Influence of inbreeding on the morphobiological characteristics of meadow clover (Trifolium pratense L.)

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Abstract. The influence of inbreeding on the morphobiological characteristics of meadow clover was revealed. Self-pollination, repeated in the number of successive generations, leads to an increase in homozygosity and to inbred depression, which increases from I1 to I3 generation and stabilizes in the I4 generation. It was found that in the I1 generation, according to the main morphobiological characteristics, there is no inward depression, but the maximum release of recessive lethal mutations is manifested, which amounted to 6.2%, and the survival rate of seedlings decreases (91.7%). By the I4 generation, the number of chlorophyll-free seedlings decreases to 1.4%. All the main morpho-biological indicators that determine the productivity of plants decrease from generation I1 to generation I3 by 1.5-2 times and stabilize in generation I4. Obtaining hybrid F1 offspring by crossing a linear material with an I4 induction level leads to the restoration of plant productivity indicators. When creating a linear material, an increase in the number of highly self-compatible genotypes from I1 to I4 generation by 60% is clearly traced. The data obtained make it possible to take into account the survival rate of seedlings, the cleavage of lethal and semi-lethal mutations, the degree of inbred depression in the formation of sample volumes when creating a linear material of meadow clover.

Keywords: meadow clover, genetic source of compatibility, induction, inbred depression, morphobiological characteristics.

1. Introduction

One of the main tasks for the fodder industry is to expand the cultivation areas of perennial legumes, among which meadow clover plays an important role. A limiting factor in the fulfillment of this task is the low seed productivity of the varieties of this crop in production. Therefore, the problem of increasing the seed productivity of meadow clover has acquired great importance [1].

As you know, meadow clover is a cross-pollinated crop with strict genetic control of the self-incompatibility reaction. Insect pollinators play a primary role in its reproduction, but the use of chemical preparations in modern agriculture has led to a significant decrease in their number, which negatively affected the productivity of meadow clover seeds [2, 3]. One of the promising ways to increase the seed productivity of this crop is the creation of multi-line varieties using the self-compatibility trait, which makes it possible to increase and stabilize the seed productivity of meadow clover [4, 5]. However, forced self-pollination, repeated in more or less successive generations, leads to an increase in homozygosity and to inbred depression, i.e. decrease in productivity, loss of power and viability of individuals, the appearance of various recessive deviations from the norm, disguised in a heterozygous state [6, 7]. The cause of inbred depression is the transition of recessive lethal genes, as
well as genes that reduce the viability of the organism, into a homozygous state [8, 9]. The manifestation of inbred depression in the process of induction has a strong influence on the approaches to the creation of linear material and the formation of breeding programs.

In this regard, the aim of the study is to assess the effect of inbred depression on the morphobiological features of meadow clover and the possibility of using inbreeding to obtain linear material.

2. Research methodology

The studies were carried out under the conditions of a selection greenhouse complex (SGC) and in chambers of an artificial climate with controlled environmental conditions according to the methodological recommendations [10, 11, 12]. A diploid variety of domestic selection Ranny 2 and a wild-growing sample No. 3000 I 2 with a self-compatibility sign were used as the starting material.

During the research, manual cross-pollination and forced self-pollination of flowers were used using additional isolation.

3. Research results

A genetic source of self-compatibility was isolated from a wild-growing population growing in mountainous Tajikistan and a sample No. 3000 I 2 was formed, the seed setting of which during self-pollination was 77% [13 14]. Under SGC conditions, reciprocal crosses were carried out between variety Ranny 2 and sample No. 3000 I 2 to create varieties with high seed productivity.

The study of the obtained hybrids according to the degree of manifestation of the self-compatibility trait made it possible to identify four groups of plants according to seed setting. For further work, genotypes with a self-compatibility level of more than 50% were selected.

In the process of research according to this scheme, artificial self-pollination was carried out, repeated in three consecutive generations, and homozygous self-compatible lines were obtained in the I 4 generation.

As a result of the analysis of the data obtained, an increase in the number of highly self-compatible genotypes from I 1 to I 4 generation by 60% is clearly traced (Figure 1).

![Figure 1](image-url)
By the I4 generation, the percentage of medium and highly self-compatible genotypes reaches 100%, with the complete absence of incompatible and weakly compatible genotypes.

Hybridization was carried out between genotypes I4 generation with a level of self-compatibility of more than 50% and a hybrid (Early 2 x No. 3000 I2) I4) F1 was obtained, the seeds of which were combined and a multi-line complex hybrid population was formed.

A comparative morphobiological assessment of the hybrid (Early 2 x No. 3000 I2) F1 in generations I1, I2, I3, I4, (I4) F1 was carried out under the conditions of SGC. The original hybrid combination was used as a standard.

Determination of seed germination and plant survival showed that the highest percentage of germination was found in the F1 and (I4) F1 generations, which amounted to 97 and 98%, respectively, and the smallest in the I1 generation (91%). Studies have shown various types of changes in the development of seedlings (chlorophyll-free seedlings, triple cotyledonous leaflet, deformed flag leaf, etc.) (Figure 2). The largest percentage of chlorophyll-free seedlings fell on generation I1 - 6.2%, which is associated with the cleavage of lethal mutations.

By the I4 generation, the number of chlorophyll-free seedlings decreases to 1.4%. Various mutations associated with homozygosity for recessive traits contributed to a decrease in the viability of genotypes from F1 to I4 generation from 97 to 76%. In the variant ((Early 2 x No. 3000 I2) I4) F1, as a result of the heterotic effect after the crosses, an increase in plant survival was noted by 10%, which amounted to 84% (Table 1).

![Figure 2. Different types of mutations associated with inbred depression](image-url)
| Variant | Laid seeds, pcs | Number of non-germinating seeds, pcs. | Germination, % Quantity chlorophyll-free seedlings, pcs. | Number of planted seedlings in vessels, pcs. | Change in cotyledon leaves1, pcs. | Changing the flag sheet2, pcs. | Survival I accounting3, pcs. | Survival II accounting4, pcs. | Survival III accounting5, pcs. |
|---------|----------------|--------------------------------------|--------------------------|---------------------------------|---------------------------------|--------------------------|---------------------|---------------------|---------------------|
| (Early 2 x № 3000) F1 | 139 | 4 | 97 | 1 | 0,7 | 120 | 2 | 4 | 118 | 98 | 116 | 97 | 116 | 97 |
| (Early 2 x № 3000) I1 | 146 | 12 | 92 | 9 | 6,2 | 120 | 5 | 11 | 104 | 87 | 99 | 82 | 98 | 82 |
| (Early 2 x № 3000) I2 | 146 | 7 | 95 | 4 | 2,7 | 120 | 4 | 18 | 109 | 91 | 108 | 90 | 108 | 90 |
| (Early 2 x № 3000) I3 | 150 | 6 | 96 | 3 | 2,0 | 120 | 4 | 14 | 110 | 92 | 102 | 85 | 96 | 80 |
| (Early 2 x № 3000) I4 | 145 | 6 | 96 | 2 | 1,4 | 120 | 2 | 15 | 105 | 88 | 96 | 80 | 91 | 76 |
| (Early 2 x № 3000) I5 | 190 | 4 | 98 | 2 | 1,1 | 120 | 1 | 8 | 115 | 96 | 111 | 93 | 101 | 84 |

1 cotyledonous leaves trifoliate
2 flag leaf:
   a) reduction;
   b) dissected;
   c) double
3 accounting when forming the 1st present sheet
4 accounting on the date of the rosette of leaves
5 accounting for the start date of flowering
As a result of hybridization and obtaining I1 offspring, a heterotic effect was observed, which was expressed in an increase in plant height by 9%, the number of heads - by 16%, and productivity - by 10% compared to the standard.

It was found that all the main morpho-biological indicators that determine the productivity of plants decrease from generation I1 to generation I3 by 1.5-2 times (table 2). So, if the height of plants in I1 was 61.3 cm, then by I3 it was only 42.1 cm, the number of heads per 1 plant decreased from 12.8 to 8.4 pcs. The induction did not have a significant effect on the formation of stems. Their number varied from 3.5 to 3.9 pieces per 1 plant, which turned out to be within the limits of the smallest significant difference.

Table 2. Assessment of morphobiological parameters of meadow clover depending on the input generations (I1 – I4)

| № in order | Plant height, cm | Number of stems per plant, pcs. | Number of heads per plant, pcs. | Foliage, % |
|------------|------------------|--------------------------------|-------------------------------|------------|
| (Early 2 x Ne 3000 I1) F1, St | 56.4 | 100 | 3.8 | 100 | 11.0 | 100 | 51 |
| (Early 2 x Ne 3000 I1) I1 | 61.3 | 109 | 3.9 | 103 | 12.8 | 116 | 48 |
| (Early 2 x Ne 3000 I1) I2 | 49.5 | 88 | 3.6 | 95 | 9.3 | 85 | 50 |
| (Early 2 x Ne 3000 I1) I3 | 41.7 | 74 | 3.5 | 92 | 8.4 | 76 | 53 |
| (Early 2 x Ne 3000 I1) I4 | 42.1 | 76 | 3.7 | 97 | 8.4 | 76 | 51 |
| (Early 2 x Ne 3000 I2) I1 | 50.2 | 89 | 4.4 | 116 | 11.3 | 103 | 50 |
| LSD05 | 2.6 | 0.4 | 1.2 |

The mass of leaves and stems decreases from I1 to I3 generation by almost 2 times. In the I1 generation, these indicators were 16.1 and 17.4 g per plant, and in the I3 generation, only 8.4 and 7.5 g per plant, respectively (Table 3).

Table 3. Influence of inbred depression on the productivity of meadow clover

| № in order | Leaves weight, g / plant | Weight of stems, g / plant | Plant weight, g / plant | % to the st |
|------------|--------------------------|---------------------------|-------------------------|------------|
| (Early 2 x Ne 3000 I1) F1, St | 15.6 | 100 | 14.9 | 100 | 30.5 | 100 |
| (Early 2 x Ne 3000 I1) I1 | 16.1 | 103 | 17.4 | 117 | 33.5 | 110 |
| (Early 2 x Ne 3000 I1) I2 | 9.7 | 62 | 9.7 | 65 | 19.4 | 64 |
| (Early 2 x Ne 3000 I1) I3 | 8.4 | 54 | 7.5 | 50 | 15.9 | 52 |
| (Early 2 x Ne 3000 I1) I4 | 8.2 | 53 | 7.8 | 52 | 16.0 | 52 |
| (Early 2 x Ne 3000 I2) I1 | 12.2 | 78 | 12.1 | 81 | 24.4 | 80 |
| LSD05 | 1.5 | 1.6 | 2.8 |

In generation I4, the performance indicators stabilize. Obtaining hybrid F1 offspring by crossing a linear material with a level of induction I4 made it possible to increase the weight of 1 plant from 16.0 g to 24.4 g, which amounted to 80% of the standard (table 3).
4. Conclusion

Thus, self-pollination, repeated in the number of successive generations, leads to an increase in homozygosity and to inbred depression, which increases from I₁ to I₃ generation and stabilizes in the I₄ generation. Besides, in the I₁ generation, according to the main morphobiological characteristics, there is no inward depression, but the maximum release of recessive lethal mutations is manifested - 6.2% and the survival rate of seedlings decreases (91.7%). Obtaining hybrid F₁ offspring by crossing a linear material with an I₄ induction level leads to the restoration of plant productivity indicators. The data obtained make it possible to take into account the survival rate of seedlings, the cleavage of lethal and semi-lethal mutations, the degree of inbred depression in the formation of sample volumes when creating a linear material of meadow clover.

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