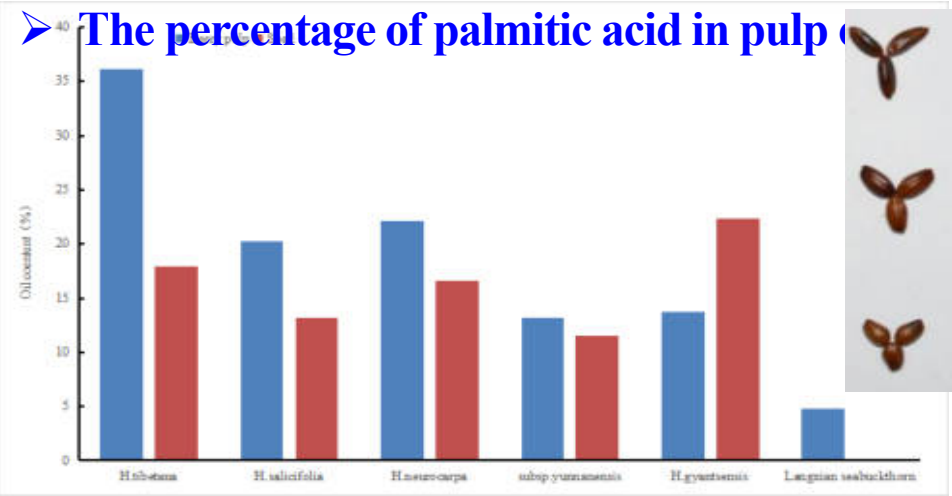
3. Formation mechanism of important traits in species of *Hippophae* genus

3.1 Important traits of species of *Hippophae* genus

- Seeds of sea buckthorn are very small, with low oil content (7-10%), which leads to seed oil yield is less than 52.5kg per ha.
- The percentage of palmitic acid in pulp o[illegible]n is up to 36%, which is not good for human health.



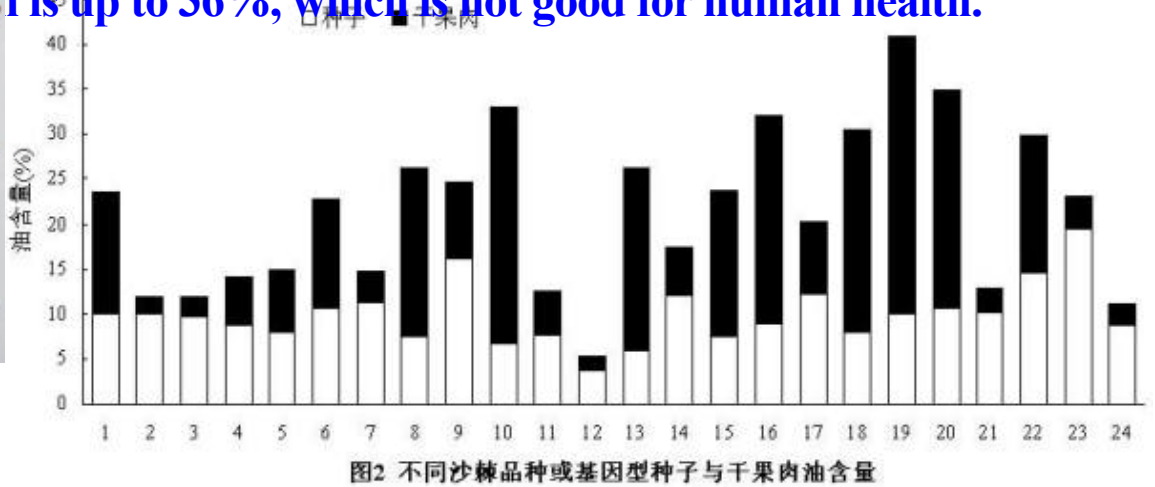
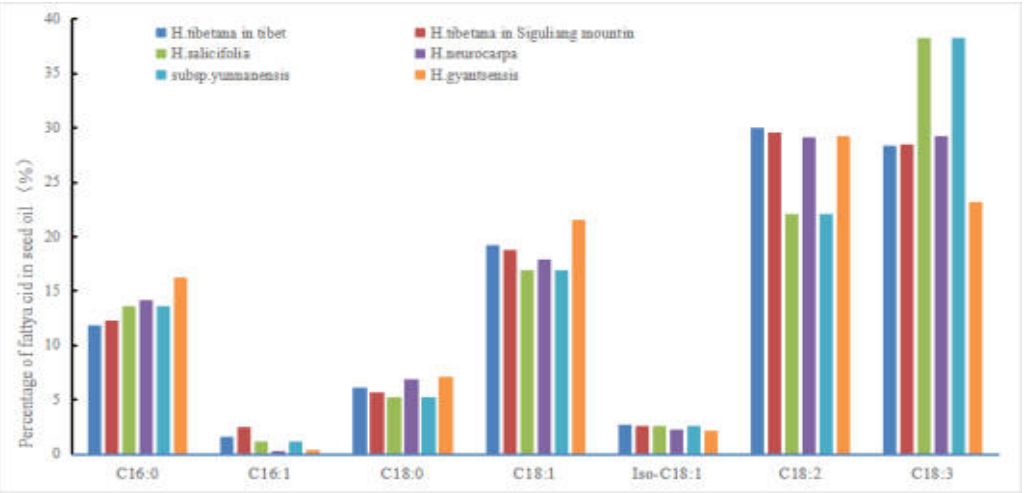
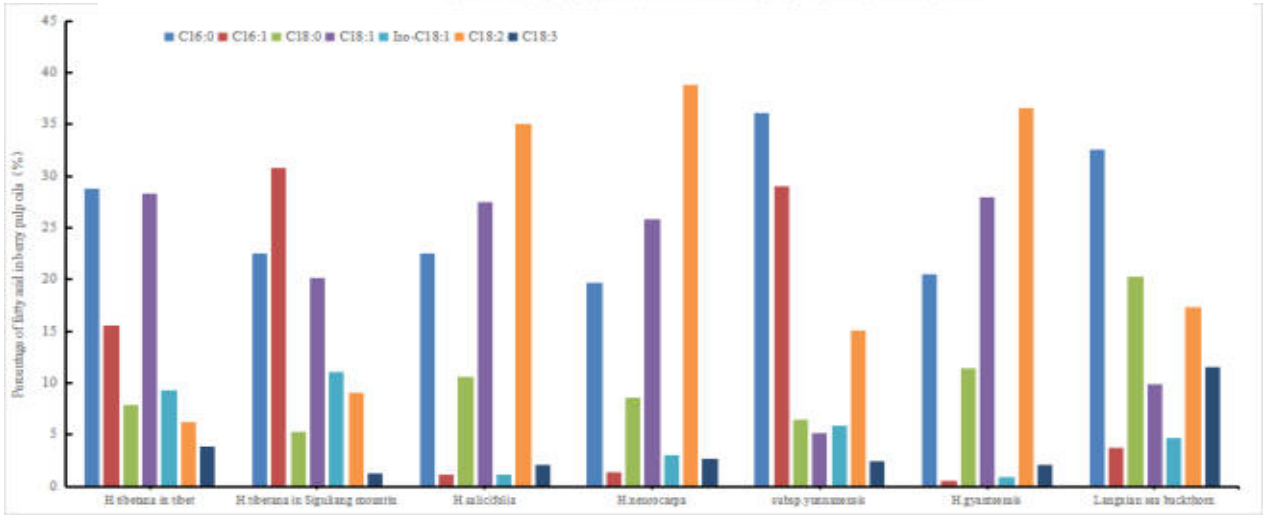


图2 不同沙棘品种或基因型种子与干果肉油含量

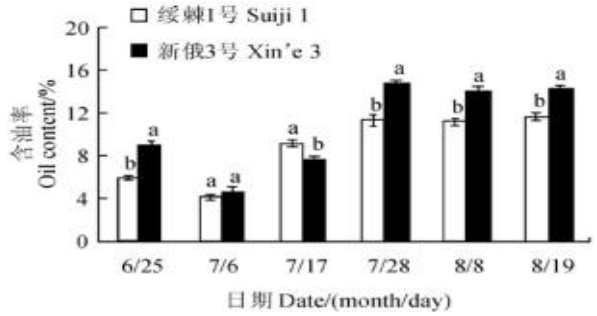


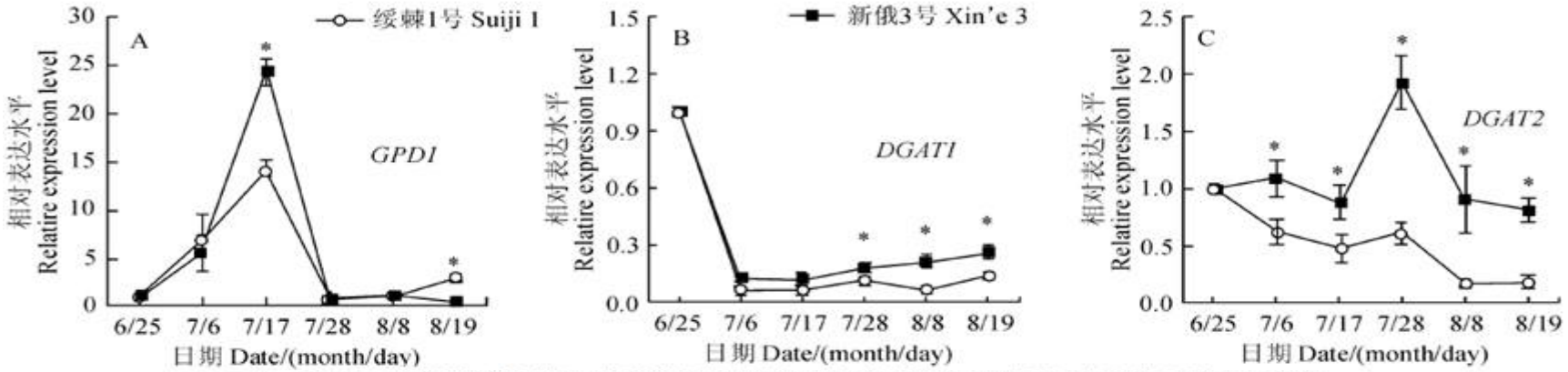


3. Formation mechanism of important traits in species of *Hippophae* genus

3.2 Co-regulation mechanism of source (*GPD1*) and pool (*DGAT*) genes for lipid biosynthesis in sea buckthorn seeds

High expression of *GPDl* gene occurred in the period of rapid lipid accumulation, which may speed up lipid biosynthesis in 'Xin'e 3' seeds through promoting the biosynthesis of glycerol-3-phosphate. The expression levels of *DGAT1* and *DGAT2* genes in line 'Xin'e 3' were higher than that line 'Suiji 1' during lipid accumulation.





* indicates significant differences of gene relative expression level between the two lines on the same harvest time at the level of 0.05

Fig. 3 Differences of *GPD*1, *DGAT*1 and *DGAT*2 genes expression in seeds between the line 'Suiji 1' and 'Xin'e 3'

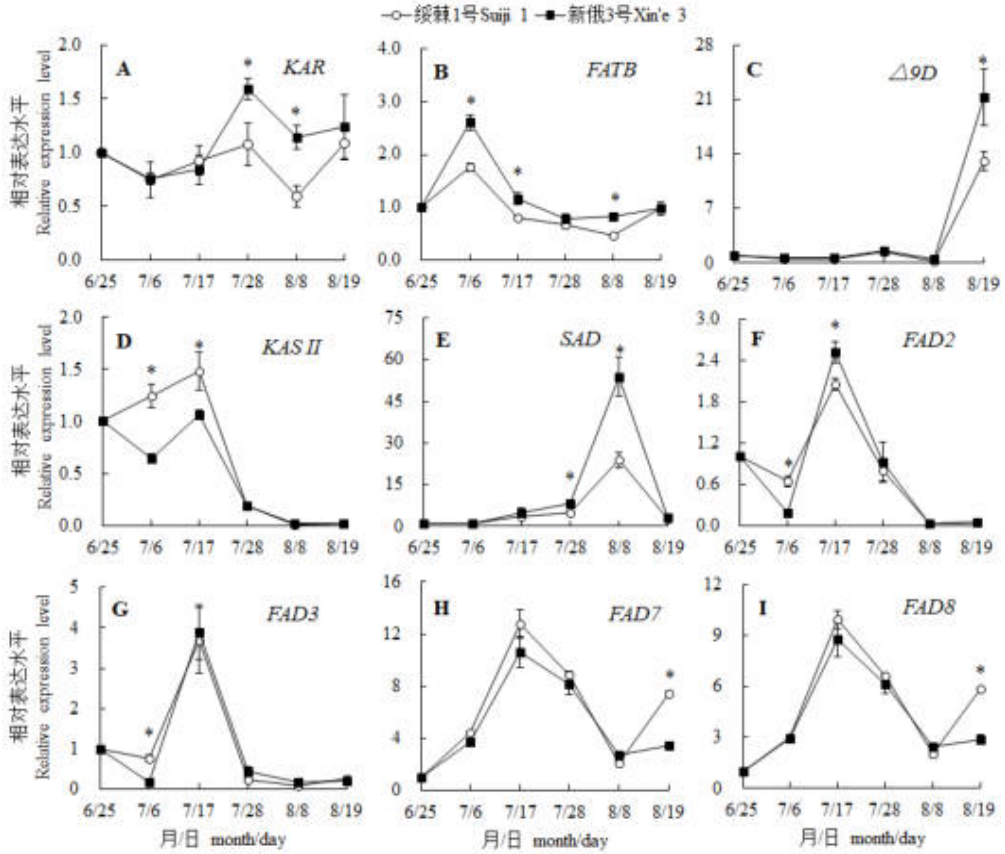
Ding *et al.Acta Bot. Boreal. -Occident. Sin.*, 2016, 102., 36(8): 1642-1647

3. Formation mechanism of important traits in species of *Hippophae* genus

3.3 Multi gene coordinated regulation of high accumulation of C18 unsaturated fatty acids in sea buckthorn seeds

High accumulation of C18 unsaturated fatty acid in sea buckthorn seed oils originates from low expression of *FATB* and *Δ 9D* genes and high co-expression of *KAR*, *KASII*, *SAD*, *FAD2*, *FAD3*, *FAD7* and *FAD8* genes.

- The down-regulation expression of *FATB* coordinated with the low expression of *Δ9D* genes decreased the metabolic biosynthesis of palmitic and palmitoleic acids transformed from C16:0-ACP. Relatively high expressions of *KAR* and KASII genes improved the biosynthesis of stearic acid, which provided more precursors for the synthesis of carbon eighteen unsaturated fatty acids.
- The continuous high expression of *SAD* gene catalyses stearic acid desaturation to synthesize oleic acid and the continuous increased radio of *SAD* to *FATB* gene expressions directly improved the fatty acid desaturation rate.
- The expression peaks of *FAD2*, *FAD3* , *FAD7* and *FAD8* genes simultaneously appeared in the periods of rapid biosynthesis of linoleic and linolenic acids and then the oleic acid was gradually desaturated to linoleic acid and linolenic acid.



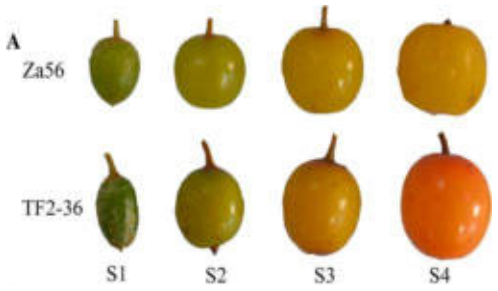
Ding *et al.* Acta Bot. Boreal. -Occident. Sin., 2017, 37(6): 1080-1089

3. Formation mechanism of important traits in species of *Hippophae* genus

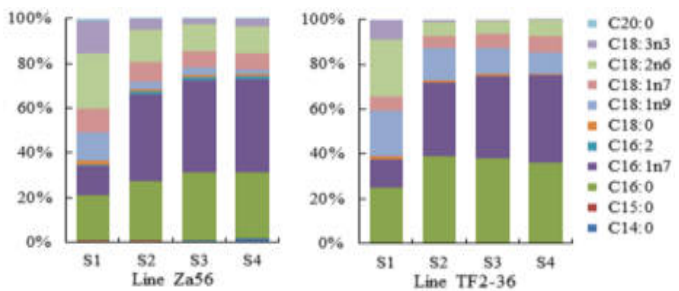
3.4 Identification of key genes involved in the high accumulation of palmitoleic acid and oil in sea buckthorn berry pulp

- The sustained high expression of *ACP-Δ9D* gene, which was related to oil, and the sustained low expression of *KASII* and *FAE1* genes, contributed to the enrichment of C16:1 fatty acids pulp.

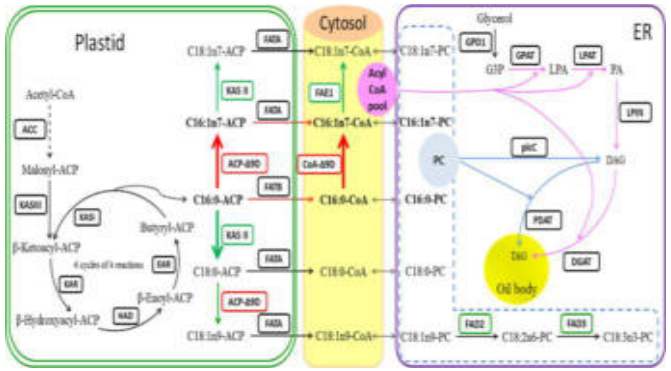
- **Non target metabonomics showed G3P, *GPD1* and *Δ 9D* contributes to high oil content and high palmitolic acid content of sea buckthorn pulp**



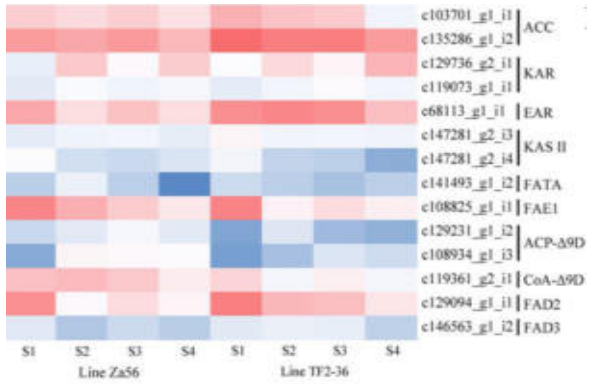
Fruits of different developmental stages



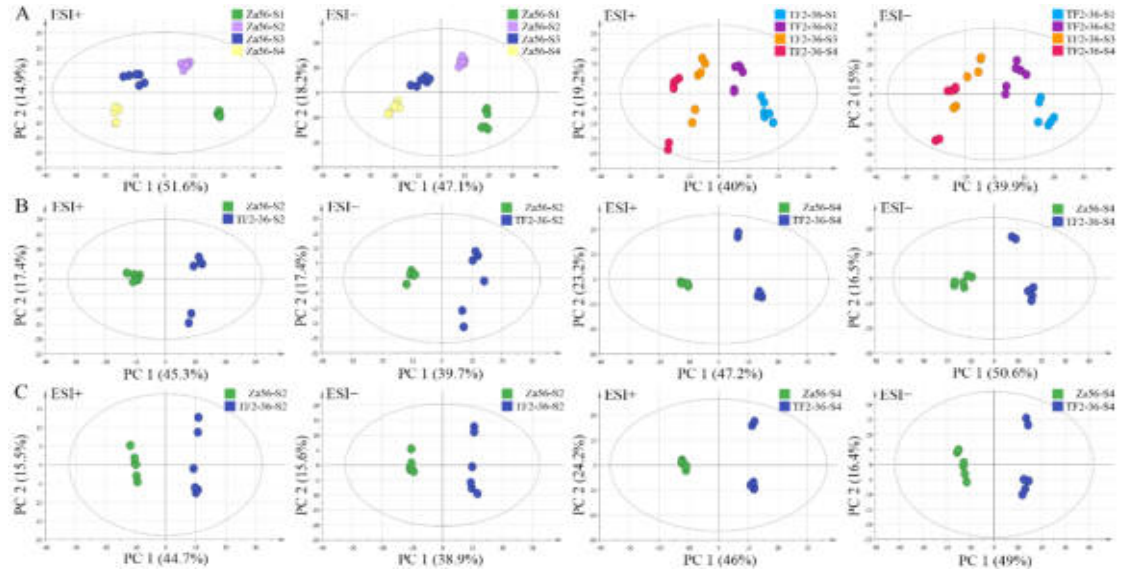
Fatty acid components of different developing pulp

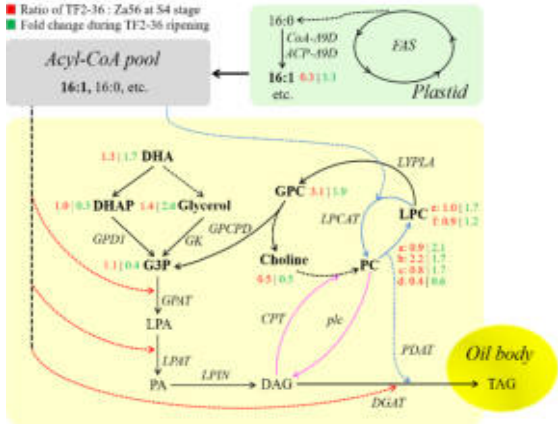


Oil synthesis and fatty acid metabolism pathway in sea buckthorn pulp



Heat map of gene expression related to fatty acid formation and accumulation in berry pulp





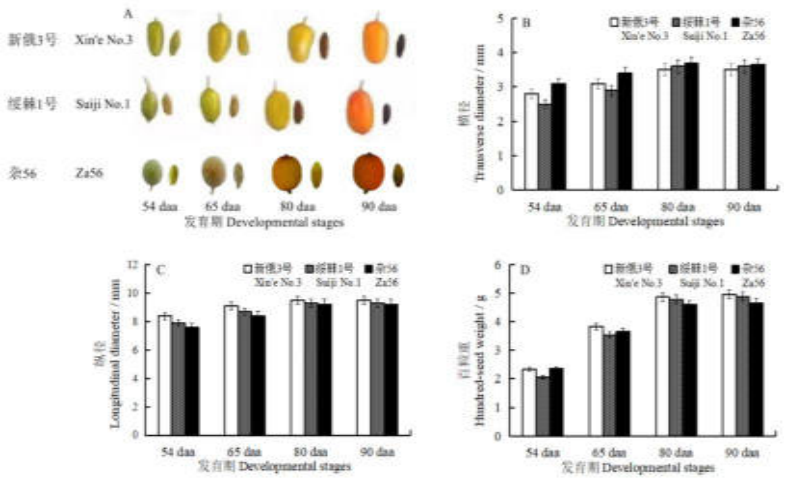
(Jian Ding, **Chengjiang Ruan***, Ying Guan, He Li, Wei Du, Shuaguang Lu, Xiufeng Wen, Ke Tang, Ye Chen. **2022**. Nontargeted metabolomic and multigene expression analyses reveal the mechanism of oil biosynthesis in sea buckthorn berry pulp rich in palmitoleic acid. ***Food Chemistry***, 374 : 131719)

(Jian Ding, Chengjiang Ruan*, Wei Du, Ying Guan. RNA-seq data reveals a coordinated regulation mechanism of multigenes involved in the high accumulation of palmitoleic acid and oil in sea buckthorn berry pulp. *BMC Plant Biology*, 2019, 19: 207)

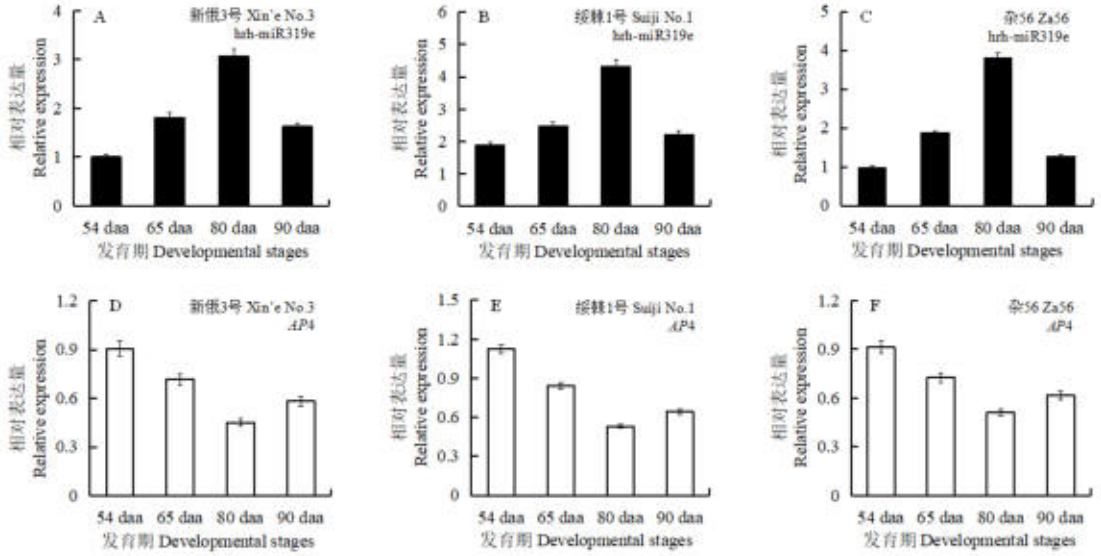
3. Formation mechanism of important traits in species of *Hippophae* genus

3.5 Sea buckthorn hrh-miR319e targeting *AP4* gene regulates seed development

- The results shows that *AP*4-3′UTR region and miR319e seed sequence were completely complementary. The vector of PCDNA3.1-hrh-miR319e+PGLOmir-AP4 can significantly inhibit the luciferase activity ($p < 0.001$).
- In developing seeds, the changes of hrh-miR319e expression level showed a trend of increasing at first and then decreasing, while the changes of *AP*4 expression level first decreased and then increased. When hrh-miR319e expression reached its highest level at 80 daa (days after anthesis) (‘Xin’e 3’—3.146, ‘Suiji 1’—4.298 and ‘Za 56’—3.892), the expression level of *AP*4 had the lowest value (‘Xin’e 3’—0.427, ‘Suiji 1’—0.526 and ‘Za 56’—0.451).
- Thus, sea buckthorn hrh-miR319e could target the 3′UTR region of *AP*4, there is negatively regulative relationship between them.



Morphological character of sea buckthorn fruits and seeds (A) and transverse (B) and longitudinal (C) diameters and hundred-seed weights (D) of seeds in different developmental stages



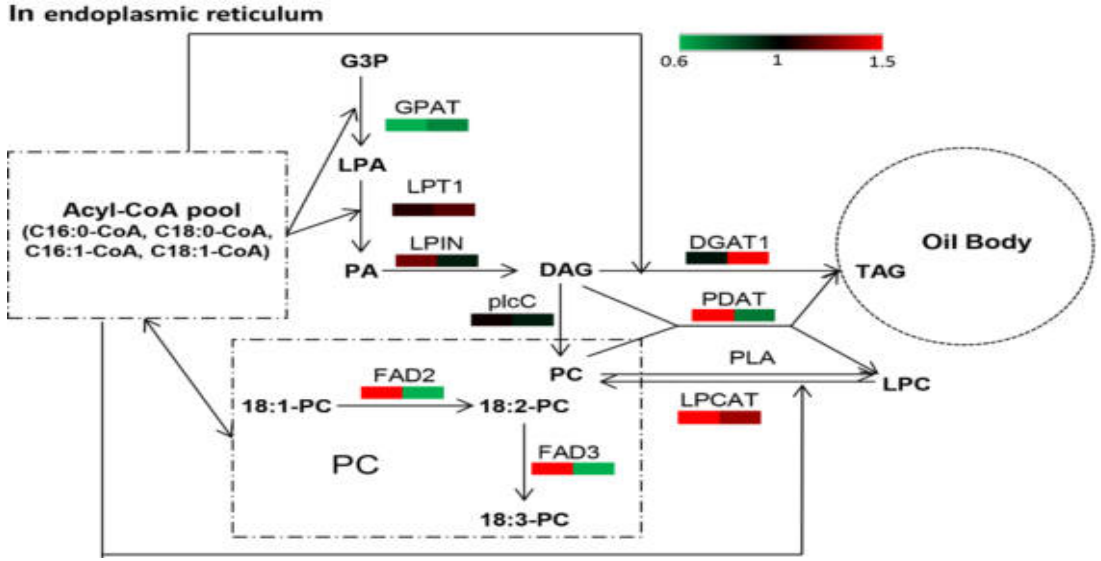
Changes of expression levels of hrh-miR319 and *AP*4 of sea buckthorn seeds at different developmental stages

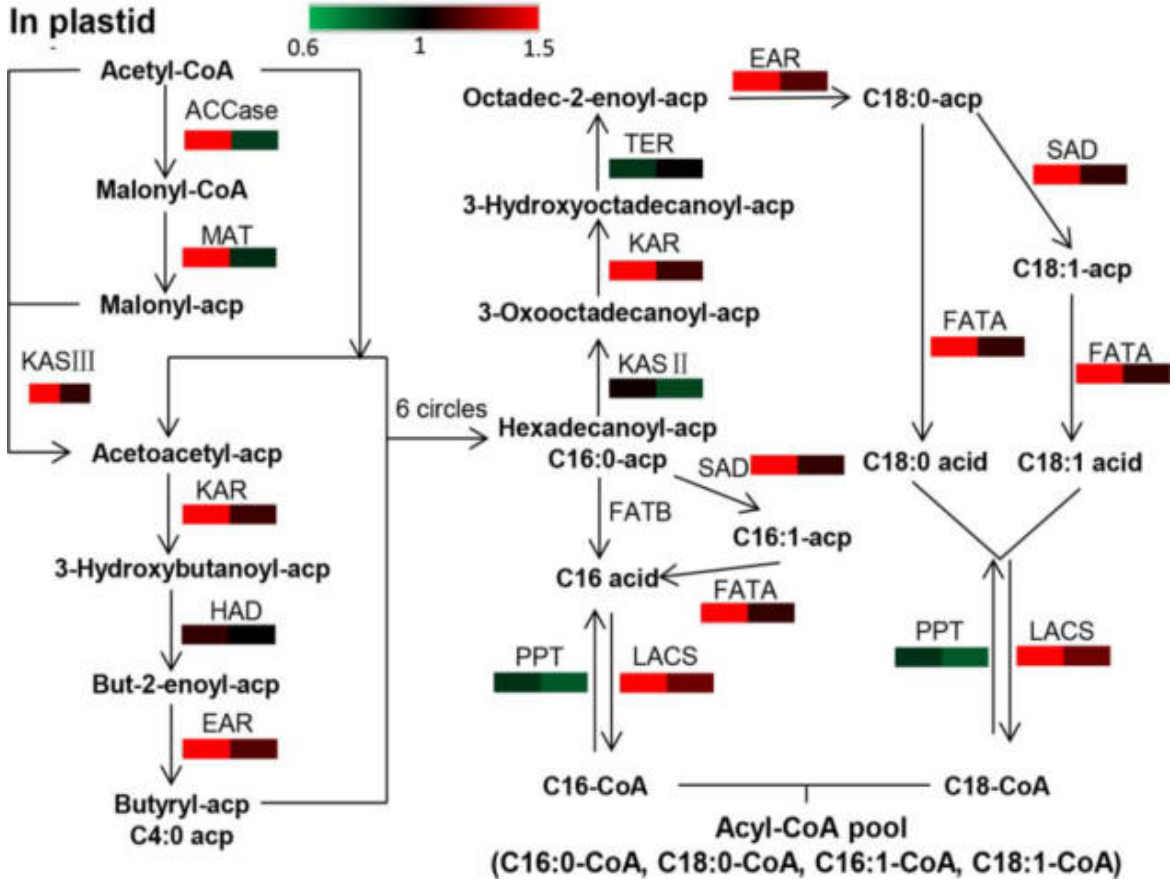
Yu et al. Chinese Bulletin of Botany 2022, **57** (5): 635–648

3. Formation mechanism of important traits in species of *Hippophae* genus

3.6 Proteomics identified several key enzymes for lipid metabolism in sea buckthorn

- Deep analysis results for the proteins identified and related pathways revealed initial fatty acid accumulation during whole-berry development.
- In the TAG metabolic pathway, **the effect of PDAT is more obvious in the early stage of fruit development**, which is conducive to increasing the proportion of unsaturated fat acids in fruit TAG, and **the DGAT is significantly increased in the late stage of development**, which is conducive to completing the transformation of fatty acids and intermediates to triglycerides.





Du Wei, Xiong Chao-Wei, Ding Jian, Nybom Hilde, Ruan Cheng-Jiang *, Guo Hai. TMT-based Quantitative Proteomics of Developing Sea Buckthorn Berries Reveals Candidate Proteins Related to Lipid Metabolism. *Journal of Proteome Research*, 2019, 18(5): 1958-1969

3. Formation mechanism of important traits in species of *Hippophae* genus

3.7 Key flavonoid and lipid synthesis proteins in the sea buckthorn pulp

- In the flavonoid synthesis pathway, CHS and F3H were the main enzymes responsible for the difference in flavonoid content between the two cultivars.
- In the lipid synthesis pathway, LPIN, plcC and MGD were the main enzymes with different contents in the middle to late stages. Higher contents of LPIN and plcC in XE than in SJ could cause DAG to generate TAG from PC, since the difference in DGAT between the two cultivars was not significant.

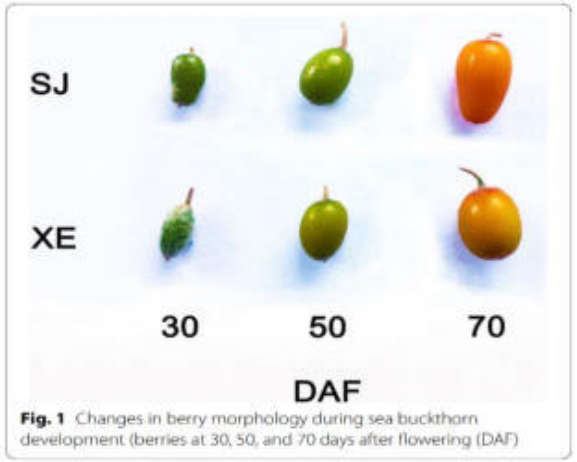


Fig. 1 Changes in berry morphology during sea buckthorn development (berries at 30, 50, and 70 days after flowering (DAF)

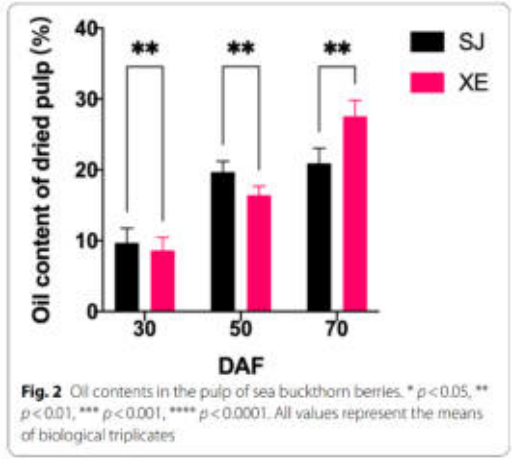


Fig. 2 Oil contents in the pulp of sea buckthorn berries. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. All values represent the means of biological triplicates

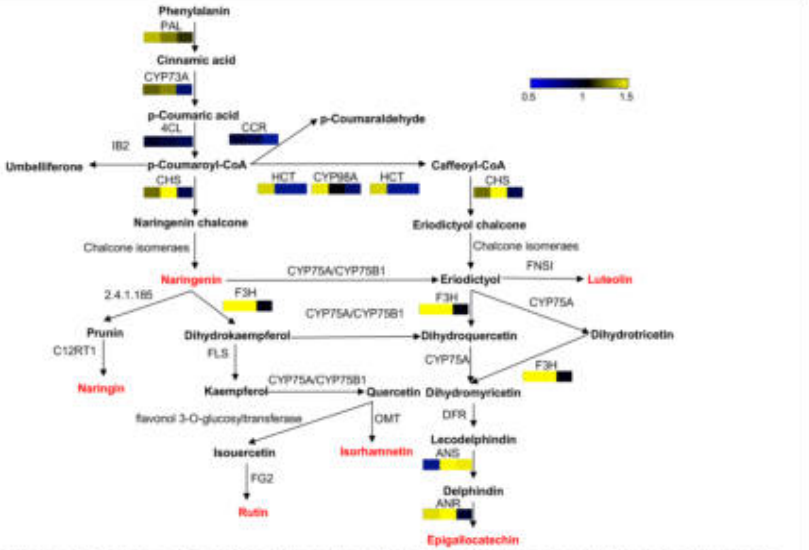


Fig. 6 Comparison of key proteins in the flavonoid synthesis pathway between SJ and XE at different developmental stages. The pattern under each enzyme is representative of the ratio of the protein content between SJ and XE cultivars. Left: XE 30 DAF/SJ 30 DAF, middle XE 50 DAF/SJ 50 DAF, right: XE 70 DAF/SJ 70 DAF. This graph was modified from a KEGG map (ko00940, ko00941 and ko00944) [23]

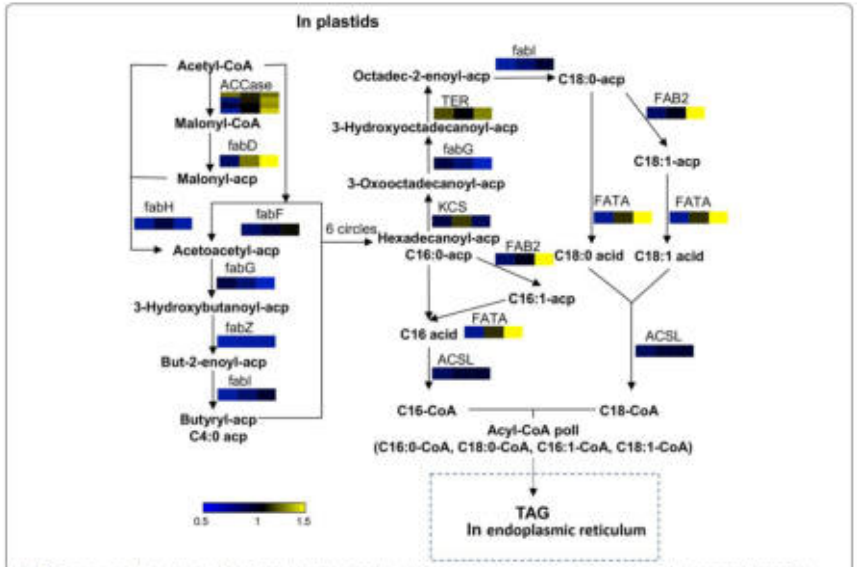
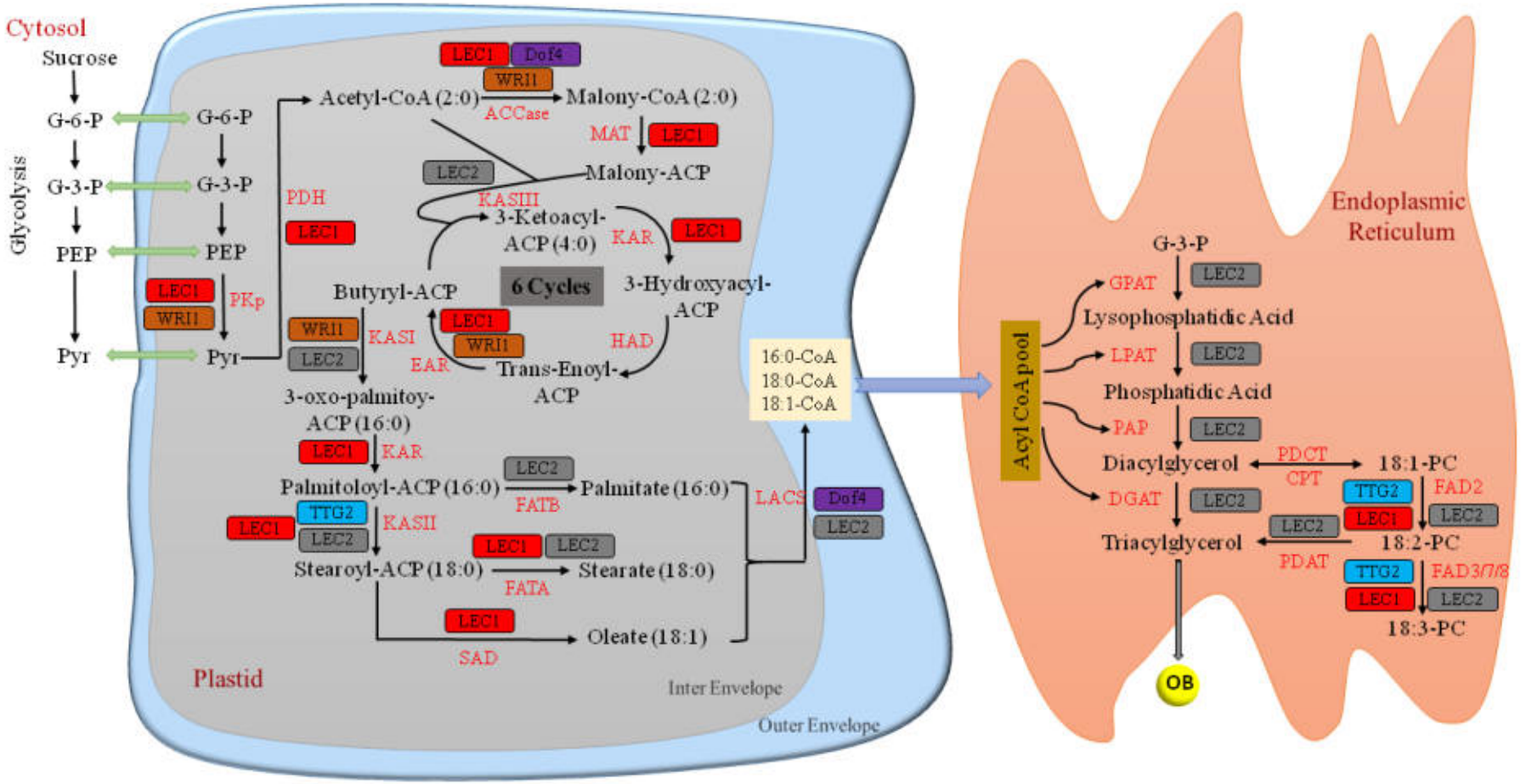


Fig. 7 Comparison of key proteins in the fatty acid synthesis pathway between SJ and XE cultivars at different berry developmental stages. The pattern under each enzyme is representative of the ratio of the protein content between SJ and XE cultivars. Left: XE 30 DAF/SJ 30 DAF, middle XE 50 DAF/SJ 50 DAF, right: XE 70 DAF/SJ 70 DAF. This graph was modified from a KEGG map (ko00061) [23]

Wei Du, Jian Ding, Shunguang Lu, Xiufeng Wen, Jianzhong Hu and **Chengjiang Ruan***. Identification of the key favonoid and lipid synthesis proteins in the pulp of two sea buckthorn cultivars at different developmental stages. ***BMC Plant Biology***, 2022, 22: 299

3. Formation mechanism of important traits in species of *Hippophae* genus

3.8 Identified transcription factor regulating key gene expression in lipid biosynthesis of sea buckthorn



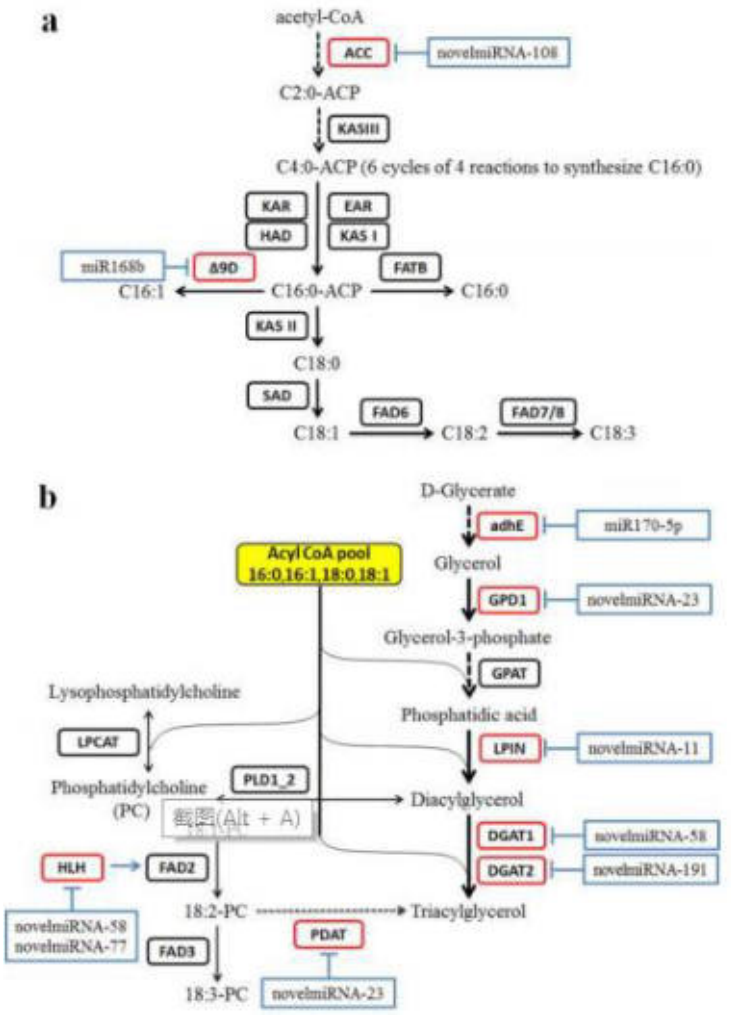
Comparing transcriptomics and co-expression analysis showed main transcription factors involved in regulating lipid biosynthesis in sea buckthorn seeds, such as ABI3, ABI4, AGL15, WRI1, FUS3, SWEET, Dof4, WRKY6, GRF8, etc.

3. Formation mechanism of important traits in species of *Hippophae* genus

3.9 Identification of miRNA regulating expression of key genes involving in lipid biosynthesis in sea buckthorn

- Screened 19 (14 known and 5 new) miRNAs involved in regulating the accumulation of sea buckthorn oil synthesis, including miR164d-*ARF2*, miR168b-*Δ9D*, novelmiRNA-108-*ACC*, novelmiRNA-23-*GPD*1, novelmiRNA-58-*DGAT1*和 novelmiRNA-191-*DGAT2*。

KEGG Pathway	miRNA name	Target ID	Annotation for targets	Gene name
Fatty acid biosynthesis	novelmiRNA-2	c141756_g2_i2	long-chain acyl-CoA synthetase	*ACSL*
	novelmiRNA-108	c103701_g1_i1	acetyl-CoA carboxylase carboxyl transferase	*ACC*
	novelmiRNA-110	c141756_g2_i2	long-chain acyl-CoA synthetase	*ACSL*
Fatty acid elongation	novelmiRNA-170	c168561_g1_i1	enoyl-CoA hydratase	*ECHS*
Fatty acid degradation	aly-miR170-5p	c60336_g1_i1	alcohol dehydrogenase	*adh*
	novelmiRNA-2	c141756_g2_i2	long-chain acyl-CoA synthetase	*ACSL*
	novelmiRNA-110	c141756_g2_i2	long-chain acyl-CoA synthetase	*ACSL*
	novelmiRNA-170	c168561_g1_i1	enoyl-CoA hydratase	*ECHS*
Biosynthesis of unsaturated fatty acids	gma-miR168b	c119361_g2_i1	delta-9-desaturase	*Δ9D*
	novelmiRNA-58	c145891_g1_i4	helix loop helix transcription factor	*HLH*
	novelmiRNA-77	c142283_g2_i1	helix loop helix transcription factor	*HLH*
Glycerolipid metabolism	gma-miR164d	c154991_g1_i1	dihydroxyacetone kinase	*DAK*
	novelmiRNA-11	c192054_g1_i1	phosphatidate phosphatase	*LPIN*
	novelmiRNA-23	c133634_g3_i5	phospholipid:diacylglycerol acyltransferase	*PDAT*
	novelmiRNA-58	c144982_g1_i2	diacylglycerol O-acyltransferase 1	*DGAT1*
	novelmiRNA-191	c220405_g1_i1	diacylglycerol O-acyltransferase 2	*DGAT2*
Glycero- phospholipid metabolism	novelmiRNA-11	c192054_g1_i1	phosphatidate phosphatase	*LPIN*
	novelmiRNA-23	c138230_g1_i3	glycerol-3-phosphate dehydrogenase	*GPD1*
	novelmiRNA-64	c176644_g1_i1	lysophospholipid hydrolase	*NTE*
Steroid biosynthesis	novelmiRNA-10	c81203_g2_i1	cycloartenol synthase	*CAS*
	novelmiRNA-58	c131674_g1_i1	sterol-4-alpha-methyl oxidase	*SMO2*
	novelmiRNA-58	c144982_g1_i3	sterol O-acyltransferase	*SOAT*
	novelmiRNA-179	c81203_g2_i1	cycloartenol synthase	*CAS*
	novelmiRNA-224	c137936_g1_i1	cycloartenol synthase	*CAS*
	novelmiRNA-224	c137936_g1_i2	lanosterol synthase	*LSS*
	novelmiRNA-232	c81203_g2_i1	cycloartenol synthase	*CAS*
Sphingolipid metabolism	zma-miR159i-3p	c262907_g1_i1	beta-galactosidase	*lacZ*
	novelmiRNA-23	c130447_g1_i3	neutral ceramidase	*ASAH2*
	novelmiRNA-108	c167130_g1_i1	sphingolipid delta-4 desaturase	*DEGS*
	novelmiRNA-151	c209847_g1_i1	beta-galactosidase	*GLB1*
	novelmiRNA-170	c199679_g1_i1	sphingomyelin phosphodiesterase 2	*SMPD2*

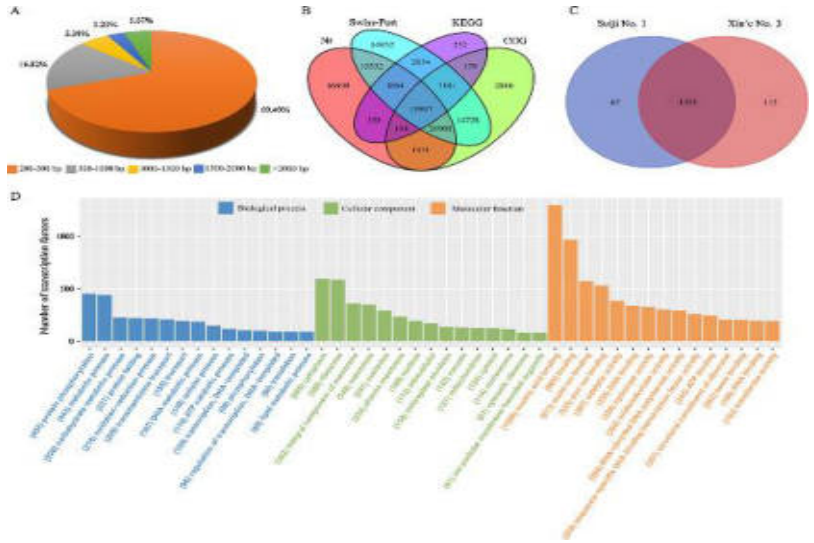


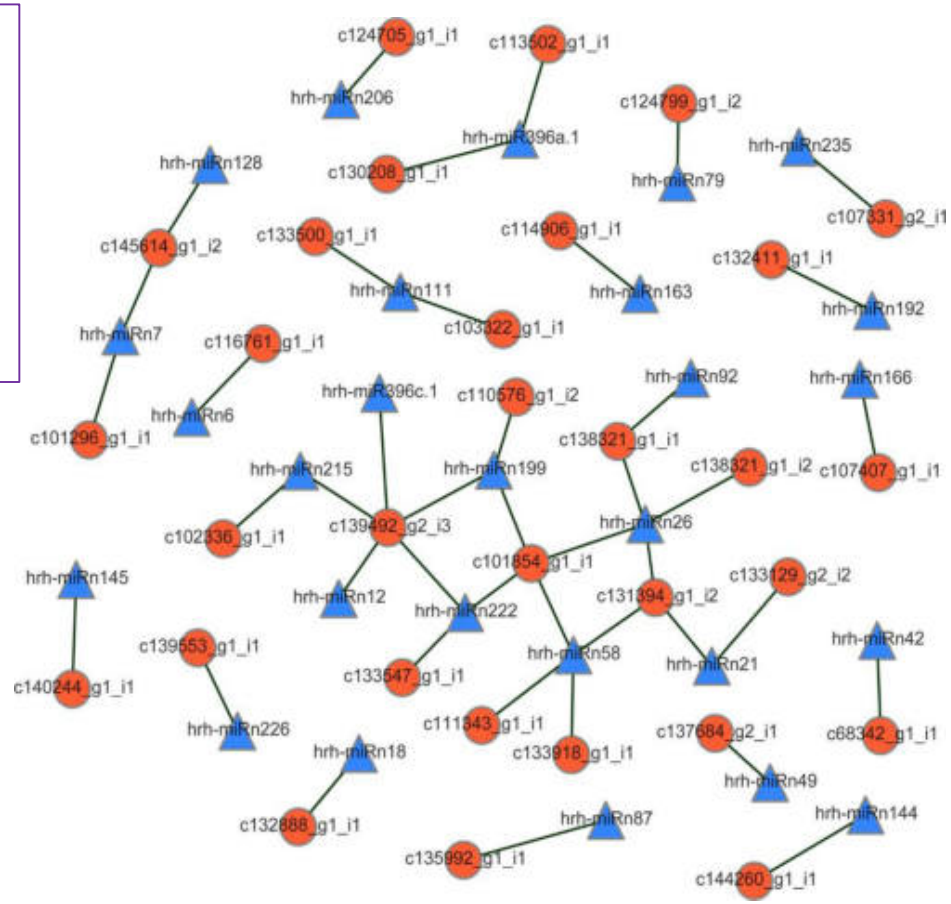
（Jian Ding, Chengjiang Ruan*, Ying Guan, Priti Krishna. Identification of microRNAs involved in lipid biosynthesis and seed size in developing sea buckthorn seeds using high-throughput sequencing. Scientific Reports, 2018, 8：4022）

3. Formation mechanism of important traits in species of *Hippophae* genus

3.10 Construction of regulatory network among miRNAs and TFs during developing seeds

- **Multiple miRNAs targeting TFs, including targeting TFs involving in lipid bio synthesis, such as** hrh-miRn215-*WRI1* (c102336_g1_i1)、miRn19- *ABI4* (c132411_g1_i1) and hrh-miRn79-*FUS3* (c124799_g1_i2).





(Jingbin Li, Jian Ding, Xue Yu, He Li, **Chengjiang Ruan***. Identification and expression analysis of critical microRNA-transcription factor regulatory modules related to seed development and oil accumulation in developing *Hippophae rhamnoides* seeds. ***Industrial Crops and Products***, 2019, 137: 33-42)

3. Formation mechanism of important traits in species of *Hippophae* genus

3.11 LncRNAs involved in the oil synthesis of different organs of sea buckthorn

- The whole transcriptome association analysis found that four lncRNAs (LINC48098, LINC48300, LINC6093 and LINC9793) and two miRNAs (ath-miR172a and ath-miR858b) were involved in the regulation of 24 transcription factors (such as MYB, AP2/ERF and TCP family) that act on the formation of sea buckthorn fruit (Figure 2.23). Through transcription factor association analysis, a total of 5762 lncRNA miRNA TF modules were obtained, including LINC39069 ath miR157a-5p-SCL32, LINC35803 ath miR156i-BH094, LINC20980 ath miR398a-3p-IDM1, and LINC7158 novel_ 186-ARFI、LINC21206-ath-miR858a-ERF6、LINC45543-novel_ The 226-WRI11 module regulates sea buckthorn fruit.

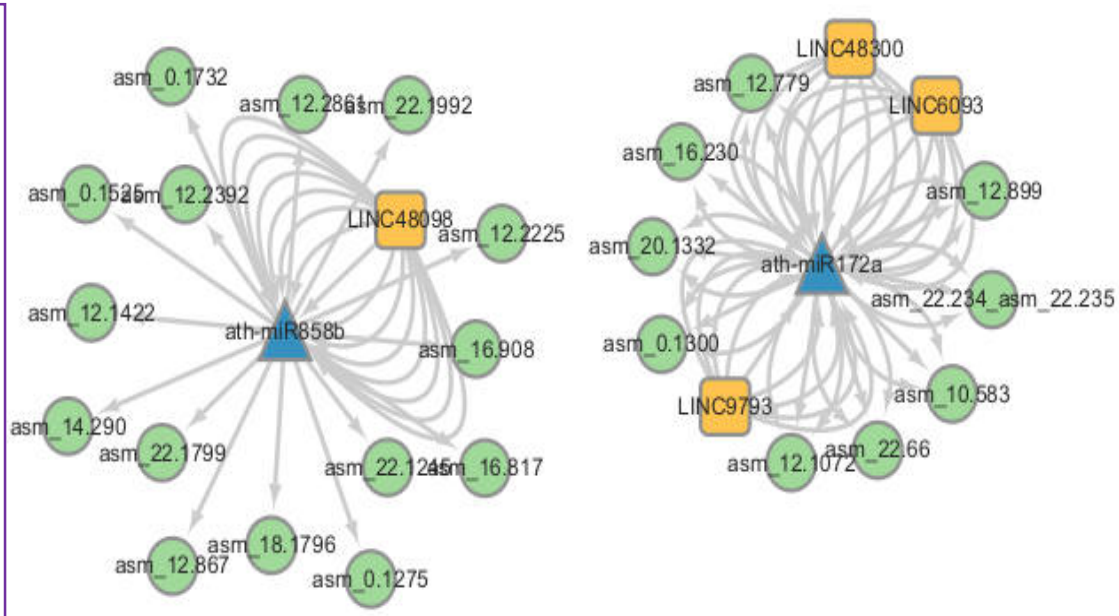


Figure 23 Regulation internet of lncRNA-miRNA-TF

Circles represent transcription factors; Triangle represents miRNA; Rectangle represents lncRNA

43 regulatory modules formated among 41 lncRNAs, 8 miRNAs, and 6 target genes are related to lipid biosynthesis. The same lncRNA-miRNA module can target multiple genes, such as LINC31812-novel_ 12 targets FATB, while LINC42952-ath-miR156i can also target FATB. Multiple functional modules related to oil synthesis and fatty acid formation and accumulation, such as LINC37656 targeting ath-miR396b-5p, LINC37732 targets ath-miR159c, LINC45223 targets ath-miR159c, Ath-miR396b-5p targeting *LACS6*, Ath-miR159c targets *FAD3*, etc.

4. Genetic improvement strategies of species in *Hippophae* genus

Improvement traits	Comparison species/subspecies
Big fruit, big seed, high grain weight	*H. tibetana*/ssp. *mongolica* vs. ssp. *sinensis*
High oil conent in seeds	*H. gyantsensis* /*H. tibetana*/*H. neurocarpa* vs. ssp. sinensis
High palmitoleic acid and low palmitic acid	ssp. sinensis vs. H. gyantsensis //
Tolerance to water logging	ssp. sinensis South of China vs. North of China; ssp. yunnanensis/H. tibetana/H. neurocarpa/H. salicifolia vs. ssp. mongolica
Tolerance to weather with high temperature and humid	ssp. mongolica/ssp. sinensis vs. ssp. yunnanensis/H. tibetana/H. neurocarpa/ H. salicifolia
Adaptation to high altitude	H. tibetana
Tolerance to drought	Different ecotypes of ssp. turkestanica

Future morden techniques for sea buckthorn breeding:

crossing, genetic transformation, gene editing……

5. Several interesting questions

① It is necessary to construct a super-pangenome of all species and subspecies in *Hippophe* genus ;

② How are species formed? Reproductive isolation, geographical isolation?

③ How hybridization forms new species？

④ How to adapt to high altitude？

⑤ How to successfully transplant high-altitude species to low altitude habitats？

⑥ How does climate change affect the population changes of seabuckthorn?

Welcome you visit my Lab.

Prof. Dr.
Chengjiang
Ruan

Associate
Prof. Dr.
He Li

Associate
Prof. Dr.
Jian Ding

Associate
Prof. Dr.
Jingbin Li

Associate
Prof. Dr.
Yuhui Hong

Engineering
Ms. Wei Du

Thank you for your attention!