Use of factors of photoperiod duration and growth regulators to increase the yield of mixoploid plants in C₀ generation in meadow clover (*Trifolium pratense* L.)

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Abstract. It has been established that the studied mode of cultivation of meadow clover under controlled environmental conditions, with continuous illumination with an intensity of 18-20 thousand lux and a constant temperature of +25 °C, causes significant morphological changes in meadow clover. Compared with the optimal (control), the proposed growing regime halves the development time of plants and by the same amount reduces the severity of morphological traits, while simultaneously causing apical dominance in 54% of plants. Spraying plants with phytohormone in the form of naphthylacetic acid in all studied concentrations reduced the growing season by 4-8 days and increased pollen fertility by 8-10%. The optimal concentration of naphthylacetic acid (NLA) was revealed to suppress the growth of secondary meristems in meadow clover under the conditions of an experimental plant growing regime. The most significant effect on the manifestation of apical dominance was shown by the use of naphthylacetic acid at a concentration of 0.001%. The combined use of the proposed growing regime and treatment with naphthylacetic acid at a concentration of 0.001% increased the yield of plants with apical dominance from 54.5% to 76%. The developed method has shown high efficiency for the creation of tetraploid forms of meadow clover. When growing clover C₀ generation and selection of mixoploid plants, the yield of mixoploids increased 5.4 times, the survival rate of seedlings increased by 21%, the time to obtain one generation and the selection of mixoploids decreased by 58%.

Keywords: meadow clover, polyploidy, mixoploids, apical dominance, photoperiod, auxin.

1. Introduction

In most regions of Russia, the main fodder crops are perennial grasses, which ensure sustainable and efficient forage production. Among perennial grasses, legumes play a leading role, due to the fact that they are the most cost-effective and have a high environment-forming ability [1-4].

In terms of economic efficiency, the main groups of forage crops are arranged in the following sequence: perennial leguminous grasses, legume-cereal mixtures, perennial grasses, corn for silage, annual grasses, fodder roots, spring and winter cereals [5]. In this regard, breeders are faced with the task of creating highly productive varieties of meadow clover, as one of the most important legumes in fodder production in Russia.

Meadow clover is distinguished by a wide modification and genetic variability, which makes it possible to create highly productive varieties. It is known that varieties-populations with high
heterozygosity have a wider rate of reaction to changing environmental conditions. Polyploidy, as a factor in increasing heterozygosity and, consequently, stress resistance and productivity, opens up broad prospects for the selection of meadow clover. A comparative study of the forage productivity of diploid and tetraploid varieties of meadow clover over a 10-year period showed that tetraploid varieties were 24–36% more productive [6].

However, when creating the initial material of meadow clover at the tetraploid level, there is a problem of identifying ploidy in the $C_0$ generation. Somatic polyploids in the $C_0$ generation are genetic chimeras with the formation of mixoploid tissues, both in the vegetative and generative spheres, which leads to the formation of haploid and diploid pollen [7,8]. The identification of polyploid tissues in the inflorescences of meadow clover is further complicated by the fact that in this species the central point of growth forms a shortened shoot (root collar), and generative shoots develop from secondary meristems [9]. Such a feature of the development of meadow clover determines the fact that when the primary apical meristems of seedlings are treated with colchicine, generative shoots do not develop from them. This leads to a decrease in the number of mixoploid generative shoots and significantly increases the time required to identify polyploid inflorescences.

The apical dominance effect can be used to overcome this problem. The phenomenon of apical dominance consists in the fact that an actively growing central point of growth (apex) has an overwhelming effect on the awakening and growth of axillary buds [10]. From the point of view of chemical regulation of the process of apical dominance, the main role belongs to auxin [10, 11]. Using synthetic analogs of auxins (AUA or NAA), this process can be largely controlled [12].

The aim of our research was to study the possibility of increasing the yield of mixoploid meadow clover plants in the $C_0$ generation based on the use of the apical dominance effect caused by round-the-clock lighting and auxin treatment.

2. Methods of the research

The studies were carried out in a selection and greenhouse complex in artificial climate chambers in accordance with the guidelines [13]. The optimal conditions for plant cultivation were used as a standard growing regime: temperature in the germination – leaf rosette phase during the day +22 °C, at night +18 °C; in the stalking phase - night + 4-5 °C, day + 12 ... 15 °C. The duration of the period is 10 days. In the budding – flowering phase, the night is + 18 °C, the day is + 25 °C, the humidity is 60-70%, the photoperiod is 18 hours, the illumination is 18-20 thousand lux.

The studies were carried out on the diploid varieties of meadow clover VIK-7 and Early 2; in each experiment, 60 genotypes were studied. To suppress the growth of secondary meristems in meadow clover, a synthetic analogue of auxin naphthylacetic acid (NAA) was used at concentrations of 0.001%, 0.002%, 0.003%, 0.004%. Clover plants were treated with phytohormone by spraying 2-3 true leaves in the initial period of growth.

Mixoploid plants of the $C_0$ generation were obtained by the action of an aqueous 0.4% solution of colchicine on the apical meristems of meadow clover seedlings with preliminary synchronization of divisions at a low temperature of + 4 °C under vacuum infiltration [14].

The selection of mixoploid inflorescences of meadow clover was carried out on the basis of cytological analysis of the size and shape of pollen grains [15]. Pollen fertility was determined by the acetocarmine method [16].

3. Results of the research

In the course of our research, we found that growing clover plants under 24-hour lighting, an air temperature of +25 °C and an illumination intensity of 18-20 thousand lux, causes significant morphological changes in plants and leads to apical dominance (Table 1).
Table 1. Comparative assessment of cultivation modes of meadow clover plants in terms of the effect on morphological characters and manifestation of the effect of apical dominance

| Mode characteristic | Plant height, cm | The coefficient of variation, V, % | Numb er of stems, pcs. | The coefficient of variation, V, % | Number of internod es, pcs. | The coefficient of variation, V, % | Time from planting to flowering, days | The number of plants with apical dominance, % |
|---------------------|-----------------|-----------------------------------|------------------------|-----------------------------------|----------------------------|-----------------------------------|------------------------------------------|-----------------------------------------------|
| Standard growing mode, St | 65,6±3,8 | 16,3 | 3,3±0,3 | 270 | 6,0±0,4 | 17,5 | 69 | 0 |
| Experimental mode: photoperiod 24 h, temperature +25 °C, constant illumination 18-20 thousand lux | 42,2±2,4 | 19,7 | 1,6±0,3 | 14,2 | 4,9±0,2 | 16,8 | 39 | 54,5 |

Analysis of the data obtained shows that the studied regime of plant growing leads to a halving of the growing season, a decrease in plant height from 66 cm to 42 cm, the number of stems from 3.3 to 1.6 per plant, the number of internodes from 6 to 4, 9 pieces and at the same time causes the effect of apical dominance in 54.5% of plants. The manifestation of apical dominance was expressed both complete - the development of one generative shoot, and incomplete, where, along with the development of the central meristem, generative shoots from secondary meristems were formed (Fig. 1).

Figure 1. Influence of the growing regime on the effect of apical dominance
1 - lack of apical dominance; 2 - incomplete apical dominance;
3 - complete apical dominance

To enhance the effect of apical dominance, the combined effect of plant growing conditions and the phytohormone naphthylacetic acid (NAA), as a factor inhibition of the growth of secondary meristems,
was studied. Clover was treated by spraying plants with an aqueous solution of NAA in the initial period of growth, in the phase of 2-3 true leaves.

Considering that many growth regulators have gametocidal properties [17], the fertility of plant pollen was determined.

For a more accurate characterization of the duration of the growing season, each phase of ontogenesis was assigned an index (seedlings - 0, rosette of leaves - 1, stemming - 2, budding - 3, flowering - 4), the reliability of differences was confirmed by mathematical processing, since it is known that auxins accelerate the formation of the generative sphere of long-day plants (Table 2).

Table 2. The combined effect of the growing regime and different concentrations of NAA on the morphological and cytological characteristics of meadow clover plants (cultivar VIK 7)

| Version | The number of plants with apical dominance, % | Plant height, cm | Quantum internodes, pcs/rast | The coefficient of variation, V, % | Pollen fertility, % | Среднее значение индекса развития | Deviation from the standard |
|---------|---------------------------------------------|------------------|-------------------------------|----------------------------------|--------------------|---------------------------------|---------------------------|
| Grow mode with apical dominance, St | 54,5 | 42,2±2,4 | 19,7 | 4,9±0,2 | 16,8 | 84,9 | 2,76 | – |
| Mode + NAA 0,001% | 76,0 | 36,7±1,6 | 15,5 | 4,1±0,1 | 9,8 | 95,6 | 3,54 | +0,78 |
| Mode + NAA 0,002% | 67,2 | 38,4±1,9 | 17,9 | 3,7±0,2 | 16,7 | 90,3 | 3,48 | +0,72 |
| Mode + NAA 0,003% | 70,0 | 41±1,7 | 15,2 | 4,1±0,1 | 11,7 | 92,4 | 3,22 | +0,46 |
| Mode + NAA 0,004% | 66,7 | 39,2±2,4 | 21,4 | 3,6±0,3 | 25,6 | 92,3 | 3,22 | +0,46 |
| НСР | 0,27 |

The studies have shown the effectiveness of using naphthylacetic acid to increase the number of meadow clover genotypes with apical dominance by 12-25%. The greatest effect of NAA was at an aqueous solution concentration of 0.001%; apical dominance in this treatment variant increased from 54 to 76%. At the same time, it was found that the use of phytohormone in all concentrations shortens the growing season by 4-8 days and increases pollen fertility by 8-10%.

The effectiveness of the developed technique was tested in the experiment on transferring the diploid cultivar of meadow clover Ranny 2 to the tetraploid level (Table 3).

The experiment confirmed that the cultivation of Co generation plants using the developed regime increases the yield of mixoploids. According to the data obtained, the number of mixoploids increased 5.4 times, the survival rate of seedlings increased by 21%, the time for growing a generation and selection of mixoploids decreased by 58%.
Table 3. Influence of growing regimes on the survival rate of seedlings, the yield of mixoploid forms and the length of the growing season of meadow clover rC0 generation (variety Early 2)

| Option processing                                      | Mode cultivation                  | Survival seedlings, % | Apical dominant plants, % | Number of mixoploids, % | Time from sowing to flowering, days |
|--------------------------------------------------------|-----------------------------------|-----------------------|---------------------------|-------------------------|-----------------------------------|
| Vacuum infiltration with 0.4% solution colchicine in the apical meristems of seedlings | Standard growing mode, St         | 25.5                  | 0.0                       | 3.3                     | 74                                |
| Experienced mode cultivation + 0.001% NAA             |                                    | 46.7                  | 69.0                      | 17.9                    | 31                                |

4. Conclusion
As a result of the studies, it was found that in order to increase the yield of mixoploid forms of meadow clover and reduce the timing of the breeding process, it is necessary to grow plants under 24-hour illumination with an intensity of 18-20 thousand lux and a constant temperature of +25 °C when plants are sprayed in the early stages of development with phytohormone in the form of naphthylacetic acid. acids at a concentration of 0.001%. The use of the proposed method in the breeding practice of creating a tetraploid source material of meadow clover, allows you to increase the yield of mixoploids by 5.4 times, the survival of seedlings by 21% and reduce the time for growing a generation and selection of mixoploids by 58%.

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