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MICROCLONAL PROPAGATION OF GRAIN LEGUME CROPS IN VITRO CONDITIONS

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Abstract

An enhanced method of microclonal propagation of grain legume seeds, including the stages of sterilization of explants of grain legumes (peas, vetch, beans, lentils, chickpeas) their introduction into in vitro conditions, actual propagation and rooting of regenerants is presented in the article.

Methods for culturing isolated plant organs, tissues and cells emerged more than half a century ago and have been constantly improved since then. Work in this direction has led to certain achievements in understanding the process of plant development.

Keywords: microclonal plant propagation, planting material, grain legume crops, in vitro.

ДӘНДІ-БҰРШАҚТЫ ДАҚЫЛДАРДЫҢ МИКРОКЛОНАЛДЫК КӨБЕЮ INVITRO ЖАҒДАЙЫНДА

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Аңдатпа

Мақалада бұршақ дақылдарының эксплланттарын (бұршақ, Ветчина, бұршақ, жасымық, ноқат) зарарсыздандыру кезеңдерін, оларды invitro жағдайларға енгізуді, регенеранттардың нақты көбеюі мен тамырлануын қамтитын дәнді-бұршақты тұқымдарды микроклоналды көбейтудің жетілдірілген әдісікелтірілген.

Оқшауланған мүшелерді, ұлпаларды және өсімдік жасушаларын өсіру әдістері жарты ғасырдан астам уақыт бұрын пайда болды және содан бері үнемі жетілдірілуде. Бұл бағыттағы жұмыс өсімдіктердің даму процесін түсінуде белгілі бір жетістіктерге әкелді.

Кілт сөздер: өсімдіктердің микроклоналды көбеюі, отырғызу материалы, бұршақ дақылдары, invitro.

МИКРОКЛОНАЛЬНОЕ РАЗМНОЖЕНИЕ ЗЕРНОБОБОВЫХ КУЛЬТУР В УСЛОВИЯХ IN VITRO

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Аннотация

В статье представлен усовершенствованный метод микроклонального размножения зернобобовых семян, включающий этапы стерилизации экспланта зернобобовых культур (горох, вика, фасоль, чечевица, нут) их введение в условия invitro, собственно размножение и укоренение регенерантов.

Методы культивирования изолированных органов, тканей и клеток растений появились более полувека назад и с тех пор постоянно совершенствуются. Работа в этом направлении привела к определенным достижениям в понимании процесса развития растений.

Ключевые слова: микроклональное размножение растений, посадочный материал, зернобобовые культуры, invitro.

Introduction

Peas, beans, legumes, soybeans, chickpeas, chickpeas, lentils, china, and lupins are all grain legume crops. Due to symbiosis with nodule bacteria, which absorb nitrogen from the atmosphere, all these crops have a high protein content in the seeds. Many important amino acids (e.g. methionine, tryptophan, valine, lysine, etc.) are present in protein Additionally, these plants are useful as food and fodder crops because their seeds contain fats (especially a lot in soybean), minerals, vitamins A, B1, B2, C, D, E, and PP. They have fiber that helps the intestines, which causes rapid satiety, and the protein that has amino acids and lysine strengthens the immune system. Legumes also have the advantage of not accumulating nitrates and toxic substances [1,2].

It was found that to obtain high-yielding, biotic and abiotic factors resistant leguminous plant hybrids and varieties, it is necessary to employ modern biotechnology methods. These methods accelerate and enhance the efficiency of the breeding process [3,4].

Effects of different explants and nutrient media composition on in vitro cultivation were studied in order to develop a technique for microclonal propagation of grain legumes. Breeders and biotechnologists can cut research and development time significantly with in-vitro cultivation.

To achieve the objectives of the study, the following tasks were set:

– to determine the optimal proportions of nutrient media for growth, development, and in-vitro rooting of the plant;

– to select a sterilising agent with the best disinfecting effect to obtain a regenerant plant.

*The research aim*is to improve the method of microclonal propagation of grain legumes in vitro.

The main objective: to determine the optimal composition of nutrient medium for growth, development and further rooting of grain legume crops.

Methods of research

The research was conducted in the “Biotechnology of agricultural plants” laboratory of ManashKozybayev North Kazakhstan University” NPLC with seeds of “Rafinad” pea variety, common bean, chickpea ‘Jubilee’, lentil ‘Vehovskaya’, vetch ‘Assorti’.

As explants for tissue culture, mature seeds of various types of leguminous crops (pea, bean, chickpea, lentil, and vetch) were utilised. Chlorhexidine, Domestos, and chlorine lime solutions were used to sterilise the explants. Various quantities of substances were employed, and the

exposure period was 30 minutes. With a few small compositional adjustments made to the Gamborg B5 nutrient medium, NUC (0.002 mg/g) was introduced, and plant germination was conducted on the new nutrient medium called "Bobik." In vitro cultivation lasted 3–4 weeks at 24 ℃ and in bright light (5–6 weeks). Three to five weeks under phytotron conditions. +20℃ is the temperature. Following the establishment of roots, regenerants are inserted into the soil, and the temperature is lowered to +18℃ before rising to +24℃ during flowering.

Research results and discussion

The results of the study showed that the effectiveness of sterilisation was a determining factor in the success of tissue culture induction. Thus, using chemicals that contained chlorine guaranteed that the material was sufficiently sterilised. After treating seeds with Domestos solution, a high level of sterilisation was also noted (95.7%). Furthermore, it was discovered that the significant damage caused to the lentil seeds during sterilisation prevented them from being propagated in vitro (Fig. 1).

Figure 1. Planting of sterile seeds on “Bobik” nutrient medium, under aseptic conditions

Figure 2. Root system formation in haricot bean on 3-4 days

The hormonal makeup of the nutrient medium is a crucial component of effective micropropagation. This is largely dependent on the amounts and combinations of phytohormones. In this instance, auxin has been employed as a phytohormone most frequently in the form of a-naphthylacetic acid (ANA) or indolyl-3-acetic acid (IAA). In our study, ANA was employed. It was found that, although the yield of rooted seedlings was only 15%, the concentration of ANA in the nutrient medium stimulates the growth of the above-ground portion of plants following root formation. maximum root formation An2,3,4). average of two or three pieces of roots, measuring 1.0 to 2.0 cm, were induced by ANA. (Fig. 2, 3, 4).

Figure 3. Root system formation of the "Yubileiny"chickpeavariety on 5-6 days

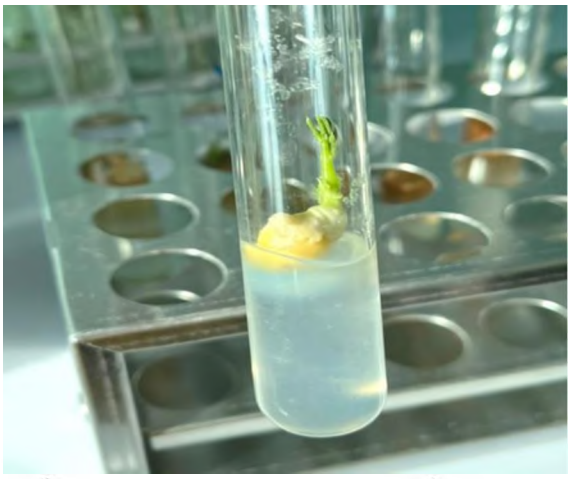

Figure 4. The 'Rafinad' pea variety with pea seedling formation

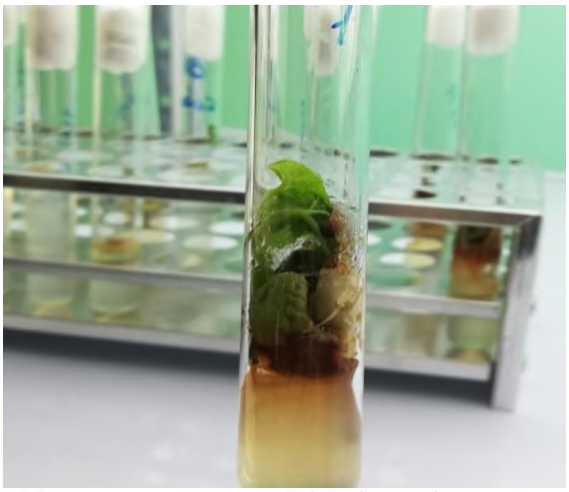

Figure 5. Shoot formation in haricot bean on 7-8 days

Figure 6. "Assorti" Vetch variety with petals formed on 2 to 3 pieces.

Figure 7. Pea plant rooted in vitro

Fig gure 8. Planting in the ground

Following planting on nutritional medium, pea and bean seedlings emerged on days three through six, chickpeas on days five through six, and lentil seeds did not sprout, according to the findings. In 7-8 days, pea and bean seedlings began to form shoots, yielding a single shoot that ranged in height from 1.5 to 3.0 cm (Figures 5, 6).

Table 1 displays the development outcomes of microgrowthsutilising the «Bobik» nutrition media.

Table 1. Morphogenesis of microplants using «Bobik» nutrient medium

Crop	Plant height	Number of internodes	Root formation		
			pcs	mm	gr
«Rafinad»pea variety	53,4	3,5	4,7	20,3	0,20
Haricot bean	30,2	1,3	3,6	15,2	0,05
«Assorti» Vetch	56,1	3,1	1,3	30,4	0,28
«Yubileiny» chickpea	-	-	-	-	-
«Vekhovskaya» Lentils	-	-	-	-	-

Grain legume crops' primary nutritional characteristic is their capacity to absorb phosphorus in forms that are difficult to reach. The metabolism of phosphorus is significantly influenced by potassium.

A sufficient amount of potassium in the nutritional medium promotes the root system's development by increasing the usage of even tiny quantities of phosphorus. Thus, it was determined that the yield of grain legume crops is positively impacted by potassium fertilisers on a nitrogen-phosphorus background.

In the future, using three to four passes will greatly increase this indicator and quickly produce the required number of plants for breeders (Fig. 7, 8).

The results obtained will serve as a basis for the development of accelerated vegetativepropagation of grain legumes.

Conclusions

1. Different ratio of leguminous crops to the composition of the “Bobik” nutrient medium was revealed. The “Rafinad” pea variety formed the largest number of internodes 3.5 pcs, and the smallest in common bean 1.3 pcs.

2. During the research the height of plants was noted, where vetch exceeded by 2.7mm, in contrast to peas.

3. Root formation of the "Rafinad" pea variety was successful at 4.7pc, less successful was the vetch at 1.3pc. The Vetch showed a fairly good result in millimetres and grams, exceeding peas by 10.1mm and 0.08g.

4. At the concentration of ANA 0.002 g/l in the nutrient medium activates the growth of the aboveground part of plants after the formation of roots. Thus, it provides the highest yield of plants from one seed.

5. The research revealed that lentils cannot withstand complete sterilisation due to their structure. At the end of sterilisation, the lentils are highly softened, which led to difficulties in transplanting and eventually failure of full development.

6. In addition, on the 3-4th day of root system formation, the "Yubileiny" chickpea variety was infected. Subsequently, it led to difficulties in full development of the plant and resulted in death.

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