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GMSEP3A GENE EXPRESSION IN SOYBEAN (*GLYCINE MAX L.*)

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SUMMARY

Soybean (*Glycine max* L.) is one of the most essential sources of high-grade protein and oil. Although growing soybeans can occur in different environmental conditions and are very productive in warm temperatures, their cultivation in cold climates, including Northern Kazakhstan, leads to considerable yield losses. The presented study sought to investigate soybean cultivars growing in the cold climate of northern Kazakhstan and find the optimal ratio of the growing season duration and crop yields. Soybean productivity and the cropping season length revealed a significant negative correlation. Early flowering is one of the relevant approaches for reducing the growing season and improving soybean seed production. A MADS-box SEPALLATA3 (*GmSEP3*) gene expression, known to promote regular and early flowering, received analysis. The *GmSEP3a* expression in soybean cultivars with high yield was also valid, with a significant positive correlation between *GmSEP3a* expression and soybean yield. The soybean cultivars, viz., Suiyang 1, Sibiryachka, No. 86, Niva, and Beidou 43 surfaced as high-yielding cultivars under environmental conditions of Northern Kazakhstan. The pertinent study revealed that the *GmSEP3a* gene can become a gene marker for screening early-ripening soybean cultivars with high yields in low-temperate climates. The promising results can also help identify potential soybean cultivars for cold-weather cultivation.

Keywords: Soybean (*Glycine max* L.), early maturing cultivars, growing season duration, productivity, plant height, transcription factors, warm and cold climate

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Key findings: The study highlights the need to enhance soybean yields in Kazakhstan, focusing on early ripening cultivars. The *GmSep3a* gene correlates with high yields and early ripening, offering a potential genetic marker for breeding. Among 90 cultivars analyzed, only five exceeded 10 t/ha, which shows promise for further improvement. This gene marker could aid in developing soybean cultivars suited for cold climates like Kazakhstan.

INTRODUCTION

Soybean (*Glycine max* L.) cultivation serves as the most valuable source of high-grade protein and oil progressively grown worldwide. Global soybean yield averages 27 t/ha, while in Russia, it is 15.9 t/ha. Soybean production centers in 90 countries, with the limit exceeding 100 million hectares worldwide. About 80% of the produced volumes served as a protein source, with 20% processed into vegetable oil, tofu, soy sauce, and pasta. Soybean acreage concentration is in the USA (about 35%–40% of the world), Brazil (20%), China (12%–13%), Argentina (12%), and India (8%). In the soybean and soy products market, the key player is China, both as a chief importer and exporter (Sun *et al.*, 2017; Wang *et al.*, 2023).

Generally, soybeans have high ecological adaptability; however, at the same time, they impose increased requirements for heat and moisture, which are limiting factors when grown under various environmental conditions. Soybean is a short-day crop that can grow in a wide range of latitudes, from equatorial to at least 50 degrees North latitude and 35 degrees South latitude (Watanabe *et al.*, 2012).

Concerning warm temperatures, soybeans have the highest need (Zhang *et al.*, 2016; Sobko *et al.*, 2020). It is a theory that the lowest limit of average daily temperature for soybean plants is at least 15 °C. The sum of active temperatures (10 °C and above) during the growing season for very early soybean cultivars should be in the range of 1700 °C–1900 °C, for early (2000 °C–2200 °C), medium–ripened (2600 °C–2750 °C), and very late maturing cultivars (3000 °C–3200 °C).

Given the climatic features, soybean cultivation is difficult in Kazakhstan (Didorenko *et al.*, 2016; Akbota *et al.*, 2017). Expanding soybean sowing areas in the northern regions of Kazakhstan sustained hindrances from stressful conditions—limited thermal and humidity resources. However, recently, the observed warm climate and enhancing active temperature in the territory allowed for the cultivation of this crop. Accordingly, the breeding focuses on the resistance to abiotic and biotic stresses, a reduced reaction to the daylight length, and adaptation to the agroclimatic conditions of the growing zone, considering the parameters of agrophytocenosis (Sidorik *et al.*, 2022).

With the highest demand for soybeans, numerous researchers are working in the direction of mapping the loci of important agronomic features, such as structural features and development of plants, quantity, structure, and quality of the crop, and resistance to abiotic stress factors and diseases (Hu *et al.*, 2013).

Soybean productivity and the growing season length revealed a significant negative correlation. For obtaining the early flowering genotypes with higher seed production, the MADS-box SEPALLATA3 (*SEP3*) gene expression underwent analysis to promote normal flower development, especially early flowering (Pelaz *et al.*, 2001; Immink *et al.*, 2009; Kaufmann *et al.*, 2009; Zeng *et al.*, 2018). The presented investigation also revealed that the *GmSEP3a* gene can serve as a gene marker for screening the early-maturing soybean cultivars with high yields in low-temperate climates. The pertinent data can help search potential soybean cultivars for cold-weather cultivation.

MATERIALS AND METHODS

Plant material

The timely research began in 2022 at the Experimental Agricultural Breeding Station, Kazakh Agrotechnical Research University, named after S. Seifullin, Akmola region, Northern Kazakhstan. The soil selected for conducting the experiments was a southern carbonate chernozem. The field used was a fallow land uncultivated for at least one year. The experiment followed agrotechniques according to the zonal cultivation technology. Sowing commenced on May 23, 2022, by the SSFK-7 breeding seeder (Omsk Experimental Company, Omsk, Russia). The object of research was 90 cultivars of soybean (*G. max*) of various ecological and geographical origins, i.e., domestic selection (19), Russian (42), Chinese (25), Canada (2), France (1), and Chuvash selection (1) (Table 1).

The layout of the field experiment was according to the methodological instructions of VIR (Vishnyakova *et al.*, 2018). Phenological observation recording of the growth and development of soybeans followed the methodology of Fehr and Cavines (1979). Cultivar purity determination analyzed sheaf samples from 25 plants. Finding the height of

attachment of lower beans had the distance measured from the place of attachment of the lower bean to the root neck. Consideration of the ripeness of seeds during these measurements allowed to minimize the loss of seeds when cut at a height of 10 cm. The selected 25 plants reached structural analysis (Korsakov *et al.*, 1968).

The soybean grain yield assessment had the separate harvesting of genotypes when fully ripened. Harvesting transpired when soybean genotypes are mature. After threshing and weighing the seeds, the seed yield in tons per hectare calculation followed.

Nucleic acid purification and RT-qPCR

Total RNA/DNA isolation from leaves of three-week soybean seedlings used the CTAB-based extraction method (Kiselev *et al.*, 2015). The cDNAs produced utilized the MMLV Reverse transcription PCR Kit with oligo (dT)15 (RT-PCR, Evrogen, Moscow, Russia), as also described by Kiselev *et al.* (2015). The mRNA transcript levels determination of the *GmSep3a* gene followed the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) with two internal controls, including *G Actin*(XM_041012802) and *Gm GAPDH*(NM_001361055) for soybean (Hu *et al.*, 2009).

Table 1. The cultivars based on the country or region of their selection.

Selection	Number of Cultivars	Cultivar Names
Domestic Selection	19	Svetlaychek, Ivushka, Nuralem-1, Nuralem-2, Ivushka 1, Nur+, Otan+, Progress, Atameken, No. 108, No. 77, No. 86, Swan 1344 I, Swan 1344 II, HanA., 111-21-1, 117-21-1, 119-21-1, 122-21-1
Russian Selection	42	SKFarta, SKElana, Bara, Pripyat', Ros', Volma, Cheremshanka, Zolotistay, Nadezhda, Alayska, Shatilovskaya17, Osmon', Zusha (1), Viktoria, Oressa, Selekt 302, Arktika, Riana, Al'ta, Viola, Belgorodskay7, Lider 10, Larisa, Sibiryachka, Omskay 4, Zaryanitsa, Kasatka, Svetlay, Dina, Eldorado, Cheremshanka 1, Scher 2, Niva, Altom, Milyausha, Mezenka, Vezelica, Lancentnay, 43-21-1, 91-21-3, 92-21-1, 93-21-2
Chinese Selection	25	Suiyang 1, Beidou 43, Beidou 26, Heihe 43, Heihe44, Beidou 51, Heihe 58, Heihe 49, Huajiong 2, Heihe 35, LongKen 336, Heihe 33, Heihe 38, Beidou 53, Kendou 60, Heike 59, 7-21-2, 13-21-1, 28-21-1, 31-21-1, 34-21-1, 35-21-1, 35-21-4, 46-21-1, 79-21-3
Canada Selection	2	OAKPrudens, 100-21-1
France Selection	1	Komandor
Chuvash Selection	1	Chara 1-3

RT-qPCR reactions proceeded in volumes of 20 µl using the real-time PCR kit (Evrogen) as described by Kiselev *et al.* (2021) containing 1x Taq buffer, 2.5 mM MgCl₂, 0.2 mM each dNTP, 0.2 µM each oligonucleotide primer, 1x SybrGreen I Real-time PCR dye, 1 µl cDNAs, and 1 unit of Taq DNA polymerase (Evrogen). Analysis ran in DTprime 4M1 Thermal Cycler (DNA-technology, Moscow, Russia) programmed for an initial denaturation step for 2 min at 95 °C followed by 50 cycles for 10 s at 95 °C and 25 s at 62 °C.

Statistical analysis

All the recorded data underwent analysis of variance (ANOVA) using Microsoft Excel and the Statistica-10 software. The means' differences comparison employed the least significant difference (LSD_{0.05}) test.

RESULTS AND DISCUSSION

Soybean cultivars with agronomic traits

The 90 soybean cultivars growing in the Akmola region, Northern Kazakhstan (Table 1) had the soybean seeds sown in a collection nursery in May 2022. Throughout the growing period, the soil had a hydrothermal moisture coefficient characteristic from 0.31 to 0.65, indicating that soybean plant growth was in arid conditions, a general feature of the Northern Kazakhstan climate.

Other important variables determined were the duration of the growing season, plant height, the number and weight of seeds, and the seed yield of different soybean cultivars using generally accepted methods. Initially, it was worth noting that the growing season duration in the studied cultivars changed in the collection nursery from 85 to 113 days (Figure 1). The shortest growing season was 85–87 days for the soybean cultivars Arctika, 7-21-2, Suiyang-1, Svetlaychek, Beidou-43, Chara-1-3, and Shatilovskay-17. However, the longest growing season (111–113 days) resulted in genotypes Riana, Viola, Bara St, Swan-1344 I, Swan 1344-II, and 43-21-1 (Figure 1).

On plant height, the tallest plants (90–94 cm) appeared in soybean cultivars 43-21-1, 13-21-1, and 93-21-2, while the cultivars with the shortest plant height (17.4–21.4 cm) were the Heihe-49, Dina, Heihe-33, and Heihe-44 (Table S1). The largest mass of seeds per plant (76.8–95.3 g) was evident in the cultivars 122-21-1, 43-21-1, and 13-21-1, with the minimum seed weight observed in the cultivars 7-21-2, Swan 1344 I, Mezenka, Belgorod-7, and Swan 1344-II.

The most influential complex trait in crop plants is seed yield, and its determination in the studied cultivars materialized. The soybean cultivars observed with the highest yield (t/ha) in existing environmental conditions were Suiyang-1 (12.2), Sibiryachka (12), No. 86 (10.8), Niva (10.2), Beidou-43 (10.1), Milyausha (9.5), Cheremshanka (9.3), Huajiong 2 (9.1), Farta (8.5), and Bara (8.4) (Figure 2). Based on the seed yield per hectare, the most productive soybean cultivars were Suiyang-1, Farta, Beidou-43, Bara, Huajiong-2, No. 86, Sibiryachka, Cheremshanka, Niva, and Milyausha (Figure 2).

Applying the GGI biplot model helped carry out effective primary data processing, which allows the assessment of soybean genotypes under various environments. Visualization of the obtained data revealed the perspectives of genotypes close to 'ideal' for the studied environments (Maniruzzaman *et al.*, 2019). Early and late-maturing soybean cultivars were samples in these models. The 'Which wins where' model characterizes the cultivars' seed yield; the horizontal axis of the biplot (PC1) showed the genotype effect, and the vertical (PC2) exhibited the genotype-environment interaction. According to the results of 'model a,' the total dispersion was 100%, with 80.1% for PC1 and 19.9% for PC2. In 'model b,' the total dispersion was also 100%, with 79.9% and 20.1% for PC1 and PC2, respectively (Figure 3).

In 'model a,' the best genotype-environment interaction (GEI) occurred in the soybean cultivar Beidou 43 for 2021 and in cultivar Niva for 2022 (Figure 3). In 'model b,' the most favorable interaction emerged for the

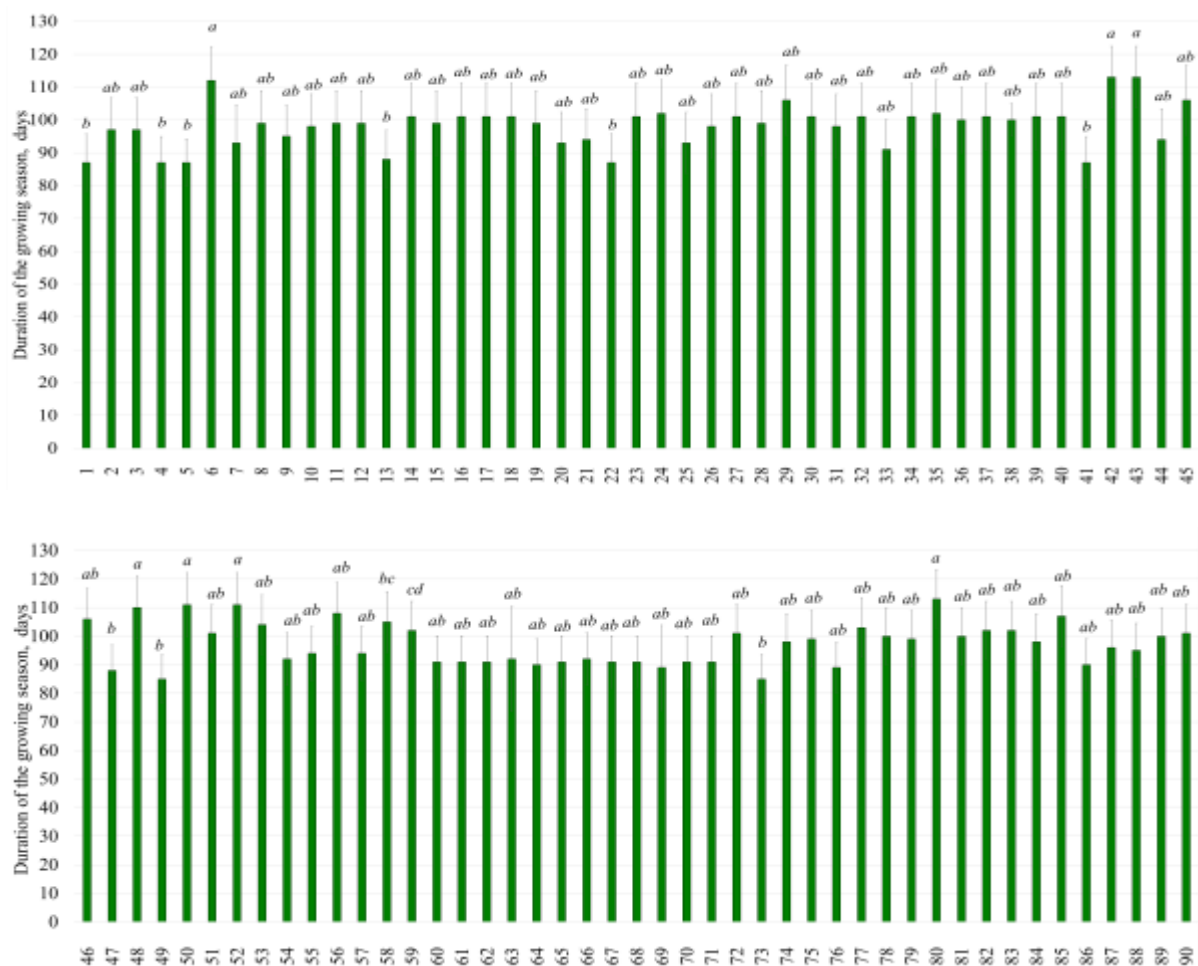


Figure 1. Duration of the growing season of the analyzed soybean *Glycine max* cultivars. 1 - Suiyang 1; 2 - SK Farta; 3 - SK Elana; 4 - Svetlaychek; 5 - Beidou 43; 6 - Bara St; 7 - Ivushka St.; 8 - Beidou 26; 9 - Heihe 43; 10 - Heihe 44; 11 - Beidou 51; 12 - Heihe 58; 13 - Heihe 49; 14 - Huajiong 2; 15 - Heihe 35; 16 - LongKen 336; 17 - Heihe 33; 18 - Heihe 38; 19 - Beidou 53; 20 - Kendou 60; 21 - Heike 59; 22 - Chara-1-3; 23 - Nuralem-1; 24 - Nuralem-2; 25 - Ivushka 1; 26 - Alayska; 27 - Pripyat'; 28 - Ros'; 29 - Volma; 30 - Cheremshanka 1; 31 - Zolotistay; 32 - Nadezhda; 33 - OAK Prudens; 34 - Nur +; 35 - Otan +; 36 - Progress; 37 - Atameken; 38 - No. 108; 39 - No. 77; 40 - No. 86; 41 - Shatilovskay 17; 42 - Akku 1344 I; 43 - Akku 1344 II; 44 - Osmon'; 45 - Zusha (1); 46 - Viktoria; 47 - Oresa; 48 - Selekt 302; 49 - Artika; 50 - Riana; 51 - Alta; 52 - Viola; 53 - Komandor; 54 - Lancentnay; 55 - Vezelica; 56 - Han A; 57 - Mazenka (1); 58 - Belogrodskay 7; 59 - Lider 10; 60 - Larisa; 61 - Sibiryachka; 62 - Omskay 4; 63 - Zaraynica; 64 - Kasatka; 65 - Svetlay; 66 - Dina; 67 - Eldorado; 68 - Cheremshanka; 69 - Sacher 2; 70 - Niva; 71 - Altom; 72 - Milyausha; 73 - 7-21-2; 74 - 13-21-1; 75 - 28-21-1; 76 - 31-21-1; 77 - 34-21-1; 78 - 35-21-1; 79 - 35-21-4; 80 - 43-21-1; 81 - 46-21-1; 82 - 79-21-3; 83 - 91-21-3; 84 - 92-21-1; 85 - 93-21-2; 86 - 100-21-1; 87 - 111-21-1; 88 - 117-21-1; 89 - 119-21-1; and 90 - 122-21-1. Means followed by the same letter were not different using Student's t test, where $p < 0.05$ was considered to be statistically significant.

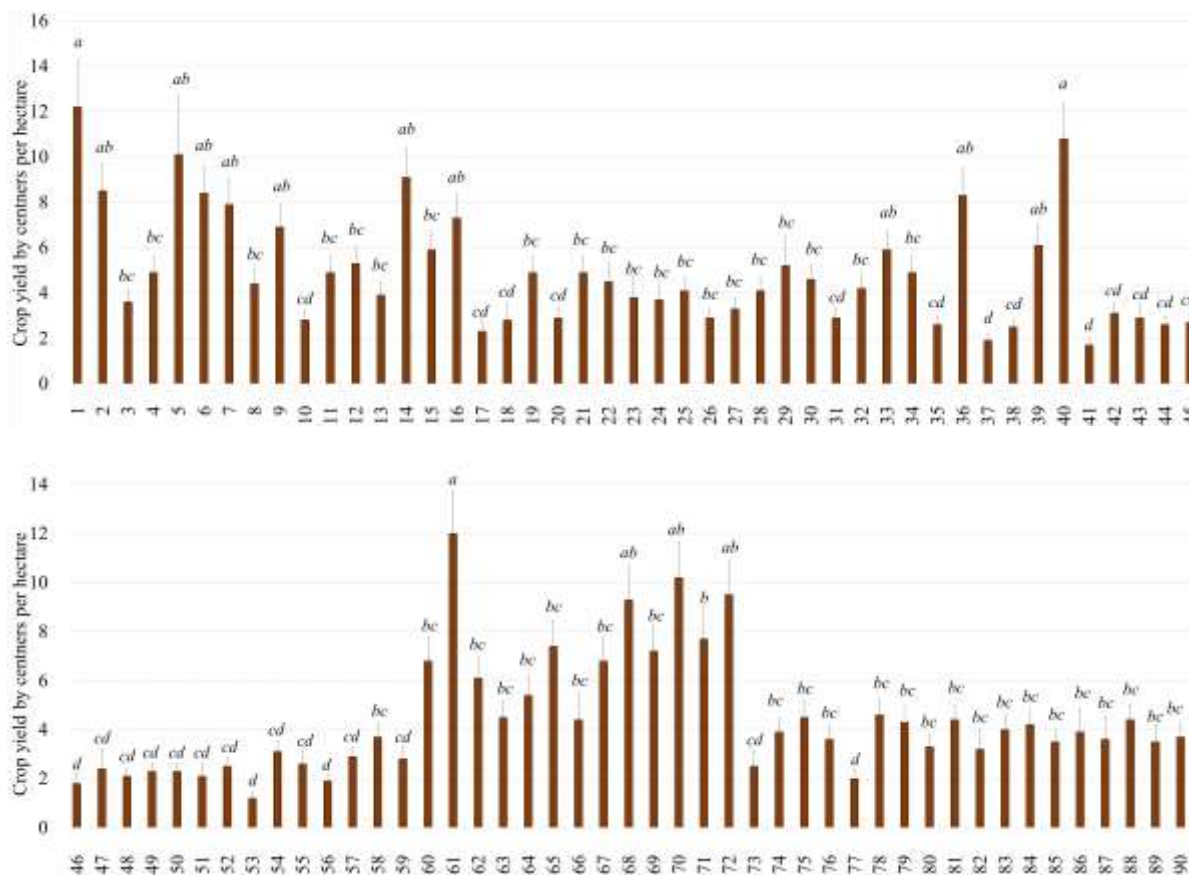


Figure 2. Crop yield by centners (100 kg/centner) per hectare of the analyzed soybean *Glycine max* cultivars. 1 - Suiyang 1; 2 - SK Farta; 3 - SK Elana; 4 - Svetlaychek; 5 - Beidou 43; 6 - Bara St; 7 - Ivushka St.; 8 - Beidou 26; 9 - Heihe 43; 10 - Heihe 44; 11 - Beidou 51; 12 - Heihe 58; 13 - Heihe 49; 14 - Huajiong 2; 15 - Heihe 35; 16 - LongKen 336; 17 - Heihe 33; 18 - Heihe 38; 19 - Beidou 53; 20 - Kendou 60; 21 - Heike 59; 22 - Chara-1-3; 23 - Nuralem-1; 24 - Nuralem-2; 25 - Ivushka 1; 26 - Alayska; 27 - Pripyat'; 28 - Ros'; 29 - Volma; 30 - Cheremshanka 1; 31 - Zolotistay; 32 - Nadezhda; 33 - OAK Prudens; 34 - Nur +; 35 - Otan +; 36 - Progress; 37 - Atameken; 38 - No. 108; 39 - No. 77; 40 - No. 86; 41 - Shatilovskay 17; 42 - Akku 1344 I; 43 - Akku 1344 II; 44 - Osmon'; 45 - Zusha (1); 46 - Viktoria; 47 - Oresa; 48 - Selekt 302; 49 - Artika; 50 - Riana; 51 - Alta; 52 - Viola; 53 - Komandor; 54 - Lancentnay; 55 - Vezelica; 56 - Han A; 57 - Mazenka (1); 58 - Belogorodskay 7; 59 - Lider 10; 60 - Larisa; 61 - Sibiryachka; 62 - Omskay 4; 63 - Zaraynica; 64 - Kasatka; 65 - Svetlay; 66 - Dina; 67 - Eldorado; 68 - Cheremshanka; 69 - Sacher 2; 70 - Niva; 71 - Altom; 72 - Milyausha; 73 - 7-21-2; 74 - 13-21-1; 75 - 28-21-1; 76 - 31-21-1; 77 - 34-21-1; 78 - 35-21-1; 79 - 35-21-4; 80 - 43-21-1; 81 - 46-21-1; 82 - 79-21-3; 83 - 91-21-3; 84 - 92-21-1; 85 - 93-21-2; 86 - 100-21-1; 87 - 111-21-1; 88 - 117-21-1; 89 - 119-21-1; and 90 - 122-21-1. Means followed by the same letter were not different using Student's t test, where $p < 0.05$ was considered to be statistically significant.

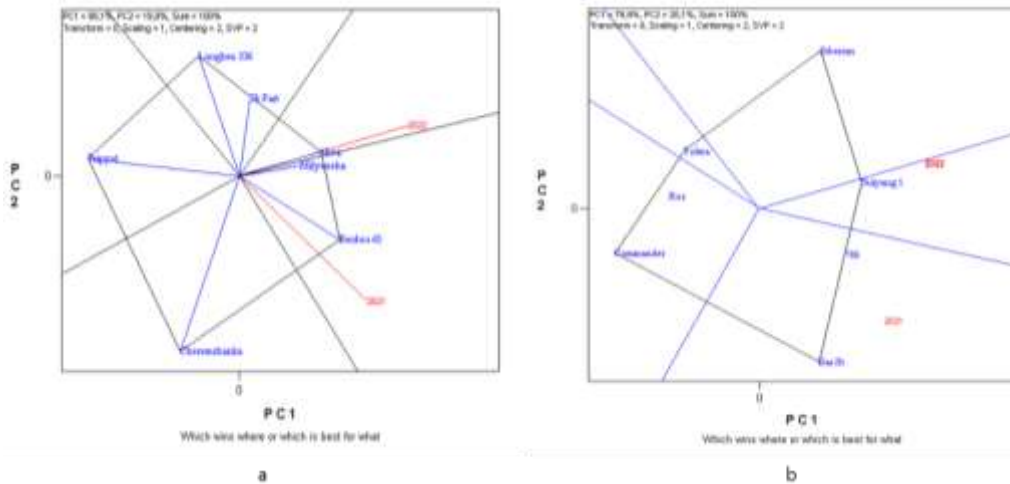


Figure 3. Biplot of the GGE model 'Who wins where or what is better for what,' different variants of soybean.

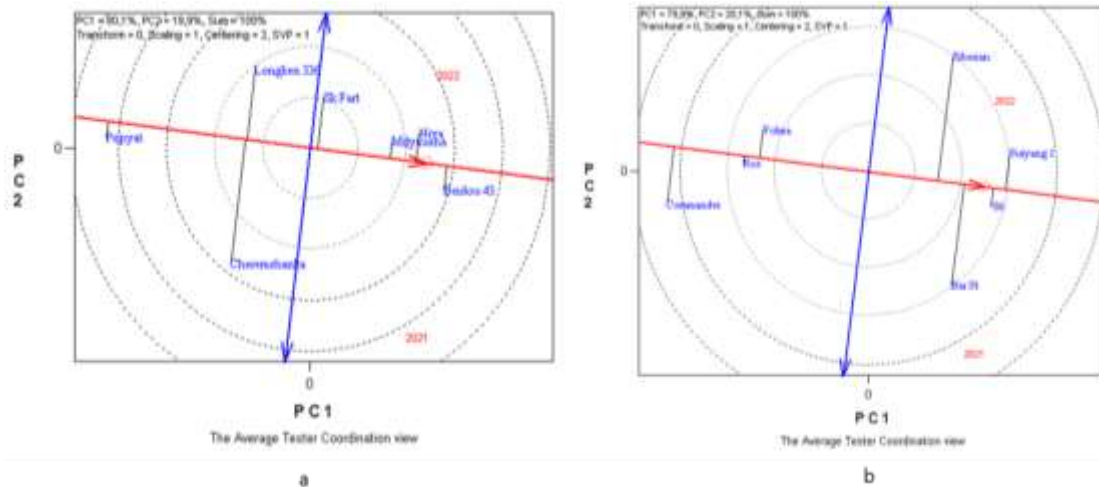


Figure 4. Biplot model GGE 'Mean against Stability,' different cultivars of soybeans.

cultivar Bar St in 2021 and Suiyang in 2022. However, the soybean genotypes SK Fart, Longken-336, Pripyat, Cheremshanka, Commander, Ros, and Volma were not part of the sectors of the studied environments. The biplot model 'Mean against Stability' displays the plasticity of the genotypes and their closeness to the straight lines to the abscissa, the more stable this sample was from the studied compartments. The crossing ordinate axis divides the region into two sectors relative to the average seed yield in the experiment (Figure 4).

According to the analysis, the early-maturing soybean cultivars proved promising

genotypes, exceeding the average yield in the experiment (Figure 2). Based on two study models, the most stable cultivars were the early-maturing genotypes Niva, Milyausha, Beidou-43, and No. 86. The biplot model ranking showed the perspective cultivars using a red arrow, with cultivars located closer to the red circle were more in perspectives than other genotypes (Figure 5). Therefore, one can identify the most perspective cultivars closest to the 'ideal genotype' in 2021–2022. Worldwide, in 2020, the average soybean seed yield was 2.7 t/ha, while in Russia, the yield was 1.59 t/ha (Stepanov *et al.*, 2020).

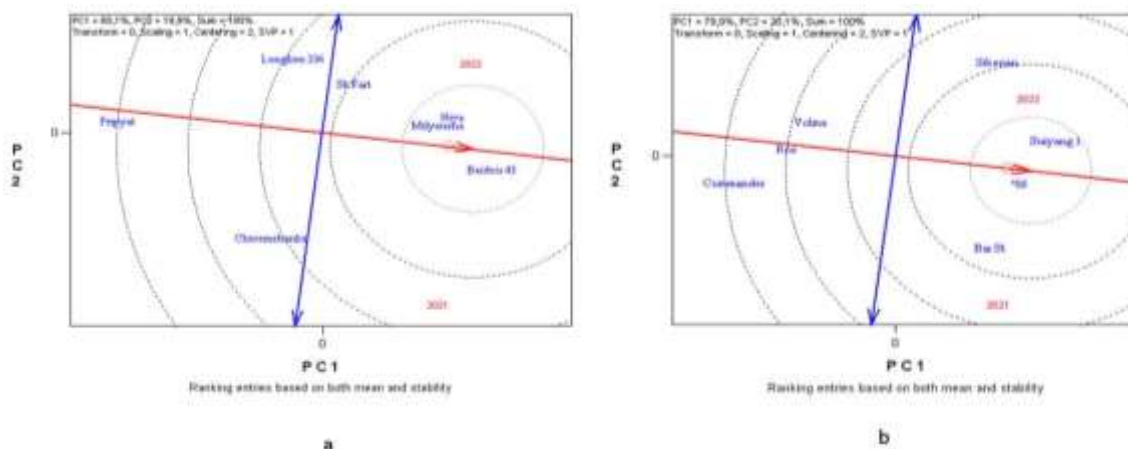


Figure 5. Biplot model 'Ranking' in different soybean cultivars.

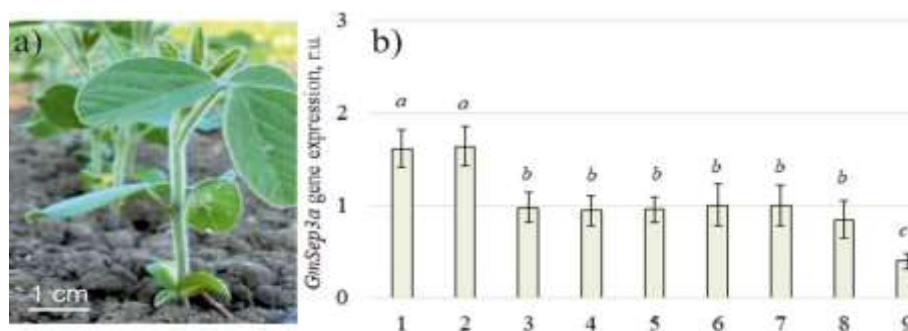


Figure 6. Soybean *Glycine max* 3-week soybean plants (a) and quantitative assessment of *GmSep3a* gene expression in different soybean cultivars by qRT PCR (b). Cultivars: 1 – Suiyang 1, 2 – Sibiryachka, 3 – No. 86, 4 – Niva, 5 – Beidou 43, 6 – Milyausha, 7 – Cheremshanka, 8 – SK Farta, and 9 – Bara. Data presented as mean \pm SE (two independent experiments with eight technical replicates). Total RNA/DNA was isolated from leaves of the 3-week soybean seedlings. Means followed by the same letter were not different using Student's t test, where $p < 0.05$ was considered to be statistically significant.

Almost all the early-maturing soybean cultivars showed absolute superiority over late-ripening ones. Among them, the closest to the 'ideal genotype' were the cultivars Niva, Milyausha, Beidou-43, Suiyang, and No. 86. Thus, the early-ripening soybean cultivars have confirmed that these genotypes were the most prospective cultivars best recommended for future breeding as base materials.

***GmSep3a* gene expression in selected soybean cultivars**

Sequencing first the part of the *GmSep3a* gene sequence in the samples of the used soybean

cultivars showed almost complete correspondence of the nucleotide sequences with the previously deposited *GmSep3a* gene sequence in the GenBank (XM_006579370). Thus, selecting the specific primers started analyzing the expression of this gene in different soybean cultivars.

The expression of the *GmSep3a* gene in three-week soybean seedlings with the most yields continued for analysis using quantitative real-time PCR (qRT PCR) with the $2^{-\Delta\Delta CT}$ method (Figure 6). Unfortunately, the cultivar Huajiong-2 did not grow into a full-fledged plant; hence, no isolated RNA came from that genotype for further analysis. The study

analyzed the *GmSep3a* gene expression in nine soybean cultivars. For regulation, the qRT PCR data used two soybean 'house-keeping' genes, i.e., *GmActin* (XM_041012802) and *GmGAPDH* (NM_001361055).

The highest expression of the *GmSep3a* gene was visible in the soybean cultivars Suiyang-1 (cv. 1) and Sibiryachka (cv. 2). In these cultivars, the expression was 1.6 to 4.2 times significantly higher than in other soybean cultivars (Figure 6b). At the same time, the lowest expression of the *GmSep3a* gene was with the cultivar-9 'Bara' (Figure 6b). The gene expression in cultivar 'Bara' was significantly lower (2.2 to 4.1 times) than in other soybean cultivars (Figure 6b).

***GmSep3a* gene correlation with soybean productivity and precocity**

Among the various soybean studied traits, a decision to compare these traits with the expression of the *GmSep3a* gene arose, and only two crucial traits were the soybean seed yield and precocity in the growing season duration of days. Thus, the highest seed yield resulted in the soybean cultivars Suiyang-1

and Sibiryachka, with the lowest yield recorded from the cultivars SK Farta and Bara (Figure 7). It is worth noting that the growing season duration was the shortest (87–91 days) in soybean cultivars Suiyang-1 and Sibiryachka and the longest (112 days) for the cultivar Bara (Figure 7).

Initially, the negative correlation ($r = -0.7$) manifested between productivity and the growing season duration, and the more precocious soybean cultivars have higher seed yield under the environmental conditions of Northern Kazakhstan. Further, a sturdy positive correlation ($r = 0.9$) was evident between the *GmSep3a* gene expression and the seed yield of the selected cultivars. However, a negative correlation ($r = -0.7$) appeared between the *GmSep3a* expression and the growing season duration of the studied soybean cultivars. Therefore, the expression level of the *GmSep3a* gene can be an influential diagnostic sign in searching for promising soybean cultivars with high yield and early maturity for cultivation in Northern Kazakhstan (Adsul *et al.*, 2016; Rauf *et al.*, 2023).

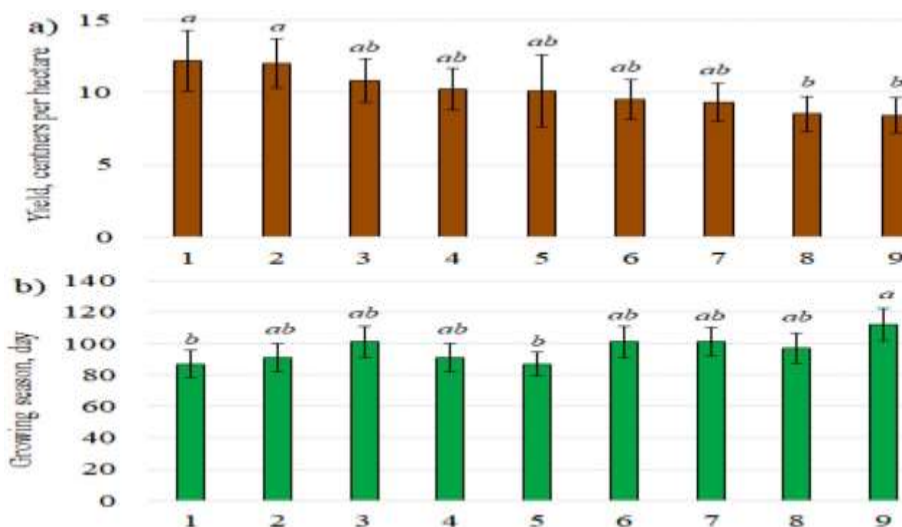


Figure 7. Indicators of yield of soybean cultivars in centners (100 kg) of seeds per hectare (a) and precocity in days of the growing season (b). Cultivars: 1 – Suiyang 1, 2 – Sibiryachka, 3 – No. 86, 4 – Niva, 5 – Beidou 43, 6 – Milyausha, 7 – Cheremshanka, 8 – SK Farta, and 9 – Bara. Means followed by the same letter were not different using Student's t test, where $p < 0.05$ was considered to be statistically significant.

CONCLUSIONS

The results revealed that five (Suiyang-1, Sibiryachka, No. 86, Niva, and Beidou-43) out of 90 soybean cultivars exceeded the seed yield by 10 t/ha. It revealed that these five cultivars are approaching the average yield of soybean cultivars grown in Russia. However, other genotypes gave 1.5 to 13 times lower yields. It proves more work on enhancing the soybean crop yield in Kazakhstan is necessary and to identify the early-maturing soybean cultivars as one of the possible directions for high yields. The presented results also identified the two most productive cultivars (Suiyang 1 and Sibiryachka), which also had the highest expression of the *GmSep3a* gene. The *GmSep3a* gene can be a helpful marker for screening early-ripening soybean cultivars with high yields in low-temperate climates. The data can be beneficial for searching potential soybean varieties for cold-weather cultivation.

FUNDING

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SUPPLEMENTARY MATERIALS

The following data are available online at www.mdpi.com/xxx/s1:

Table S1: Duration of the growing season of the analyzed soybean *Glycine max* cultivars.

Table S2: Plant height, the height of attachment of the lower bean, the weight of 1000 seeds, the number of seeds per 1 plant, the weight of seeds from one plant of the analyzed soybean *Glycine max* cultivars.

Table S3: Yield by centners per hectare of the analyzed soybean *Glycine max* cultivars.

Table S4: Primers used for real-time PCR analysis of *GmSep3a* gene.

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