

## Assessment of genetic diversity in sesame germplasm as an initial material for Bulgarian breeding programs

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### Abstract

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The purpose of this study is to identify and evaluate sesame genotypes for their suitability for mechanized harvesting for the needs of the crop hybridization program. 47 introduced sesame samples of diverse ecological and geographical origin were studied. Subjective independent evaluation method, Sesaco method and field testing were used. The genetic diversity is represented by a hierarchical cluster analysis dendrogram. The results reveal the wide genetic basis of the gene pool studied in terms of the ability of individual samples to retain seed in the capsule during physiological maturity and harvesting. The working collection is characterized by samples that retain their seeds on the field during ripening and those that retain the seeds during threshing. The study identified donors which could produce offspring with excellent retention of the seeds on the field until plant maturation and during threshing, when properly combined.

*Keywords:* *Sesamum indicum* L.; genotype; introduction; mechanized harvesting

### Introduction

The wide range of the plant gene pool provides the basis for genetic improvement of cultivated plants. The large collections and databases of plant genetic resources are difficult to manage, evaluate and use (Holden, 1984). The creation of work collections with the appropriate research direction is a powerful tool for using and exploiting genetic diversity opportunities (Hodgkin et al., 1995; Zhang et al., 2011).

The suitability of a sample for mechanized harvesting is determined by the ability to retain the seeds in the capsules up to deliver in a rigging mechanism (Langham & Wiemers, 2002; Georgiev et al., 2008; Ishpekov et al., 2019a; Ishpekov et al., 2019b; Nobre et al., 2019)

Indeterminate habit's plant and its seeds ripen unevenly (Day et al., 2002; Anon., 2004; Ashri, 2007; Mazzani et al., 2007). Thus, plants with fully mature seeds in open fruit capsules and closed capsules with unripe seeds are found in physiological maturity on the field (Figure 1). The period of

full maturity (that is at the same time as harvesting) is different to each genotype and depends on environmental conditions. During this period, the seeds in fully mature plants are under various dynamic effects and stresses. The wind shakes the plants, causing mechanical inertia and impact on the seeds, letting them leave the capsules (Ishpekov et al., 2016). Meteorological conditions in the autumn are accompanied by periods of high humidity or drought. Therefore the capsules shrink and widen periodically around the clock. These deformities cause tearing of the placenta and membranes, resulting in a large number of seeds scattered in the field (Langham & Wiemers, 2002).

Another set of factors causing seed loss are provoked from the mechanical effects of existing heads of harvesters and threshers. The crank curves the plants before bending them to the cutting machine, and it shakes them several times before cutting the stems. Conventional tip machines traumatize the seeds mechanically because their maturity under Bulgarian conditions does not fall below 12%, in which they

are not resistant to mechanical effects (Stamatov & Deshev, 2018; Ishpekov, 2019).

To assess the ability of individual sesame genotypes in the working collection to retain their seeds in the field until entering the final mechanism, Bulgarian methods of subjective-independent evaluation and field test were applied, as well as the Sesaco evaluation methodology (Langham, 1998; 2001; Ishpekov & Stamatov, 2015; Stamatov et al., 2016).

The purpose of this study is to identify and evaluate genotypes of *Sesamum indicum* L. in terms of their suitability for mechanized harvesting for the needs of the hybridization program in sesame.

## Material and Methods

### Plant material

47 introduced samples of sesame with diverse ecological and geographical origin were received by international germplasm free exchange with the gene bank of Griffin, USA, the Macedonian gene bank and through personal correspondence with scientists from the Polytechnic Institute in Beja, Portugal.

On 10 selected capsules in physiological maturity, whose carpels are still enclosed by the middle part of the plant, three geometric dimensions are considered—length, width and thickness. Four anatomical indices of the capsules – degrees of cleavage, opening, contraction of the walls and closure of the membrane were evaluated on a scale borrowed from Sesaco. The scores that samples form according to the anatomical features of the capsule are grouped into four groups. Weak evaluation—samples that do not have anatomical attachments to retain their seeds receive 0 to 14 points. 15 to 24 points receive the samples from an average grade; those that have satisfactory rating receive 25 to 29 points. With a maximum score (30–32 points) are samples that have excellent anatomical features to retain their seeds.

The mass of seeds in a capsule is an indicator related to the productivity of the genotype, but it is not relevant to the retention of the seeds.

### Methods of analysis

#### *Method of subjectively independent assessment*

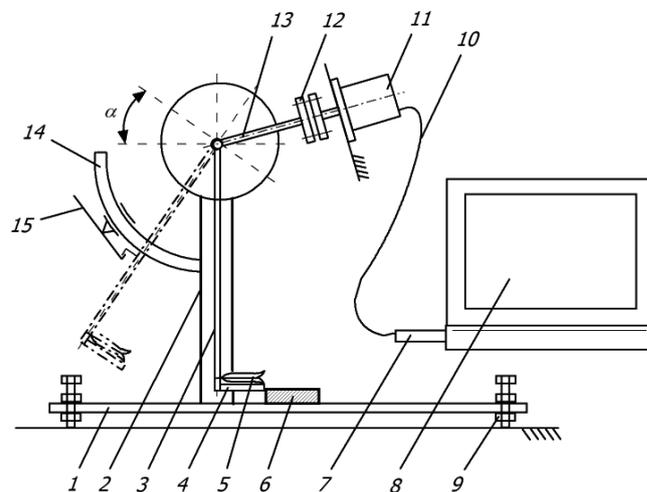
The ability of the various genotypes to retain the seeds in the capsule after inertial action is determined by a Pendel apparatus stand and an electronic data collection system (Figure 1). The placenta has an inertial force equal to  $F_{in} = 5.5E - 4 N$  and acceleration  $a_{in} = 133.8 m/s^2$ . Thus, the load on each seed capsule is 5.64 g.

The susceptibility assessment of sesame capsules for mechanized seed harvesting is evaluated using a specially

developed subjectively independent methodology. Three dimensionless indices are determined for each genotype tested:

- $i_1$  – numerical indicator of seed dispersal when shaking plants;
- $i_2$  – indicator of seed retention in the capsule due to the peculiarities of its shape;
- $i_3$  – indicator of the strength of the relationship between the seeds and the placenta.

Genotypes with a high index value of  $i_1$  are not susceptible to mechanized harvesting due to seed dispersal. Those with a high index value of  $i_2$  are suitable for harvesting by crushing the capsule. The high value of the index  $i_3$  indicates the suitability for seed collection by inertial action and without breaking the capsules.



**Fig. 1. Test stand for inertial separation of the seeds from the sesame seeds:**

- 1 – base, 2 – stand, 3 – pendulum rod, 4 – pendulum plate,
- 5 – fruit capsule tested, 6 – counter, 7 – QSB-D counter,
- 8 – computer, 9 – horizontal screws, 10 – cable, 11 – Photo-bit converter (FRF), 12 – connector, 13 – pendulum shaft, 14 – scale,
- 15 – pendulum trigger

### *Sesaco Method*

It includes several tests. The first is to investigate the proportion of seed retained in the capsules, depending on the fragmentation of the chambers, placenta, and membranes upon maturation (Shattering test). This test involves two steps:

- *Test of capsules in physiological maturity (Green test)*

It includes the following steps:

G1: Cut a capsule from the middle of ten plants.

G2: Allow the capsule to dry completely.

G3: Turn each box with the top down without squeezing it in order not to narrow the opening. The fallen seeds are collected and weighed.

G4: Each box is tripped from a height of 0.1 m. The separated seeds are weighed.

G5: The remaining seeds in the boxes are separated and also weighed.

G6: Sums of seeds from steps G3, G4 and G5 are summed:

$$G6 = G3 + G4 + G5$$

G7: The following are calculated:

$$SR1_{\text{green}} = (G4 + G5) / G6; SR2_{\text{green}} = G5 / G6$$

– *Full matured test (Dry test)*

It includes the following steps:

D1: Performs G1 at full maturity of sesame plants. It is recommended to close the top of the cut off capsule with your thumb and index finger.

D2: Allow the capsules to dry completely.

D3: Each box is rotated top down and the individual seeds are drawn.

D4: Separate remaining seeds in capsules and weigh.

D5: Determine the seed dispersed by dependency:

$$D5 = G6 - (D3 + D4)$$

D6: Sum the seed masses from steps D3, D4 and D5:

$$D6 = D3 + D4 + D5$$

D7: The following are calculated:

$$SR1_{\text{dry}} = (D3 + D4) / D6 \quad SR2_{\text{dry}} = D4 / D6$$

The described procedure is carried out in at least three repetitions for each tested variety and the Shatter Resistance Average is calculated using the formula:

$$SRA = (SR1_{\text{green}} + SR2_{\text{green}} + SR1_{\text{dry}} + SR2_{\text{dry}}) / 4 * 100\%$$

Depending on the value of the obtained SRA, the varieties are classified as follows:

– SUS (super shattering) – varieties destroy their capsules completely when ripe and retain less than 10% of the seeds;

– SHA (shattering) – capsules of these varieties are partially destroyed and retain from 10% to 40% of the seeds;

– NSH (non-shattering) – with non-shattering capsules that hold from 40% to 80% of the seeds;

– DC (direct combine) – with capsules that hold over 80% of the seeds;

– ID (indehiscent)–These varieties have capsules that are closed when ripe and hold all the seeds.

*Field method*

The capsules are wrapped in permeable fabric and hang on a rope, freely swayed by the wind and warmed by the sun (Figure 2). When the seeds ripen and the capsules burst, they are removed. The loose seeds and the seeds retained in the capsules are weighing. In this way, the proportion of spilled seeds is taken into account until the entire crop is fully ripe

and ready to be combined. This test simulates the effects of the sun and wind sesame plants and their fruit capsules have on the field.



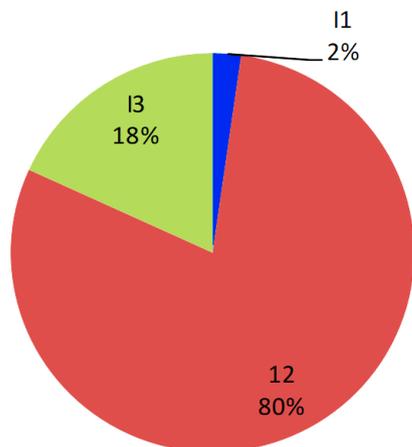
**Fig. 2. Field test in IPGR Sadovo**

According to this test, samples that retain 0 to 30% of their seeds were evaluated as spraying. From 30 to 60% seed retention classifies the samples as poorly retained. From 60 to 75% have samples with average retention. Those with satisfactory retention rates of up to 99, and excellent retention of 100% of their seeds, are satisfactorily retained.

## Results and Discussion

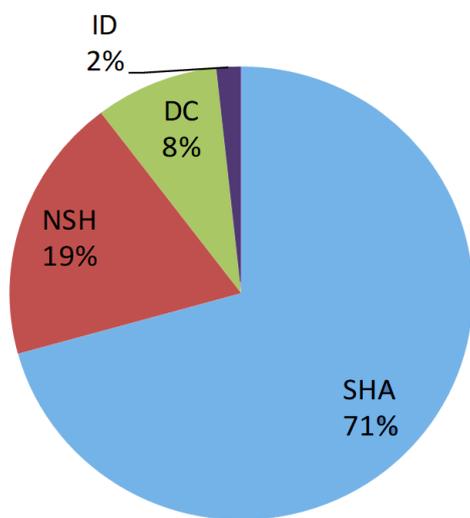
The results of the subjective-independent evaluation are visualized in Figure 3. They show that 80% of the tested samples have the anatomical features of the capsule to retain

most of their seeds ( $i_2$ ). Another 13% samples retain some of their seeds due to the attached placenta ( $i_3$ ). Fully dispersing their seeds without any ability to retain them are 2% of the tested genotypes ( $i_1$ ).



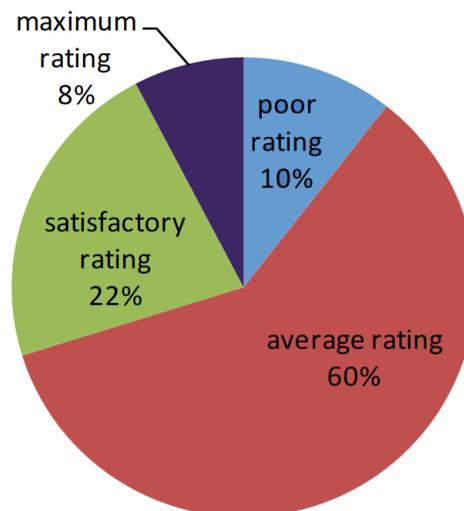
**Fig. 3. Indexes of subjective-independent method**

The Sesaco test (Figure 4) shows that 71% of the test samples were classified as dispersal. They retain up to 40% of their seeds. Non-destructive samples that hold their seeds up to 80% are estimated to be 19% of the samples. Suitable for direct combining, retaining over 80% of their seeds, are 8% of the samples. Non-sprayed (indehiscent) varieties that retain 100% of their seeds are 2% of the tested genotypes.



**Fig. 4. Sesaco test**

The field test, the results of which are shown in Figure 5, estimated 10% of samples as those that scatter their seeds when ripe. With average seed retention are 60%. 22% of the tested genotypes satisfactorily retain their seeds. 8% of the samples are characterized with excellent seed retention.



**Fig. 5. Graph of the field test**

The genetic diversity within the work collection is shown in Figure 6. The dendrogram shows that the samples are divided into two cluster groups according to their genetic distance.

The first cluster group at the smallest Euclidean distance includes 18 samples characterized by a high value of the field test and low values of the subjective-independent evaluation. These genotypes would not lose seeds until the plants ripening in the field, but the pre-combining would result in significant losses.

The second cluster group, at the larger Euclidean distance, contains the other samples. The farthest is the genotype with catalogue № B800069, originating from Turkey, having the highest values of the subjective-independent method of assessment. The other samples in this group are characterized by a low value of the field test, but high value in the subjectively independent evaluation and the Sesaco test. They lose the seeds of the field to the ripening of the plants, but when they are combined produce minimal losses. In the future breeding program, parents from both cluster groups should be used to produce offspring with low seed loss while the plants are in the field at physiological maturity and when combined.

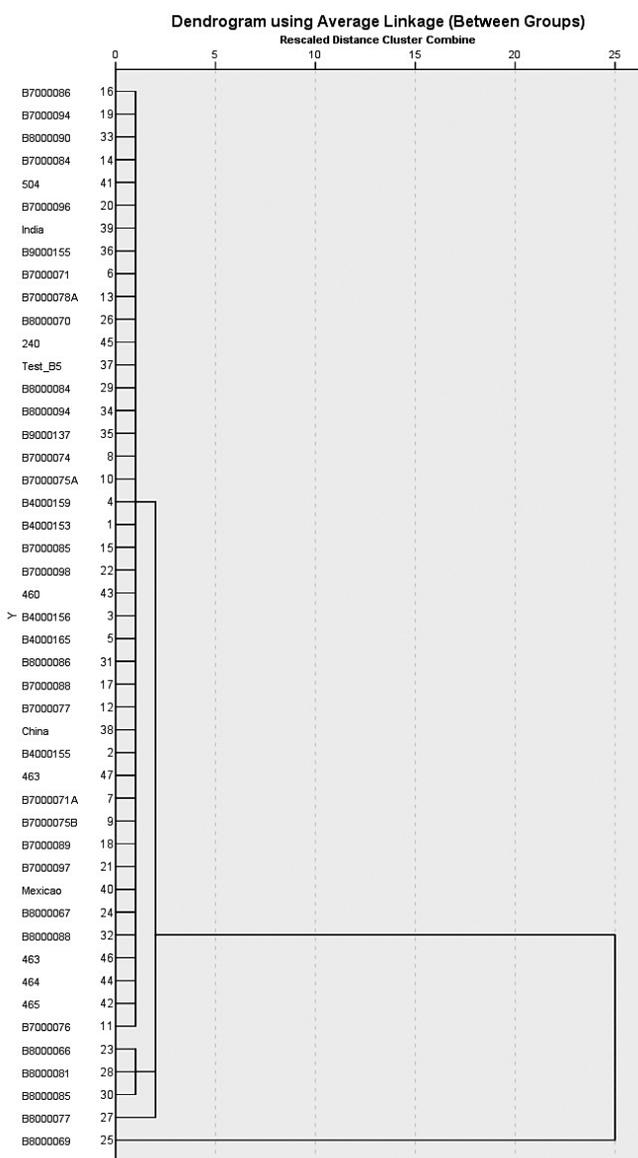


Fig. 6. Dendrogram of cluster analysis

## Conclusions

Samples that have different attachments to hold their seeds in the capsules and in the harvesting mechanism have been introduced.

A broad basis of genetic diversity in terms of the ability of individual samples to retain seed in the capsules during physiological maturity and harvesting has been evaluated.

The work collection of IPGR Sadovo is characterized by samples that retain their seeds in the field during the ripening period and those that retain the seeds during threshing.

Donors, that if they are suitably combined, could produce offspring with excellent retention the seeds until plant maturation and during threshing on field, have been identified.

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