás KK**, Taller J *The effect of quinone outside inhibitors fungicides on powdery mildew in a grape vineyard in Hungary.* SCIENTIA HORTICULTURAE (2013) 161 pp. 233-238.) **IF:1,504**

Gorji MA, **Mátyás KK,** Dublecz Zs, Decsi K, Cernák I, Hoffmann B, Taller J, Polgár Zs *Investigation of in vitro osmotic stress tolerance in tetraploid potato and identification of major QTLs.* AMERICAN JOURNAL OF POTATO RESEARCH 89 (2012): 6 pp. 453-464. **IF:1,250**

Mátyás KK, Taller J, Cseh A, Poczai P, Cernák I *Development of a simple PCR-based assay for the identification of triazine resistance in the noxious plant common ragweed (Ambrosia artemisiifolia) and its applicability in higher plants.* BIOTECHNOLOGY LETTERS 33 (2011): 12 pp. 2509-2515. **IF:1,683**

Poczai P, **Mátyás KK**, Szabó I, Varga I, Hyvönen J, Cernák I, Mosapour Gorji A, Decsi K, Taller J *Genetic Variability of Thermal Nymphaea (Nymphaeaceae) Populations Based on ISSR Markers: Implications on Relationships, Hybridization, and Conservation.* PLANT MOLECULAR REPORTER 29 (2011): 4 pp. 906-918.) **IF: 2,453**

Poczai P, **Mátyás K**, Taller J, Szabó I *Study of the origin of the rarely cultivated edible Solanum species: morphological and molecular data.* BIOLOGIA PLANTARUM 54 (2010) : 3, pp. 543-546. **IF: 1,582**

National Publications (In Hungarian)

Taller J, Nagy E, Decsi K, Kutasy B, **Mátyás KK**, Farkas E, Kolics B, Barta E, Virág E *A transzkriptomika hasznosítása a gyomkutatásban. Esettanulmány a legelterjedtebb gyomnövényünkkel, az ürömlevelű parlagfűvel (Ambrosia artemisiifolia* L.*).* MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 17 (2016) 5-15.

Mátyás KK, Vignesh M, Taller J *Előzetes eredmények az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) Kárpát-medencei populációgenetikai vizsgálatáról.* MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 13 (2012) 21-36.

Conference articles (In English)

Mátyás KK, Kolics B, Csép A, Nagy E, Taller J *Isolation and preliminary analysis of the variability of the ALS (Acetolactate synthase) gene from common ragweed (Ambrosia artemisiifolia L.).* In: Bene Sz (szerk.) 20th Youth Scientific Forum (2014): University of Pannonia Georgikon Faculty Keszthely, Magyarország, Elektronikus kiadvány *pp. 386-396.*

International Conferences

Kolics B, Sajtos Zs, **Mátyás K**, Kolics É, Taller J, Baranyai E. *"Lithium Chloride - Hazard or Possibility?"* In 46th Apimondia International Apicultural Congress Montréal Québec - Canada, 8-12 September 2019, Elektronikus kiadvány p. 285.

Kolics B, **Mátyás K**, Kutasy B, Parrag T, Házi F, Kolics É, Orbán L, Taller J *Novel method to identify the sex alleles of the honey bee,* In 46th Apimondia International Apicultural Congress Montréal Québec Canada, 8-12 September 2019 Elektronikus kiadvány p58.

Mátyás KK, Poczai P, Cernák I, Benoit DL, Taller J *Identification of linuron resistance in the common ragweed (Ambrosia artemisiifolia L.)* The 10th International Ph.D. Student Conference on Experimental Plant Biology (2012) Brno, Czech Republic, September 3-5 2012 Bulletin-Book of Abstracts 117 p.

Mátyás KK, Murthy V, Gorji AM *Population Genetic analysis of Common ragweed (Ambrosia artemisiifolia L.) in Europe using DNA – Based Molecular Markers* In: Bohren C, Bertossa M, Schoenenberger N, Rossinelli M, Conedera M (szerk.) 3rd International Symposium Environmental Weeds and Invasive Plants (2011): European Weed Research Society (EWRS) p127.

National Conferences (In Hungarian)

Solti I, Farkas E, **Mátyás K**, Székvári K, Kolics B, Kutasy B, Nagy E, Kálmán N, Hegedűs G, Taller J, Virág E *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) főbb allergénjeinek génkifejeződés vizsgálata*. MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 20 (2019): 1 p. 87.

Nagy E, Virág E, **Mátyás K**, Kutasy B, Solti I, Kolics B, Taller J *Mikroszatellitek az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) kloroplasztisz genomjában* MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 20 (2019:) 1 p. 85.

Nagy E, **Mátyás K**, Kolics B, Virág E, Kutasy B, Taller J *Molekuláris genetikai vizsgálatok a virágzás szabályozottságának feltárására az ürömlevelű parlagfűben (Ambrosia artemisiifolia L.)* MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 16 (2015): 1 p. 67

Varga I, Nagy V, Baltazár T, **Mátyás KK** *A fehér fagyöngy (Viscum album L.) elleni herbicides védekezés hatékonyságának vizsgálata,*In: XXII. Keszthelyi Növényvédelmi Fórum (2012) Keszthely, 2012.január 25-27., Magyarország: Pannon Egyetem Georgikon Kar p. 124

Taller J, Cernák I, **Mátyás KK**, Poczai P, Cseh A *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) atrazin és ALS-gátló hatóanyagú herbicidekkel szembeni rezisztenciájának molekuláris genetikai vizsgálata.* In: Lehoczky É, Budai P, Kadlicskó S, Marczali Zs, Nádasy M, Nádasyné Ihárosi E, Takács A (szerk.) XX. Keszthelyi Növényvédelmi Fórum (2010): 20 éves a Keszthelyi Növényvédelmi Fórum 1990-2010, Keszthely, 2010. január 27-29, Magyarország p. 151

Conference articles (In Hungarian)

Solti I, Farkas E, **Mátyás K**, Székvári K, Kolics B, Kutasy B, Nagy E, Kálmán N, Hegedűs G, Taller J, Virág E *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) allergénjeinek génkifejeződés vizsgálata.* In: Dr. Bene Szabolcs (szerk.) XXV. Ifjúsági Tudományos Fórum (2019) Pannon Egyetem Georgikon Kar, Keszthely, 2019. május 23. CD kiadvány ISBN száma: 978-963-9639-98-0.

Kutasy B, **Mátyás K**, Virág E, Decsi K, Nagy E, Farkas E, Hegedűs G, Kolics B, Taller J *Herbicid célgének in silico azonosítása az ürömlevelű parlagfűben (Ambrosia artemisiifolia L.).* In: Bene, Szabolcs (szerk.) XXIII. Ifjúsági Tudományos Fórum (2017), Pannon Egyetem Georgikon Kar, Keszthely, 2017.május26., CD kiadvány, ISBN 978-963-9639-87-4.

Kutasy B, Virág E, Decsi K, Nagy E, Farkas E, **Mátyás K**, Kolics B, Taller J *Herbicid célgének vizsgálata az ürömlevelű parlagfűben (Ambrosia artemisiifolia L.).* In: Nagy Zita Barbara (szerk.) LVIII. Georgikon Napok: Felmelegedés, ökolábnyom, élelmiszerbiztonság. 462 p., Keszthely, Magyarország, 2016.09.29-2016.09.30. pp. 486-496. ISBN:978-963-9639-85-0

Farkas E, Decsi K, Nagy E, **Mátyás K**, Kolics B, Kutasy B, Virág E, Taller J *Pollen allergének transzkriptomikai vizsgálata az ürömlevelű parlagfűben (Ambrosia artemisiifolia L.)* In: Nagy Zita Barbara (szerk.) LVIII. Georgikon Napok: Felmelegedés (2016), ökolábnyom, élelmiszerbiztonság. 462 p., Keszthely, Magyarország, 2016.09.29-2016.09.30., pp. 92-99. ISBN:978-963-9639-85-0

Nagy E, Virág E, **Mátyás K**, Kolics B, Kutasy B, Taller J *Az ürömlevelű parlagfű (Ambrosia artemisiifolia L.) kloroplasztisz genomjának vizsgálata* In: Nagy Zita Barbara (szerk.) LVIII. Georgikon Napok (2016): Felmelegedés, ökolábnyom, élelmiszerbiztonság. 462 p., Keszthely, Magyarország, 2016.09.29-2016.09.30., pp. 479-485. ISBN:978-963-9639-85-0

Nagy E, **Mátyás K**, Kolics B, Virág E, Kutasy B, Taller J *Molekuláris genetikai vizsgálatok a virágzás szabályozottságának feltárására az ürömlevelű parlagfűben (Ambrosia artemisiifolia L.)* In: Pannon Egyetem Georgikon Kar Állattudományi és Állattenyésztéstani Tanszék (szerk.) XXI. Ifjúsági Tudományos Fórum (2015). Konferencia helye, ideje: Keszthely, Magyarország, 2015.05., 6 p. ISBN:978-963-9639-78-2

Tóth G, **Mátyás KK**, Buda B *A szombathelyi Isis-szentélyben feltárt emberi csontmaradványok vizsgálatának előzetes eredményei* In: Újlaki Pongrácz, Zsuzsánna (szerk.) Hadak útján: Népességek és iparok a népvándorlás korában: A népvándorláskor fiatal kutatóinak XVI. Konferenciáján (Nagykovácsi, 2005. szeptember 26-28.), Nagykovácsi, Magyarország: Pars Kft., (2006) pp. 33-36., 4 p

Appendix

Appendix 1. Average weight of samples.

Sample	Average weight (mg)
Male flower stage 1 (Figure 9 M A)	0,3
Male flower stage 2 (Figure 9 M B)	2,1
Male flower stage 3 (Figure 9 M C)	8
Male flower stage 4 (Figure 9 M D1)	0,9
Male flower stage 5 (Figure 9 M D2)	1,8
Male flower stage 6 (Figure 9 M D3)	3
Male flower stage 7 (Figure 9 M D4)	8,6
Female flower stage 1 (Figure 9 F a)	0,1
Female flower stage 2 (Figure 9 F b)	0,2
Female flower stage 3 (Figure 9 F c)	0,4
Female flower stage 4 (Figure 9 F d)	0,5
Female flower stage 5 (Figure 9 F e)	0,7
Female flower stage 6 (Figure 9 F f)	0,9
Female flower stage 7 (Figure 9 F g)	1
Female flower stage 8 (Figure 9 F h)	1,2
Female flower stage 9 (Figure 9 F i)	1,8

Appendix 2. Primers used for q-RT PCR validation.

Genes	Forward primer sequences	Reverse primer sequences
LAP6	CGAGGTTACGAAGAAGAATGC	ATGGATTTTGGGAATGTGAGTAG
STIG1	CATCCCATAGGCACAAGACTC	AAGGGAAGTGTTTTGATGTGTC
SUPERMAN	CAAATGGGAAAGCAACAACTAC	AAGAGAGGGTGGTGAAGACTG
TCP12	AGCTTTTGGAATTCACTTGAGTC	GAATAGTCACCAACCCAATGC
PI	AGAACACAAACAACAGGCAAG	GTGTTAGGGCTGCAATACTCA

Validation of F, M and L transcriptomes were performed by using Q-RT-PCR. Genes expressed characteristically in different transcriptomes were selected based on *in silico* analysis. Primers used for qPCR were designed based on *in silico* predicted sequences.

Appendix 3. RPKM values of reference genes in each library.

	1M	2M	1F	2F	L	N	H
Ref Gene	RPKM	RPKM	RPKM	RPKM	RPKM	RPKM	RPKM
GAPDH	4795,676	4914,222	3321,383	2480,102	3611,886	2676,673	3565,147
TUA	539,292	584,526	756,759	884,490	724,105	41,835	183,369
TUB	805,383	721,719	811,335	348,121	19,985	160,036	263,883

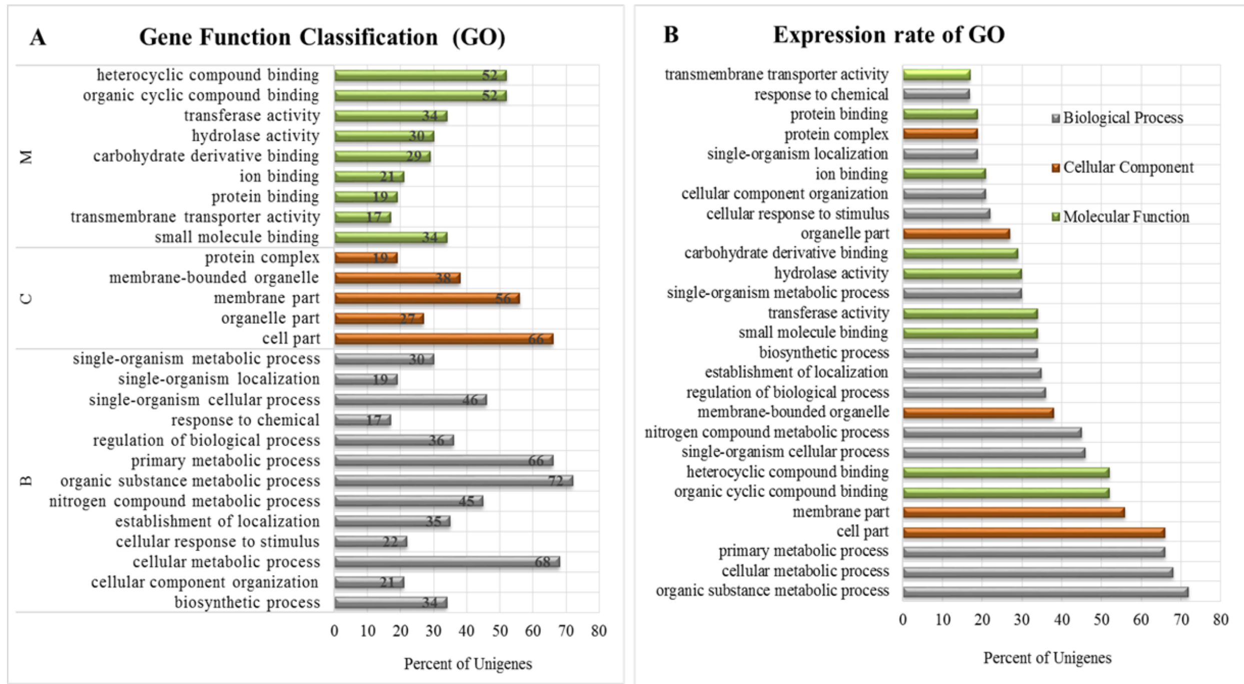
The color scale means green low, white intermediate, and red are high expression values.

Appendix 4. Sequencing and assembly statistics of *A. artemisiifolia* flower libraries.

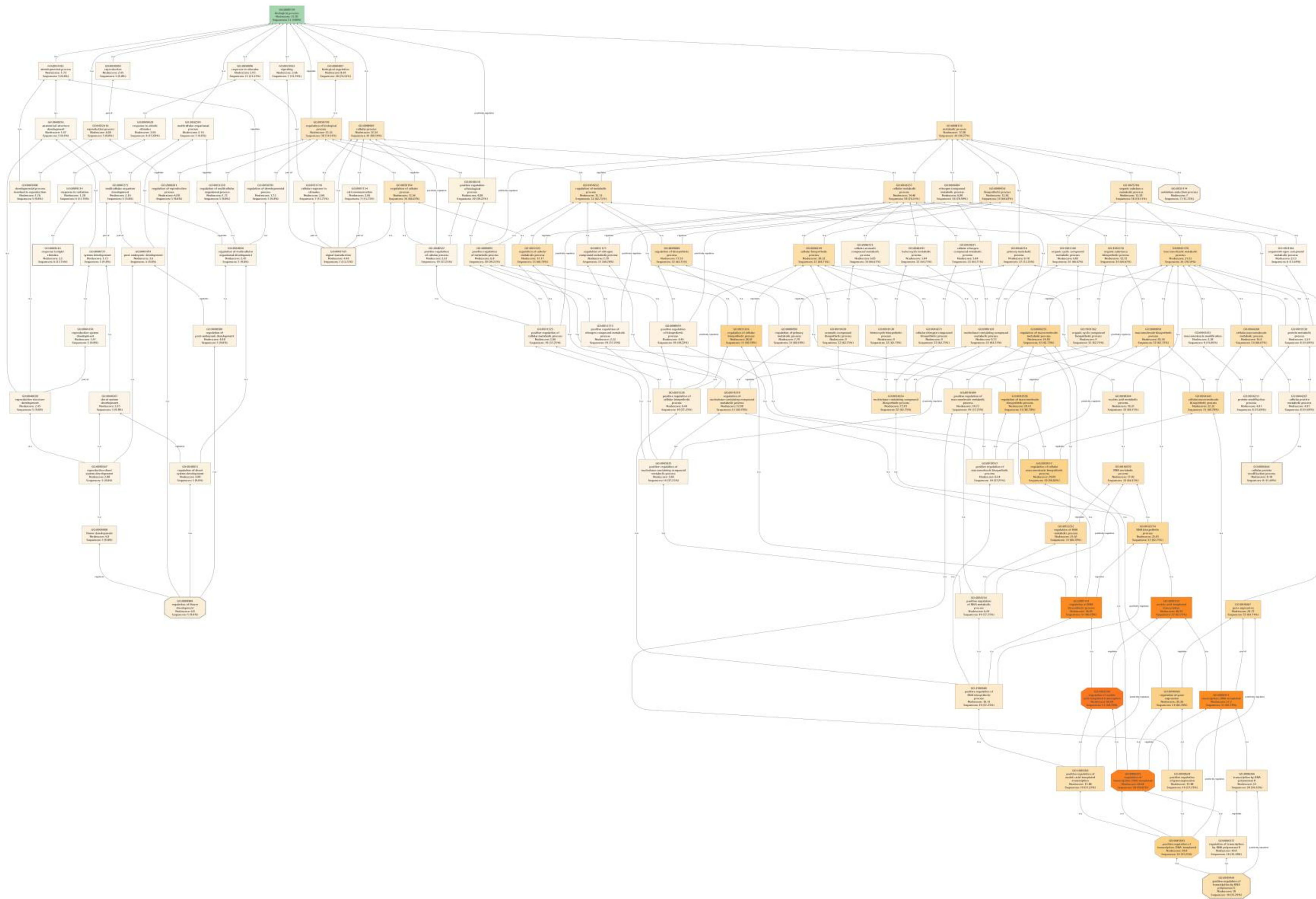
	Sample	**M**	**L**	**F**	**1F**		**2F**	
Sequencing	Illumina platform	HiSeq2000	HiSeq2000	HiSeq2000	NextSeq500		NextSeq500	
	Number of raw reads	24,110,256	24,330,693	23,264,636	39,664,366		37,127,852	
	Number of clean reads	18,472,374	17,435,976	15,290,201	36,491,216		34,157,623	
	Average read length (bases)	2*100	2*100	2*100	2*80		2*80	
Accession number	NCBI SRA	SRR3995704	SRR3995705	SRR3995703	SRR5965731		SRR5965732	
	NCBI TSA	GEZL00000000			GFWB00000000		GFWS00000000	
Assembly	**strategy**	**Combined reads for de novo assembly (used as reference)**			**de novo**	**ref. guided**	**de novo**	**ref. guided**
	assembler	Trinity			Trinity	Bowtie2	Trinity	Bowtie2
	total assembled reads	1,377,646			20,421,456	33,108,438	18,032,538	30,611,092
	Number of contigs	229,116			109,452	147,457	97,239	141,588
	Range of contig length	224 -14,368			201 -8,854	210 - 14,375	201 -6,882	2015 - 14,370
	N50 length	774			669.7	697.5	635	626.9

Libraries were generated from gender specific RNA pools. Nominations: male flowers for eight developmental stages (M), young leaves (L), and female flowers for eight developmental phases (F), classified into early (1F) and late (2F) stages. In order to represent both vegetative and generative transcriptomes a combined library from M, L and F samples was generated. In order to analyse expression in detail pistillate organogenesis the nine female flower phenophases were classified into two libraries and aligned to the *A. artemisiifolia* reference transcriptome.

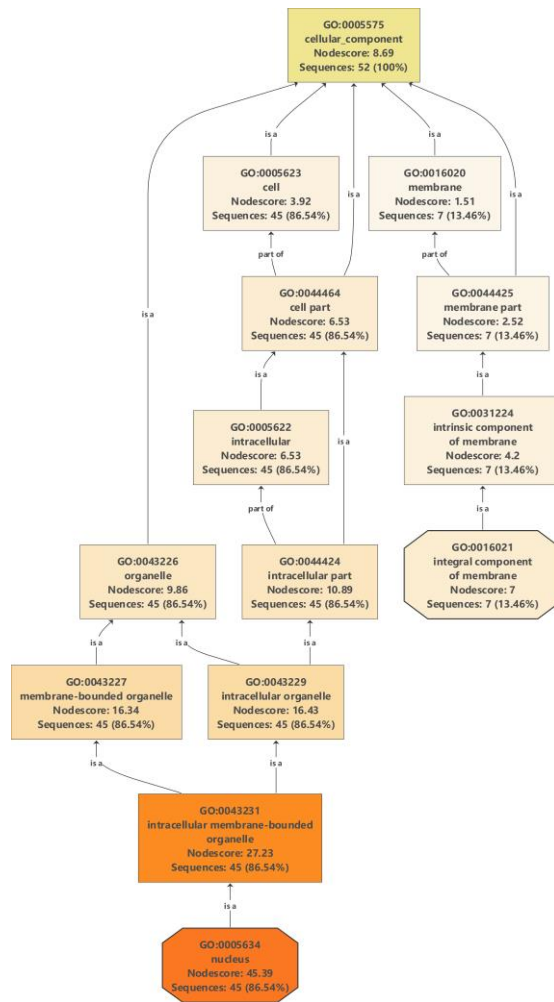
Appendix 5.



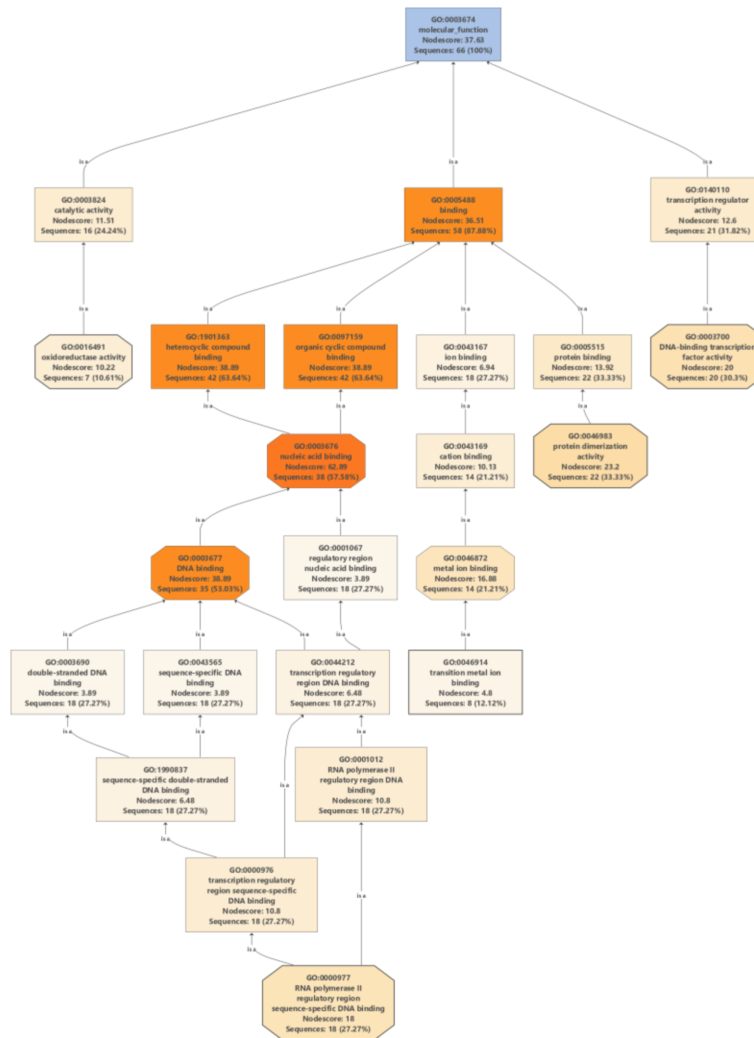
Functional classification of *A. artemisiifolia* unigenes in reproductive organs. **A.** Expression pattern of transcript products within the category of (C) cellular component, (M) molecular function, and (B) biological process sub-ontologies. **B.** Sub ontologies were arranged according to Expression rate. The most intense gene expressions were found to be in metabolic processes, catalytic and binding activity ontology.



Appendix 6. GO graph of biological process. GO graph performed based on GO table of 80 discussed genes.



Appendix 7. GO graph of cellular component. GO graph performed based on GO table of 80 discussed genes.



Appendix 8. GO graph of molecular function. GO graph performed based on GO table of 80 discussed genes.

Acknowledgements

First of all, I would like to thank my supervisor, Eszter Virág PhD, for her great support and help during the experiments and the careful attention provided by publishing activities playing a decisive role in my professional development.

I'm also pleased to János Taller, PhD for the initiation of my PhD studies, and providing the labor environment in the Biotechnology Lab involving the technical and financial background for all the molecular experiments.

Furthermore, I would like to thank Balázs Kolics PhD who has been for my great assistance for both the laboratory experiments and in the field in technical details and helped in English editing of preparation of manuscripts.

I would like to thank my colleagues, Erzsébet Nagy PhD, Eszter Farkas Péterné, Izabella Solti, Barbara Kutasy and Éva Kolics for providing a helpful work atmosphere in the lab. Also, I would like to thank Kincső Decsi PhD, István Cernák PhD, and Péter Poczai PhD for their help during my first years in the lab years.

I also thank Géza Hegedűs, Tamás Parrag and Zoltán Farkas for IT help.

I would like to thank Nikoletta Kálmán PhD for her help in the RT-qPCR experiments.

I express our thanks to our retired colleague Csaba Pintér PhD for the pictures in Figure 2-3-4-5-9 and for enabling a good insight into the stereomicroscopic focus stacking technique.

I would like to thank my colleague Judit Bódis, PhD for field trips concerning flower morphology.

I also thank Márton Juha for the graphical representation in Figure 16.

I would like to thank all the current and former staff of the Department of Plant Science and Biotechnology for their administrative help.

Last but not least, I would like to express my special thanks to my family for their patience and their emotional support.

This work/research was supported by the Hungarian Government and the European Union, with the co-funding of the European Regional Development Fund in the frame of the Széchenyi 2020 Programme **GINOP-2.3.2-15-2016-00054 project**.