

Pod Shattering and Seed Shedding Resistance Indicators in the Soybean Collection and Hybrid Populations

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Abstract: Early pod shatter is a critical factor contributing to soybean yield loss, with potential losses ranging from 34 to 99%. When shatter-resistant genotypes are grown in different agro-climatic zones, there is often a loss of this resistance. Therefore, there is a need for a more detailed critical analysis of breeding methods and strategies to reduce soybean pod shatter. It has been established that pod shattering typically begins at the ventral suture, followed by a rupture along the dorsal suture, causing a longitudinal tear in the central vein. Selection of soybean varieties for increased strength at the ventral suture has not resulted in the development of varieties that are guaranteed to resist premature shattering. Thus, studying the structure of the seed peduncle is of practical significance for future research. The aim of this study was to develop a cultivar resistant to seed shedding. Of the 428 varieties examined, 103 were prone to shedding, while 70 exhibited resistance to shedding, as indicated by the presence of a white eye on the rib. Among the maturity groups, the ultra-rapid, early-ripening, mid-early, and early-ripening groups showed the highest number of shedding varieties. A positive correlation was observed between traits of non-shedding and resistance to shattering. The seed peduncle, fused with the seed leaves, was found to be a dominant trait, controlled by a single gene. The inheritance of shattering was either dominant or exhibited an intermediate mode of inheritance. In terms of yield, the average yield of the control plot with the fused seed peduncle (43.6 kg/ha) was lower than that of the plot without this trait (47.5 kg/ha).

Keywords: Soybean, Pod Shattering, Seed Shedding, Yield, Hybrids, Selection

Introduction

Soybean is one of the most important crops globally due to its high vegetable protein content and extensive use in food production and animal husbandry. It belongs to the genus *Glycine* from the subfamily Papilionoideae of the family Leguminosae (Yarantsev, 2021). Soybean ranks among the top ten crops with the largest cultivated area worldwide. In 2016, global soybean production reached 334.8 million tonnes, covering over 120 million hectares, with the largest production areas in the United States, Brazil, and Argentina (Fadeeva and Vorobyova, 2017).

Despite its economic significance, soybean cultivation faces several challenges, including yield losses due to pod shattering and seed shedding. Pod shattering occurs when

mature pods split open prematurely, leading to significant seed losses. This problem is particularly pronounced in susceptible varieties and can be exacerbated by environmental conditions such as drought and high temperatures (Ibragimov, 2018; Messina & Messina, 2010). Addressing this issue is crucial for improving soybean yield stability and economic returns. Soy protects people from various diseases, and its antioxidant effect is beneficial for human health (Ali *et al.*, 2020; Rizzo, 2020).

Breeding efforts have focused on artificial hybridization to develop soybean varieties with desirable agronomic traits. One key breeding objective is to enhance resistance to pod shattering and seed shedding. Traditional selection methods have primarily targeted structural pod traits, such as the thickness of the pod wall



and the strength of the ventral suture. However, recent studies suggest that additional morphological and anatomical features may play a role in seed retention (Maity *et al.*, 2021; Parker *et al.*, 2021; Krisnawati *et al.*, 2021). In particular, the presence of a fused seed peduncle has been associated with increased resistance to seed shedding, though the underlying genetic mechanisms remain insufficiently explored.

Research on leguminous crops has shown that shattering resistance is influenced by multiple anatomical and morphological factors, including pod wall thickness, vascular bundle structure, and fiber orientation relative to the pod axis. These traits are believed to be governed by a polygenic inheritance pattern, making resistance improvement a complex breeding challenge (Zelentsov *et al.*, 2015; Zelentsov and Moshnenko, 2014; Zelentsov *et al.*, 2016; Zelentsov and Moshnenko, 2014; Kataliko *et al.*, 2019). Identifying stable genetic markers associated with pod-shattering resistance is essential for developing improved soybean varieties adapted to diverse agro-climatic conditions (Didorenko *et al.*, 2022; Krisnawati *et al.*, 2020; Bara *et al.*, 2013; Licht, 2014).

The present study aims to develop soybean lines resistant to seed shedding by investigating morphological traits associated with pod-shattering resistance. The specific objectives of this research include: 1) examining the soybean collection for traits related to pod shattering and seed shedding 2) analyzing the inheritance patterns of shattering and shedding resistance 3) developing soybean lines with enhanced seed retention capacity.

Materials and Methods

The material of the research was varieties of domestic selection, as well as the international collection provided by the head of the Department of Leguminous Crops of VNIIR Vishnyakova M.A., the curator of the soybean collection Seferova I.V. (2008-2018), the head of the Department of Soybean Breeding of VNIIMK Zelentsov S.V. (2015), the senior researcher of "Eco-Niva Seeds" Rosenzweig V., and employees of VNIISOi (Ukraine, Poltava region). In total, 428 varieties from 33 countries were studied (Fig. 1).

Hybrid populations obtained by crossing shattering and shedding-resistant forms were also studied.

Climatic Conditions of the Study Area

The studies were carried out near the city of Kaskelen in the Karasai district of the Almaty region. The study area was ca 740 m above sea level. In the region, the frost-free period lasts for 170-180 days, which makes it possible to evaluate the collection material with a large range of harvesting time, from ultra-rapid to late-ripening varieties

(85-160 days). In the study area, the amount of precipitation during the growing season is not sufficient to cultivate soybeans without irrigation. Therefore, irrigation is organized in the summer season. The average temperatures of the summer months are 23-26°C.

The studies were conducted in 2019-2022. The summers of 2021 and 2022 were dry, with 210.4 and 253.5 mm of precipitation, respectively, during the growing season, while the long-term average is 366.3 mm. Also, these years were characterized by relatively high average air temperature.

Methods

Sowing was carried out in the third decade of April under favorable weather conditions. Seeds from collections were sown manually to a depth of 4 cm in the amount of 25 seeds per 1 linear meter. The experiments were not replicated. Phenological observations recorded key developmental phases every two days, and the main phases of development were noted (appearance of seedlings, flowering, bean formation, and ripening). Watering was carried out in the third decade of July, the second decade of July, and the first decade of August. The degree of shattering and shedding was determined as the varieties matured on the 5th, 10th, 15th, 20th, and 25th day after maturation (Zhapayev *et al.*, 2023). The following points were assigned for resistance to shattering and shedding: 5 points 0%; 4 points 1-20%; 3 points 20-40%; 2 points 40-60%; 1 point 60-80%; 0 points 80-100%.

The degree of attachment of the seed peduncle to the seed leaves was estimated by the presence of a characteristic white eye on the seed rib (Fig. 2).

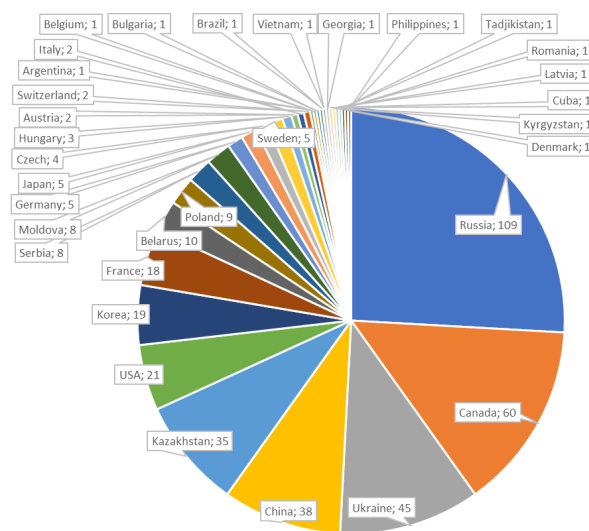


Fig. 1. Collections of soybean varieties by country of origin

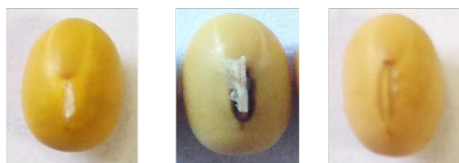


Fig. 2. Scar with a fused seed peduncle. Rib type: 1– with an eye, two without an eye

Inter-variety hybridization was carried out to obtain new breeding material. Hybridization was carried out according to the method of Didorenko (Kurmanbayeva *et al.*, 2021; Burkitbayev *et al.*, 2021; Ospanbayev, 2017). Domestic soybean varieties Almaty, Zara, and the variety Odesa 150 of the Ukrainian breeding were used as maternal lines. A distinctive feature of these varieties was the presence of a white eye on the rib; that is, they were presumed to be non-shedding. The Birlik KV variety did not have this feature. The selected varieties had white flowers, which helped in further identification of the breeding material. All paternal forms had a purple corolla and different resistance to shattering and shedding.

The choice of parental varieties was based on previous studies indicating their non-shedding or shedding-prone characteristics, ensuring a controlled approach to hybridization.

Results and Discussion

In the first stage of the project, the soybean collection was screened for signs of resistance to shattering and for morphological signs of fusion of seeds with seed leaves. Collected varieties were divided into maturity groups depending on the length of the growing season and the required sum of positive temperatures per growing season: 000 (up to 80 days) – 2 varieties, 00 (81-90 days) – 30 varieties, 0 (91- 110 days) – 158 varieties, I (111-125 days) -146 varieties, II (126-135 days) – 40 varieties, III (136-150 days) – 38 varieties, and IV (156- and more days) – 14 varieties.

Of the 428 varieties studied, 103 varieties were prone to shattering, and 70 varieties were resistant to shedding (according to the presence of a white eye on the rib).

Depending on the length of the growing season, the number of shattering forms varied. Thus, the varieties of the first four groups had the largest number of shattering forms (Fig. 3).

There was a positive correlation between the signs of shattering and the signs of non-shedding. The largest number of varieties with a fused seed peduncle (the presence of an eye on the rib) was also observed in the first four groups (Fig. 4).

Fig. 5 illustrates the distribution of soybean collection varieties based on their resistance to pod shattering. The majority of varieties in the collection exhibit resistance to cracking (represented in blue), while a smaller proportion is not resistant (represented in orange).

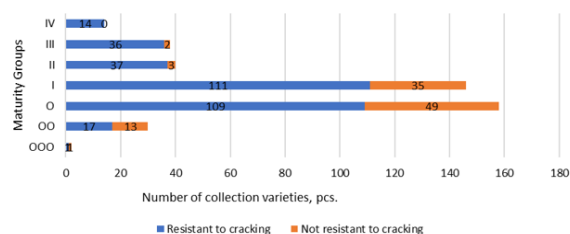


Fig. 3. Shattering and shatter-resistant forms of soybean by maturity groups

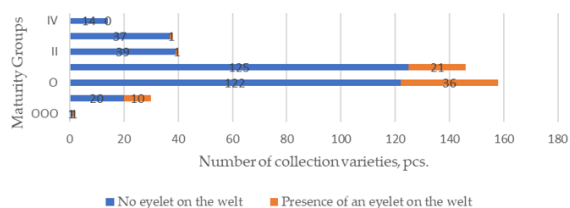


Fig. 4. Shedding and non-shedding forms of soybean by maturity groups



Fig. 5. Shattering forms of soybeans in the whole collection and in the collection of non-shedding varieties

A key observation is that within the total collection, resistant varieties significantly outnumber non-resistant ones, indicating that pod-shattering resistance is a common trait among the studied soybean genotypes. However, in the subset labeled "Unfilled moulds," there is a relatively higher proportion of non-resistant varieties, suggesting potential environmental or genetic factors affecting this trait.

This distribution highlights the importance of further research into the genetic and morphological factors contributing to pod-shattering resistance. Additionally, breeding programs should aim to enhance this trait while ensuring that yield and other agronomic characteristics remain optimal.

To assess the degree of shattering from the beginning of ripening, observations were made, and after full ripening, shattering resistance scores were assigned. One selection number from the collection, Fora (Russia), began to shatter as soon as the pods closest to the ground turned brown; therefore, the resistance to shattering was already at 0 points at the stage of incomplete ripeness. Observations were also carried out after maturation on days 5, 10, 15, 20, and 25. With each subsequent observation, the degree of shattering increased (Fig. 6). Thus, by the end of the

observations, resistance to shattering was: 5 points – 0 cultivars, 4 points – 14 cultivars, 3 points – 32 cultivars, 1 point – 22 cultivars, and 0 points – 24 cultivars.

To better understand the impact of time on the susceptibility of soybean varieties to shattering, measurements were taken at 5, 15, and 25 days after pod maturation. The obtained data are presented in Figure 6. As shown in the diagram, the degree of pod shattering varies over time: after 5 days. Most varieties exhibit a high tendency to shatter (scoring 5 or 4 points). This confirms the presence of genetically predisposed lines prone to shattering. After 15 days, the number of varieties with the highest shattering scores decreases while the proportion of intermediate values (3 and 2 points) increases. This suggests a gradual development of pod damage in less susceptible varieties. After 25 days, the number of varieties with severe shattering (5 points) further declines. At the

same time, resistant varieties (scoring 0–2 points) maintain pod integrity, indicating the presence of lines capable of withstanding this factor.

The analysis of trends shows that the susceptibility to pod shattering varies among the studied varieties. Rapid shattering in certain lines indicates genetic predisposition, while resistant varieties maintain pod integrity even after 25 days. Further research should focus on identifying morphological and genetic factors influencing resistance, as well as balancing this trait with yield potential.

Inter-varietal hybridization was carried out to obtain a new starting material. The varieties of domestic selection and the Ukrainian variety Odesa 150 were used as maternal varieties. Varieties of domestic, Russian, Ukrainian, Chinese, French, and Czech breeding were used as paternal forms. Varieties were characterized by varying degrees of resistance to shattering and different types of seed peduncle (Table 1).

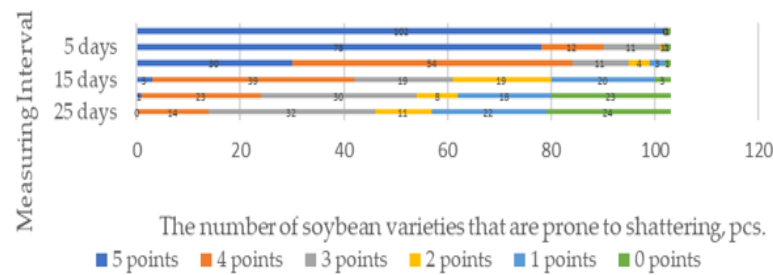


Fig. 6. Degree of shattering of soybean varieties depending on the time since maturation

Table 1. Characteristics of parental forms in terms of resistance to pod shattering and seed shedding

Parental variety	Country of origin	Shattering resistance, points	Signs of non-shedding, presence/absence
Maternal varieties			
Almaty	Kazakhstan	3	+
Zara	Kazakhstan	3	+
Odesa 150	Ukraine	3	+
Birlik KV	Kazakhstan	4	-
Paternal varieties			
Maleta	Russia	3	+
Bara	Russia	4	-
Memory of SGC	Kazakhstan	4	-
Ustja	Ukraine	5	-
Rana	Czech Republic	5	-
Selecta 302	Russia	3	-
Zhansaya	Kazakhstan	4	-
Harbin	China	4	-
Safran	France	5	-
Korsak	Ukraine	5	-
Cheremosh	Ukraine	4	-

The obtained hybrids of the first generation were evaluated according to the degree of shattering and the presence of a seed peduncle fused with the seed leaves.

As a result of the research, it can be concluded that regardless of the presence of a fused seed peduncle in the maternal or paternal form, all hybrids of the first generation had this feature. Thus, this is a dominant trait. In subsequent generations, this trait was split in accordance with the laws of G. Mendel, suggesting that it is controlled by a single gene.

Regarding the sign of shattering, the results were less conclusive. When crossing shattering and shatter-resistant forms, in the first generation it was difficult to assess the degree of shattering. We believe that either this trait is dominant or has an intermediate type of inheritance.

Because of the negative selection for lines with signs of shattering, in older nurseries, there may be single selection numbers with a shattering score of at least 4. At this stage, forms with morphological signs of resistance to shedding are of greater interest because the newly created variety resistant to shattering in the breeding zone may lose this resistance when cultivated in other agroclimatic zones. Initially, hybrid and breeding nurseries carried out selection for high yields. Therefore, plants with signs of free attachment of the seed peduncle were not rejected. As a result, plants from the same hybrid combination but with a different structure of the seed peduncle were included in the control variety testing.

In 2022, 38 individuals were studied in the control nursery, 14 of which had a sign of non-shedding, i.e., the presence of an eye on the rib. Of the total number of studied varieties of the control nursery, 24 had an unfused, and 14 had a fused seed peduncle. Of the 38 samples of the control nursery studied, 11 selection numbers without a rib and five numbers with a rib exceeded the standard yield. The average yields of selection numbers with a fused seed peduncle (43.6 c/ha) were lower than the yields of selection numbers without this feature (47.5 c/ha). A similar situation was observed in relation to the selection numbers of competitive variety testing (Fig. 7).

The analysis of pod shattering and seed shedding resistance revealed key patterns in the inheritance and distribution of these traits among soybean varieties. The presence of a fused seed peduncle was found to contribute to reduced seed shedding, suggesting its potential role in breeding programs aimed at improving harvest stability. However, while this trait enhances resistance, its impact on overall yield must be considered.

As shown in Figure 7, soybean lines with a fused ovule demonstrated lower average yields compared to those without this feature. In control variety testing, the yield of fused ovule variants was 43.6 c/ha, whereas

standard varieties yielded 47.5 c/ha. A similar trend was observed in competitive variety testing, where the fused ovule lines produced 43.8 c/ha, compared to 48.4 c/ha in standard varieties. These findings highlight the trade-off between resistance traits and productivity, emphasizing the challenge of balancing yield stability and economic viability in soybean breeding.

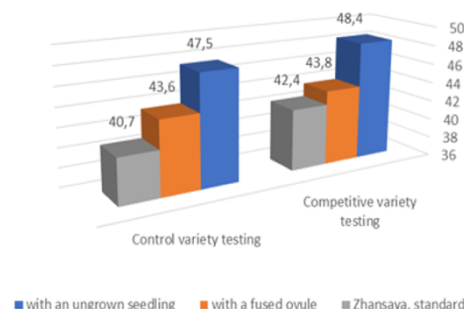


Fig. 7. The average yield (c/ha) of soybean selection numbers of the control and competitive variety testing

Future breeding strategies should focus on optimizing this balance by integrating resistance-enhancing traits without compromising yield potential. Identifying genetic markers linked to both high productivity and resistance to shedding will be essential for developing soybean cultivars that meet agricultural demands under varying environmental conditions.

In three (Zara/Desna, Zara/Zhansaya, Odessa 150/Safrana) of the five hybrid populations, a higher yield was observed in selection numbers with a fused seed peduncle than in their counterparts with a free seed peduncle. In the productivity traits, the smallest variability within each hybrid combination was observed in the plant height, lower pod attachment height, number of lateral branches, and number of productive nodes.

The number of seeds per plant, the weight of seeds per plant, and the weight of 1000 seeds were lower in most selection numbers with a fused seed peduncle in comparison with the shedding selection numbers (Table 2).

In the nursery of competitive variety testing, out of 26 studied selection numbers, 3 were characterized by a sign of non-shedding, an eye on the rib (IT 24/2, IT 24/4 – Za-ra*Cheremosh; I-23/7 – Zara*Korsak) with a yield range of 27.1-54.2 centners per hectare. Of the 26 selection numbers that did not have a fused seed peduncle, 15 selection numbers with an average yield of 48.4 centners per hectare exceeded the standard yield. Only one of the three selection numbers with a fused seed peduncle (I-23/7 – Zara*Korsak) turned out to be more productive than the standard Zhansaya variety. The average yield of this selection number was 43.8 centners per hectare.

Table 2. Productivity traits, yield, and growing season length of selection numbers of soybeans of the control variety obtained by crossing with non-shedding varieties

Selection number	Type of seed peduncle	Height (cm)	No. of productive nodes (pcs)	No. of beans per plant (pcs)	Weight of seeds from one plant (g)	Weight of 1000 seeds (g)	Yield, (dt/ha)
Zara/Selecta 302							
E 28/1	Free	65.4	11.6	58.4	15.2	154.0	37.5
E 28/3	Fused	70.8	12.2	49.4	13.8	161.0	34.4
Zara/Desna							
K 28/4	Free	69.4	12.2	59.2	20.9	191.0	38.5
K 28/3	Fused	82.8	12.4	60.4	18.7	142.0	45.8
K 28/6	Fused	72.8	11.4	45.2	15.2	177.0	41.7
Zara/Zhansaya							
KT 41/3	Free	68.4	13.2	47.0	14.4	196.0	38.5
K 41/1	Fused	79.6	13.4	51.4	17.3	195.0	44.8
Odessa 150/Harbin							
K 15/9	Free	79.8	12.6	64.8	18.2	165.0	45.8
K 15/7	Fused	81.6	10.4	48.0	12.0	181.0	37.5
Odessa 150/Safrana							
KT-46/6	Free	77.2	12.8	52.4	16.8	197.0	34.4
K 46/4	Fused	69.0	8.4	39.0	15.9	176.0	38.5
K 46/5	Fused	86.2	14.4	70.6	22.1	181.0	39.6

Conclusion

Our study assessed a soybean collection for pod shattering and seed shedding resistance. The highest frequency of shattering susceptibility was observed among the early-maturing varieties (ultra-rapid, rapid, mid-early, and early-ripening). A positive correlation was found between pod-shattering and non-shedding traits. The fused seed peduncle trait exhibited dominance, suggesting it may be controlled by a single gene. Additionally, our results indicate that the inheritance of shattering resistance follows either a dominant or intermediate pattern.

The study also revealed a trade-off between yield and resistance traits. On average, soybean lines with a fused seed peduncle had lower yields (43.6 c/ha) compared to those without this trait (47.5 c/ha). This finding highlights the need for breeding strategies that balance resistance improvements with maintaining high yields.

Our results underscore the importance of developing soybean varieties with improved resistance to seed shedding, which can significantly reduce harvest losses. Further research should focus on identifying genetic markers for shattering resistance and exploring ways to enhance resistance without compromising yield potential. These insights will aid in the development of resilient soybean cultivars suited to various agro-climatic conditions.

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Author Contributions

Sagit Islambek: Conceptualization, Methodology, Writing Review and Editing.

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Rinat Kasenov: Investigation, Visualization.

Dalibaeva Almagul: Software, Resources.

Dina Karabalayeva: Validation, Data Curation.

Meruyert Kurmanbayeva: Writing-Original Draft Preparation, Supervision.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript, and no ethical issues are involved.

Conflict of Interest

The authors declare no conflicts of interest.

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