ECOCYCLES
ISSN 2416-2140

OPEN ACCESS
Scientific journal of the
European Ecocycles Society

Ecocycles, Vol. 11, No. 1, pp. 46-58 (2025)
DOI: 10.19040/ecocycles.v11i1.497

RESEARCH ARTICLE

Mutational and evolutionary dynamics of *Brassicaceae* plant organs

Gábor Zs. Gyulai[1], Renée P. Malone[2], Gábor Gyulai[1,3], K.A. Tóth-Lencsés[3*]
[1]*PhD Schools of MATE University, Gödöllő, H-2100, Hungary*
[2]*Technological University of Dublin, Grangegorman, Dublin 7, Ireland*
[3]*Institute of Genetics and Biotechnology, MATE University, Gödöllő, H-2100, Hungary*

**Corresponding Author: toth-lencses.andrea.kitti@uni-mate.hu*

Abstract – The plant family of *Brassicaceae* (*Syn., Cruciferae* after the four petals in cruciform arrangement) comprises over 3,700 species of 338 genera including vegetables, crops, fodder crops and wild species. The most species-rich genera are the wild plants *Draba* (440 species), *Erysimum* (261 species), *Lepidium* (234 species), *Cardamine* (233 species), and *Alyssum* (207 species) (http://www.theplantlist.org/1.1/browse/A/; Simoncsics, 2017). The families of *Armoracia*, *Raphanus*, *Sinapis*, *Wasabia*, *Arabidopsis* (Rédei, G. [1921-2008], 1975; TAIR - www.arabidopsis.org) with over 50 species (http://www.theplantlist.org), and *Thlaspi jankae* (pennycress Janka, described in Hungary by Janka, V. [1837-1890]) also belongs to *Cruciferae*. Genus *Brassica* comprises 37 species and numerous subspecies (*ssp*.) and over 3.000 registered cultivars (*cv.*) growing globally. Here we analyze genomes and genes of *BRASSICACEAE* species based on *in silico* data mining (www.ncbi.nlm.nih.gov) to reveal further rationale for the extreme levels (Li *et al*., 2024) of plant organs mutations, molecular diversity, phenotypic plasticity, diversification, domestication, evolution, selection, speciation and breeding of *BRASSICACEAE* species.

Keywords – *BRASSICACEAE*, plant organs modification, genome evolution, sequence alignments, dendrogram/cladogram analysis

***JEL classification*:** C

Received: October 24, 2024 Accepted: December 3, 2024

BACKGROUND

The main *Brassica* species (Fig. 1) with genome symbols and basic chromosome numbers are *B. nigra* [2n(2x)=16, BB]; *B. oleracea* [2n(2x)=18, CC]; *B. campestris* (*Syn., B. rapa*) [2n(2x)=20, AA]; and the three natural (allo)tetraploid species of *B. carinata* [2n(4x)=34, BBCC]; *B. juncea* [2n(4x)=36, AABB]; and *B. napus* [2n(4x)=38, AACC] (Dixon, 2006; https://brassibase.cos.uni-heidelberg.de).

Cultivars of *Brassica* species (Fig. 1, Table 1) include bok choy (*B. rapa, var. chinensis*), brown mustard (*B. juncea*), broccoli (*B. oleracea var. italica*), Brussels sprouts (*B. o. var. gemmifera*), cabbage (*B. o. var. capitata*), cauliflower (*B. o. var. botrytis*), collard (*B. o. var. acephala*), kales (*B. o. var. sabauda*, leafy and ornamental kales), kohlrabi (*B. o. var. gongyloides*), napa-cabbage (*B. rapa var. pekinensis*), rapeseed (*B. napus var. napus*), rutabaga (*B. napus var. napo-X-brassica*), and turnip (*B. rapa var. rapa*) (El-Esawi *et al*., 2012; Gamba *et al*., 2021).

Brassica species live annual, biennial or perennial life cycles including two main groups of acephala ('leafy') and cephala ('headed') forms with numerous plant organs modifications (Fig. 1, Table 1).

Medicinal ingredients

The main metabolites of *BRASSICACEAE* vegetables are phenolic compounds of flavonoids (Rusznyák and Szent-Györgyi, 1936), anthocyanins, hydroxycinnamic acids, carotenoids, and terpene derivatives (*e.g.*, phytosterols).

The sulphur containing glucosinolates, sulforaphane (CH_3-SO-$(CH_2)_4$-N=C=S), and phenylthiocarbamide (PTC) are responsible for the sharp taste (Ramirez *et al*., 2020).

In radish (*Raphanus sativus*) the percentage of the main compounds showed the highest content in flavonoids (38.8%), glucosinolates (5.6%), and sulphur-containing compounds (1.8%) (Gamba *et al*., 2021).

A *phytoalexin* type compound (Kőmíves and Király, 2019) of ascorbigen (*i.e.*, indol-3-ylmethyl-ascorbate) in

fresh-cut cabbages shows important roles of *Brassica* vegetables not only in human health but also in plant protection (Kátay *et al.*, 2011; Yin *et al.*, 2024).

Figure 1. Illustrations of characteristic plants of BRASSICACEAE family. *(X) Brassica cretica* the probable closest ancestor of *B. oleracea* (Mabry *et al*., 2021) with the *Ancestral Crucifer Karyotype* (ACK, n = 8) (Shen *et al.*, 2023).
- FLOWER BUD MUTANTS: *(1)* broccoli (*B. o. italica*) and the purple variety; *(2)* broccolini (*B. o. italica* × *B. o. alboglabra*); *(3)* Chinese broccoli '*k(g)ai-lan*' (*B. o. alboglabra*); *(4)* cauliflowers (*B. o. botrytis*) with differently colored curds; *(5)* Romanesco-cauliflower (*B. o. botrytis 'Romanesco'*).
- LEAF BUD MUTANTS: cabbages *(B. oleracea)* [white- (*6a)*, red- (*6b)*, cone- *(6c, 6d)* and flat head cabbage *(6e)*]; (*7*) Chinese kale/cabbage *(B. rapa Pekinensis)*; *(8)* pak-choy (*B. rapa 'pak-choy'*); *(9)* komatshuna *(B. r. Pekinensis var. 'komatshuna')*; *(10)* Savoy-headed kale (*Brassica o. sabauda*) with the wild ancestor leafy kale and ornamental kale (*B. o. acephala*); *(11)* Brussels sprouts *(B. o. gemmifera).*
- ROOT MUTANTS: *(12)* rutabaga (*B. napus napobrassica*); *(13)* turnip (*B. rapa rapa*); (*14.a, b, c*); radish cultivars (*Raphanus sativus*) with different sizes and colors; and *(15)* kohlrabi (*B. o. gongyloides*)

Mutation, Domestication and Breeding

Brassicaceae mutations include haploids (Fig. 2), plant genome duplications and triplications, coupled with events of paleo-polyploidy occurred 160 million years ago, MYA, (Zhang, L. *et al.*, 2020) resulted in series of plant morphological diversity. In addition, molecular evolution was found to driven continuously by natural DNA substitutions (*i.e.*, mutations) of the genomes.

The rate (Kimura, 1968) of mutation [Hugo de Vries (1848-1935), 1901-1903] of DNA was determined in a range from 5.9 - 6.5 to 7.1 × 10^{-9} DNA bp changes per bp site / per generation / per year in *A. thaliana* (Ossowski *et al*., 2010).

The majority of mutations of DNA were determined to be mainly at G≡C to A=T transitions (Salser, 1978; Ossowski *et al.*, 2010), which weakens DNA from the three hydrogen-bridge bonded rigid G≡C DNA to the 'softer' two hydrogen bonded A=T DNA. The DNA mutations (*i.e.*, '*G≡C suppression*'; Cooper and Gerber-Huber, 1985) take place during ontogeny and phylogeny due to the spontaneous *deamination* of the methylated/silenced ^{5m}C (5-methylcytosine) DNA site, which spontaneously turns to thymidine (T) during the subsequent DNA replication. Finally, it results in DNA base pair (bp) changes through cascade reactions of G≡C → G≡^{5m}C → A=T (Cooper and Gerber-Huber, 1985).

Theoretically, this molecular change of DNA allows quicker transcription reaction from DNA to mRNA and resulted in translated proteins, which therefore, facilitates the ability of plants to respond to the environmental changes/stresses (Yim *et al.*, 2022).

Mutations in the vegetative tissues of meristems of *Brassica* plants have resulted in wide ranges of organs modifications (Fig. 1, Table 1).

Modifications of plant organs of *BRASSICACEAE* species are not unique. Extreme levels of fruit diversity of apples, cherries, pears (*ROSACEAE*); cucurbits (*CUCURBITACEAE*); *Capsicum* fruits and potato tubers (*SOLANACEAE*); leafy head varieties of lettuce (*Lactuca sativa, e.g., 'butterhead lettuce'* and *'iceberg lettuce'*); and '*Radicchio*' of chicory (*Cichorium intybus; ASTERACEAE/COMPOSITAE*) are also significant (LettuceGDB; https://www.lettucegdb.com; Barcaccia *et al.*, 2016).

Table 1. The main plant organs modifications of *BRASSICACEAE* species

Plant Organs modified	Roots / Corm / Tuber Rhizome / Taproot / Tuber	Hypocotyl-tuber / enlarged hypocotyl	Epicotyl-tuber /Stem -tuber	Leaf buds	Leaves (*some samples*)	Shoot apical meristem	Flowers / Inflorescence	Seeds
Brassica oleracea			Kohlrabi	Brussels sprouts	Kale	Cabbage	Cauliflower	
					Collard green	Kale	Romanesco	
							Broccoli	
Brassica rapa		Turnip			Chinese cabbage			
					Pak-choy			
					Komatshuna			
					Asian greens			
					Mizuma			
B. rapa oleifera								Oilseed
B. napus								Rapeseed
B. napus napobrassica	Rutabaga							
B. juncea napiformis	Root mustard							
Raphanus sativus		Radish						Oilseed radish
			Daikon					
Armoracia rusticana	Horseradish							
Wasabia japonica	Wasabe							

Genome constitutions

Haploid (n=2x) genotypes

Anther and pollen cultures of *Brassica* biotechnology have resulted in mail haploids (n=2x=19) and dihaploids (DH; nn=4x=38) of *B. napus* genotypes through pollen embryogenesis (Lee, *et al.*, 2017; Bhaskara *et al.*, 2018).

In *Brassica* biotechnolog the methodology included responsive flower buds selection with optimal ratio between anther and filaments (Fig. 2./B, -C, -D), proper tissue culture media, and microscopically monitored samplings (Fig. 2) (Gyulai *et al.*, 1992; Marchant and Walbot, 2022)

Diploid (2n=2x) genotypes

B. o. oleracea (*Mediterranean cabbage*, 2n=18, CC) had developed from wild mustard/cabbage (*B. o. oleracea*; 2n=18, CC) and from *Brassica cretica* the probable closest ancestor (Mabry *et al.*, 2021), and had diversified to numerous cultivated plants (Fig. 1, Table 1).

A wild species of *Brassica macrocarpa* has also been studied in detail (Gray, 1982; Dixon, 2006; El-Esawi *et al.*, 2012; Gamba *et al.*, 2021).

B. rapa (*turnip rape / black mustard / Asian cabbage*, 2n=20, genome AA) also comprises species with modified *LEAFY* organs, as in Chinese kale/cabbage (*B. rapa pekinensis*) and the recently identified intraspecific varieties of *komatsuna* (*B. rapa perviridis*), *mizuna*, *taichai*, *zicaitai*, *wutacai*, and *pak/bok-choi* (*B. rapa chinensis*); and *ROOT* organs of *turnip* (*B. rapa*) and oilseed (*B. rapa oleifera*) (Fig. 1, Table 1).

B. napus (*rapeseed*) also has a variety (Fig. 1, Table 1) with enlarged roots (*B. n. napobrassica*) ('rutabaga') which has the highest drought tolerance among crops (L. Waters Jr., Auburn Univ, Alabama, U.S.A., personal communication).

Raphanus raphanistrum sativus a root vegetable of radish (2n=18; genome size 0.4334 Gbp, equal to 433.4 Mbp /10^6 bp/ DNA) (NCBI ID#: GCA_000801105.3) and the cultivars also show high levels of root morphological diversity (Fig. 1./14a, -b, -c) (Swaamy, 2023).

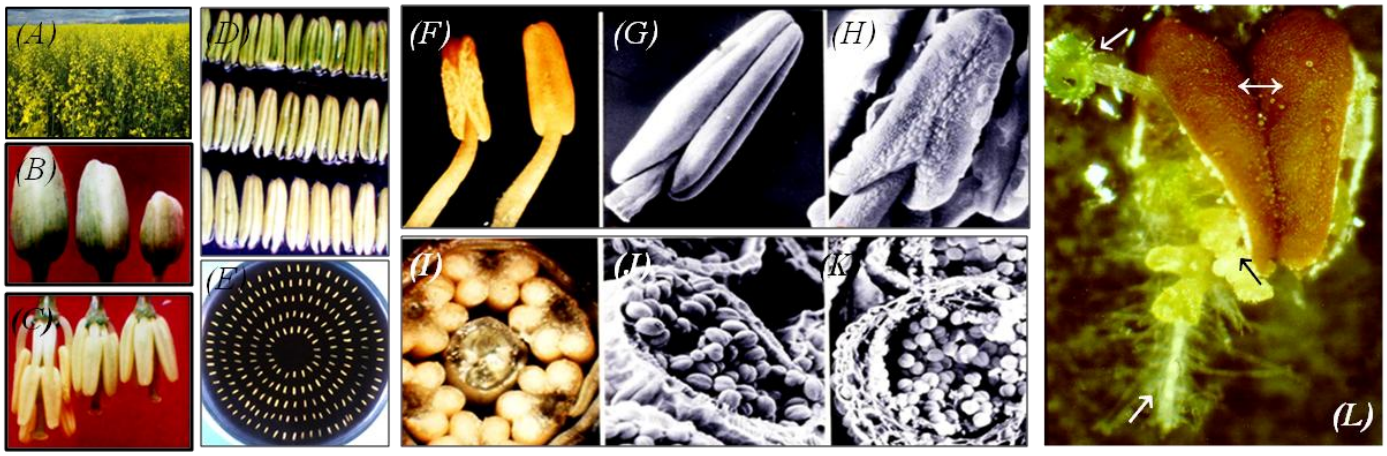

Figure 2. Methodology of haploid (n=2x=19) plant development from pollen grains in anther cultures of *Brassica napus* (2n=4x=38). Flower buds were collected from field grown plants (*A*), selected (*B, C*) at three different anther developments including the proper size - in the middle row (*D*), laid down on to the surface of aseptic tissue culture media in designed arrangement (*E*). The processes were monitored by LM- (*E, F, I, L*); and SEM analysis (*G, H, J, K*) [the sizes of *Brassica* pollen grains developed in pollen sacs are 90-100 µm / >0.1 mm (*I, J, K*)]. Haploid plant developed from pollen grain sprouting from the anther (*L*); the shoot and root of haploid plantlet, pollen grains and the two parts of anther wall (↔) are indicated with arrows.

Triploid (2n=3x) genotypes

In triploid breeding, like in the case of seedless watermelon breeding (Tóth *et al.*, 2007), no report has been published in *Brassica*s.

Tetraploid (2n=4x) genotypes

The fundamental *triangle* genomes model of *Brassica* species (Nagaharu/U, 1935) revealed genetic linkages among three genome-additive, interspecific, (allo)tetraploid hybrids of *B. carinata* (*Ethiopian mustard*, 2n=4x=34, BBCC); *B. juncea* (*Indian mustard*, 2n=4x=36, AABB); and *B. napus* (*rapeseed*, 2n=4x=38, AACC).

The tetraploid hybrids were found to be developed via natural hybridization between two of the three diploid progenitor species of *B. nigra* (black mustard, 2n=16, BB), *B. oleracea* (wild cabbage, 2n=18, CC), and *B. rapa* (turnip / field mustard, 2n=20, AA) through the following steps (Nagaharu/U, 1935) of:

(a) [*B. rapa* (turnip / field mustard), 2n=20, AA] x [*B. o. oleracea* (wild cabbage), 2n=18, CC] → *Brassica napus* (rapeseed), 2n=4x=38, AACC;

(b) [*B. rapa* (turnip / field mustard), 2n=20, AA] x [*B. nigra* (black mustard), 2n=16, BB] → *B. juncea* (Indian mustard), 2n=4x=36, AABB; and

(c) [*B. nigra* (black mustard), 2n=16, BB] x [*B. o. oleracea* (wild cabbage), 2n=18, CC] → *B. carinata* (Ethiopian mustard), 2n=4x=34, BBCC (Nagaharu/U, 1935; Yim *et al.*, 2020; Yin *et al.*, 2022).

Interspecific tetraploid *Brassica* hybrid of *Raphanobrassica* was also developed between *Raphanus sativus* and *Brassica* species recently (Lee *et al.*, 2017) following the fundamental work of Karpechenko, G.D. [1889-1941] (1928).

The cultivated horseradish (*Armoracia rusticana*) (2n=4x=32), which is the main source of horseradish peroxidase (HRP); the aquatic herb watercress (*Nasturtium officinale*) (2n=4x=32) (Mandáková and Lysak, 2019), and the Japanese horseradish (wasabe) (*Wasabia japonica*, *Syn*., *Eutrema japonicum)* (2n=4x=28) are also tetraploid (Table 1) species (Tanaka *et al.*, 2023).

During hybrid development huge numbers of gene loss occurs. When horseradish *(Armoracia rusticana)* developed 5 MYA a total of 2,653 genes were predictably lost (1,563 genes from the A-subgenome and 1,090 genes from the B-subgenome) (Shen *et al.*, 2023). This result indicates the genetic potential of *reciprocal crossings* used in plant breeding (Mendel, 1865; Ordás *et al.*, 2008).

Hexaploid (2n=6x) genotypes

(Allo)hexaploid (2n=6x=54, AABBCC) *Brassica*s was developed by breeding steps of crossings, back crossings and selections among three cultivated (allo)tetraploid *Brassica* species (*B. napus*, *B. juncea*, *B. carinata*) by Zhang *et al*. (2021).

Octoploid (2n=8x) genotypes

Octoploid *B. napus* (2n=8x=76, AAAACCCC) was developed recently (Yin *et al.*, 2020).

The one-rowed watercress (*Nasturtium microphyllum*) is also an octoploid wild species (Shen *et al.*, 2023).

Evolution

The level of species diversity of a plant genus was postulated to show positive correlations with the geographical origin, *i.e.*, Vavilovian areas (Vavilov, N. [1887-1943], 1926, 1928; *In:* Janick, 2015). The theory

shows two centres of *Brassica* species in the Mediterranean region of Europe and in China.

Unlike crops of barley (*Hordeum* ssp.), wheat (*Triticum* ssp.), rice (*Oryza* ssp.) or soybean (*Glycine* ssp.), the domestication of *Brassica* species - similar to maize (*Zea ssp*.) may have occurred more recently (Qu *et al*., 2023).

However, samples of archaeogenetics (Gyulai, 2011), *e.g.*, of *Brassica*/*Sinapis* seeds excavated from the 10th millennium B.P. in Syria (Willcox, 2002) show a more ancient evolutionary track.

B. napus (rapeseed) also shows a relatively recent hybrid development <7,500 years ago (Chalhoub *et al*., 2014).

B. oleracea including the leaf-head type cabbages and kales were domesticated recently in the Middle Ages about 500 years ago, but in different cultures and different geographical locations (East Asia, and Western Europe). The divergence among the main *Brassica* species was estimated to have occurred about 5 MYA (Cheng *et al.*, 2017).

By using a calculations of mutations (Ossowski *et al*., 2010), the split of speciation between two hypocotyl tuberous species (Table 1) of radish (*Raphanus raphanistrum*) and turnip (*Brassica r. rapa*) may have happened 13.19 MYA (Moghe *et al.*, 2014).

B. juncea (Indian mustard) has most likely an ancient, single origin developed in West Asia 8,000 - 14,000 years ago and diverged in to four subspecies of *B. j. juncea* (seed mustard), *B. j. integrifolia* (leaf mustard), *B. j. napiformis* (root mustard), and *B. j. tumida* (stem mustard) (Kang *et al*., 2021).

Tuberous turnip (*B. r. rapa*) (Fig. 1./13) and kohlrabi (*B. o. gongyloides*) (Fig. 1./15) - both selected in Europe, were suggested to be the result of a convergent (*i.e*., parallel) selection (Cheng *et al.*, 2017).

B. oleracea underwent a sub/genomic triplication - including WGDs (*whole genome duplications*) which contributed to plant organ mutations (Parkin *et al.*, 2014; Guo *et al*., 2024).

In the analysis of plant evolutionary history, the perennial *B. cretica* ssp. *aegaea* (Heldr. & Halácsy) (2n=18) was found to be the closest ancestor of *B. oleracea* (Mabry *et al.*, 2021).

Kohlrabi (*B. o. gongyloides*) was reported to appear first in the Middle Ages in Europe (Dixon, 2006).

An sequence analysis of 378 single-copy genes of *Armoracia* of *BRASSICACEAE* and the related *CARDAMINEAE* species showed 5 MYA estimated divergence time (Shen *et al.*, 2023).

METHODOLOGY

Illustrations of *BRASSICACEAE* species (Fig. 1) were collected from web sites (alamy.com; Britannica.com; monaconature-encyclopedia.com; and Wikipedia.org).

Anther cultures (Fig. 2) were developed and monitored by Light Microscopy (LM) and Scanning Electron Microscopy (SEM) according to Gyulai *et al.* (1992).

Genome (Fig. 3) and gene sequences of *Brassica* species (Fig. 4, 5, 6) were downloaded from NCBI server (www.ncbi.nlm.nih.gov).

Sequences were aligned by computer program BioEdit (Hall, 1999).

Molecular cladograms were edited by MEGA7 computer program (Kumar *et al.*, 2016) following the edition ways of Szabó *et al.*, (2023) and Gyulai *et al.* (2024).

Statistical bootstrap analysis was applied with x1000 repetitions by using MEGA7 computer program (Kumar *et al.*, 2016).

RESULTS AND DISCUSSION

Genome sizes

For genome size analysis, genomes of *Brassica* species available at gene banks (59 to date) were compared (Fig. 3). Of them, *B. rapa pekinensis* (chinese kale/cabbage) showed the smallest genome (244.9 x 10^6 bp DNA) compared to *B. carinata* (Abyssinian mustard) with the largest genome (1,087.0 x 10^6 bp).

Entries of *B. o. oleracea* (wild mustards/cabbages) showed middle size genomes (555 - 581 x 10^6 DNA bp) (Fig. 3).

Gene sequences

For gene sequence analysis, *gap*C-mRNA of the highly conserved nuclear gene *gapdh* (*glyeraldehyde-3-phosphate dehydrogenase*) sequences of *Brassica* species were analyzed (Fig. 4).

The gene *gap*C is one of the eight *gapdh* genes, which encode housekeeping enzymes and playing central roles in carbon metabolism, *e.g*., in the glycolysis.

The *gapdh* genes encode for proteins of GAPDHs (GAPA-1, GAPA-2, and GAPB), chloroplast glycolytic GAPDHs (GAPCp-1 and GAPCp-2), cytosolic glycolytic GAPDHs (GAPC-1 and GAPC-2), and the NADP-dependent non-phosphorylating cytosolic GAPDH (NP-GAPDH) (Henry *et al.*, 2015).

Null mutants of tDNA insertion lines of *Arabidopsis*, which were deficient in *gap*C1, have been selected (*gap*c1, NCBI# SALK_010839) coupled with incomplete seed fertility (Rius *et al.*, 2008).

In the analysis of *gap*C-mRNA sequences *Brassica* species showed two distinct clades at high (x1000) bootstrap level (Fig. 4). The data of chloroplast encoded *gap*Cp-mRNA sequences were excluded.

To compare *gap*C-mRNA sequences (Fig. 4) the ancient one-of-the first land plant (a fern *Selaginella lepidophylla*; Wang, J. *et al*., 2020); the one-of-the first flowering land plants of the basal ANITA angiosperm species of *Trimenia argyroplaga;* and a sample of Gymnosperm larch tree *(Larix gmelinii*) (http://www.theplantlist.org/1.1/browse/G/) were included to compare to Angiosperm *Brasssica*s (Fig. 4).

Root node development

Genes of *nsp*-1 (*nodulation signaling pathway*) of nitrogen-fixing *Rhizobium* bacteria that form symbiotic root nodes of Legumes have also been identified and sequenced in *Brassica*s genomes, however, without gene expression activity (Hayward *et al.*, 2012).

Based on the highly conserved DNA sequences of *nsp*-1, analyzed here, two *nsp* clades were identified in

BRASSICACEAE species (Fig. 5a). In the analysis, wild species of *Matthiola* (*BRASSICACEAE*) showed closer distance to *Brassica* species than *Sinapis* (*BRASSICACEAE*) (Fig. 5a). Silent- and amino acid change mutations were also determined (Fig. 5b).

Head- and Curd development - Flower bud mutations

The development of cabbages heads was found to be a partial control of cytokinin hydroxylase-like gene (CYP735A2; cytochrome P450 family 735, subfamily A, polypeptide 2; 1,407 nt mRNA; http://www.translatomedb.net).

The *Me*FT-1 gene (*Manihot esculenta* flowering terminal locus-1) (Rehman *et al.*, 2023) (*Syn.*, Terminal Flower-1 – *TFL*1), and *TFL*1 of *B. rapa* (NCBI# GCF_000309985.2) have been found to contribute to the transition of terminal/apical vegetative shoot meristem to flower bud meristem (Liu *et al*., 2021; Fan *et al*., 2024). *TFL*1 genes were also found to be under epigenetic control of photoperiod, vernalization, temperature, and plant hormones (Azpeitia *et al.*, 2021).

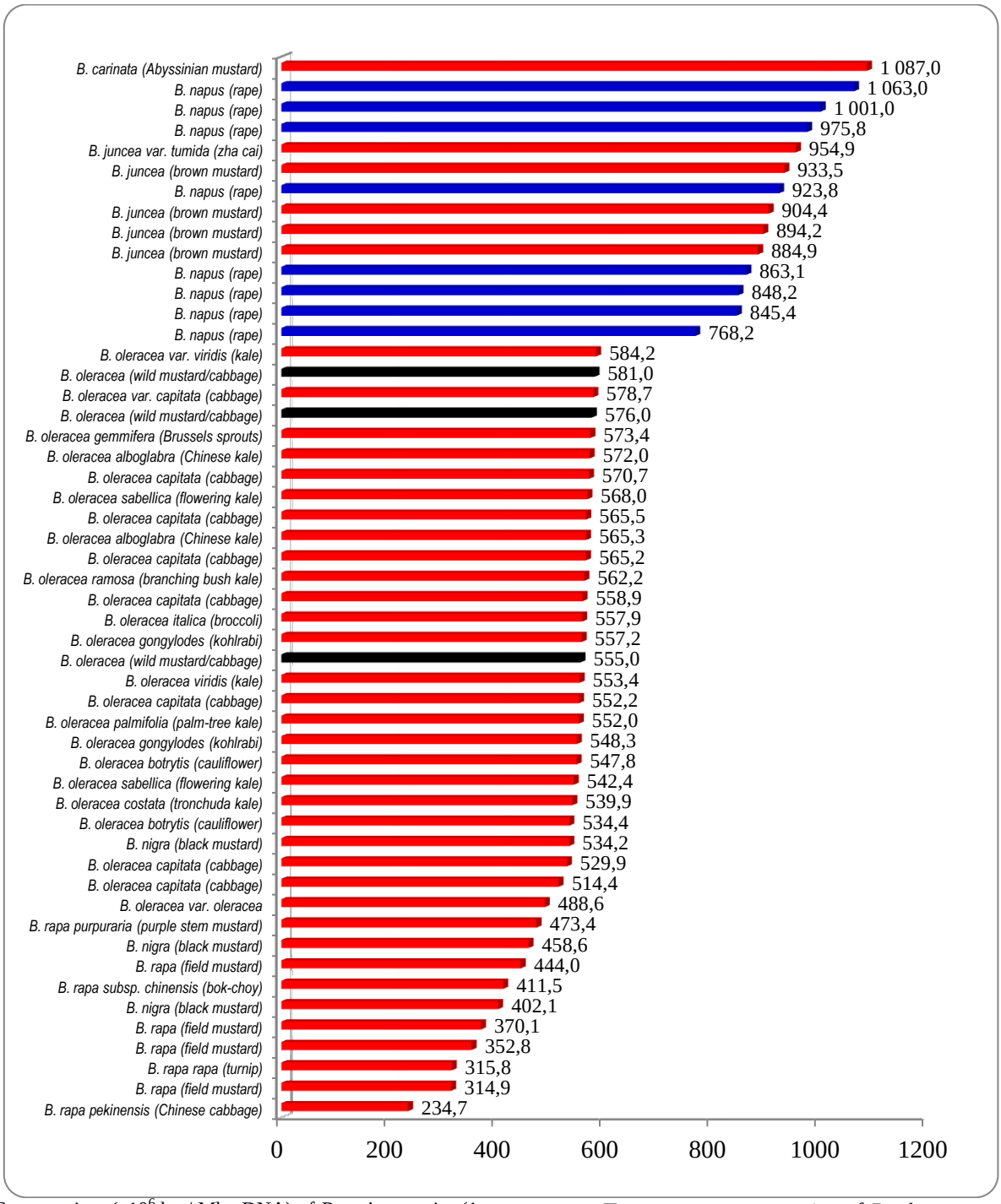

Figure 3. Genome sizes (x10^6 bp / Mbp DNA) of *Brassica* species (1-59) available of the total 98 *BRASSICACEAE* species to date (www.ncbi.nlm.nih.gov). Entries of *B. napus* (*blue columns*) and wild progenitors *B. o. oleracea* (wild mustard/cabbage) (*black columns*) are indicated. To compare, genome size of *Raphanus raphanistrum sativus* (2n=18) is 433.4 Mbp /10^6 bp/ DNA.

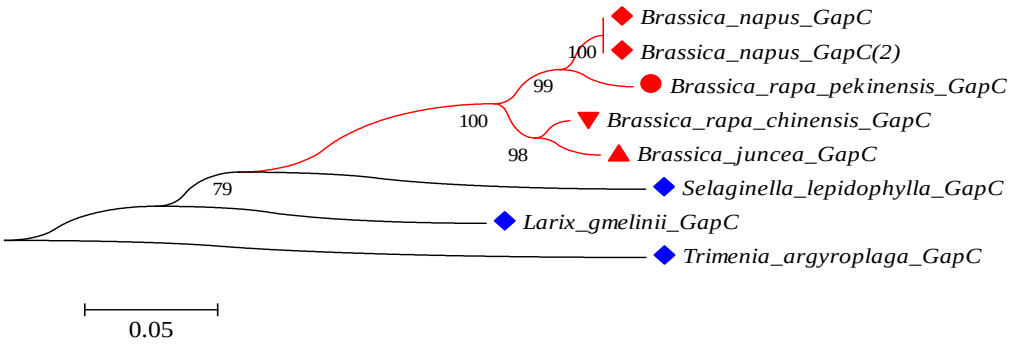

Figure 4. Cladogram (NJ; MEGA7; Kumar *et al.*, 2016) of *gap*C-mRNA sequences with statistical bootstrap (79 – 100) analysis (x1000 repetitions) transcribed from the nuclear gene *glyeraldehyde-3-phosphate dehydrogenase* (*gap*C) of *Brassica* and related species. The unit of genetic distance (0.05) is indicated which gives the numbers of nucleotide substitutions along a 100 bp mRNA stretch.

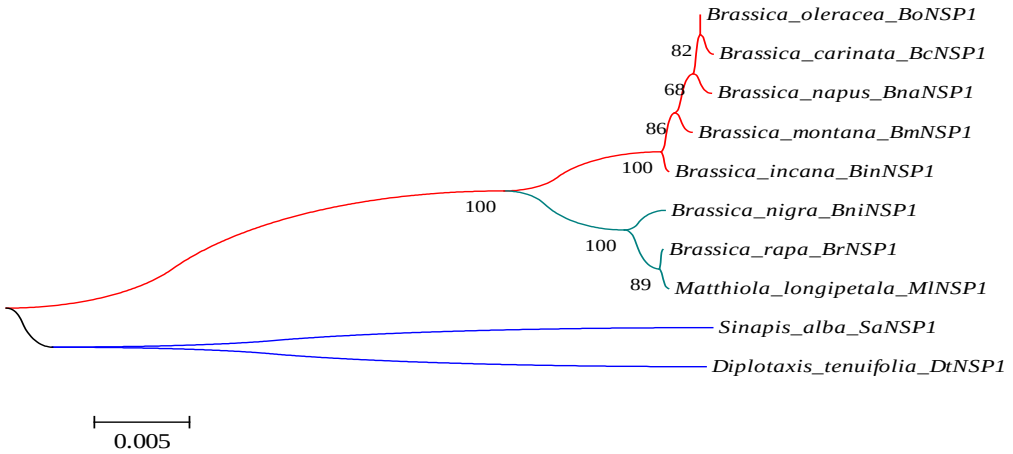

Figure 5a. Cladogram (NJ; MEGA7; Kumar *et al.*, 2016) of *Bonsp*-1 (*Brassica oleracea nodulation signaling pathway*) genes (Hayward *et al.*, 2012) of *BRASSICACEAE* species with statistical bootstrap (68 – 100) analysis (x1000 repetitions) (*Bonsp*1 gene, complete cds 1,533 bp; NCBI ID# JX242546). The unit of genetic distance (0.005) shows high level of DNA sequence similarity.

```
                      180         190         200         210         220         230
                     |.. ... ... |.. ... ... .|. ... ... ..| ... ... ... |.. ... ... ..| ... ... ..|
B. napus BnaNSP1     GGA TGT GAT TTT CTT CAT AGC TAT AGT CAA GAT CTT GAT GCA TAT ATA GGT GGT GAA GCA
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Gly Glu Ala
B. montana BmNSP1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Gly Glu Ala
B. incana BinNSP1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Gly Glu Ala
B. carinata BcNSP1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Gly Glu Ala
B. oleracea BoNSP1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Gly Glu Ala
B. rapa BrNSP1       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G T.. ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Cys Glu Ala
B. nigra BniNSP1     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G T.. ... ...
                     Gly Cys Asp Phe Leu His Ser Tyr Ser Gln Asp Leu Asp Ala Tyr Ile Gly Cys Glu Ala
```

Figure 5b. Sequence alignments of *nsp*-1 genes (180 – 230 nt) (upper rows) and NSP-1 translated proteins (60 aa stretches, of the total 571 aa; lower rows) translated by BioEdit computer program (Hall, 1999) by using 3-letters codes (NCBI ID# JX242546; 1,533 bp; Hayward *et al.*, 2012) of *Brassica* species.

Silent (*i.e.*, synonymous; GGT/Gly→GGG/Gly DNA mutations (*i.e.*, substitutions) of T→G of Gly (glycine aa), and amino acid change mutations are indicated by pink boxes (GGT/Gly → TGT/Cys) (glycine → cysteine change) according to Balázs and Dudits (2017).

The *TFL*1 gene family was found to be component of *'florigen'* (Chailakhyan, 1936; *In:* Liu *et al.*, 2021) with concerning the Mendel's 6th of the seven growth 'factors' of green pea (*Pisum sativum*) (1865; *In*: Corcos and Monaghan, 1990; Putterill and Varkonyi-Gasic, 2016).

Flower bud mutants of cauliflowers (*B. o. botrytis*) with differently colored curds (Fig. 1./4) and '*Romanesco*'

cauliflower (*B. o. botrytis 'Romanesco'*) (Fig. 1./5), broccoli (*B. o. italica*) (Fig. 1./1), broccolini (*B. o. italica* × *B. o. alboglabra*) (Fig. 1./2), and Chinese broccoli (*B. o. alboglabra*) (Fig. 1./3) have been selected (Azpeitia *et al.*, 2021). In curd development of cauliflower (Fig. 1./4) MADS-box genes and zinc-finger proteins were also identified (Chen *et al.*, 2024).

MADS box *TF* genes (with short DNA bp length which encode small 56-60 amino acid long DNA-binding proteins) also play central role in flowering time control, inflorescence and floral morphotypes, and seed development of *BRASSICACEAE* species (Song *et al.*, 2023; Adhikari and Kasahara, 2024).

[The name of MADS box acronym comes from the four basic *TF* genes of *mcm*1 (*mini chromosome maintenance*) of the budding yeast (*Saccharomyces cerevisiae*); the floral *agamous* mutants (*i.e., agamous* → 'without gametes' / parthenogenic) of the thale cress (*Arabidopsis thaliana*) (TAIR# AT4G18960); the floral *deficiens* mutants (*i.e.*, changes of petals to sepals, and stamina to carpels) of snapdragon (*Antirrhinum majus*) (Summer *et al.*, 1990), and the *srf* (*serum response factor*) of the human genome (*Homo sapiens*) (Gene/6722[uid]/ NCBI)].

TF genes of MADS-box superfamily (Adhikari and Kasahara, 2024) - a total of 1.430 genes to date, have been analyzed in *Brassica* species, and four floral organ mutants of *wtl (wild type like)*, *feml (female flower like)*, *aglf*-1, and *aglf*-2 (*agamous-like flower*) were identified (Song *et al.*, 2023).

Red coloration - Transcription factors (TFs) activity

The expression of bHLH type (*i.e., basic Helix-Loop-Helix* DNA) *TF*s (*transcription factors*) genes encoding for DNA binding proteins was identified first in red-seeded maize by Ludwig *et al.* (1989), and recently in turnip (NCBI# HQ337791; 1.566 nt mRNA) and other *Brassica* species (Nesi *et al*., 2000).

In *Brsassica rapa* the DNA binding protein of *Br*TT8-(*transparent testa* 8 gene)-bHLH-*TF* is 521 aa long (#XP_009113574.1) and causes red coloration of turnip epidermal tissues (Fig. 1./13) (Zhang, Y. *et al.*, 2020).

In the analysis of mRNA sequences (1.566 nt) of bHLH TT8 *TF*s of *BRASSICACEAE* species *Raphanus* and *Arabidopsis* showed closer sequence similarity then that of *B. o. botrytis* (Fig. 6).

Light-responding anthocyanin production was also observed in epidermal tissues of purple broccoli (Fig. 1./1) (Liu *et al.*, 2020).

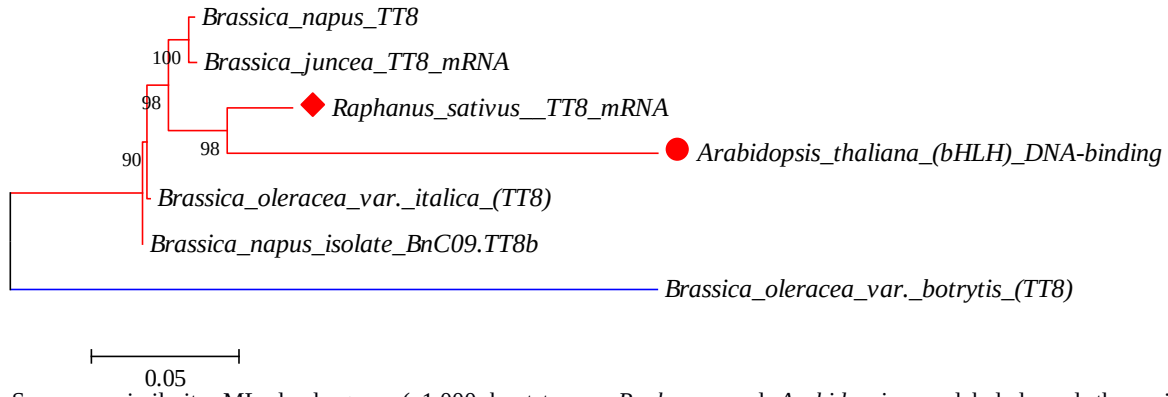

Figure 6. Sequence similarity ML dendrogram (x1.000 bootstrap analysis) of mRNAs (1.566 nt) of *bHLH TT8 TFs* of DNA-binding proteins of *BRASSICACEAE* species edited by MEGA7 computer program (Kumar *et al.*, 2016) of mRNA sequences (1.566 nt). *Raphanus* and *Arabidopsis* are labeled, and the unit of genetic distance (0.05) is indicated which gives the numbers of nucleotide substitutions along a 100 bp mRNA stretch.

Transposons activity

Transposons of both groups of DNA- and Retro-transposons, account for huge part of all eukaryotic genomes from 40% of the human genome to 50% – 90% of the important agricultural crops of maize, wheat, barley, rye, and sugar beet (McClintock, 1948; Schulman and Kalendar, 2005; Walker *et al.*, 1997; Ivics *et al.*, 2009; Kalendar and Schulman, 2014).

Transposons do not code for house-keeping proteins (*i.e.*, 'junk DNA'), however they play an important role in genome function by jumping in to the DNA of gene sequences and down-regulating it (Alzohairy *et al*., 2013).

Horseradish genome analysis suggested that DNA methylation plays a sentral role in regulating and maintaining the stability of LTR-RT regions (Shen *et al.*, 2023).

The up-regulation of *purple locus* in maize (on chromosome 10) by insertion of DNA transposons was identified in the locus of *R-p* complex of the anthocyanin biosynthetic pathway causing purple coloration of maize seeds (Ludwig *et al.*, 1989) which was named later as PIF (*p instability factor*; Wicker *et al.*, 2007).

Changes in purple/red coloration coupled with anthocyanin accumulation were also described in the *pap*1-D (*production of anthocyanin pigmentation1-Dominant*) of *Arabidopsis* (Borevitz *et al.*, 2000; Shi and Xie, 2010), *ant*-1 (*anthocyanin*-1) in tomato (Menconi *et al*., 2024), and *red-flesh* apple (Espley *et al.*, 2007) as the result of DNA transposition of insertions in to anthocyanin related genes (Chen *et al*., 2023; Menconi *et al.*, 2024).

In *Brassica*, the purple coloration of cauliflowers (Fig. 1./4) was identified first by the insertion ('jumping') of *harb* DNA transposon (5.382 bp; Kapitonov and Jurka, 1999) by jumping in to the upstream regulatory region of the purple(*Pr*)-D MYB-

allele and, by this way up-regulating it (Chiu *et al.*, 2010; Chiu and Li, 2012).

In *B. napus*, high numbers of 227 MYB-related (*Bn*MYBR/*Bn*$_1$R-MYB) transposons, 429 R_2R_3-MYB (*Bn*$_2$R-MYB) genes, 22 $R_1R_2R_3$-MYB (*Bn*$_3$R-MYB) genes, and two $R_1R_2R_2R_{1/2}$-MYB (*Bn*$_4$R-MYB) genes have been identified (Li *et al.*, 2020).

Seed coat color analysis of six *Brassica* species including *B. napus* for the selection of the preferred yellow canola seed type, a high number of 1.119 anthocyanin-related genes were identified coupled with *Bn*MYB$_5$ activity (Chen *et al.*, 2023).

Epigenetic effect of drought stress also induces red coloration in plant leaves. In *Chaenomeles speciosa* (*ROSACEAE*) the expression of TF proteins of *Cs*MYB-123 and *Cs*bHLH-111 were found to be increased under drought stress.

The *TF* proteins can also activate the anthocyanin-related *Cs*CHI gene (*Cucumis sativus chalcone-flavanone isomerase*) by binding to the gene promoter region (Ren *et al.*, 2023).

An R_3-MYB TF repressor protein (*Bn*CPC; *Brassica napus cysteine peptidase* C; 86 aa) (cds – *coding sequence*: *Bn*aC04g50810D) was also identified in *B. napus* by repressing anthocyanin biosynthetic *Bn*DFR gene (*dihydroflavonol 4-reductase*) blocking at the promoter region (Xie, T. *et al.*, 2022).

The regulatory switch on-and-off of MYB *TF*s, similar to MYC *TF*s [named after MYeloCytomatosis, an avian virus] (*e.g.*, UniProt# P10395) also was found to activate DNA binding proteins [*e.g.*, in Human; UniProt# P10242, 640 aa].

The gene copy numbers of *myb tf* homologous genes were found to be numerous in plants, *e.g.*, in grapevine (*Vitis vinifera*) (order VITALES) 265 *Vv*-MYB (Wang *et al.*, 2023), and in *Casuarina equisetifolia* (woody FAGALES) 182 *Ceq*-MYB (R_2R_3-MYB) genes were identified recently (Wang *et al.*, 2021).

Retrotransposons can also be active in *Brassica* species. In total 262 intact LTR (*long terminal repeat*) retrotransposons were identified in *Brassica* genomes (Nouroz *et al.*, 2015).

In addition to red coloration, *Br*MYB-108 was also reported to confer resistance to vascular fungal disease *Verticillium* wilt in *Brassica rapa* by activating ROS (*reactive oxygen species*) generations (Su *et al.*, 2023).

Heat stress can also block anthocyanin production, *e.g.*, in red coloration of apple fruit temporarily (Wang, W. *et al.*, 2020), and of red leaf hazel (*Corylus avellana*) genotype stably turning it back to wild type green leaf as it occurred recently in summer 2024 (above daily temperature 40 °C for two to three months) in Gödöllő (Hungary, Europe).

Roles of smallRNAs and siRNAs

SmallRNAs of both groups of microRNAs (miRNAs) and phased siRNAs (phasiRNAs); and short interfering RNAs (siRNAs) including tasiRNAs and casiRNAs according to the mode they target genes in trans (tasiRNAs) or in cis (casiRNAs) positions also contribute in gene expression by cleaving or inhibiting the transcribed mRNAs through post-transcriptional gene silencing (Wang, F *et al.*, 2012; Wang, W.-Q. *et al.*, 2020).

In Chinese cabbage (*Brassica rapa pekinensis*) huge numbers of microRNAs were found (11,210) belonging to 321 conserved miRNA families and 228 novel miRNAs (Wang, F *et al.*, 2012).

In horseradish (*Armoracia rusticana*, $2n = 4x = 32$) 557 miRNAs were sequenced (Shen *et al.*, 2023).

In *Brassica campestris* mirRNAs (*bra*-miRn9, and *bra*-miRn10-1) were found to act in the regulation of pollen (Fig. 2) development (Jiang *et al.*, 2014).

Tuber formation

BRASSICACEAE species of turnip (*B. rapa*), rutabaga (*B. napus* x *napobrassica*), radish (*R. sativus*), horsredish (*Armoracia rusticana*), root mustard (*B. juncea napiformis*) and wasabi (*W. japonica*) (Table 1) develop enlarged tubers.

Tuberization also occurs in plant families of *UMBELLIFERAE/APIACEAE* species of carrot (*Daucus carota*) [2n=2x=18, with a genome size of 473 Mb], parsley (*Petroselinum crispum*), parsnip (*Pastinaca sativa*), celery (*Apium graveolens*), lovage (*Levisticum officinale*), sugarbeet (*Beta vulgaris*) and red beetroot (*B. rubra*) both *AMARANTHACEAE*.

Red beetroot tissue pattern is similar to secondary stem thickening of woody plants (Toldi *et al.*, 1996; Zierer *et al.*, 2021; Székely and Máté, 2022).

Tubers of cassava (*Manihot esculenta*; *EUPHORBIACEAE*); corm of taro (*Colocasia esculenta*) (*ARACEAE*), batata/sweet potato (*Ipomoea batatas*; *CONVOLVULACEAE*), and potato (*Solanum tuberosum*; *SOLANACEAE;* [2n=2x=24; 2n=4x=48; 2n=5x=60]) are also significant. This indicates poly- and paraphyletic genetic origin of plant organ modifications (Greguss, 1964; Soó, 1965; Cornwell *et al.*, 2014).

Tuber development does not depend on a single gene function. A *Weigthed Coexpression Network Analysis* (WGCNA) indicated 59 co-expressed genes which are involved in transcriptional regulation of hypocotyl-tuber formation of turnip (*B. rapa*) tuber (Fig. 1./13, Table 1). Among the genes, the increased level of trans-zeatin – a form of cytokinin type plant hormones (Gyulai *et al.*, 1995) was found to be the most significant (Liu *et al.*, 2019).

CONCLUSION

Genome (*BRASSICACEAE*) and gene sequence analyses (*gap*C-mRNA, *nsp* and *bHLH TT8* TFs) of *Brassica* species were carried out here by *in silico* data mining. The results provide new information to the molecular background of domestication of *BRASSICACEAE* species.

Epigenetic effect of heat stress to plant anthocyanin production, and the tools of *Brassica napus* biotechnology were also indicated.

Genetic background of the extreme levels of morphological diversity of *BRASSICACEAE* species, and the role in agro-ecocycles was also aimed to analyze.

The data presented could be of potential use in identifying the most adaptive *Brassica* crops to grow under the current climate changing and global warming (Rezaei *et al.*, 2023) conditions.

REFERENCES

Adhikari, P.B., & Kasahara, R.D. (2024). An overview on MADS box members in plants: a meta-review. *International Journal of Molecular Sciences*, 25, 8233. pp.25.
DOI: org/10.3390/ijms25158233

Alzohairy, A.M., Gyulai, G., Jansen, R.K., & Bahieldin, A. (2013). Transposable elements domesticated and neofunctionalized by eukaryotic genomes. *Plasmid*, 69:1–15.
DOI: 10.1016/j.plasmid.2012.08.001

Azpeitia, E., Tichtinsky, G. , Le Masson. M., + 10, & Parcy, F. (2021). Cauliflower fractal forms arise from perturbations of floral gene networks. *Science*, 373(6551):192–197.
DOI: 10.1126/science.abg5999.

Balázs, E., & Dudits, D. (2017). Precíziós nemesítés – Kulcs az agrárinnovációhoz. *Agroinform*, Budapest, *Ch.* 4., pp.41–51. ISBN 978-615-5666-09-4

Bhaskara, G.B. (2018). Basic principles and recent advances in anther/pollen culture for crop improvement. *In.* Plant biotechnology, Vol.1. *Ch*. 5. pp. 87–123. *Eds*.: Prasad, B.D., Sahni, S., Kumar, P. & Siddiqui, M.W. ISBN 9781774631102

Borevitz, J.O., Xia, Y., Blount, J., Dixon, R.A., & Lamb, C. (2000). Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. *The Plant Cell*, 12(12): 2383–2394.
DOI: 10.1105/tpc.12.12.2383

Branca, F., & Cartea, M.E. (2011). *Brassica*. *In*: Wild crop relatives: genomic and breeding resources, oilseeds. (*Ed*.), Kole, C., *Ch.* 2., pp. 17–36. Springer-Verlag, Berlin, Heidelberg, D.
DOI: 10.1007/978-3-642-14871-2_2

Chalhoub, B., Denoeud, F., Liu, S., +78, & Wincker, P. (2014). Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science*, 345(6199): 950–953.
DOI: 10.1126/science.1253435

Chen, D., Chen, H., Dai, G., +5, & Tan, C. (2023). Genome-wide identification and expression analysis of the anthocyanin-related genes during seed coat development in six *Brassica* species. *BMC Genomics*, 24(1):103.
DOI: 10.1186/s12864-023-09170-2

Chen, R., Chen, K., Yao, X., +18, & Sun, D. (2024). Genomic analyses reveal the stepwise domestication and genetic mechanism of curd biogenesis in cauliflower. *Nature Genetics*, 56: 1235–1244.
DOI: 10.1038/s41588-024-01744-4

Cheng, F., Liang, J., Cai, C., Cai, X., Wu, J., & Wang, X. (2017). Genome sequencing supports a multi-vertex model for *Brassicaceae* species. *Current Opinion in Plant Biology*, 36: 79–87.
DOI: 10.1016/j.pbi.2017.01.006

Chiu, L.W., Zhou, X., Burke, S., Wu, X., Prior, R.L, & Li, L. (2010). The purple cauliflower arises from activation of a MYB transcription factor. *Plant Physiology*, 154(3): 1470–80.
DOI: 10.1104/pp.110.164160

Chiu, L.W., & Li, L. (2012). Characterization of the regulatory network of BoMYB2 in controlling anthocyanin biosynthesis in purple cauliflower. *Planta*, 236: 1153–1164.
DOI: 10.1007/s00425-012-1665-3

Cooper, D.N., & Gerber-Huber, S. (1985). DNA methylation and CpG suppression. *Cell Differentiation*, 17(3): 199–205.
DOI: 10.1016/0045-6039(85)90488-9

Corcos, A.F. & Monaghan, F.V. (1990). Mendel's work and its rediscovery: A new perspective. *Critical Reviews in Plant Sciences*, 9(3): 197–212.
DOI: 10.1080/07352689009382287

Cornwell, W.K., Westoby, M., Falster, D.S., + 22, & Zanne, A.E. (2014). Functional distinctiveness of major plant lineages. *Journal of Ecology*, 102(2): 345–356.
DOI: 10.1111/1365-2745.12208

Dixon, G.R. (2006). Vegetable *Brassica*s and related crucifers. *Crop Production Science in Horticulture Series*, Vol. 14. *Editor* J. Atherton, pp. 327; CABI, Wallingford, UK, ISBN: 978-0-85199-395-9.

El-Esawi, M., Bourke, P., Germaine, K., & Malone, R. (2012). Assessment of morphological variation in Irish *Brassica oleracea* species. *Journal of Agricultural Science* 4(10): 20–34.
DOI: 10.5539/jas.v4n10p20

Espley, R.V., Hellens, R.P., Putterill, J., Stevenson, D.E., Kutty-Amma, S., & Allan, A.C. (2007). Red coloration in apple fruit is due to the activity of the MYB transcription factor *md*MYB10. *The Plant Journal*, 49: 414–427.
DOI: 10.1111/j.1365-313X.2006.02964.x

Fan, L., Zhu, Z., Lin, X., Shen, X., Yang, T., Wang, H., & Zhou, X. (2024). Comparative genomic analysis of PEBP genes in Cucurbits explores the interactors of cucumber *Cs*PEBPs related to flowering time. *International Journal of Molecular Sciences*, 25(7): 3815.
DOI: 10.1002/sstr.202000125

Gamba, M., Asllanaj, E., +7, & Muka, T. (2021). Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. *Trends in Food Sci. & Technol.*, 113: 205–218.
DOI: 10.1016/j.tifs.2021.04.045

Gray, A.R. (1982). Taxonomy and evolution of broccoli (*Brassica oleracea* var. *italica*). *Economic Botany*, 36: 397–410.

Greguss, P. (1964). The phylogeny of sexuality and triphyletic evolution of the land plants. *Acta Biologica*, 10(1-4): 3–51. http://acta.bibl.u-szeged.hu/id/eprint/21589

Guo, N., Wang, S., Wang, T., +12, & Liu, F. (2024). A graph-based pan-genome of *Brassica oleracea* provides new insights into its domestication and morphotype diversification. *Plant Communications*, 5(2): 100791, pp. 18.
DOI: 10.1016/j.xplc.2023.100791

Gyulai, G., & Heszky, L.E. (1992). Biotechnology of rapeseed (*Brassica napus* L.). *Acta Agronomica Hungarica*, 41:277–287. https://real-j.mtak.hu/897/1/ACTAAGRONOMICA_1992_41.pdf

Gyulai, G., Jekkel, Z., Kiss, J., & Heszky, L.E. (1995). A selective auxin and cytokinin bioassay based on root and shoot formation *in vitro*. *Journal of Plant Physiology*, 145: 379–382.
DOI: 10.1016/S0176-1617(11)81907-3

Gyulai, G. (*Ed*.) (2011). Plant archaeogenetics. *Nova Science Publisher* Inc. New York, USA, pp. 168. ISBN 978-1-61122-644-7

Gyulai, Zs.G., Malone, R.P., Gyulai, G., Ali, A.M., & Tóth, Z. (2024). *Monilia* and *Monilinia* – *In silico* genetic analysis of plant pathogenic fungi. *Ecocycles*, 10(1): 84–94.
DOI: 10.19040/ecocycles.v10i1.401

Hayward, A., Vighnesh, G., Delay, +5, & Batley, J. (2012). Second-generation sequencing for gene discovery in the *Brassicaceae*. *Plant Biotechnology Journal*, 10(6): 750–759.
DOI: 10.1111/j.1467-7652.2012.00719.x

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95–98.
DOI: 10.14601/phytopathol_mediterr-14998u1.29

Henry, E., Fung, N., Liu, J., Drakakaki, G., & Coaker, G. (2015). Beyond glycolysis: GAPDHs are multi-functional enzymes involved in regulation of ROS, autophagy, and plant immune responses. *PLoS Genet*ics, 11(4): e1005199.
DOI: 10.1371/journal.pgen.1005199

Ivics, Z., Li, M.A., Mátés, L., Boeke, J.D., Nagy, A., Bradley, A., & Izsvák, Zs. (2009). Transposon-mediated genome manipulation in vertebrates. *Nature Methods*, 6: 415–422.
DOI: 10.1038/nmeth.1332

Janick, J. (2015). Nikolai Ivanovich Vavilov: Plant geographer, geneticist, martyr of science. *HortScience*, 50: 772–776.
DOI: 10.21273/HORTSCI.50.6.772

Jiang, J., Lv, M., Liang, Y., Ma, Z., & Cao, J. (2014). Identification of novel and conserved miRNAs involved in pollen development in Brassica campestris ssp. chinensis by high-throughput sequencing and degradome analysis. *BMC Genomics*, 15:146.
DOI: 10.1186/1471-2164-15-146

Kalendar, R., & Schulman, A.H. (2014). Transposon-based tagging: IRAP, REMAP, and iPBS. *Methods in Molecular Biology.* 1115: 233–255.
DOI: 10.1007/978-1-62703-767-9_12

Kang, L., Qian, L., Zheng, M., +24, & Liu, Z. (2021). Genomic insights into the origin, domestication and diversification of *Brassica juncea*. *Nature Genetics*, 53: 1392–1402.
DOI: 10.1038/s41588-021-00922-y

Kapitonov, V.V., & Jurka, J. (1999). Molecular paleontology of transposable elements from *Arabidopsis thaliana*. *Genetica*, 107(1-3): 27–37. PMID: 10952195

Kátay, G., Tyihák, E., & Kátay, E. (2011). Effect of ascorbigen and 1'-methylascorbigen on disease resistance of bean plants to *Uromyces phaseoli*. *Natural Product Communications*,6(5): 611-615. PMID: 21615018

Karpechenko, G.D. (1928). Polyploid hybrids of *Raphanus sativus* × *Brassica oleracea*. *Bulletin of Applied Botany*, 17: 305–408.

Kimura, M. (1968). Evolutionary rate at the molecular level. *Nature*, 217: 624–626.
DOI: 10.1038/217624a0

Kőmíves, T., & Király, Z. (2019). Disease resistance in plants: The road to phytoalexins and beyond. *Ecocycles*, 5(1): 7–12.
DOI: 10.19040/ecocycles.v5i1.132

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33: 1870–1874.
DOI: 10.1093/molbev/msw054

Lee, S.-S., Hwang, B.H., Kim, T.Y., +4, & Belandres, H.R. (2017). Developing stable cultivar through microspore mutagenesis in ×*Brassicoraphanus koranhort*, Inter-generic allopolyploid between *Brassica rapa* and *Raphanus sativus*. *American Journal of Plant Sciences*, 8: 1345–1356.
DOI: 10.4236/ajps.2017.86091

Li, P., Wen, J., Chen, P.,+6, & Du, H. (2020). MYB superfamily in *Brassica napus*: Evidence for hormone-mediated expression profiles, Large expansion, and functions in root hair development. *Biomolecules*, 10(6): 875.
DOI: 10.3390/biom10060875

Li, X., Wang, Y., Cai, C., +15, & Cheng, F. (2024). Large-scale gene expression alterations introduced by structural variation drive morphotype diversification in *Brassica oleracea*. *Nature Genetics*, 56: 517–529.
DOI: 10.1038/s41588-024-01655-4

Liu, M., Bassetti, N., Petrasch, S., +4, & Bonnema, G. (2019). What makes turnips: anatomy, physiology and transcriptome during early stages of its hypocotyl-tuber development. *Horticulture Research*, *Oxford Academic*, 6: 38.
DOI: 10.1038/s41438-019-0119-5

Liu, C., Yao, X., Li, G., Huang, L., & Xie, Z. (2020). Transcriptomic profiling of purple broccoli reveals light-induced anthocyanin biosynthetic signaling and structural genes. *PeerJ Life and Environment*, 8: e8870.
DOI: 10.7717/peerj.8870

Liu, L., Xuan, L., Jiang, Y, & Yu, H. (2021). Regulation by flowering locus T and terminal flower 1 in flowering time and plant architecture. *Small Structures*, 2000125.
DOI: 10.1002/sstr.202000125

Ludwig, S.R., Habera, L.F., Dellaporta, S.L., & Wessler, S.R. (1989). *Lc*, a member of the maize *R* gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the *myc*-homology region. *Proceedings of the National Academy of Sciences of the United States of America*, 86: 7092–7096.
DOI: 10.1073/pnas.86.18.7092

Mabry, M.E., Turner-Hissong, S.D., Gallagher, E.Y., + 14, & Pires, J.C. (2021). The evolutionary history of wild, domesticated, and feral *Brassica oleracea* (*Brassicacea*e). *Molecular Biology and Evolution*, 38(10): 4419–4434.
DOI: 10.1093/molbev/msab183

Mandáková, T., & Lysak, M.A. (2019). Healthy roots and leaves: Comparative genome structure of horseradish and watercress. *Plant Physiology*, 179(1): 66–73.
DOI: 10.1104/pp.18.01165

Marchant, D.B., & Walbot, V. (2022). Anther development – The long road to making pollen. *The Plant Cell*, 34(12): 4677–4695.
DOI: 10.1093/plcell/koac287

McClintock, B. (1948). Mutable loci in maize. *In*: Annual report of the director of the department of genetics, *Carnegie Institution of Washington Year Book*, 47: 155–169. Carnegie Institution of Washington, Cold Spring Harbor, New York, URL: https://archive.org/stream/yearbookcarne47194748ca...

Menconi, J., Perata, P., & Gonzali, S. (2024). In pursuit of purple: anthocyanin biosynthesis in fruits of the tomato clade. *Trends in Plant Sci*ence, 29(5): 589–604.
DOI: 10.1016/j.tplants.2023.12.010

Mendel, J.G. (1865). Versuche über Pflanzenhybriden. *Verhandlungen des naturforschenden Vereines in Brünn*, Bd. IV für das Jahr, 1865 Abhandlungen (1866): 3–47. [for English translation: www.mendelweb.org/Mendel.plain.html].

Moghe, G.D., Hufnagel, D.E., Tang, H., +4, & Shiu, S.H. (2014). Consequences of whole-genome triplication as revealed by comparative genomic analyses of the wild radish *Raphanus raphanistrum* and three other *Brassicaceae* species. *The Plant Cell*, 26(5): 1925–1937.
DOI: 10.1105/tpc.114.124297

Nagaharu, U. (1935). Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *The Journal of Japanese Botany*, 7: 389–452.

Nesi, N., Debeaujon, I., Jond, C., Pelletier, G., Caboche, M., & Lepiniec, L. (2000). The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in *Arabidopsis* siliques. *Plant Cell*, 12(10): 1863–78.
DOI: 10.1105/tpc.12.10.1863

Nouroz, F., Noreen, S., & Heslop-Harrison, J.S. (2015). Identification and evolutionary genomics of novel LTR retrotransposons in *Brassica. Turkish Journal of Biology*, 39: 740–757.
DOI: 10.3906/biy-1501-77

Ordás, B., Malvar, R.A., Ordás, A., Revilla, P. (2008). Reciprocal differences in sugary x sugary enhancer sweet corn hybrids. *Journal of the American Society for Horticultural Science*. 133(6): 777-782.
DOI:10.21273/JASHS.133.6.777

Ossowski, S., Schneeberger, K., +5, & Lynch, M. (2010). The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science*, 327: 92–94.
DOI: 10.1126/science.1180677

Parkin, I.A.P, Koh, C., Tang, H., +28, & Sharpe, A.G. (2014). Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. *Genome Biology*, 15(6): R77.
DOI: 10.1186/gb-2014-15-6-r77

Putterill, J., & Varkonyi-Gasic, E. (2016). FT and florigen long-distance flowering control in plants. *Current Opinion in Plant Biology*, 33: 77–82.
DOI: 10.1016/j.pbi.2016.06.008

Qu, C., Zhu, M., Hu, R. +34, & Li, J. (2023). Comparative genomic analyses reveal the genetic basis of the yellow-seed trait in *Brassica napus*. *Nature Communications*, 14(1): 5194.
DOI: 10.1038/s41467-023-40838-1

Ramirez, D., Abellán-Victorio, A., Beretta, V., Camargo, A., & Moreno, D.A. (2020). Functional ingredients from *Brassicaceae* species: Overview and perspectives. *International Journal of Molecular Sciences*, 21, 1998.
DOI: 10.3390/ijms21061998

Rezaei, E.E., Webber, H., +5, Asseng, S. (2023). Climate change impacts on crop yields. *Nature Reviews Earth & Environment*, 4: 831–846.
DOI: 10.1038/s43017-023-00491-0

Rédei, G.P. (1975). *Arabidopsis* as a genetic tool. *Annual Review of Genetics*, 9: 111–127.
DOI: 10.1146/annurev.ge.09.120175.000551
Rehman, S., Bahadur, S., & Xia, W. (2023). An overview of floral regulatory genes in annual and perennial plants. *Gene*, 885: 147699.
DOI: 10.1016/j.gene.2023.147699

Ren, Y., Zhang, S., Zhao, Q., Wu, Y., & Li, H. (2023). The *CsMYB123* and *CsbHLH111* are involved in drought stress-induced anthocyanin biosynthesis in *Chaenomeles speciosa*. *Molecular Horticulture*, 3: 25.
DOI: 10.1186/s43897-023-00071-2

Rius, S.P., Casati, P., Iglesias, A.A., & Gomez-Casati, D.F. (2008). Characterization of *Arabidopsis* lines deficient in GAPC-1, a cytosolic NAD-dependent glyceraldehyde-3-phosphate dehydrogenase. *Plant Physiology*, 148(3): 1655–1667.
DOI: 10.1104/pp.108.128769

Rusznyák, I., & Szent-Györgyi, A. (1936). Vitamin P: flavonols as vitamins. *Nature*, 138: 27.
DOI: 10.1038/138027a0

Salser, W. (1978). Globin mRNA sequences: analysis of base pairing and evolutionary implications. *Cold Spring Harbor Symposia on Quantitative Bioliology*, 42 Pt 2:985–1002.
DOI: 10.1101/sqb.1978.042.01.099

Schulman, A.H., & Kalendar, R. A. (2005). Movable feast: diverse retrotransposons and their contribution to barley genome dynamics. *Cytogenetic and Genome Research*, 110(1-4): 598–605.
DOI: 10.1159/000084993

Shen, F., Xu, S., Shen, Q., Bi, C., & Lysak, M.A. (2023). The allotetraploid horseradish genome provides insights into subgenome diversification and formation of critical traits. *Nature Communication*, 14: 4102.
DOI: 10.1038/s41467-023-39800-y

Shi, M.-Z., & Xie, D.-Y. (2010). Features of anthocyanin biosynthesis in *pap*1-D and wild-type *Arabidopsis thaliana* plants grown in different light intensity and culture media conditions. *Planta*, 231(6): 1385–1400.
DOI: 10.1007/s00425-010-1142-9

Simoncsics, Pál (2017). Növénynevek magyarázó szótára /*Dictionary of plant names/. Tilia* XVIII. *Ed.* by Bartha, D., & Gyulai, G., *Language Lector* Simoncsics, Péter. Szeged-Sopron-Gödöllő. LővérPrint Sopron. pp. 458. ISSN 1219–3003

Song, M., Zhang, Y., Jia, Q., +5, & Hu, S. (2023). Systematic analysis of MADS-box gene family in the U's triangle species and targeted mutagenesis of *BnaAG* homologs to explore its role in floral organ identity in *Brassica napus*. *Frontiers in Plant Science*, 13:1115513.
DOI: 10.3389/fpls.2022.1115513

Soó, R. (1965). Fejlődéstörténeti növényrendszertan (*'Evolutionary plant taxonomy'*). Tankönyvkiadó, Budapest, pp. 560. ISBN 2399973334235

Sommer, H., Beltrán, J.P., Huijser, P., Pape, H., Lönnig, W.E., Saedler, H., & S.-Sommer, Z. (1990). Deficiens, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. *The EMBO J.*, 9: 605–613.
DOI: 10.1002/j.1460-2075.1990.tb08152.x

Szabó, Z., Balogh, M., Domonkos, Á., Csányi, M., Kaló, P., & Kiss, Gy.B. (2023). The *bs5* allele of the susceptibility gene *Bs5* of pepper (*Capsicum annuum* L.) encoding a natural deletion variant of a CYSTM protein conditions resistance to bacterial spot disease caused by *Xanthomonas* species. *Theor and Appl Genetics*, 136(64): 1–16.
DOI: 10.1007/s00122-023-04340-y

Székely, D., & Máté, M. (2022). Red beetroot (*Beta vulgaris* L.). *Advances in Root Vegetables Research*, IntechOpen, pp. 1–22.
DOI: 10.5772/intechopen.106692

Su, T., Wang, W., Wang, Z., +9, & Yu, S. (2023). *Br*MYB108 confers resistance to *Verticillium* wilt by activating ROS generation in *Brassica rapa*. *Cell Reports*, 42(8): 112938.
DOI: 10.1016/j.celrep.2023.112938

Swaamy, K.R.M. (2023). Origin, distribution, genetic diversity and breeding of radish (*Raphanus sativus* L.). *International Journal of Development Research*, 13(02): 61657–61673.
DOI: 10.37118/ijdr.26289.02.2023

Tanaka, H., Hori, T., Yamamoto, S., Toyosa,A., Yano, K., Yamane, K., & Itoh, T. (2023). Haplotype-resolved chromosomal-level assembly of wasabi (*Eutrema japonicum*) genome. *Scientific Data*, 10: 441.
DOI: 10.1038/s41597-023-02356-z

Toldi, O., Gyulai, G., Kiss, J., Tamás, I.A., & Balázs, E. (1996). Antiauxin enhanced microshoot initiation and plant regeneration from epicotyl-originated thin-layer explants of sugarbeet (*Beta vulgaris* L.). *Plant Cell Report***,** 15: 851–854.
DOI: 10.1007/BF00233155

Tóth, Z., Gyulai, G., Horváth, L., Szabó, Z., & Heszky, L. (2007). Watermelon (*Citrullus l. lanatus*) production in Hungary from the Middle Ages. *Hungarian Agricultural Research***,** 2007/4: 14–19.

Xie, M., Zuo, R., Bai, Z., +8, & Liu, S. (2022). Genome-wide characterization of serine/arginine-rich gene family and its genetic effects on agronomic traits of *Brassica napus*. *Frontiers in Plant Science*, 13: 829668.
DOI: 10.3389/fpls.2022.829668

Xie, T., Zan, X., Chen, X., Zhu, H., Rong, H., Wang, Y., & Jiang, J. (2022). An R3-MYB repressor, *Bn*CPC forms a feedback regulation with MBW complex to modulate anthocyanin biosynthesis in *Brassica napus*. *Biotechnology for Biofuels and Bioproducts*, 15(1): 133.
DOI: 10.1186/s13068-022-02227-6

Yim, W.C., Swain, M.L., Ma, D., +16, & Zhang, X. (2022). The final piece of the triangle of U: Evolution of the tetraploid *Brassica carinata* genome. *Plant Cell*, 34(11): 4143–4172.
DOI: 10.1093/plcell/koac249

Yin, L., Zhu, Z., Luo, X., +12, & Fu, S. (2020). Genome-wide duplication of allotetraploid *Brassica napus* produces novel characteristics and extensive ploidy variation in self-pollinated progeny. *G3 Genes Genomes Genetics*, 10: 3687–3699.
DOI: 10.1534/g3.120.401493

Jin, W., Jiang, Q., Zhao, H., Su, F., Li, Y., & Yang, S. (2024). Transcriptome analysis of ethylene-related genes in chlorine dioxide-treated fresh-cut cauliflower. *Genes* (Basel), 15(8): 1102.
DOI: 10.3390/genes15081102

Zhang, L., Wu, S., Chang, X., +7, & Chen, F. (2020). The ancient wave of polyploidization events in flowering plants and their facilitated adaptation to environmental stress. *Plant Cell* & *Environment*, 43: 2847–2856.
DOI: 10.1111/pce.13898

Zhang, Y., Wang, G.J., Li, L.X., Li, Y.H., Zhou, B., & Yan, H.F. (2020). Identification and expression analysis of *Br*TT8 during anthocyanin biosynthesis and exposure to abiotic stress in turnip (*Brassica rapa* subsp. *rapa* '*Tsuda*'). *Scientia Horticulturae*, 268:109332.
DOI: 10.1016/j.scienta.2020.109332

Zhang, K., Mason, A.S., +6, & Zhou, W. (2021) Challenges and prospects for a potential allohexaploid *Brassica* crop. *Theoretical and Applied Genetics*,134(9): 2711–2726.
DOI: 10.1007/s00122-021-03845-8

Zierer, W., Rüscher, D., Sonnewald, U., & Sonnewald, S. (2021). Tuber and tuberous root development. *Annual Review of Plant Biology*, 72 (1). Annual Reviews: 551–580.
DOI: 10.1146/annurev-arplant-080720-084456

Walker, E.L., Eggleston, W.B., Demopulos, D., Kermicle, J., & Dellaporta, S.L. (1997). Insertions of a novel class of transposable elements with a strong target site preference at the r locus of maize. *Genetics*, 146(2): 681–93.
DOI: 10.1093/genetics/146.2.681

Wang, F., Li, L., Liu, L., Li, H., Zhang, Y., Yao, Y., Ni, Z., & Gao, J. (2012). High-throughput sequencing discovery of conserved and novel microRNAs in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Molecular Genetics and Genomics*, 287: 555–563.
DOI: 10.1007/s00438-012-0699-3

Wang, J., Yu, J., Sun, P., +19, & Wang, X. (2020). Paleo-polyploidization in Lycophytes. *Genomics Proteomics Bioinformatics*, 18: 333–340.
DOI: 10.1016/j.gpb.2020.10.002

Wang, W., Celton, J.-M., Buck-Sorlin, G., Balzergue, S., Bucher, E., & Laurens, F. (2020). Skin color in apple fruit (*Malus* × *domestica*): genetic and epigenetic insights. *Epigenomes*, 4(3), 13. pp. 18.
DOI: 10.3390/epigenomes4030013

Wang, W.-Q., Allan, A.C., & Yin, X.-R. (2020). Small RNAs with a big impact on horticultural traits. *Critical Reviews in Plant Sciences*, 39: 30–43.
DOI: 10.1080/07352689.2020.1741923

Wang, Y., Zhang, Y., Fan, C., Wei, Y., Meng, J., Li, Z., & Zhong, C. (2021). Genome-wide analysis of MYB transcription factors and their responses to salt stress in *Casuarina equisetifolia*. *BMC Plant Biol*ogy, 21: 328. pp.17.
DOI: 10.1186/s12870-021-03083-6

Wang, X., Zhao, S., Zhou, R., Liu, Y., Guo, L., & Hu, H. (2023). Identification of *Vitis vinifera* MYB transcription factors and their response against grapevine berry inner necrosis virus. *BMC Plant Biology*, 23: 279. pp.13.
DOI: 10.1186/s12870-023-04296-7

Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J.L., +8, & Schulman, A.H. (2007). A unified classification system for eukaryotic transposable elements. *Nature Reviews Genetics*, 8(12): 973–982.
DOI: 10.1038/nrg2165

Willcox, G. (2002). Charred plant remains from a 10th millenium B.P. kitchen at Jerf el Ahmar (Syria). *Vegetation History and Archaeobotany*, 11: 55–60.
DOI: 10.1007/s003340200006