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The IIIVmrMLM method uncovers new genetic variants associated with resistance to Fusarium wilt in flax

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Abstract. Flax (*Linum usitatissimum*) is an important agricultural crop grown for fiber and oil production, playing a key role in various industries such as production of paints, linoleum, food, clothes and composite materials. Fusarium wilt caused by the fungus *Fusarium oxysporum* f. sp. *lini* is a reason of significant economic damage in flax cultivation. The spores of the fungus can persist in the soil for a long time, so obtaining resistant varieties is important. Here we used data on the resistance of 297 flax accessions from the collection of the Federal Center for Bast Crops in Torzhok (Russian Federation) to infection by a highly virulent isolate of the fungus MI39 in 2019–2021. Genotype resistance to infection was assessed by calculating the DSI index, a normalized proportion of genotypes with the same disease symptoms. The IIIVmrMLM program in Single_env mode was used to search for regions of the flax genome associated with resistance. The IIIVmrMLM model was designed to address methodological shortcomings in identifying all types of interactions between alleles, genes and environment, and to unbiasedly estimate their genetic effects. Being a multilocus MLM model, it estimates the effects of all genes as well as the effects of all interactions simultaneously. A total of 111 QTNs were found, of which 34 fell within the body of a known gene or were located in flanking regions within 1,000 bp. The genes into which the detected variants fell were associated with resistance to abiotic and biotic stresses, root, shoot and flower growth and development. Ten of the QTNs found mapped to regions of previously identified QTLs controlling the synthesis of palmitic, oleic, and other fatty acids. QTN Chr1_1706865/Chr1_1706872 and QTN Chr8_22542741 mark regions identified previously in an association search by the GAPIT program. The allelic effect was confirmed for all the QTNs found: a Mann–Whitney test was performed, which confirmed significant differences between the DSI index value in carriers of the reference and alternative allele. An increase in the number of alleles with negative effects in the genotype leads to a statistically significant decrease in the DSI value for all three years of testing. The groups of varieties with a large number of alleles reducing the DSI index had the best resistance. A total of 5 varieties were selected from the collection for which the number of alleles reducing the DSI index value did not exceed the number of alleles with the opposite effect for all three years. These varieties can be used further in breeding programs.

Key words: flax; *Linum usitatissimum*; GWAS; Fusarium wilt; *Fusarium oxysporum* f. sp. *lini*

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Метод IIIVmrMLM обнаруживает новые генетические варианты, связанные с устойчивостью к фузариозному увяданию у льна

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Аннотация. Лен (*Linum usitatissimum*) – важная сельскохозяйственная культура, выращиваемая для получения волокна и масла. Лен используют для производства красок, линолеума, в пищевой промышленности, для производства одежды и композитных материалов. Значительный экономический ущерб при выращивании льна наносит фузариозное увядание, вызываемое грибом *Fusarium oxysporum* f. sp. *lini*. Споры гриба могут долгое время сохраняться в почве, поэтому получение устойчивых к заражению сортов имеет большое значение.

Здесь мы использовали данные об устойчивости 297 образцов льна из коллекции Федерального научного центра лубяных культур в Торжке (Россия) к заражению сильно вирулентным изолятом гриба MI39 в 2019–2021 гг. Устойчивость генотипа к заражению оценивали путем вычисления индекса DSI – нормализованной пропорции генотипов с одинаковыми симптомами болезни. Для поиска районов генома льна, ассоциированных с устойчивостью, использовали программу IIIVmrMLM в режиме Single_env. Модель IIIVmrMLM была разработана для устранения методологических недостатков в выявлении всех типов взаимодействий между аллелями, генами и средой и для несмещенной оценки их генетических эффектов. Поскольку это мультилокусная MLM-модель, она оценивает эффекты всех генов, а также эффекты всех взаимодействий одновременно. Всего было найдено 111 QTN, из которых 34 были локализованы в последовательности известного гена или расположены во фланкирующих районах на расстоянии, не превышающем 1 т. п. н. Гены, в которые попадали обнаруженные варианты, были связаны с устойчивостью к абиотическим и биотическим стрессам, с ростом и развитием корня, побега и цветка. Десять из найденных QTN картировались в областях ранее идентифицированных QTL, контролирующих синтез пальмитиновой, олеиновой и других жирных кислот. QTN Chr1_1706865/Chr1_1706872 и QTN Chr8_22542741 маркируют районы, идентифицированные нами ранее при поиске ассоциаций программой GAPIT. Для всех найденных QTN был подтвержден аллельный эффект: произведен тест Манна–Уитни, который подтвердил значимые различия между значением DSI у носителей референсного и альтернативного аллеля. Увеличение в генотипе числа аллелей с негативным эффектом приводит к статистически значимому уменьшению величины DSI для всех трех лет тестирования. Группы сортов с большим количеством аллелей, уменьшающих индекс DSI, имели наилучшую устойчивость. Всего из коллекции было выбрано пять сортов, для которых число аллелей, уменьшающих величину DSI, не превышало число аллелей с обратным эффектом по всем трем годам. Эти сорта могут быть использованы в дальнейшем в селекционных программах.
Ключевые слова: лен; *Linum usitatissimum*; GWAS; фузариозное увядание; *Fusarium oxysporum* f. sp. *lini*

Introduction

Flax (*Linum usitatissimum*) is an important crop grown for both fiber and oil. Flaxseed oil is used in the food industry as a source of unsaturated fatty acids and is also used as the main component of varnishes, paints and linoleum. Flax fiber is used in textiles, composites and insulation materials (Goudenhooft et al., 2019). Fusarium wilt caused by the fungus *Fusarium oxysporum* f.sp. *lini* limits flax production (Dean et al., 2012). This disease lowers fiber quality and can lead to yield loss in the absence of proactive measures.

Primary fungal infection occurs through the roots. The pathogen enters the xylem and blocks the flow of water and nutrients, causing wilting, stem damage and eventually plant death. The spores of the fungus can persist in infested soil for up to 50 years and are very difficult to eliminate (Houston, Knowles, 1949).

Control of Fusarium wilt is possible through various agricultural practices, such as the use of pesticides (Rashid, Kenaschuk, 1993), but the possible harmfulness of pesticides to human health leads to the preference of using varieties resistant to infection, which is an alternative option to control yield loss caused by *F. oxysporum* (Ondrej, 1993; Rozhmina, Loshakova, 2016).

Resistance to the disease has been acquired through breeding, but the mechanisms of resistance remain incompletely understood. Modern flax varieties have high to medium resistance to Fusarium wilt (Rozhmina, Loshakova, 2016; Rozhmina, 2017). However, co-evolution of pathogen and plant can lead to the emergence of strains with higher aggressiveness or to the loss of resistance in varieties, determined by a small number of genes. Therefore, breeding new varieties with different combinations of genes determining resistance is important for long-term effects. Transcriptomics experiments have shown that cell wall components, transcription factors, secondary metabolites and antioxidants play a prominent role in the response of flax to infection by *F. oxysporum* f.sp. *lini* (Galindo-González, Deyholos, 2016; Dmitriev et al., 2017; Boba et al., 2021).

The search for new genomic variants associated with disease resistance and the identification of new genes affecting resistance to fungal infection play a key role in breeding programs. The use of classical GWAS identified QTNs (Quantitative Trait Nucleotides) associated with resistance to Fusarium wilt (Kanapin et al., 2021) and located mainly on chromosome 1, as well as on chromosomes 8 and 13. A large number of QTNs are localized on chromosome 1 within 640 kb (Kanapin et al., 2021; Cloutier et al., 2024).

Plant resistance to disease may also be determined by multiple indirect factors related not only to resistance to fungal infection but also to other plant characteristics, e. g. fatty acids in plants are known to be involved in defense mechanisms against various stressors, including fungal infection (Kachroo et al., 2008; He, Ding, 2020).

Classical MLM-type models help eliminate effects introduced by population structure and sample relatedness, but suffer from the Bonferroni correction for multiple testing, which is too stringent to detect associations with complex traits (Zhang Y.M. et al., 2019). To address this problem, multi-locus MLM models have been proposed that can detect QTNs with marginal effects for which the significance threshold set by the Bonferroni correction is too stringent.

One such method using multi-locus models is the mrMLM method (Zhang Y.-M. et al., 2020) implemented in the IIIVmrMLM package (Li M. et al., 2022). In this paper, we applied this method to search for genomic associations with resistance assessed in infected plants in 2019, 2020 and 2021, which allowed us to identify novel genetic variants not previously detected by classical methods. These variants fell within resistance-related genes as well as within quantitative trait loci (QTL, Quantitative Trait Locus) published previously and related to fatty acid production (You, Cloutier, 2020).

Materials and methods

297 flax samples from the collection of the Federal Scientific Centre for Bast Crops were grown in Torzhok, Russia. 180 accessions were fiber flax varieties, and 117 belonged to oilseed flax. Of the oilseed samples, 98 belonged to the intermediate type, 4, to large-seeded varieties, and 15, to the crown type.

Resistance of accessions to *F. oxysporum* f. sp. *lini* was evaluated under infection-provocation nursery conditions with controlled irrigation but not controlled temperature. Evaluations were conducted in 2019, 2020 and 2021 (Rozhmina et al., 2022). Each variety was replicated 16 times by sowing all seeds in cross rows of containers. The dimensions of the containers were 550 × 85 × 20 cm. Two genotypes, AP5 and I-7, were used as susceptible and resistant genotypes to control Fusarium wilt. The infection background was established by introducing 400 g of pure culture of *F. oxysporum* f. sp. *lini* strain MI39. Seeds were planted on the 12th day after inoculation with pure culture of the fungus.

Pure culture was prepared by preliminary cultivation of strain MI39 on agar-agar medium with beer wort and subsequent incubation on oat grain substrate (grain/water ratio 1 to 1.75) for 3–4 weeks, until complete infection of oats by the fungus, after which the pathogen was introduced into the soil. The indicator of reliability of the infection background was the reference varieties (resistant and susceptible genotypes), which were sown at the edges and in the middle of each container (16 seeds each). Disease severity was assessed using the Disease Severity score (DSS). The DSS scores ranged from 0 to 3, where 0 was a healthy plant, 1 was a partially blighted plant or stem blight on one side, 2 was a completely blighted plant with seed pods, and 3 was a completely blighted plant that died before pod formation. Based on the DSS, disease severity index (DSI) was calculated using the formula adopted in phytopathology (Guidelines for the Phytopathological Assessment, 2000): $DSI = (\Sigma ab/3A) \times 100$ %, where a is the number of plants with the same DSS, b is the DSS score; A is the total number of plants, and 3 is the highest DSS score.

DNA was isolated from leaves using the DNeasy Plant Mini Kit (Qiagen). Whole-genome sequencing of DNA was performed in BGI using the Illumina protocol, which generates paired-end reads of 150 base pairs in length. Comparison with the NCBI ASM22429v2 reference genome assembly (Wang Z. et al., 2012) was performed using bwa-mem (Li H., Durbin, 2009). Variant prediction was performed using NGSEP (Tello et al., 2019) version 4.0; from the 3,416,829 SNPs obtained, 72,526 SNPs were retained after filtering by MAF = 0.05 and conditioning on the presence of the variant in at least 85 % of genotypes. An annotation of the flax genome with the indicated Arabidopsis orthologous genes was provided by the Cloutier group (You, Cloutier, 2020).

Using the IIIVmrMLM package (Li M. et al., 2022) in Single_env mode, GWAS analyses were performed on genetic data filtered by MAF = 0.05. TASSEL (Bradbury et al., 2007) and PLINK (Purcell et al., 2007) with standard settings were used for the necessary data transformation.

The additive effect calculated by the IIIVmrMLM package was used to identify genotypes with high performance. An allele with a negative effect led to a decrease in the DSI in its carriers, while an allele with a positive effect increased the DSI. Varieties were selected in which the number of negative-effect alleles exceeded the number of positive-effect alleles.

Linkage disequilibrium decay (LD) was estimated using the square of the Pearson correlation coefficient (r^2). PopLDdecay version 3.4.1 (Zhang C. et al., 2019) was run to calculate r^2 in a 300 kb window. LD decay was calculated based on r^2 and distance for each SNP pair using the R script.

Results

Environmental characteristics may influence disease development. Plants were grown under the infection-provocation nursery conditions with regular irrigation but not controlled temperature. According to the weather station at the growing site, the temperature in the first decade of May was above average in 2019 and 2020 and below average in 2021 (Table 1). In the second decade of May, the temperature was above average in 2019 and 2021 and below average in 2020.

The analysis of variance showed that the Fusarium wilt infection depends on the year of cultivation and genotype (Table 2). When considering the influence of temperatures, it was found that only the temperature in the 1st decade of May has a significant influence on the variation ($F > 1$, $Pr(>F) < 0.05$); moreover, its influence on the Fusarium wilt infection is almost identical to the influence of the year, as can be seen from the values of the root mean square of the residuals in the analysis of variance in Table 2, whereas other environmental characteristics made only a small contribution.

On average, the difference between the maximum and minimum DSI values for genotype in different years is 25.9. Nevertheless, the differences in the DSI for the whole population under consideration from one year to another do not show sufficient significance: when comparing the 2019 and 2020 data, the p-value was 0.996, the 2019 and 2021 data, $p = 0.113$, the 2020 and 2021 data, $p = 0.12$.

In other words, despite the large influence of growing conditions, the main interest of the study continues to be the effect of variety (genotype) on disease resistance.

GWAS identified 111 QTNs (Supplementary Materials, Table S1)[1] associated with the DSI in different years, of which 35 were associated with 2019 data, 37, with 2020 data, and 40, with 2021 data. QTNs associated with data from different years are located on all chromosomes, of which 44 fell within known QTLs (You, Cloutier, 2020; Cloutier et al., 2024) or appeared to be localized in the gene sequence or less than 1,000 bp away from genes (Fig. 1*a–c*). The distribution of all found QTNs in the genome is shown in Figure 1*d*. The allelic effect was confirmed for all found QTNs: a Mann–Whitney test was performed, which confirmed significant differences between the DSI value in carriers of the reference and alternative allele (Table S1).

The largest number of QTNs found for each year's infection data were located on chromosomes 1, 2, 8 and 15. In a previous study that used the GAPIT package to find associations with resistance to Fusarium wilt, QTNs were also located on chromosomes 1 and 8 (Kanapin et al., 2021). In total, all the

[1] Tables S1, S2 and Figure S1 are available at:
https://vavilov.elpub.ru/jour/manager/files/Suppl_Duk_Engl.xlsx

Table 1. Average temperature of the growing seasons 2019–2021 (according to Torzhok meteorological station)

Month	Decade	Average temperature, °C			Average, long-term
		2019	2020	2021	
May	1	10.1	11.3	7.9	9.9
	2	14.5	8.0	16.7	11.5
	3	16.3	11.1	12.6	12.9
June	1	18.8	15.8	15.8	15.3
	2	17.9	19.6	19.2	15.6
	3	16.0	18.9	22.5	16.5
July	1	14.2	18.2	20.5	16.9
	2	13.8	15.8	23.2	17.4
	3	17.1	16.8	17.9	17.5
August	1	13.3	17.7	18.4	17.2
	2	16.1	–	–	15.2
	3	15.7	–	–	13.9

QTNs explain more than 50 % of the variation, at most one QTN explains about 5 % of the variation for one year, as can be seen in Table 3.

Only one QTN was found in the data of two years and three pairs of QTNs found for different years appeared to be located quite close to each other, as shown in Table 4. Table 4 also presents the mean non-normalized DSI values for carriers of the reference and alternative allele for a given QTN. It can be seen that carriers of the alternative allele for all the indicated QTNs showed much lower DSI values than the reference allele carriers. However, of the mentioned QTNs, only one QTN common to the 2020 and 2021 data fell within the sequence of a gene, the function of which, however, is not known, while the other three pairs were located more than 1 kb away from the nearest genes. It can also be noted that QTNs Chr1_1706865/Chr1_1706872 fell into the previously identified region on chromosome 1, with coordinates 1213418–1854337, associated with resistance to Fusarium wilt (Kanapin et al., 2021).

Of the 111 QTNs associated with Fusarium wilt resistance in different years, 34 were localized within the gene body or were located at a distance of less than 1 kb from the gene (Table 5).

Within the protein-coding genes and their 1-kb flanking regions, we found 34 QTNs (Table 5), of which 12 had an alternative allele with an effect of decreasing the value of the DSI and 22 with an effect of increasing this value.

10 QTNs fell within the QTLs published previously in (You, Cloutier, 2020), of which two were near a known gene (marked as ** in Table 5). In addition, one QTN fell within a region associated with resistance to Fusarium wilt on chromosome 1 (Table 6), published in (Kanapin et al., 2021; Cloutier et al., 2024).

Eleven QTNs whose positions overlap with previously identified QTLs from (You, Cloutier, 2020; Cloutier et al., 2024) are shown in Table 6. Most of these QTL are associated with the production of fatty acids: palmitic acid, oleic acid, linolenic acid, etc., and only two QTNs, Chr1_17552378 and Chr1_2540379, fell into QTLs associated with plant immunity. In nine out of eleven cases, the presence of the alternative QTN allele in the plant resulted in an increase in the DSI value, and only in two cases the alternative allele resulted in a decrease in the DSI value compared to the reference allele carriers.

Table 2. Dispersion analysis

Source of variance	Mean Sq	F	Pr(>F)
DSI ~ genotype + year			
Genotype	2931.5	10.016	<2e–16
Year	1927.7	6.586	0.00148
Residuals	292.7		
DSI ~ genotype + temperature in the 1st decade of May			
Genotype	2931.0	10.01	<2e–16
Temperature in the 1st decade of May	3396.0	11.590	0.000707
Residuals	293.0		

To assess variety performance, the number of alleles with a negative effect (reduction of the DSI value) and with a positive effect was counted among the QTNs found from each year's data (Table S2). The number of negative and positive alleles affecting the DSI for each year is different, but an increase in the total number of alleles with a negative effect in the varieties leads to a statistically significant decrease in the DSI value for all three years, as can be seen in Figure 2.

Table 7 shows the varieties for which the number of alleles that increased the DSI value did not exceed the number of alleles with the opposite effect in all three years.

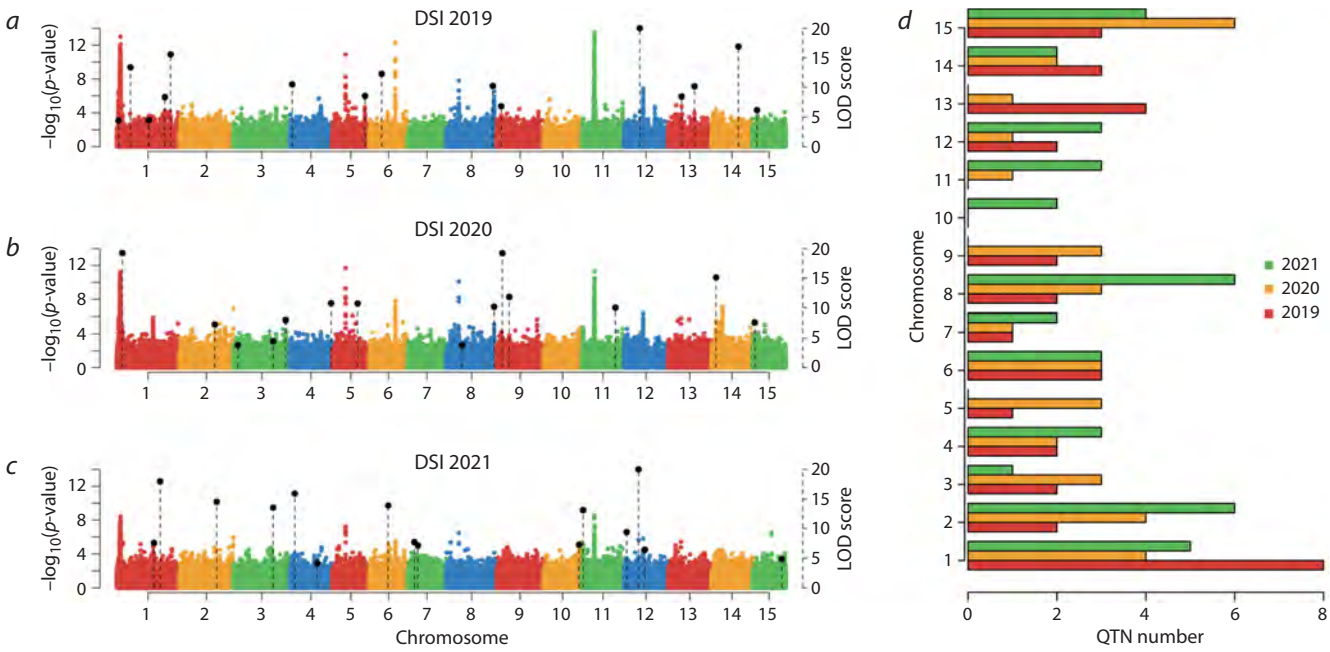

Fig. 1. Location of QTNs associated with fusarium wilt relative to chromosomes in flax.

a –c – Manhattan plots of GWAS results using the IIIVmrMLM package; black shows QTNs that fell in the QTL or were located near genes along with their LOD score value, which is used in IIIVmrMLM to assess significance; *d* – distribution of QTNs found for the DSI for the three years data, by chromosome.

Table 3. Cumulative percentage of variation in each year's data explained by QTNs

Data	Total r^2, %	QTNs with the largest r^2, %	Largest r^2, %
DSI 2019	55.99	Chr3_18720497	5.31
DSI 2020	58.50	Chr2_15253612	4.43
DSI 2021	69.84	Chr12_10144355	5.35

Note. QTN names are formed as ChrX_N, where X is the chromosome number and N is the position in the chromosome.

Table 4. Co-localized QTNs across the years

QTN	Chr3_18671763	Chr1_1706865/ Chr1_1706872	Chr15_7067724/ Chr15_7067662	Chr2_25600109/ Chr2_25600116
Year	2020/2021	2019/2021	2020/2021	2020/2021
Distance between QTNs	0 bp	7 bp	62 bp	7 bp
r^2, %	1.14/3.47	4.24/3.17	1.05/0.75	4.25/4.66
Average DSI for REF	41.55/36.92	43.12/38.47	41.24/36.31	41.85/38.7
Average DSI for ALT	25.18/21.02	10.55/9.52	24.75/20.56	14.53/15.06
p-value of the Mann–Whitney test	0.0014/0.0021	5.42e–19/1.16e–13	0.0026/0.0027	1.56e–06/2.92e–10
Nearest gene	*Lus10033807*	*Lus10025819*	*Lus10001477*	*Lus10003500*
QTN location relative to the gene	Within the gene	1,026/1,033 bp downstream	3,605/3,543 bp downstream	23,760/23,753 bp upstream
Gene annotation	Protein with an unknown function (DUF1664)	2-Oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	Remorin family protein	Basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Ortholog in *Arabidopsis*	*AT1G04960.1*	*AT3G21360.1*	*AT5G23750.2*	*AT3G21330.1*

Note. The corresponding lines show data for different years separated by "/"; bp – base pairs; REF – reference allele; ALT – alternative allele.

Table 5. QTNs located within protein-coding genes and their 1-kb flanking regions

QTN	r^2, %	Average DSI for REF / Average DSI for ALT	QTN position relative to a gene	Annotation	Ortholog in *Arabidopsis*
2019					
Chr1_740951	0.51	32.67 / 46.32*	*Lus10036050*, gene body	Calcium-dependent protein kinase 34	*AT5G19360.1*
Chr1_6391647	2.21	35.67 / 43.75*	*Lus10034284*, gene body	Sodium/calcium exchanger family protein	*AT5G17850.1*
Chr1_15073726	0.90	41.12* / 31.36	*Lus10015586*, gene body	Prolyl oligopeptidase family protein	*AT1G50380.1*
Chr1_22688905	0.64	41.63* / 8.42	*Lus10014640*, 573 bp downstream	Major facilitator superfamily protein	*AT2G39210.1*
Chr1_25377570	3.15	41.97* / 30.21	*Lus10027990*, gene body	Oxidoreductase, 2OG-Fe(II) oxygenase family protein	*AT4G02940.1* (Duan et al., 2017)
Chr4_1087234	1.82	35.08 / 46.28*	*Lus10030349*, 155 bp upstream	DZC (Disease resistance/ zinc finger/chromosome condensation-like region) domain-containing protein	*AT1G31880.1* (Depuydt et al., 2013; Rodriguez-Villalon et al., 2014)
Chr5_15553508 **(QPAL-Lu5.2, PAL)	1.24	33.95 / 45.51*	*Lus10024055*, gene body	(Ortholog *Arabidopsis*: nitric oxide synthase interacting protein)	*AT5G65030.1*
Chr6_5732293	1.86	36.49 / 48.47*	*Lus10036674*, 132 bp downstream	Homeobox 1	*AT3G01470.1* (Aoyama et al., 1995)
Chr9_2114668	0.68	35.09 / 52.45*	*Lus10017493*, 464 bp upstream	–	*AT4G34630.1*
Chr13_6339069	1.71	35.05 / 53.25*	*Lus10002083*, 88 bp downstream	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	*AT5G08790.1* (Delessert et al., 2005; Wang X. et al., 2009; Wang X., Culver, 2012)
Chr13_12427556	1.21	34.20 / 49.92*	*Lus10010801*, 894 bp upstream	Cytochrome P450, family 721, subfamily A, polypeptide 1	*AT1G75130.1*
Chr14_12732300	1.08	41.14* / 21.40	*Lus10008367*, 263 bp upstream	ARM repeat superfamily protein	*AT3G08960.1* (Jia et al., 2023)
Chr15_2097827	0.88	33.09 / 49.75*	*Lus10007320*, gene body	RING/FYVE/PHD-type zinc finger family protein	*AT1G29800.1* (Kim et al., 2023)
2020					
Chr1_2540379 **(Lu1_2500703, DSI)	2.02	36.98 / 58.99*	*Lus10025924*, 539 bp upstream	Sec14p-like phosphatidylinositol transfer family protein	*AT3G24840.1*
Chr2_16849610	1.01	35.50 / 50.85*	*Lus10016310*, gene body	Cytochrome P450, family 721, subfamily A, polypeptide 1	*AT1G75130.1*
Chr3_1992356	1.34	35.70 / 58.40*	*Lus10037255*, gene body	Solute:sodium symporters, urea transmembrane transporters	*AT5G45380.1* (Liu et al., 2003; Kojima et al., 2007)
Chr3_24632490	1.27	43.84* / 28.25	*Lus10037741*, gene body	Lipoamide dehydrogenase 1	*AT3G16950.2* (Lutziger, Oliver, 2000)

Table 5 (end)

QTN	r^2, %	Average DSI for REF / Average DSI for ALT	QTN position relative to a gene	Annotation	Ortholog in *Arabidopsis*
2020 г.					
Chr4_19469341	0.79	40.45* / 21.40	*Lus10039825*, 945 bp upstream	–	*AT4G28290.1*
Chr8_7404794	0.81	40.23* / 15.74	*Lus10021849*, gene body	Cysteine-rich RLK (receptor-like protein kinase) 8	*AT4G23160.1*
Chr9_2644458	3.16	36.05 / 54.10*	*Lus10010491*, gene body	Immunoglobulin E-set superfamily protein	*AT3G07880.1* (Carol et al., 2005)
Chr11_15810790	0.29	37.57 / 64.13*	*Lus10023622*, gene body	ADC synthase superfamily protein	*AT1G74710.1* (Wildermuth et al., 2001; Strawn et al., 2007)
Chr14_2163238	1.74	42.23* / 23.94	*Lus10025537*, gene body	PAZ domain-containing protein/piwi domain-containing protein	*AT5G21030.1*
Chr15_1044247	0.95	34.39 / 43.97*	*Lus10011210*, 33 bp upstream	F-box and associated interaction domains-containing protein	*AT1G32420.1*
2020, 2021					
Chr3_18671763	1.14	41.55* / 25.18	*Lus10033807*, gene body	Protein of unknown function (DUF1664)	*AT1G04960.1*
2021					
Chr1_20417569	0.57	35.92* / 14.00	*Lus10015886*, gene body	Nucleotidyl-transferase family protein	*AT4G00060.1*
Chr2_17726495	2.95	30.63 / 45.43*	*Lus10033187*, gene body	K-box region and MADS-box transcription factor family protein	*AT3G54340.1* (Krizek, Meyerowitz, 1996)
Chr4_2301676	3.27	29.94 / 48.51*	*Lus10029444*, gene body	Peptide chain release factor 1	*AT3G62910.1* (Motohashi et al., 2007)
Chr4_12925693	2.22	32.24 / 55.99*	*Lus10015799*, gene body	Leucine-rich repeat protein kinase family protein	*AT1G67510.1*
Chr6_8828608	2.89	31.06 / 44.86*	*Lus10036278*, 568 bp downstream	RNA-directed DNA polymerase (reverse transcriptase)	*AT5G04050.1*
Chr7_3147157 **(QPAL-Lu7.3, PAL)	0.87	31.48 / 48.22*	*Lus10023551*, 387 bp upstream	–	*AT5G66440.1*
Chr10_16815632	1.08	29.87 / 43.13*	*Lus10022764*, 483 bp upstream	ABI five binding protein 3	*AT3G29575.1* (Garcia et al., 2008)
Chr11_575034	0.49	32.80 / 59.28*	*Lus10027253*, gene body	Ortholog *Arabidopsis*: GPI- anchor protein	*AT3G18050.1*
Chr12_9853001	1.96	36.04* / 20.76	*Lus10024259*, gene body	Aldehyde dehydrogenase 2C4	*AT3G24503.1* (Nair et al., 2004)
Chr15_13834579	0.87	35.60* / 16.58	*Lus10037970*, gene body	Plant U-box 14, flowering regulation	*AT3G54850.1* (Andersen et al., 2004)

Note. REF – reference allele; ALT – alternative allele. * The largest of the mean DSI values in carriers of the reference or alternative allele; ** QTNs localized both in the gene body and known QTL.

Table 6. QTNs located within previously identified QTLs

QTN	r^2, %	Average DSI for REF Average DSI for ALT	QTL	Trait	QTL position
Chr5_15553508	1.24	33.95 45.51*	QPAL-Lu5.2	PAL	13796740–15667804
Chr8_21862725	2.47	36.27 51.36*	QOLE-Lu8.1	OLE	21781910–23526575
Chr12_7449738	1.06	36.35 60.51*	QOIL-Lu12.6	OIL	4591134–7490902
Chr1_2540379	2.02	36.98 58.99*	Lu1_ 2500703	DSI	2500703–2636369
Chr5_12086840	1.77	37.19 53.09*	QPAL-Lu5.1	PAL	12061283–12181348
Chr8_22542741	2.77	41.64* 23.63	QOLE-Lu8.1	OLE	21781910–23526575
Chr1_17552378	0.90	28.86 41.23*	QPM-crc-LG1	PM	16920407–18739647
Chr7_3147157	0.87	31.48 48.22*	QPAL-Lu7.3	PAL	624439–5423600
Chr7_4787639	1.57	35.91* 18.53	QPAL-Lu7.3	PAL	624439–5423600
Chr12_1240570	0.81	32.95 55.79*	QIOD-Lu12.3/QLIN-Lu12.3/QLIO-Lu12.3	IOD/LIN/LIO	489561–2981562
Chr12_6862107	3.27	32.31 55.52*	QOIL-Lu12.6	OIL	4591134–7490902

Note. REF – carriers of the reference allele, ALT – carriers of the alternative allele. * The largest of the mean DSI values in carriers of the reference or alternative allele. Abbreviations of trait names from (You, Cloutier, 2020): PAL (Palmitic %) – palmitic acid content; OLE (Oleic %) – oleic acid content; OIL (Oil content %) – oil content, PM (Powdery mildew rating) – powdery mildew rate; IOD (Iodine value) – iodine content; LIN (Linoleic %) – linoleic acid content; LIO (Linolenic %) – linolenic acid content.

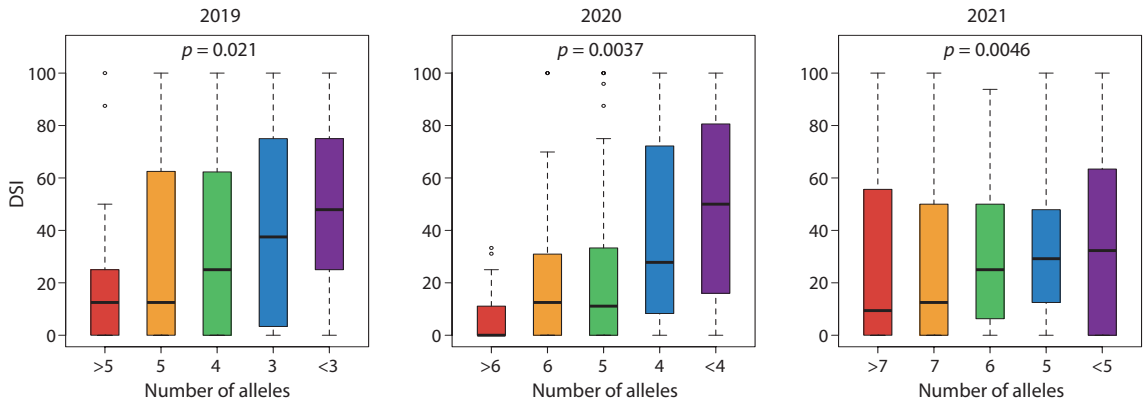

Fig. 2. Distribution of DSI values in different years for accessions containing different numbers of alleles that have a negative effect on the DSI value.

The upper part of the graphs shows the *p*-value of the statistical test.

Table 7. Varieties that had the best combination of alleles with positive and negative effects in all three years

DSI			Morphotype	Country	Breeding	Name
2019	2020	2021				
0	8.3	0	Fiber	Japan	Line	Honkei35, k-5396
0	0	0	Intermediate	Czechia	Line	AGT987, k-7225
0	8.3	0		France	Cultivar	Eolle, k-7034
19	8.3	0		Russia	Line	VNIIL, LM92, k-6672
0	0	0			Cultivar	Voronezhskij 1308/138, k-3052

Discussion

In this paper, we used the IIIVmrMLM program to find genomic regions controlling resistance to Fusarium wilt in flax. A total of 111 QTNs associated with the disease severity index (DSI) value in data from different years were found.

QTNs Chr1_1706865, Chr1_1706872, and Chr8_22542741 apparently point to regions on chromosomes 1 and 8 previously found using the GAPIT package, as published in (Kanapin et al., 2021), being less than the average LD for the corresponding chromosomes, which was 16 and 45 kb for chromosomes 1 and 8, respectively (Fig. S1).

Many of the QTNs fall into or near genes with important functions, and it is possible that these genes are casual (Table 5). Among the QTNs found to fall into genes, there are QTNs that have a favorable effect on a trait. For example, the alternative allele of the QTN Chr1_25377570 in the *Lus10027990* gene decreases the DSI which is a favorable effect for this value. The orthologue of this gene in *Arabidopsis AT4G02940.1* encodes a dioxygenase that demethylates m^6A in mRNA. Mutations in this gene affect the mRNA stability of the flowering time regulators FT, SPL3, and SPL9 and delay the transition from vegetative growth to flowering (Duan et al., 2017). The alternative allele of QTN Chr3_1992356 (Table 5) located in the *Lus10037255* gene also increases the DSI value. The *Lus10037255* ortholog encodes the urea proton symporter DUR3, which is involved in urea transport across the plasma membrane into root cells (Liu et al., 2003; Kojima et al., 2007). Since *F. oxysporum* infects plants via roots, the transport of metabolites in roots may influence the susceptibility of the plant to infection.

Some QTNs fall into genes associated with plant immunity (Table 5). For example, QTN Chr15_2097827 with a positive effect (ALT allele increases the DSI) is localized in the *Lus10007320* gene, the orthologue of which in *Arabidopsis* regulates autophagy (Kim et al., 2023). In contrast the alternative alleles of QTNs in the genes *Lus10021849, Lus10008367*, and *Lus10024259* decrease the DSI value. *Lus10021849* is an orthologue of *Arabidopsis CRK8*, which encodes a receptor-like protein kinase. The *Arabidopsis* orthologue *Lus10008367* encodes the effector Ran KA120. This effector prevents autoimmune activation in the absence of pathogens and restricts the activity of the *SNC* gene, which encodes a TIR-NB-LRR-like receptor involved in the salicylic acid-mediated immune response (Jia et al., 2023). The *Lus10024259* orthologue in *Arabidopsis* is involved in the biosynthesis of ferulic and synapic acids (Nair et al., 2004), which are important for plant resistance to biotic and abiotic stresses.

In many cases, the presence of the alternative allele resulted in an increased DSI value in its carriers. Many of the genes that harbored such QTNs were associated with root or leaf growth. For example, *Lus10030349* (Table 5) (orthologue *AT1G31880.1*) encodes the BREVIS RADIX protein, which regulates cell elongation and differentiation in the root and shoot (Depuydt et al., 2013; Rodriguez-Villalon et al., 2014). *Lus10036674* (orthologue *AT3G01470.1*) encodes the HAT5 protein with homeobox and leucine zipper domains that is involved in the mechanism of leaf growth regulation (Aoyama et al., 1995). *Lus10010491* (orthologue *AT3G07880.1*) encodes RhoGDI, an inhibitor of GDP dissociation from Rho GTPase. This inhibitor spatially restricts the sites of growth to a single point on the trichoblast and regulates activation of the RHD2/AtrbohC NADPH oxidase, which is required for root hair growth (Carol et al., 2005).

Mutations in genes related to plant immunity and stress response can also have a negative effect on plant resistance to Fusarium wilt (Table 5). For example, *Lus10002083* (orthologue of *AT5G08790.1*) encodes the ATAF2 protein, which is involved in the regulation of basal defense responses of the host plant against viral infection (Delessert et al., 2005; Wang X. et al., 2009; Wang X., Culver, 2012). *Lus10023622* (ortholog *AT1G74710.1*) encodes chloroplast isochorismate synthase 1, which is involved in the synthesis of salicylic acid, essential for plant defense against pathogens (Wildermuth et al., 2001; Strawn et al., 2007). The *AT1G67510.1* orthologue, *Lus10015799*, encodes an RLK protein kinase rich in leucine repeats. Many RLK kinases are involved in cell response processes to pathogens and abiotic stresses (Lease et al., 1998; Gish, Clark, 2011; Yan et al., 2023). The orthologue of *AT3G29575.1*, *Lus10022764*, acts as a negative regulator of abscisic acid (ABA) and stress response (Garcia et al., 2008).

Also, some QTNs are located in genes related to energy metabolism and flower growth. For example, *AT3G16950.2*, the orthologue of the *Lus10037741* gene (Table 5), encodes a dehydrogenase that is a component of the plastid pyruvate dehydrogenase complex (PDC) (Lutziger, Oliver, 2000). This complex is involved in glycolysis. *Lus10037741* contains the Chr3_24632490 QTN, in which the alternative allele reduces the DSI value (Table 5). Conversely, the alternative QTN alleles Chr4_2301676 and Chr2_17726495 in the genes *Lus10029444* and *Lus10033187* increase the DSI value (Table 5). *AT3G62910.1*, the orthologue of the *Lus10029444* gene, encodes the chloroplast peptide chain release factor APG3, which is required for normal chloroplast development (Motohashi et al., 2007). *AT3G54340.1,* the orthologue of

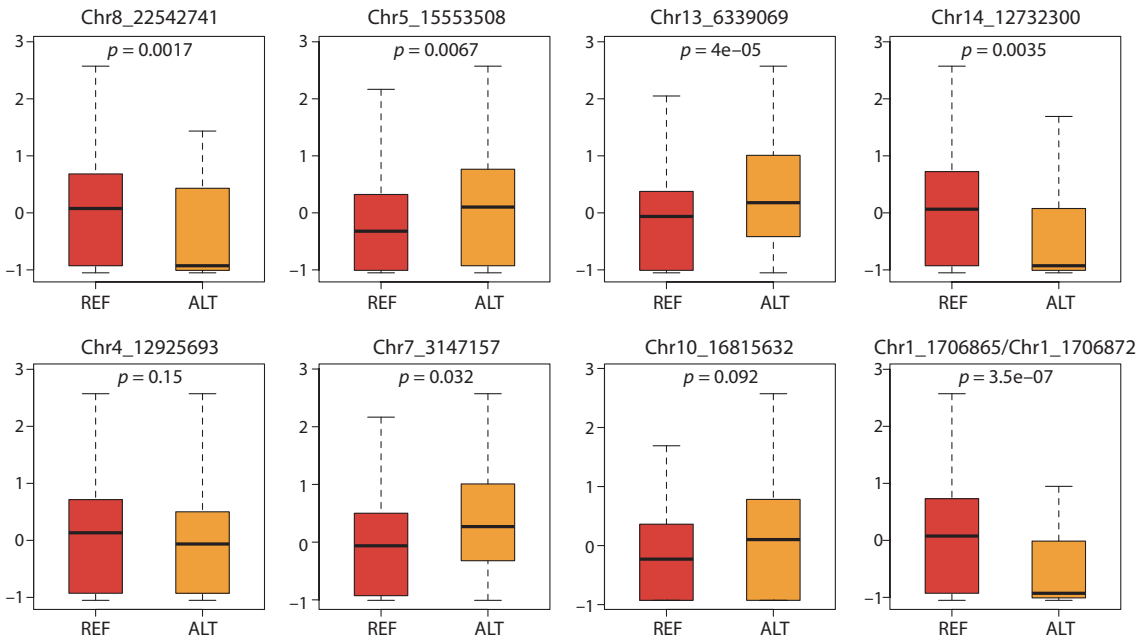

Fig. 3. Normalized DSI values in carriers of the reference (REF) and alternative (ALT) alleles in an independent dataset of 100 samples for some QTNs common to both datasets.

The *p*-values of the Mann–Whitney test are shown.

Lus10033187, encodes the homeobox protein APETALA 3, which regulates flower development (Krizek, Meyerowitz, 1996). On the other hand, QTN Chr15_13834579 in the *Lus10037970* gene, orthologous to the flowering regulator *AT3G54850.1*, has a positive effect on resistance to *F. oxysporum*, reducing the DSI value in carriers of the alternative allele.

It is also interesting to note that 11 of the QTNs found overlapped with previously published functional QTL regions, but only two of these regions were associated with plant immunity, while the rest were related to fatty acid production (Table 6). Fatty acids in plants act as a defense against pathogens and abiotic stresses (Kachroo et al., 2008; He, Ding, 2020); in addition, palmitic acid has been shown to reduce *Fusarium* infection in other plants (Ma et al., 2021). Thus, QTNs located in regions associated with fatty acid production may influence plant resistance to Fusarium wilt. Four QTNs fell into regions associated with palmitic acid (Table 6), which may indicate an important role of this acid in defense against Fusarium wilt in flax. The Chr8_22542741 QTN overlapped with the QOLE-Lu8.1 QTL associated with oleic acid production, and the Chr8_2256060236 and Chr8_2256060290 QTNs previously found with the GAPIT package (Kanapin et al., 2021) also fell within this region, indicating the possible importance of oleic acid production in protecting the plant against Fusarium wilt. One QTN, Chr1_2540379, also overlapped with a recently published region associated with flax resistance to Fusarium wilt (Cloutier et al., 2024).

We also tested on an independent dataset of 100 accessions the validity of the detected associations between QTNs and the DSI value (Fig. 3). This dataset grown under the same conditions was previously sequenced separately from the dataset under consideration and does not overlap with the dataset used in this study. It can be noted that the Chr5_15553508 and Chr7_3147157 QTNs, which fell into the palmitic acid-related regions, and the Chr8_22542741 QTN, which fell into the oleic acid-related region, demonstrate a significant difference in the DSI value between carriers of the reference and alternative alleles in this dataset (Fig. 3). Also, a significant allelic effect is seen in QTNs located in genes involved in plant immunity and stress response (Tables 5 and 6): Chr13_6339069 (*Lus10002083*), Chr14_12732300 (*Lus10008367*), Chr4_12925693 (*Lus10015799*), and Chr10_16815632 (*Lus10022764*). This suggests that these genes may also be involved in the defense of flax plants against infection.

We identified five varieties with the largest number of alleles decreasing the DSI (Table 7). The DSI of these varieties is much lower than the average DSI value, which for 2019, 2020 and 2021 was 38.7, 38.9 and 34.4, respectively. These varieties can be integrated into modern breeding programs.

Conclusion

As a result of application of the new multilocus model IIIVmrMLM to search for genomic associations with flax resistance to Fusarium wilt wilt, new genomic variants located in important regulatory regions were identified. Varieties with these variants showed greater resistance to the disease and can be used in breeding programs.

References

Andersen P., Kragelund B.B., Olsen A.N., Larsen F.H., Chua N.H., Poulsen F.M., Skriver K. Structure and biochemical function of a prototypical Arabidopsis U-box domain. *J Biol Chem.* 2004; 279(38):40053-40061. doi 10.1074/jbc.M405057200

Aoyama T., Dong C.H., Wu Y., Carabelli M., Sessa G., Ruberti I., Morelli G., Chua N.H. Ectopic expression of the Arabidopsis transcriptional activator Athb-1 alters leaf cell fate in tobacco. *Plant Cell.* 1995;7(11):1773-1785. doi 10.1105/tpc.7.11.1773

Boba A., Kostyn K., Kozak B., Zalewski I., Szopa J., Kulma A. Transcriptomic profiling of susceptible and resistant flax seedlings after *Fusarium oxysporum* lini infection. *PLoS One*. 2021;16:e0246052. doi 10.1371/journal.pone.0246052

Bradbury P.J., Zhang Z., Kroon D.E., Casstevens T.M., Ramdoss Y., Buckler E.S. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*. 2007;23(19):2633-2635. doi 10.1093/bioinformatics/btm308

Carol R.J., Takeda S., Linstead P., Durrant M.C., Kakesova H., Derbyshire P., Drea S., Zarsky V., Dolan L. A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature*. 2005; 438(7070):1013-1016. doi 10.1038/nature04198

Cloutier S., Edwards T., Zheng C., Booker H.M., Islam T., Nabetani K., Kutcher H.R., Molina O., You F.M. Fine-mapping of a major locus for Fusarium wilt resistance in flax (*Linum usitatissimum* L.). *Theor Appl Genet*. 2024;137(1):27. doi 10.1007/s00122-023-04528-2

Dean R., Van Kan J.A., Pretorius Z.A., Hammond-Kosack K.E., Di Pietro A., Spanu P.D., Rudd J.J., Dickman M., Kahmann R., Ellis J., Foster G.D. The Top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol*. 2012;13(4):414-430. doi 10.1111/j.1364-3703.2011.00783.x

Delessert C., Kazan K., Wilson I.W., Van Der Straeten D., Manners J., Dennis E.S., Dolferus R. The transcription factor ATAF2 represses the expression of pathogenesis-related genes in Arabidopsis. *Plant J*. 2005;43(5):745-757. doi 10.1111/j.1365-313X.2005.02488.x

Depuydt S., Rodriguez-Villalon A., Santuari L., Wyser-Rmili C., Ragni L., Hardtke C.S. Suppression of *Arabidopsis* protophloem differentiation and root meristem growth by CLE45 requires the receptor-like kinase BAM3. *Proc Natl Acad Sci USA*. 2013;110(17): 7074-7079. doi 10.1073/pnas.1222314110

Dmitriev A.A., Krasnov G.S., Rozhmina T.A., Novakovskiy R.O., Snezhkina A.V., Fedorova M.S., Yurkevich O.Y., Muravenko O.V., Bolsheva N.L., Kudryavtseva A.V., Melnikova N.V. Differential gene expression in response to *Fusarium oxysporum* infection in resistant and susceptible genotypes of flax (*Linum usitatissimum* L.) *BMC Plant Biol*. 2017;17(Suppl.2):253. doi 10.1186/s12870-017-1192-2

Duan H.C., Wei L.H., Zhang C., Wang Y., Chen L., Lu Z., Chen P.R., He C., Jia G. ALKBH10B is an RNA N^6-methyladenosine demethylase affecting Arabidopsis floral transition. *Plant Cell*. 2017;29(12): 2995-3011. doi 10.1105/tpc.16.00912

Galindo-González L., Deyholos M.K. RNA-seq transcriptome response of flax (*Linum usitatissimum* L.) to the pathogenic fungus *Fusarium oxysporum* f. sp. *lini*. *Front Plant Sci*. 2016;7:1766. doi 10.3389/fpls.2016.01766

Garcia M.E., Lynch T., Peeters J., Snowden C., Finkelstein R. A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. *Plant Mol Biol*. 2008;67(6):643-658. doi 10.1007/s11103-008-9344-2

Gish L.A., Clark S.E. The RLK/Pelle family of kinases. *Plant J*. 2011; 66(1):117-127. doi 10.1111/j.1365-313X.2011.04518.x

Goudenhooft C., Bourmaud A., Baley C. Flax (*Linum usitatissimum* L.) fibers for composite reinforcement: exploring the link between plant growth, cell walls development, and fiber properties. *Front Plant Sci*. 2019;10:411. doi 10.3389/fpls.2019.00411

Guidelines for the Phytopathological Assessment of the Resistance of Fiber Flax to Diseases. Moscow, 2000 (in Russian)

He M., Ding N.Z. Plant unsaturated fatty acids: multiple roles in stress response. *Front Plant Sci*. 2020;11:562785. doi 10.3389/fpls.2020.562785

Houston B.R., Knowles P.F. Fifty-years survival of flax fusarium wilt in the absence of flax culture. *Plant Dis Rep*. 1949;33:38-39

Jia M., Chen X., Shi X., Fang Y., Gu Y. Nuclear transport receptor KA120 regulates molecular condensation of MAC3 to coordinate plant immune activation. *Cell Host Microbe*. 2023;31(10):1685-1699.e7. doi 10.1016/j.chom.2023.08.015

Kachroo A., Fu D.Q., Havens W., Navarre D., Kachroo P., Ghabrial S.A. An oleic acid-mediated pathway induces constitutive defense signaling and enhanced resistance to multiple pathogens in soybean. *Mol Plant Microbe Interact*. 2008;21(5):564-575. doi 10.1094/MPMI-21-5-0564

Kanapin A., Bankin M., Rozhmina T., Samsonova A., Samsonova M. Genomic regions associated with Fusarium wilt resistance in flax. *Int J Mol Sci*. 2021;22(22):12383. doi 10.3390/ijms222212383

Kim J.H., Jung H., Song K., Lee H.N., Chung T. The phosphatidylinositol 3-phosphate effector FYVE3 regulates FYVE2-dependent autophagy in *Arabidopsis thaliana*. *Front Plant Sci*. 2023;14:1160162. doi 10.3389/fpls.2023.1160162

Kojima S., Bohner A., Gassert B., Yuan L., von Wirén N. AtDUR3 represents the major transporter for high-affinity urea transport across the plasma membrane of nitrogen-deficient Arabidopsis roots. *Plant J*. 2007;52(1):30-40. doi 10.1111/j.1365-313X.2007.03223.x

Krizek B.A., Meyerowitz E.M. The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. *Development*. 1996;122(1):11-22. doi 10.1242/dev.122.1.11

Lease K., Ingham E., Walker J.C. Challenges in understanding RLK function. *Curr Opin Plant Biol*. 1998;1(5):388-392. doi 10.1016/s1369-5266(98)80261-6

Li H., Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760. doi 10.1093/bioinformatics/btp324

Li M., Zhang Y.W., Xiang Y., Liu M.H., Zhang Y.M. IIIVmrMLM: the R and C++ tools associated with 3VmrMLM, a comprehensive GWAS method for dissecting quantitative traits. *Mol Plant*. 2022; 15(8):1251-1253. doi 10.1016/j.molp.2022.06.002

Liu L.H., Ludewig U., Frommer W.B., von Wirén N. AtDUR3 encodes a new type of high-affinity urea/H^+ symporter in Arabidopsis. *Plant Cell*. 2003;15(3):790-800. doi 10.1105/tpc.007120

Lutziger I., Oliver D.J. Molecular evidence of a unique lipoamide dehydrogenase in plastids: analysis of plastidic lipoamide dehydrogenase from *Arabidopsis thaliana*. *FEBS Lett*. 2000;484(1):12-16. doi 10.1016/s0014-5793(00)02116-5

Ma K., Kou J., Khashi U., Rahman M., Du W., Liang X., Wu F., Li W., Pan K. Palmitic acid mediated change of rhizosphere and alleviation of Fusarium wilt disease in watermelon. *Saudi J Biol Sci*. 2021; 28(6):3616-3623. doi 10.1016/j.sjbs.2021.03.040

Motohashi R., Yamazaki T., Myouga F., Ito T., Ito K., Satou M., Kobayashi M., Nagata N., Yoshida S., Nagashima A., Tanaka K., Takahashi S., Shinozaki K. Chloroplast ribosome release factor 1 (AtcpRF1) is essential for chloroplast development. *Plant Mol Biol*. 2007;64(5):481-497. doi 10.1007/s11103-007-9166-7

Nair R.B., Bastress K.L., Ruegger M.O., Denault J.W., Chapple C. The *Arabidopsis thaliana REDUCED EPIDERMAL FLUORESCENCE1* gene encodes an aldehyde dehydrogenase involved in ferulic acid and sinapic acid biosynthesis. *Plant Cell*. 2004;16(2):544-554. doi 10.1105/tpc.017509

Ondrej M. Evaluation of flax genepool according to resistance to Fusarium wilt of flax and to mildew. *Plant Genet. Resour*. 1993;92:54-58

Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A., Bender D., Maller J., Sklar P., de Bakker P.I., Daly M.J., Sham P.C. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575. doi 10.1086/519795

Rashid K.Y., Kenaschuk E.O. Effect of trifluralin on fusarium wilt in flax. *Can J Plant Sci*. 1993;3:893-901. doi 10.4141/cjps93-117

Rodriguez-Villalon A., Gujas B., Kang Y.H., Breda A.S., Cattaneo P., Depuydt S., Hardtke C.S. Molecular genetic framework for protophloem formation. *Proc Natl Acad Sci USA*. 2014;111(31):11551-11556. doi 10.1073/pnas.1407337111

Rozhmina T.A. Identification of effective genes of resistance to Fusarial wilt at variety of fibre-flax. *Biology in Agriculture*. 2017;4: 10-12 (in Russian)

Rozhmina T.A., Loshakova N.I. New sources of effective resistance genes to Fusarium wilt in flax (*Linum usitatissimum* L.) depending on temperature. *Sel'skokhozyaistvennaya Biologiya = Agric Biol.* 2016;51(3):310-317. doi 10.15389/agrobiology.2016.3.310eng

Rozhmina T., Samsonova A., Kanapin A., Samsonova M. An account of Fusarium wilt resistance in flax *Linum usitatissimum*: the disease severity data. *Data Brief*. 2022;41:107869. doi 10.1016/j.dib.2022.107869

Strawn M.A., Marr S.K., Inoue K., Inada N., Zubieta C., Wildermuth M.C. *Arabidopsis* isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits properties consistent with a role in diverse stress responses. *J Biol Chem*. 2007;282(8):5919-5933. doi 10.1074/jbc.M605193200

Tello D., Gil J., Loaiza C.D., Riascos J.J., Cardozo N., Duitama J. NGSEP3: accurate variant calling across species and sequencing protocols. *Bioinformatics*. 2019;35(22):4716-4723. doi 10.1093/bioinformatics/btz275

Wang X., Culver J.N. DNA binding specificity of ATAF2, a NAC domain transcription factor targeted for degradation by Tobacco mosaic virus. *BMC Plant Biol*. 2012;12:157. doi 10.1186/1471-2229-12-157

Wang X., Goregaoker S.P., Culver J.N. Interaction of the Tobacco mosaic virus replicase protein with a NAC domain transcription factor is associated with the suppression of systemic host defenses. *J Virol*. 2009;83(19):9720-9730. doi 10.1128/JVI.00941-09

Wang Z., Hobson N., Galindo L., Zhu S., Shi D., McDill J., Yang L., Hawkins S., Neutelings G., Datla R., Lambert G., Galbraith D.W., Grassa C.J., Geraldes A., Cronk Q.C., Cullis C., Dash P.K., Kumar P.A., Cloutier S., Sharpe A.G., Wong G.K., Wang J., Deyholos M.K. The genome of flax (*Linum usitatissimum*) assembled *de novo* from short shotgun sequence reads. *Plant J*. 2012;72(3):461-473. doi 10.1111/j.1365-313X.2012.05093.x

Wildermuth M.C., Dewdney J., Wu G., Ausubel F.M. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*. 2001;414(6863):562-565. doi 10.1038/35107108

Yan J., Su P., Meng X., Liu P. Phylogeny of the plant receptor-like kinase (RLK) gene family and expression analysis of wheat RLK genes in response to biotic and abiotic stresses. *BMC Genomics*. 2023;24(1):224. doi 10.1186/s12864-023-09303-7

You F., Cloutier S. Mapping quantitative trait loci onto chromosome-scale pseudomolecules in flax. *Methods Protoc*. 2020;3(2):28. doi 10.3390/mps3020028

Zhang C., Dong S.-S., Xu J.-Y., He W.-M., Yang T.-L. PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics*. 2019;35(10):1786-1788. doi 10.1093/bioinformatics/bty875

Zhang Y.M., Jia Z., Dunwell J.M. Editorial: the applications of new multi-locus GWAS methodologies in the genetic dissection of complex traits. *Front Plant Sci*. 2019;10:100. doi 10.3389/fpls.2019.00100

Zhang Y.-W., Tamba C.L., Wen Y.-J., Li P., Ren W.-L., Ni Y.-L., Gao J., Zhang Y.-M. mrMLM v4.0.2: an R platform for multi-locus genome-wide association studies. *Genomics Proteomics Bioinformatics*. 2020;18(4):481-487. doi 10.1016/j.gpb.2020.06.006

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