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STUDYING THE DIVERSITY OF LENTIL VARIETIES BY ALLELES OF THE EARLY FLOWERING GENE *LcELF3* USING MOLECULAR MARKERS

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Abstract

Lentils are one of the most widespread leguminous crops in the world and now Kazakhstan is also experiencing a significant expansion of acreage. Howev-er, late sowing is unacceptable for this crop, since in this case the photoperiod effect and drought lead to loss of crop samples if the flowering period of lentil plants is not optimized. The genes controlling early flowering (*EFL3*) are associ-ated with the rate of growth and the beginning of flowering and are independent of the length of daylight. In this study, the presence of the early flowering gene *LcELF3* was analyzed in 76 collection samples of lentils. As a result of PCR am-plification and sequencing of the *LcELF3* gene using the Sanger method, genetic polymorphism was detected and codominant SNP (Single Nucleotide Polymor-phism) markers were developed. Further testing of collection samples of lentils by ASQ genotyping allowed us to identify 36 samples with homozygous geno-type *aa* (late flowering) and 40 samples with homozygous genotype *bb* (earlier flowering). The use of factor analysis of the productivity elements of lentil sam-ples bearing favorable bb alleles of the early flowering gene *LcELF3* made it pos-sible to conduct a visual analysis for the studied years. Thus, using the example of lentils, the possibility of sampling samples containing favorable *bb* alleles of the *LcEFL3* gene, controlling early flowering, which are necessary to increase its yield in conditions of prolonged spring and short summer season in the dry steppe zone of Northern Kazakhstan, is shown.

Keywords: lentils; earlier flowering; factor analysis; allele-specific q-PCR, SNP genotyping; fluorescent tags; genetic markers.

Introduction

Lentil (*Lens culinaris* Medik) is a diploid species (2n = 2x= 14) with a hap-loid genome size of 4063 Mb [1], and an important legume crop, especially com-mon in India, North Africa, Asia, Southern Europe, America and Australia [2]. Lentil seeds are very nutritious and their protein content ranges from 20 to 36% [3]. Additionally, like all legumes, lentil plants are able to fix atmospheric nitro-gen [4] and improve the fertility of degraded soils [5, 6]. Due to the gradual de-cline in the profitability of wheat, Kazakhstan is gradually increasing crops and switching to other promising crops. In this regard, in the future, lentils may be-come a particularly important crop [5] and promising for replacing wheat in crop rotations. However, currently the zoned varieties of lentils are characterized by low yields. One of the main reasons for this situation is the limited genetic base of the available cultivated varieties of lentils with low yield potential. In this regard, research on lentil breeding is carried out in Kazakhstan, including by the authors of this study [6-8], in order to develop and study the source material for new va-rieties adapted to specific natural and climatic conditions. The studied lentil culti-vars were obtained from International Genetic Centers. When studying lentil cul-tivars, great attention was paid to phenotypic features that can be influenced by environmental conditions and geographical areas of plant cultivation (for exam-ple, soil condition, nutrient deficiency, lack of moisture, extreme temperatures, pest infestation and technological operations) [9, 10]. Modern and convenient molecular markers were used to identify various genotypes and carry out their genotyping. Currently, molecular markers are widely used for plant genotyping, assessing genetic diversity among species, population genetics, phylogenetic studies, biotechnology, creating genetic maps, mapping specific genes, and per-forming marker-assisted selection (MAS) [11-14]. First, it is necessary to deter-mine which type of molecular marker is best to use based on the following crite-ria: number of markers needed and their variability, codomant or other type of inheritance, requirements for the DNA to be tested, efficiency of the practical analysis, reproducibility of the results, technical support needed and its cost. Mo-lecular markers of several types were used to determine the allelic composition in various genetic collections of lentils [15-21]. The presented work uses ASQ markers (Allele-specific qPCR) developed on the basis of allele-specific quantita-tive real-time PCR (q-PCR) [22]. This marker system consists of two universal probes with fluorophores, as well as a third probe with a attenuator [23]. Addi-tionally, the modified ASQ method turned out to be significantly cheaper than other similar methods based on allele-specific interactions between fluorophores and a attenuator, since all three molecular probes in the ASQ method are repre-sented by short linear oligonucleotides, and the fluorophores and the attenuator are located at their ends [24]. This genotyping method is simple, affordable and low-cost for use in practical plant breeding based on SNP, using ASQ molecular markers.

Lentil plants usually show sensitivity to vernalization, and the development of signs such as stem elongation and branching of shoots play an important role in the transition from a vegetative state to flowering and, ultimately, affect plant productivity. In many species of flowering plants, determining the photoperiod is the key to synchronizing the reproductive stage with seasonal changes, which leads to maximum reproduction success. Sensitivity to photoperiod is the main agricultural feature chosen by breeders to increase yields or adapt crop varieties to different latitudes [25, 26]. In this regard, *ELF* (Early flowering time) genes are of particular importance, controlling the early flowering and reaction of plants to environmental changes and affecting the time of the beginning of flowering of leg-umes such as lentils. *ELF* genes are the main regulator for switching photoperiodic flowering with alternating cycles in time (circadian rhythms) and therefore they are given special attention in breeding for rapid plant development and crop improvement [27].

Circadian rhythms of plants are associated with the change of day and night and are important for the adaptation of plants to diurnal fluctuations in pa-rameters such as temperature, lighting and humidity. Plants exist in an ever-changing world, so circadian rhythms are important so that they can provide a quick response to abiotic stress. Changing the position of leaves during the day is just one of many constantly changing processes in plants. During the day, parameters such as enzyme activity, gas exchange intensity and photosynthetic activity fluctuate. A comprehensive study of one gene from this group, ELF3, in various crops demonstrates its sensitivity to early flowering in plant species such as Arabidopsis [28], rice [29], pea [30, 31], soybean [32], barley [33], chickpea [34] and many others.

The purpose of this study is to apply ASQ genotyping technology in the assessment of lentil cultivars and to identify the most valuable genotypes with *bb* alleles of the *LcELF3* gene controlling early flowering trait.

Materials and methods

The present study was conducted for three years (2020-2023) in the exper-imental fields of A.I. Barayev Research and Production Centre of Grain Farming. The experimental plant material consisted of 76 samples of the germplasm collec-tion of lentil from ICARDA (International Center for Agricultural Research in Ar-id Areas, Syria) and VIR (Vavilov Research Institute of Plant Industry, Russia). About half of them (43 samples) belong to the microsperma subspecies (small seed size), while the remaining 33 samples belong to the macrosperma subspecies (large seed size). Of these, 7 lentil samples were selected to confirm the genetic diversity: with an early flowering period (FLIP-1996-48L, FLIP-1992-36L, Niva-95 and Syrian Local) and late-flowering samples (ILL-1552, Krapinka and Vekhovskaya-1).

Data on the yield structure were evaluated on plots with an area of 1 m2, the distance between rows was 15 cm. The Shyraily variety was adopted as the standard for large seed size lentil, and the Krapinka variety for small seed size lentil. The study of the germplasm collection was carried out according to the methodology of studying the collection of legume crops [35].

Laboratory experiments were conducted on the basis of the Scientific Re-search Platform of Agricultural Biotechnology at S. Seifullin Kazakh AgroTech-nical Research University and in the Laboratory of Plant Genomics and Bioin-formatics at the National Center of Biotechnology.

The genomic DNA of lentil was isolated from the leaves of young plants [36]. The quality of the obtained DNA was checked by electrophoresis in 1% agarose gel with the addition of ethidium bromide. DNA concentration was measured using a NanoDrop spectrophotometer when analyzing the ratio of ab-sorption wavelengths of nucleic acids [36]. During PCR amplification, denatura-tion was performed at 94 °C for 15 seconds, the annealing temperature was set at 56°C, the elongation was at 72 °C for 1 min, and it was carried out for 39 cycles. The *LcELF3* gene in the study was fully amplified using two pairs of primers (Table 1). The amplified fragments were sequenced by Sanger to identify SNPs between genotypes. The obtained nucleotide sequences were analyzed and com-pared using the Sequencher program.

Table 1 – List of primers used in the work

№	Gene name	F/R	Sequence (5'-3')	Amplicon (bp)
1	*LcEFL3*	F1	CAGGCAGTCCAAAACAGTTAGG	863
		R1	CATGGTTGGGACACTTGAACAG	
2		F2	CACTGGGGCATGTTTCCTTGTA	853
			CTCTTCCAGATGGATCTACTCC	

The ASQ method with molecular markers was used for plant genotyping [37]. Allele-specific primers F1 and F2 were design to target each SNP, as well as one common reverse primer R. These primers did not contain fluorescent tags. In addition, universal molecular probes (Uni-1 and Uni-2) with fluorescent tags at the end, FAM and HEX (VIC), respectively, were added to the reaction mixture and bound to the PCR product based on primers F1 and F2 during amplification. The third universal probe (Uni-Q) had a Dabcyl quencher that absorbed unreal-ized fluorescence in amplification. Universal probes and allele-specific primers were synthesized at Sigma Aldrich (USA). SNP genotyping of lentil samples was performed on the amplifiers of the QuantStudio 7 Real-Time PCR System (Ap-plied Biosystems, USA) and Bio-Rad Real-time CFX96 Touch (USA). To ana-lyze SNP-based genotyping, PCR was performed in a 10 µl reaction mixture con-taining 10 ng of genomic DNA, 1 µl of 10× buffer, 1 µl of 2.5 mM dNTP mixture, 0.6 µl of 2.5 mM MgCl2, 0.8 µl DMSO (dimethyl sulfoxide), 10 µM of universal probes, 5 µM of each SNP-specific primer and 0.065 µl of Taq-polymerase (Si-bEnzyme, Russia) in each reaction. The ASQ technology on the real-time quantitative PCR (qPCR) instrument shows the distribution of alleles according to the fluorescence level of the two dyes used. SNP genotyping was performed automatically by detecting both types of *aa* and *bb* homozygotes for alleles 1 (FAM) and 2 (HEX-VIC), respectively. Heterozygotes were genotyped by two fluorescence signals at approximately the same level. To assess

the sensitivity of the reaction, sterile water containing no DNA (NTC, No template control) was used as a negative control. To prevent evaporation of the mixture, PCR was prepared on a special cooler, in 96-well microplates and sealed with an ultra-clear adhesive film.

Statistical analysis was carried out using Excel software based on the indi-cators of the elements of the crop structure: plant height, height to fist pod, plant dry weight, mass and number of seeds per plant. The identified 38 early flowering genotypes were analyzed during three years of study: 2021-2023. The data were processed using the 'Main Components' and Varimax method for a two-dimensional coordinate system using the SPSS Statistics Program. Data for the formation of two macrofactors coordinate axes are presented, which displayed correlations with them of crop structure for the studied years [38].

Results

Progress in the field of modern biology and plant breeding is based mainly on the development and application of molecular genetic methods based on the analysis of DNA polymorphism, which can be determined by various types of molecular markers. The use of any type of molecular markers for the correct interpretation of genotyping results is associated with the optimization of genotyping. Genotyping using molecular markers makes it possible to more accurately assess the genetic diversity of the studied cultivars, their purity, similarities and differences, as well as to evaluate possible genetic linkages of molecular markers with potential genes controlling economically valuable traits. Obviously, the genotype cannot be predicted by the appearance of a plant in the field, and genotyping of plants using molecular markers can only be carried out by a specialist in the laboratory.

Genotypes with different flowering periods were selected for genetic analy-sis: FLIP-1992-36L, FLIP-1996-48L, Krapinka, ILL-1552, Syrian Local, Vekhovskaya-1, Niva-95. Initially, PCR amplification was performed to se-quence the *LcELF3* gene by Sanger. As a result of amplification of the first fragment in the intron zone of the gene using a pair of specific primers *LcEFL3-F1* and *LcEFL3-R1*, a specific PCR product of the gene with a size of 863 bp was obtained. When comparing the sequencing results of this fragment, a genetic polymorphism was found between the studied lentil samples, where the presence of SNP on chromatograms is summarized in Table 2.

Table 2 – The SNP identification according to the results of nucleotide sequence of intron genetic fragments of the *LcELF3* gene with primers (F1 and R1) in seven genotypes of lentil

Genotypes	Flowering time	The position of the SNP (bp) in the PCR product of the F1R1 fragment in intron of the *LcELF3* gene					
		242	247	331	524	564	781
FLIP-1996-48L	early	T	T	A	A	T	-
ILL-1552	late	C	A	T	A	T	C
FLIP-1992-36L	early	T	T	A	G	G	T
Niva-95	early	T	T	A	G	G	T
Krapinka	late	C	A	T	A	T	C
Syrian Local	early	T	T	A	A	T	
Vekhovskaya-1	late	C	A	T	A	T	C

Based on the results of comparing the nucleotide sequence of the *LcELF3* fragment presented in Table 1, SNPs were identified that exactly corresponded to genotypes from different groups: early flowering (FLIP-1996-48L, FLIP-1992-36L, Niva-95 and Syrian Local) and late flowering (ILL-1552, Krapinka and Vekhovskaya-1). At the same time, the following combinations of SNPs were ob-served for six isolated SNPs, characteristic of genotypes with early and late flow-ering periods, respectively: T/C, T/A, A/T, G/A, G/T and T/C. However, only the first three SNPs (242, 247 and 331 bp, respectively), highlighted in Table 1, showed a complete coincidence of the obtained and expected results. These data are of particular interest and importance for the continuation of this work.

The results for the presence of the fourth and fifth SNP (524 and 564 bp, respectively) differed from the rest and did not completely coincide with the ex-pected results. This fact requires additional study and verification. In conclusion, the sequences of amplified PCR products in two samples, FLIP-1996-

48L and Syrian Local, turned out to be shorter than the others. Therefore, it is impossible to conclude that these samples have a sixth SNP (781 bp).

Based on the results of sequencing of lentil samples, one SNP-3 was select-ed for the development of allele-specific primers and genotyping of lentil samples according to the alleles of the *LcELF3* gene controlling early flowering trait. The design of the developed allele-specific primers used for molecular markers based on the identified SNP-3 for the assessment of genetic polymorphism by the *LcELF3* gene is shown in Figure 1.

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TTAAATAGGAATGTTTGGTAGTCCGATGATTTGATTTTTGTTATTTTGGTGGTTTATTGGTA
TTTTCTCCTACCTTCTTTTTTATATTTTCTTGCATTTATCATTAGCCAGGGCTGGGTTTGAA
AATATATATGAAAAWATCCTTTGAAGAGAAGAAATGTAGGACCAGGTAATGTGAATTATAGG
GACACTCCTATCTAACGTTGCCATTTTTTCCCTTACCTTTTAAAGCATCTTTTATGCATCCT
TAATTAAA
[W = A/T]

LcEFL3-SNP3-F1: CTACATTTCTTCTCTTCAAAGGATTT
26 bp, 31%GC, Tm=51.7C
(Reverse-compliment): AAATCCTTTGAAGAGAAGAAATGTA

LcEFL3-SNP3-F2: CTACATTTCTTCTCTTCAAAGGATAT
26 bp, 31%GC, Tm=51.7C
(Reverse-compliment): ATATCCTTTGAAGAGAAGAAATGTA

LcEFL3-SNP3-R: GGAATGTTTGGTAGTCCGATG
21 bp, 48%GC, Tm=52.4C
Amplicon size = 155 bp
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Figure 1 – Fragment of amplification and design of allele-specific primers based on SNP-3 in the *LcEFL3* gene, where positions of SNPs are indicated in red

The modified ASQ method allows changing completely any component, including the design and composition of allele-specific primers and universal probes, as well as the amplification program, temperature and duration of each stage. The modified ASQ method is widely used in plant genotyping [24].

For the general verification of ASQ technology and optimization of PCR stages, 19 experiments were conducted using molecular markers to study alleles of the *LcEFL3* gene, which controls flowering time trait in lentil plants. The quantitative PCR equipment used has been carefully prepared and tested in ac-cordance with the instructions provided. A quantitative PCR program was used, which gave positive results, but it can also be modified and optimized to achieve the best results (Figure 2).

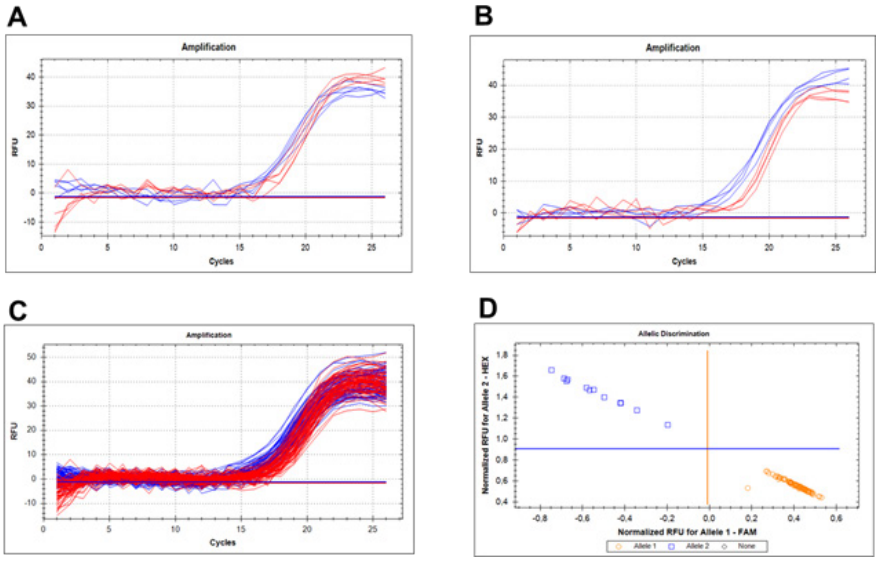

Figure 2 – Amplification curves and a graph for determining (discrimination) of alleles during genotyping of lentil samples by the *LcEFL3* gene: (A) homozygous *aa* genotypes demonstrating FAM amplification, red; (B) homozygous *bb* genotypes with HEX amplification, blue; (C) total fluorescence level during largescale genotyping (94 DNA samples and 2 NTC); (D) allele discrimination among genotypes

Using ASQ technology, the results of SNP genotyping are presented in a simple automatic way using computer software embedded in the qPCR device, similar to other methods based on fluorescence analysis. Graphs of allelic discrimination of fluorescence levels of the two dyes used are presented in graphical and tabular formats. The X- and Y-axes were automatically set to relative fluorescence units (RFU) for FAM and HEX/VIC, respectively. SNP genotyping is performed automatically by detecting both types of *aa* and *bb* homozygotes for alleles 1 (FAM) and 2 (HEX), respectively. Heterozygotes *ab* can be genotyped by two fluorescence signals at approximately the same level. In addition, genotypes showing mixed fluorophore amplification with clear discrimination should be classified as 'undetermined' or 'mixed', and such genotypes require further analysis. The use of control samples of both genes in SNP genotyping is necessary at each stage of genotyping for the accuracy of allele determination. The results of SNP genotyping are presented in the form of a graph of allele discrimination in different colors, normalized by NTC (Figure 2D).

As a result of the ASQ method application and optimization of the amplification protocol for SNP genotyping using primers developed for alleles of the *LcEFL3* early flowering gene, 36 homozygous *aa* alleles (late flowering) and 40 homozygous *bb* alleles (early flowering) were identified (Table 3).

Table 3 – Results of using a molecular marker for alleles of the LcEFL3 gene, which controls the sign of flowering time, for SNP genotyping of lentil from the International germplasm collection

№	Genotypes of plants	
	aa (late flowering)	bb (early flowering)
1	Krapinka, standard	Shyraily, standard
2	Sakura, Russia	PI 451764, Australia
3	ILL-474, Australia	PI-468898, Australia
4	FLIP 1996-15 L, ICARDA	ILL-485, Australia
5	K-2717, Mexico	FLIP 1996-48L, ICARDA
6	E-157, K-2708, Ecuador	PI-557499, Australia
7	K-2796, Ecuador	FLIP 1992-36L, ICARDA
8	K-924, Syria	FLIP 1986-51L, ICARDA
9	Vekhovskaya-1, Russia	PRECOZ, Argentina
10	K-2721, Colombia	E-149, K-2712, Ecuador
11	K-1084, Italy	E-112, K-2713, Ecuador
12	Giansha, Italy	K-2715, Ecuador
13	89ZPR-8, K-2843, Canada	K-2720, Colombia
14	Odnotsvetkovaya, Hungary	Richelea, Canada
15	K-1532, Russia	89-12, K-2845, Canada
16	Niva-95, Russia	Chiflik-7, Bulgaria
17	Sel97-39L, 98S029, Australia	Slavyanka, Russia
18	ILL-5725, Australia	Rozovaya, Russia
19	PI-509335, Australia	FLIP 1997-6L, ICARDA
20	PI-509334, Australia	PI-543920, Australia
21	FLIP 1990-41L, ICARDA	FLIP 1995-30L, ICARDA
22	FLIP1989-63L, ICARDA	PI-509333, Australia
23	FLIP 1987-56L, ICARDA	ILL-4611, Australia
24	Syrian local, Syria	ILL-1464, Australia
25	Local, K-903, Armenia	Lentil Pardina, Italy
26	Local, K-894, Russia	Lebanese Local, ICARDA
27	Roze, K-2846, Canada	Local, K-2707, Mexico

Continuation of Table 3

28	PR-86-385, K-2834, Canada	Local, K-408, Palestine
29	VIR, K-482, Armenia	Local, K-883, Palestine
30	VIR, K-452, Georgia	Local, K-2589, Armenia
31	VIR, K-538, Turkey	81S15, K-5883, Georgia
32	ILL-4349, Australia	VIR, K-474, Armenia
33	PI 471916 Australia	VIR, K-664, Azerbaijan
34	L1278, India	VIR, K-910, Armenia
35	78S 26002, Australia	VIR, K-907, Armenia
36	VIR, K-905, Armenia	VIR, K-467, Armenia
37		ILL-5714, Australia
38		Petrovskaya-Yubileynaya, Russia
39		FLIP 88-10L, ICARDA
40		k-2706, Bolivia

Discussion

Monitoring the fluorescence of each of the universal probes used is very convenient using a quantitative real-time PCR instrument that automatically rec-ords the fluorescence after each amplification cycle. However, as a rule, the am-plification of any fluorescence begins no earlier than 14-15 cycles, and even after 20 cycles. Non-typical or 'abnormal' amplified samples must be removed or re-amplified to avoid incorrect evaluation. For example, such a situation may occur if the fluorescent signal began to manifest very early, after several cycles, or if amplification is not the main exponential curve, since this is a requirement of all methods based on PCR with fluorescent tags.

To study the phenotypic features, a factor analysis of the indicators of productivity elements of 40 genotypes of lentils bearing favorable *bb* alleles of the early flowering gene *LcELF3* was carried out, and the results of three-year studies were presented in 2D format. The average values for each feature are shown in Table 4.

Table 4 – Indicators of economically valuable traits in 40 lentil germplasms with favorable *bb* alleles of the early flowering gene *LcELF3* by year, 2021-2023

Year	Plant height, cm	The height of the lower bean attachment, cm	Dry weight, g	The number of seeds per plant, pcs.	Weight of seeds per plant, g
2021	29.28	11.56	6.24	128.43	5.88
2022	25.02	13.22	2.59	19.94	0.85
2023	25.30	15.21	3.54	28.72	1.36
Average	26.53	13.33	4.12	59.03	2.70

When constructing the model, the reliability of the results is proved by the KMO criterion, which showed a result above 0.6 with a statistical significance of $p<0.05$ (Table 5).

Table 5 – Indicators of the adequacy measure and the Bartlett criterion

Measure of sampling adequacy Kaiser-Meyer-Olkin (KMO)		0.697
Bartlett's sphericity criterion	Approximate chi-squared	593.790
	Standard deviation	36
	Significance	0.000

Two components have been selected for a two-dimensional coordinate sys-tem, and the Varimax rotation method is necessary to display the correlation of economic characteristics with only one of them. If the first component (PC1) on the abscess axis was characterized by morphological features

(plant height and attachment height of the lower bean), then the second (PC2), on the ordinate axis, by plant productivity (dry weight, number and weight of seeds per plant). On the graph, the indicators of the crop structure are displayed close to the correlating axis. In this case, a perpendicular line is constructed from the medium under study to the feature under consideration. This takes into account the location of the projection relative to the origin; zero is the average (Figure 3).

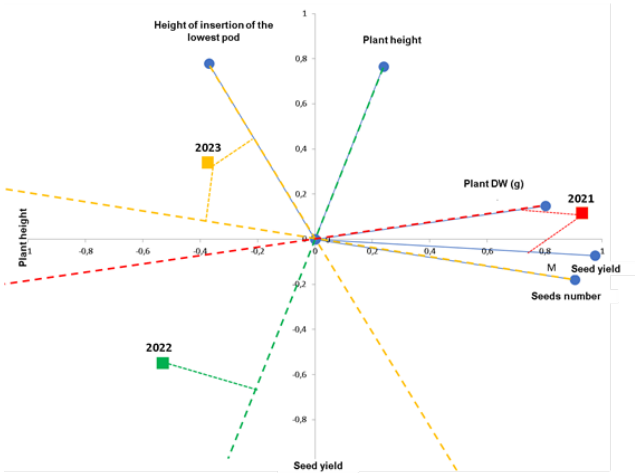

Figure 3 – Model of changes in the elements of the crop structure for the studied years

Table 4 shows that the average value of the seed weight (5.88 g) and the dry weight of the plant (6.24 g) were noticeably higher in 2021. Therefore, the projection on this axis in the form of a small segment is most distant from the origin in the positive direction. Similar, in 2022, plant height value stood out – the average estimate of 25.02 cm out of 26 possible is the closest to the average value, but downwards. According to the results of observations in 2023, the indi-cators of the number of seeds per plant (28.72) and height to first pod (15.21 cm) prevailed.

Conclusion

Although the ASQ genotyping method is relatively new, it is cost-effective, but in order to achieve accurate results using this technology, it is necessary to optimize the composition and mode of PCR, to determine the relationship between the selected primers and universal probes. In addition, it was found that the use of DMSO as a control variant in a DNA-free sample led to a significant decrease in the fluorescence of the Uni-FAM and Uni-HEX/VIC, and NTC probes, as well as improved the genotyping of SNP.

A genetic analysis of seven genotypes with different flowering periods was carried out: early flowering (FLIP-1996-48L, FLIP-1992-36L, Niva-95 and Syrian Local) and late flowering (ILL-1552, Krapinka and Vekhovskaya-1). Initially, PCR amplification was performed by Sanger sequencing of the *LcELF3* gene. As a result of PCR amplification in the intron region of the gene using a pair of specific primers *LcEFL3-F1* and *LcEFL3-R1*, a specific PCR product of the gene with a size of 863 *bp* was obtained. When comparing Sanger sequencing results of the *LcELF3* gene, the genetic polymorphism was found in this fragment between the studied lentil samples. Three isolated SNPs (on the positions 242, 247 and 331 bp, respectively) showed a complete coincidence of the obtained and expected results. In this study, one SNP-3 was selected for the development of allele-specific primers in lentil genotypes for alleles of the *LcELF3* gene that control early flowering trait.

For the general verification of ASQ technology and optimization of PCR stages, 19 experiments were conducted. In this study, the testing of ASQ technology showed a positive result using a molecular marker for the *LcEFL3* gene, which controls flowering time trait in 76 samples of the International lentil germplasm collection: 36 samples with the homozygous *aa* genotype had later flowering, and 40 samples with the homozygous *bb* genotype had earlier flowering.

Additionally, analysis of productivity elements in 40 lentil genotypes carrying favorable aa alleles of the early flowering gene *LcELF3* allowed a quick interpretation of the averages. At the same time,

based on the factor analysis of the results of field tests of lentil genotypes, a compact and informative diagram in a two-dimensional coordinate system was obtained. As a result of the analysis, it was found that the weather conditions of 2021 had a favorable effect on the value of the seed weight (5.88 g) and dry weight of plant (6.24 g), and in 2022 tall plants prevailed. Despite the arid conditions in 2023, the indicators of height to first pod (15.21 cm) and seed number per plant (28.72) prevailed, which proves the presence of the *ELF* gene in these lentil samples, which control early flowering and the reaction of plants to environmental changes.

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МОЛЕКУЛЯРЛЫҚ МАРКЕРЛЕРДІ ҚОЛДАНУ АРҚЫЛЫ LcELF3 ЕРТЕ ГҮЛДЕУ ГЕНІНІҢ АЛЛЕЛІНЕ СӘЙКЕС ЖАСЫМЫҚ СОРТ ҮЛГІЛЕРІНІҢ ӘРТҮРЛІЛІГІН ЗЕРТТЕУ

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Түйін

Жасымық дүние жүзінде кең тараған бұршақ тұқымдас дақылдардың бірі болып табылады және қазір Қазақстанда да егіс алқаптары айтарлықтай кеңейіп жатыр. Алайда, бұл дақыл үшін кеш егуге жол берілмейді, өйткені бұл жағдайда фотопериодтық эффект пен құрғақшылық жасымық өсімдіктерінің гүлдену кезеңі оңтайландырылмаған жағдайда үлгі өнімділігінің жоғалуына әкеледі. Ерте гүлденуді бақылайтын гендер (EFL3) өсу жылдамдығымен және гүлденудің басталуымен байланысты және күн ұзақтығына тәуелсіз. Бұл зерттеуде *LcELF3* ерте гүлдену генінің болуы жасымықтың 76 коллекция үлгісінде талданған. ПТР күшейту және *LcELF3* генін Сэнгер әдісімен секвенирлеу нәтижесінде генетикалық полиморфизм ашылды және кодоминантты SNP (Single Nucleotide Polymorphism) маркерлері жасалды. ASQ генотиптеу

әдісін қолдану арқылы коллекциялық жасымық үлгілерін одан әрі сынау гомозиготалы *aa* генотипімен (кеш гүлдену) 36 үлгіні және гомозиготалы *bb* генотипімен (ерте гүлдену) 40 үлгіні анықтауға мүмкіндік берді. Ерте гүлдейтін *LcELF3* генінің қолайлы *bb* аллельдерін алып жүретін жасымық үлгілерінің өнімділік элементтеріне факторлық талдауды қолдану зерттелетін жылдарға визуалды талдау жүргізуге мүмкіндік берді. Осылайша, Солтүстік Қазақстанның дала зонасында жасымық мысалын пайдалана отырып, біз *LcEFL3* генінің қолайлы *bb* аллельдері бар үлгілерді таңдау мүмкіндігін көрсетеміз, олар ерте гүлденуді бақылайды, бұл құрғақшылықта ұзақ көктем және қысқа жаз мезгілі жағдайында оның өнімділігін арттыру үшін қажет.

Кілт сөздер: жасымық; ерте гүлдену; факторлық талдау; аллельге тән q-ПТР, SNP генотиптеу; флуоресцентті тегтер; генетикалық маркерлер.

ИЗУЧЕНИЕ РАЗНООБРАЗИЯ СОРТООБРАЗЦОВ ЧЕЧЕВИЦЫ ПО АЛЛЕЛЯМ ГЕНА РАННЕГО ЗАЦВЕТАНИЯ *LcELF3* С ИСПОЛЬЗОВАНИЕМ МОЛЕКУЛЯРНЫХ МАРКЕРОВ

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Аннотация

Чечевица – одна из наиболее распространенных зернобобовых культур в мире и сейчас в Казахстане также наблюдается значительное расширение посевных площадей. Однако, поздний посев неприемлем для данной культуры, так как в этом случае эффект фотопериода и засуха приводят к потере урожая образцов, если не оптимизировать период цветения растений чечевицы. Гены, контролирующие раннее цветение (EFL3), связаны со скоростью роста и началом зацветания и независимы от длины светового дня. В данном исследовании был проанализировано наличие гена раннего зацветания *LcELF3* у 76 коллекционных образцов чечевицы. В результате ПЦР-амплификации и секвенирования гена *LcELF3* по методу Сэнгера был обнаружен генетический полиморфизм и разработаны кодоминантные SNP (Single Nucleotide Polymorphism) маркеры. Дальнейшее тестирование коллекционных образцов чечевицы методом ASQ-генотипирования позволило выделить 36 образцов с гомозиготным генотипом aa (позднее зацветание) и 40 образцов с гомозиготным генотипом *bb* (ранее зацветание). Применение факторного анализа

элементов продуктивности образцов чечевицы, несущих благоприятные *bb* аллели гена раннего зацветания *LcELF3*, позволила провести визуальный анализ по исследуемым годам. Таким образом, на примере чечевицы показана возможность отбора образцов, содержащие в своем составе благоприятные *bb* аллели гена *LcEFL3*, контролирующие раннее зацветание, которые необходимы для увеличения ее урожайности в условиях затяжной весны и короткого летнего сезона в сухостепной зоне Северного Казахстана.

Ключевые слова: чечевица; ранее зацветание; факторный анализ; аллель-специфическая q-ПЦР, SNP-генотипирование; флуоресцентные метки; генетические маркеры.