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Advances in Molecular Techniques Used Flax Research in China

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Abstract

In this paper, the major achievements in the application of molecular methods in flax research in China are discussed. Some advanced biological technologies in flax breeding program are mentioned that were utilized widely in flax studies, which were also in employed in the fiber crop sector around the world. Special attention has been focused on the new results of flax disease investigations performed through molecular methods, particularly those related to powdery mildew and wilt. A conclusion was drawn that powdery mildew resistance is inherited via a single dominant gene. A label bank was made containing 20000 SAGE LABLE separating from cDNA, through SAGE analysis of genes resistant to powdery mildew. AFLP analysis was performed on wilt. Specific bands, AG/CAG and FuJ7(t), of the wilt resistance gene were found to be closely linked, with a genetic distance of 5.2 cm between them. The AG/CAG segments were recovered, cloned, sequenced, and successfully transformed into a SCAR marker, used for molecular detection and marker-assisted selection breeding. A flax genetic linkage map was constructed with 12 linkage groups. The results revealed that the markers on the map were distributed evenly, and the co-dominant markers in SRAP and SSR were more suitable for the construction of a genetic map in flax. All of the above findings have established a solid foundation for further flax research in China.

Keywords: Flax; Molecular; Biological technology; Diseases; Genetic map

Flax, which is widespread in the temperate zone, is used for the production of oil and fiber. The contents of unsaturated fatty acids in linseed seed can reach 45%; it is also rich in lignan, glue, vitamins A and B, etc., which makes its supplementations very valuable for human health. Flax fiber possesses air permeability, hygroscopicity, and antibacterial characteristics, which makes flax textile products exceedingly popular in the international market [1].

Flax textile was advanced in China, but more than 70% raw material needed to be imported, because flax growing lagged behind compared with France and some european country. The main reason was short of flax accessions, so we have not developed cultivars with high yield and resistance to diseases, recent years we introduced french flax variety. Now our modern varieties with narrow genetic back ground may be succeptible to certain flax major diseases, what happened in China have proved this, for example, powdery mildew, no cultivars were resistant to it completely.

Flax growing in China has a history of no more than 100 years, but only 20 years ago, crossing became was the main flax breeding method, but in the recent years, along with the development of biotechnology, advanced molecular techniques have been utilized , resulting in a quickly increased breeding level. Here, we introduce the achievements we have made in this field of flax research.

Biotechnology Utilization in Flax Breeding Programs

Flax gene transfer system

A flax gene transfer system was established by Agrobacterium tumefaciens – mediated method. According to the specific procedures, flax hypocotyl is used as an explant and MS medium as the optimal medium for flax transgenosis, with the addition of 50 mg/L kanamycin for selection pressure [2,3].

Callus is selected via passages through induction medium, regeneration culture medium, and rooting medium. After transferring the resistant genes and the gene responsible for the synthesis of cellulose synthase into the explants, GMO plants containing the target gene have been acquired [4,5].

In experiments conducted with the cultivars Heiya 11 and Heiya 9, Kang qinghua, obtained transformed callus containing the gene Bar which is responsible for herbicide resistance. Wangyufu transferred this gene into Heiya 11 and later confirmed through PCR that the gene had been integrated into flax genome . The resistance test evidenced that the transformed plants were resistant to the herbicide Basta [6].

Haploid utilization

(1) **Distant hybridization:** In a study, after employing cultivated flax as the male parent and wild flax as the female parent (*Limum grandiflorum* L.), inflate bolls containing green embryos without endosperm were obtained in only 30% crosses. Young embryos were peeled off at the optimal time (8–20 days after crossing) and were transferred onto MS culture medium [7]. At present, a number of lines have been obtained, and the breeding time was shortened to 4–5 years.

(2) Polyembryonic seed utilization: Haploid breeding is the main method in flax breeding programs. We are the first country to perform experiments on flax haploidization via anther culture. Each polyembryonic seed can produce at least two seedlings, one of which is commonly haploid, whereas the other is diploid. The aim of our earlier examination was to double the haploid one. A series of high-quality polyembryonic lines were obtained with good, with a polyembryonic rate of more than 10% [8].

(3) Anther and microspore culture: F1 plants were used as experimental material. When microspores were at the late-uninucleate stage, the buds were disinfected. Then, the culture media for callus

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induction and differentiation were selected. Further, rooting was induced, and plants were subsequently transplanted. Flax anther culture technique has been used for many years, but the technologically difficult point was how to improve transfer efficiency.

Exogenous DNA induction

Exogenous DNA induction work was to obtain DNA of proper concentration, purity, and length. Then, pollen tunnel technology was utilized, and DNA was injected into the ovary, to realize certain gene transfer. An exceedingly valuable variety, Heiya No.14, was developed by this method at the Institute of Industrial Crops of Heilongjiang Academy of Agricultural Sciences [9].

Transcriptome use in flax

Dr Yu used the method to conduct a study on flax tolerance to salt and alkaline soil. Salinization and alkalization of soil are widespread environmental problems, and alkaline salt stress is more destructive than neutral salt stress [10]. Therefore, understanding the mechanism of plant tolerance to saline-alkaline stress has become a major challenge. However, little attention has been paid to the mechanism of plant alkaline salt tolerance. In an investigation, the transcriptome method was used. Gene expression profiling of flax under alkaline-salt stress (AS2), neutral salt stress (NSS), and alkaline stress (AS) was analyzed by digital gene expression [11]. Three-week-old flax seedlings were placed in 25 mM Na_2CO_3 (pH 11.6) (AS2), 50 mM NaCl (NSS), and NaOH (pH 11.6) (AS) for 18 h. There were 7736, 1566, and 454 differentially expressed genes in AS2, NSS, and AS compared to CK, respectively. The GO category gene enrichment analysis revealed that photosynthesis was particularly affected in AS2, carbohydrate metabolism was particularly affected in NSS, and the response to biotic stimulus was particularly affected in AS. We also analyzed the expression pattern of five categories of genes, including transcription factors, signaling transduction proteins, phytohormones, reactive oxygen species proteins, and transporters under the action of these three stress conditions. Some key regulatory gene families involved in abiotic stress, such as WRKY, MAPKKK, ABA, and PrxR, as well as ion channels were differentially expressed. Compared with NSS and AS, AS2 triggered more differentially expressed genes and special pathways, indicating that the mechanism of AS2 was more complex than that of NSS and AS. To the best of our knowledge, this was the first transcriptome analysis of flax in response to saline-alkaline stress. These data indicate that common and diverse features of saline-alkaline stress provide novel insights into the molecular mechanisms of plant saline–alkaline tolerance and offer a number of candidate genes as potential markers of tolerance to this kind of stress exposure.

Molecular Research on Flax Diseases

Powdery mildew is a minor disease in west countries, but in China, all varieties introduced from west countries are susceptible to this disease.

A study on Inheridity of resistance to powdery mildew

Flax line 9801-1 which is resistant to powdery mildew and the susceptible cultivars Ilona, Venus, and Diane were used in an examination.

Line 9801-1was a natural mutant found in 1998 and derived through selection from the variety Heiya 11 during the period 1999–2004, finally led to the development of a resistant plant [12].

According to the analysis of data from the in the experimental period (1999-2004), Line the grade of resistance of line 9801-1 was 0, perform HR, ILONA, VENUS, DIANE were sensitive and 4 grade, disease occurrence rate 100%(HS) (Table 1).

Line 9801-1 was crossed with plants of the cultivars Ilona, Venus, and Diane. Then, the parents, F1, and F2 were sown in the field. Pathogenic bacterium was multiplied in the greenhouse during the fast growing period of flax plants. Further, the seedlings in the field were inoculated with conidia. Disease occurrence was investigated in the technical maturity period.

Reciprocal crossing was performed between 9801-1 and the cultivars Ilona, Venus, and Diane. The plants in F1 generation expressed HR, which indicated that the trait of resistance of line 9801-1 was completely dominant and was transferred via nucleus inheritance (Table 2).

In F2 generation, the ratio of resistant to susceptible plants was nearly 3:1. Therefore, after summarizing the performance in F1, we drew the conclusion that powdery mildew resistance should be a trait of dominant single gene inheritance.

Powdery mildew gene RAPD markers in Line 9801-1:

RAPD analysis was conducted employing the DNA mixed pool as a template constructed by F2 population and 240 random primers. There were amplified bands of 203 primers, others not, accounted for 15.4% of total number, obtained 1201 bands in mixing pool [13].

The average number of bands per primer was 5.9. Through 3-fold selection, only the primer opp02 (TCGGCACGCA) could amplify stable polymorphism in parents and pools and displayed a specific amplification pattern of 792bp, named opp02792. Further analysis showed that the marker was co-separated with the powdery mildew gene.

The specific fragments opp02792 were recycled and connected by the pMD18-T vector, transferred into *Escherichia coli* (*E. coli*) DH5. DNA was extracted from the recombinant plasmid. Through PCR detection and plasmid DNA double enzyme electrophoresis, it was found that the specific fragments opp02792 have been transferred into the *E. coli* bacteria.

HR	No symptom	0
R	No scrab on leaves, white powder distributed	1
M	No scrab on leaves, thin white powder distributed	2
S	Sporadic scrab , thicker white powdery material	3
HS	More scrab on leaves, thicker white powdery material, leaves begin to become yellow and necrosis	4

HR: high resistance, R: resistance, M: medium resistance, S: succeptible , HS: high succeptible.

Table 1: Resistance standard.

No.	F_2	(R)	(S)	R: S
1	YK0501 (short)	858	269	3.2: 1
2	YK0501 (tall)	728	240	3.03: 1
3	YK0502 (short)	1150	289	3.98: 1
4	YK0502 (tall)	582	165	3.53: 1
5	YK0503	583	180	3.34: 1
6	YK0504	1135	367	3.09: 1
7	YK0505	1228	457	2.69: 1
8	YK0506	936	289	3.24: 1

R: resistance, S: succeptible, R:S: resistance:succeptible.

Table 2: Resistance data in F_2.

SAGE analysis of gene responsible for resistance to powdery mildew:

The whole RNA repeat was extracted, and the optimal method was developed. The concentration, purity, and integrity met the SAGE test standard. An oligo-nucleotide sequence of specific length (21 bp) was used to represent each transcript in a transfer system with highly specific I-SAGETM LONGKIT. Made a label bank containing 20000 SAGE LABLE separating from cDNA. The results will be used together with SAGE map to compare data from different sources in expression database. The reserve gene expresses data for analysis of gene expression difference resistant to powdery mildew.

Molecular markers for flax wilt resistance gene

Flax wilt disease is one of the main flax diseases worldwide, caused by *Fusarium oxysporum* linen [14]. The research work on molecular markers related to genes responsible for resistance to high wilt disease, not only contribute to the development of the work on wilt resistance marker-assisted selection and improve the efficiency of flax disease resistance breeding, but also lay the foundation for the isolation and cloning of wilt resistance genes.

In China, the flax wilt disease resistance genes FuJ7(t) was analyzed through AFLP markers by Dr. Bo, who crossed as parents the highly resistant to wilt flax variety 'Jin Ya 7' and the highly susceptible to wilt variety 'Jin Ya 1' [15,16]. F1 and F2 reciprocal cross segregation population were identified by inoculation. It was found that 'Jin Ya 7' was controlled by two dominant genes for wilt resistance; thus, it was transferred via nucleus inheridity [17].

AFLP analysis with 48 primers was conducted on the resistance and susceptibility gene pool in F2 population and their parents ('Jin Ya 7' and 'Jin Ya 1'). There were three stable differences in the totally amplified identifiable bands (approximately 3300). These three specific bands and the target gene linkage relationships were analyzed using F2 segregation population.

The specific bands AG/CAG and FuJ7(t) wilt resistance gene were found to be closely linked, and the genetic distance between them was 5.2 cM. The AG/CAG segments were recovered, cloned, sequenced, and successfully transformed into a SCAR marker [18].

At present, the specific SCAR marker genes FuJ7(t) are used for molecular detection and marker-assisted selection breeding.

Genetic Linkage Map Construction

This genetic map is reported for the first time in China. There were three foreign related publications [19], but the maps described in them were not integrated and could not be used in marker-assisted practice. Therefore, precondition for the successful QTL analysis on flax, is that a high density and proper genetic map must be constructed, aimed at laying the foundation for determination of gene location.

Spielmeyer [20] investigated wilt resistance QTL by constructing an AFLP linkage map. Eighteen linkage groups were built, covering 1400 cM, and the average distance was 10 cM. Among the 60 AFLP loci, 28% of the markers deviated considerably from the expected segregation ratio. As confirmed by flax AFLP markers, the two QTL loci that exert substantial effects on flax resistance to Fusarium wilt are located in two separated linkage groups [19-21].

Western scholars also constructed a map of QTLs responsible for fatty acid composition traits of flax [19].

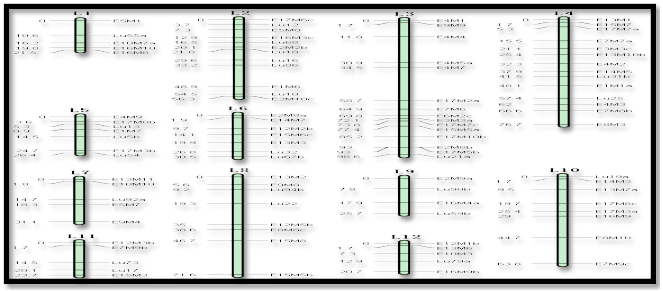
A flax genetic linkage map which contained 71 SRAP markers and 24 SSR markers was constructed based on a F2 segregation population in China. The map was with a total length of 546.5 cM, with 12 linkage groups, and an average distance of 5.75 cM between markers. The results indicated that the markers on the map were distributed evenly in the 12 linkage groups, and the co-dominant markers in SRAP and SSR were more suitable for the construction of a genetic map in flax [22,23] (Figure 1).

The map contained 12 major linkage groups, each with 4 to 15 markers. The ranges of genetic distance and marker numbers varied largely in the different linkage groups. The linkage group length was from 20.6 to 98.7 cM (Table 3).

By QTL mapping of flax plant height, we obtained the QTL on 8, 9, and 10 linkage group. The largest LOD value of 3.09 accounted for 17.14% of the contribution rate. The findings reported in this study revealed that the great probability in the section was related to flax plant height. In the next step, more target gene information would be obtained associated with plant height through the encryption technique of local tags in maps, and laid a foundation for the flax plant height breeding [24] (Table 4).

Technology Prospect

The study on innovative methods in flax breeding is one of the key factors to promote industry development. The results of these investigations are of a high significance to the enhancement of the basic innovation capacity. In recent years, molecular biotechnology techniques, such as gene transfer, molecular-assisted selection, and



The map contained 12 major linkage groups.

Figure 1: Map of linkage groups.

Linkage group	Number of marker	Distance (cM)	Average distance (cM)
LG1	5	21.6	4.32
LG2	12	56.3	4.69
LG3	15	98.7	6.58
LG4	14	76.7	5.48
LG5	7	26.5	3.79
LG6	7	30.6	4.37
LG7	5	31.1	6.22
LG8	8	71.5	8.94
LG9	4	25.6	6.4
LG10	8	63.7	7.96
LG11	5	23.7	4.74
LG12	5	20.6	

The linkage group length was from 20.6 to 98.7 cM. each group with 4 to 15 markers.

Table 3: Distribution of linkage groups marker.

Traits	LG	Marker Interval	Position	LOD value	Additive	R^2
Plant	8	E12M5b - E8M8c	37.9	2.9	6.76	16.04%
Height	9	- Lu19a	0.23	3.09	0.23	17.14%
	10	E8M10 - E7M9c	58.8	2.5	7.65	6.71%

The QTL on 8, 9, and 10 linkage group. The largest LOD value of 3.09 accounted for 17.14% of the contribution rate.

Table 4: QTL mapping for plant height in flax.

gene mapping have contributed to the development of new flax cultivars.

To improve the breeding efficiency, in the coming years we focus on the utilization of the haploidization method and will strive to increase polyembryonic rates and the density of genetic maps. By employing the target gene we have obtained, we will produce GMO plants that are resistant to powdery mildew and wilt. By the utilization of the genetic map, some functional genes will be located, cloned, and transferred.

With different advanced biological tools we will establish a highly efficient breeding system which can save time and costs. New flax cultivars possessing traits, such as high productivity, excellent quality, and resistance to major diseases will be bred in the future.

References

1. Qiao Rui Qing, Long Song Hua (2013) Research Progress of Molecular Biology on Flax 5: 265-269.
2. Song Shumin (2009) Application of the Genetic Engineering in Breeding for Flax Disease Resistance. Chinese Agricultural Science Bulletin 25: 227-229.
3. Ji Yanru, T ian Yujie, Song Shumin, Guan Xiangjun (2005). Preliminary Creation on Regenerational Flax System of Shoot Differentiate Directly from Explant. Biotechnology bulletin 3: 41-46.
4. Wu Jianzhong (2014) Cloning the Key Gene of Cellulose Synthase (LusiCesA1) in Flax.Crops 6: 35-38.
5. Wen Xin, Shao Tiemei, Li Xue Jiao, Zhan Kong, Weina An Shengjun, et al. (2011). Progress on Genetic Transformation of Foreign Gene in Flax. Biotechnology bulletin 12: 14-20.
6. Wang Yu-fu, Kang Qing-hua (2008) Inspetion of flax transfer gene resistant to herbicide. Plant Fiber Sciences in China 1: 13-16.
7. Cheng Li li, Guan Feng zhi (2015). Interspecific Hybridization and Immature Embryo Rescure between *Linum usitatissimum* and *Linum grandiflorum*. Plant fiber sciences in China 1: 1-4.
8. Kang Qinghua, Xuhan, Study on Cold and Salt Resistance Gene Transfermation of flax Haploid. Plant fiber sciences in China 6: 291-296.
9. Wang Yufu, Liu Yan, Yang Xue, Kang Qinghua (1999) Study on Herdity and Variation of Progenies Derived by Introducing Exogenous DNA in Flax. China's fiber crops 3: 7-11.
10. Yu Ying, Chen Hongyu, Cheng Lili (2015) Cloning and expression analysis of MAPK gene under saline-alkaline stress in flax. Journal of Northeast Agricultural University 46: 21-28.
11. Yu Y, Huang W, Chen H, Wu G, Yuan H, et al. (2014) Identification of differentially expressed genes in flax (*Linum usitatissimum* L.) under saline-alkaline stress by digital gene expression. Gene 549: 113-122.
12. Yang Xue, Zhao Yun, Guan Feng-Zhi (2008) Genetic Analysis Of Resistance To Powdery Mildew In Flax Line 9801-1. Acta Phytopatholog Ica Sin Ica. 38: 656-658.
13. Liu Li yan, Yang Xue, GUAN Feng- zhi (2009). Optimizing and Primers Screening of RAPD Reaction System for Resistance to Powdery Mildew of Flax. Plant Fiber Sciences in China 2: 130-134.
14. Boba A, Kulma A, Kostyn K (2011) The influence of carotenoid biosynthesis modification on the Fusarium culmorum and Fusarium oxysporum resistance in flax [J]. Physiological and Molecular Plant Pathology76: 39-47.
15. Bo Tian- yue, Yang Jian- cun, Ren Yun- ying, Chen Jin- Xiu (2006) Evaluation of flax germplasm for resistance to wilt. Chinese journal of oil crop sciences 28: 470- 475.
16. Zhao Nian –li (2012) Research on Occurrence of Blight in Flax. Horticulture and Seed 4: 64-66.
17. Bo Tian yue, Ye Hua zhi, Li Xiao bing, ZHU Li Huang (2003). Identification of Molecular Markers Linked to the Wilt Resistance Gene FuJ7 (t) in Flax. Scientia Agricultura Sinica 36: 287-291.
18. Hao Dongmei, Qiu Caisheng (2011) Construction of Flax Molecular ID Card System by RAPD Marker and Genetic Diversity Analysis. Chinese Agricultural Science Bulletin 27: 168-174.
19. Cloutier S, Ragupathy R, Niu ZX (2011) SSR - based linkage map of flax (*Linum usitatissimum* L.) and mapping of QTLs underlying fatty acid composition traits [J]. Mol Breeding 28: 437-451.
20. Spielmeyer W, Green AG, Bittisnich D (1998) Identification of quantitative trait loci contributing to Fusarium wilt resistance on an AFLP linkage map of flax (*Linum usitatissimum*) [J]. Theoretical and applied genetics 97: 633-641.
21. Oh TJ, Gorman M, Cullis CA (2000). RFLP and RAPD mapping in flax (*Linum usitatissimum*) [J]. Theoretical and applied genetics 101: 590–593.
22. Wu Jian-Zhong, Huang Wen-Gong, Kang Qing-Hua (2013) Construction of a Genetic Linkage Map in Flax (*Linum usitatissimum L.*), Acta Agronomica Sinica 39: 1134-1139.
23. Wang Bin, Dang Zhanhai (2009) Optimization of SRAP System in Flax. Genomics and Applied Biology 28: 760-764.
24. Wu Jian-zhong, Huang Wen-Gong (2011). Optimization of SRAP Reaction System and Screening of Polymorphic SRAP Markers in Flax. Plant fiber sciences in China 6: 281-284.