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OPTIMIZATION OF MAIZE SEEDLINGS PRODUCTION FOR DNA EXTRACTION

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Наведено результати впливу умов пророщування на насіння кукурудзи для отримання проростків і виділення з них ДНК для полімеразної ланцюгової реакції. Встановлено оптимальні умови для пророщування насіння, а саме: використання стерильної дистильованої води, стерильного фільтрувального паперу та поверхнево простерилізованого насіння для пророщування при 25 °С про-тягом 8 діб, що забезпечує отримання нормально розвинених неконтамінованих проростків для виділення ДНК.

Ключові слова: *пророщування насіння, стерилізація, проростки, кукурудза, ДНК.*

The increasing demand for modern methods of DNA diagnostics has driven the development and the use in practice of highly sensitive methods utilizing polymerase chain reaction methodology (PCR) [1–4]. Studies at the DNA level, in addition to molecular biology, are required in modern investigations on breeding, taxonomy, biodiversity, evolution, the genetic basis of regulations of physiological processes and resistance to biotic and abiotic environmental factors, biotechnology, bioengineering, etc. [2, 5–10].

A key step to obtaining of high-precision results of molecular genetic analysis by PCR is obtaining high-quality DNA preparations. Methods of extraction of plant DNA must be adapted to certain types of plant tissues through a substantial range of variability of the chemical composition of plant cells and structural features of their cell walls [1]. Plant DNA is predominantly extracted from young tissues due to the low content of polysaccharides, polyphenols and other secondary metabolites, which are precipitated from the DNA during isolation process, preventing its dissolution [11, 12]. Extraction of DNA from the seedlings is optimal for PCR analysis.

Maize (*Zea mays* L.) is one of the most common and productive cereal crops in global agricultural production, including in Ukraine. Despite the high level of molecular genetic studies on maize, certain methodological aspects remain unexplored. Thus, at the stage of germination of maize seeds, there are a lot of difficulties associated with contamination of seeds during germi-nation. Therefore, the optimization of maize seeds sterilization and germination conditions to prevent the ingress of extraneous DNA into a sample of target DNA is of considerable interest.

The aim of the given work was the selection of optimal conditions for obtaining non-contaminated packages of maize seedlings for DNA extraction and use in PCR analysis. In the experiment the effects of such factors as surface seeds sterilization, the application of sterile filter paper and sterile water for wetting on the production of uncontaminated and viable maize seedlings were being estimated.

As the material for the study the seeds of the maize inbred DK267 were used. The germination of seeds was performed in Petri dishes, of 10 caryopses per 1 Petri dish, using filter paper soaked in distilled water according to [13]. Seeds were germinated in the dark at 25 ºC. The experi-ment was replicated three times. The general scheme of the experiment is presented in Table 1.

1. The scheme of the experiment on optimization of obtaining uncontaminated viable seedlings of maize

Factor / Option number	Filter paper	Water for wetting	Seeds
1	2	3	4
1	Sterile	Sterile	Surface sterilized

2			Intact
3			Without seeds
4		Non-sterile	Surface sterilized
5			Intact
6			Without seeds
1	2	3	4
7	Non-sterile	Sterile	Surface sterilized
8			Intact
9			Without seeds
10		Non-sterile	Surface sterilized
11			Intact
12			Without seeds

Filter paper and distilled water were sterilized by autoclaving at pressure of 1 ATM during 1 hour. Surface sterilization of seeds was performed according to the modified method [13] in a saturated solution of sodium hypochlorite for 20 min followed by washing three times in sterile distilled water.

Analysis of the results was performed on the 4^{th} and 8^{th} days of germination in the following parameters: 1) seed germination (%), which was defined as the percentage ratio of the number of seeds germinated to total number of seeds analyzed; 2) contamination of seedlings (%), which was defined as the percentage of contaminated seedlings to the total number of seedlings received; 3) the average number of microbial spots (pieces) on the surface of the filter paper in one Petri dish, which was defined as ratio of the total number of microbial spots with a diameter of 1 cm to the total number of Petri dishes analyzed. Statistical processing of experimental data was performed according to [14]. Data in tables are presented as $x \pm mt$, where x is the arithmetic mean, m is the error of the arithmetic mean, t is the Student coefficient at the level of significance 0.05.

Data on the influence of seed growing factors on their germination, contamination and the specific number of microbial spots on the 4^{th} day of observation are presented in Table 2.

Analysis of summarized data on the effect of sterile filter paper for germination has allowed to establish that the germination of seeds on sterile filter paper was significantly lower than on non-sterile one. It could appear owing to the specific of paper modification under sterilization and the release of specific substances into the germination space. Sterility or non-sterility of filter paper had no significant effect on the contamination of seedlings. Also the number of microbial spots on paper did not differ significantly between variants, therefore the effect of sterility of filter paper on the contamination of 4-day-old seedlings was not reliably established. The sterility of distilled water for wetting also did not affect significantly the germination of seeds, seedling contamination and the number of microbial stains on filter paper on the 4^{th} day of observation.

2. The influence of the experimental conditions on the emergence of seedlings and their contamination (at the 4^{th} day of the study)

Paper	Water	Seeds	Option number	Number of seeds analyzed	Seed germination, %	Contamination of seedlings, %	Average number of microbial spots on paper, pieces
Sterile	Sterile	Surface sterilized	1	30	83,3	0	0
		Intact	2	30	26,7	100	0
		Without seeds	3	0	-	-	0
	Non-sterile	Surface sterilized	4	30	80,0	0	0
		Intact	5	30	60,0	100	1,7
		Without seeds	6	0	0	-	0
Non-sterile	Sterile	Surface sterilized	7	30	90,0	0	0
		Intact	8	30	66,7	96,7	0

		Without seeds	9	0	-	-	0
	Non-sterile	Surface sterilized	10	30	96,7	0	0
		Intact	11	30	70,0	86,7	0
		Without seeds	12	0	-	-	0
Total with sterile paper				120	62,5 ± 8,8	50,0 ± 9,1	0,3
Total with non-sterile paper				120	80,8 ± 7,2	45,9 ± 9,1	0
Total with sterile water				120	66,7 ± 8,6	49,2 ± 9,1	0
Total with non-sterile water				120	74,2 ± 8,0	46,7 ± 9,1	0,3
Total with surface sterilized seeds				120	88,0 ± 6,0	0	0
Total with intact seeds				120	55,8 ± 9,6	95,6 ± 3,7	0,3
Total without seeds				0	-	-	0

The estimation of the effect of surface seeds sterilization on the 4th day of germination showed that the germination in the variants with superficially sterilized seeds was significantly higher than in the variants with intact seeds. In variants with surface sterilized seeds contaminated seeds were not observed at all. The surface sterilization of seeds made no significant effect on the number of microbial spots on paper.

8-day-old maize seedlings were obtained according to variants of the experiment that were shown in Table 1. The results of studies on the effect of germination factors on the seeds to arise, seedling contamination and the number of microbial spots on filter paper at the 8th day of the study are shown in Table 3.

Analysis of the effect of the sterility of filter paper on the germination of seeds has allowed to establish that the level of the contamination of seedlings at the 8th day in the variants with sterile filter paper was significantly lower than in the variants with non-sterile one. The average number of microbial spots on filter paper showed that the paper sterility also had no effect on this parameter.

The use of sterile/non-sterile water had no significant influence on the contamination of seeds at the 8th day of germination. This allowed us to conclude that non-sterile water did not course the contamination of seedlings. However, the number of microbial spots on filter paper was significantly lower when using sterile water to moisten the paper in comparison with the variants with non-sterile water.

The results of the influence of surface seeds sterilization on their germination on the 8th day of observation showed a reduction of contamination in variants with surface sterilized seeds. The number of microbial spots on paper was also significantly lower after surface seeds sterilization than in the variants with intact seeds. Besides in the samples with intact seeds there was a significant microbial contamination of both seedlings and filter paper. The contamination resulted in the inhibition of obtained seedlings, and sometimes in their death after. In the samples obtained from variants with intact seeds such contaminants as fungi *Penicillum notatum* and *Aspergillus niger* were detected.

3. The influence of the experimental conditions on the emergence of seedlings and their contamination (at the 8th day of the study)

Paper	Water	Seeds	No. option	Number of seeds analyzed	Seed germination, %	Contamination of seedlings, %	Average number of microbial spots on paper, pieces
Sterile	Sterile	Superficially sterilized	1	30	100	6,7	0
		Intact	2	30	100	100	8,0
		Without seeds	3	0	-	-	0
	Non-sterile	Superficially sterilized	4	30	100	20,0	0
		Intact	5	30	100	100	14,0
		Without seeds	6	0	-	-	0
Non-sterile	Sterile	Superficially sterilized	7	30	100	100	2,7
		Intact	8	30	100	100	8,0

		Without seeds	9	0	-	-	5,7
	Non-sterile	Superficially sterilized	10	30	100	70,0	2,7
		Intact	11	30	100	100	4,3
		Without seeds	12	0	-	-	0
Total with sterile paper				120	100	56,7 ± 9,0	3,7 ± 0,8
Total with non-sterile paper				120	100	92,5 ± 4,8	3,9 ± 0,8
Total with sterile water				120	100	76,7 ± 7,7	4,1 ± 0,9
Total with non-sterile water				120	100	72,5 ± 8,2	1,5 ± 0,5
Total with surface sterilized seeds				120	100	49,2 ± 9,1	1,4 ± 0,5
Total with intact seeds				120	100	100	8,6 ± 1,0
Total without seeds				0	-	-	0,5

Thus, the results of the research of the factors influencing the preparations of maize seeds for germination (sterility of filter paper, sterility of distilled water, surface sterilization of seeds) to obtain high-quality uncontaminated seedlings for molecular genetic analysis by PCR, allows us to draw the following conclusions:

1. When used 4-day-old seedlings of maize for DNA extraction for seeds germination to apply surface sterilized seeds, but in order to reduce costs for the investigations non-sterile filter paper and non-sterilized distilled water for wetting may be utilized.

2. When used 8-day-old seedlings of maize for extraction of DNA it is necessary to apply surface sterilized seeds, sterile filter paper and sterile distilled water for wetting the filter paper.

References

1. Методы выделения растительной ДНК из растений и продуктов питания на их основе / *Л. А. Остроумова, А. Ю. Просеков, А. Н. Архипов, О. В. Мудрикова* // Известия Самарского науч. центра Российской акад. наук. – 2010. – Т. 12, № 4 (3). – С. 722–724.
2. Molecular characterization of global maize breeding gernplasm based on genome-wide single nucleotide polymorphisms / [Y. Lu, J. Yan, C. T. Cuimaraes et al.] // Theor. Appl. Genet. – 2009. – Vol. 120. – P. 93–115.
3. *Єфименко В. Г.* Молекулярно-генетична характеристика генотипів кукурудзи за складом мікросателітних локусів / *В. Г. Єфименко, Н. Е. Кожухова, Ю. М. Сиволап* // Вісн. Укр. товариства генетиків і селекціонерів. – 2006. – Т. 4, № 1. – С. 21–30.
4. Song R. Mosaic organization of orthologous sequences in grass genomes / R. Song, V. Llaca, J. Messing // Genome Research. – 2002. – Vol. 12. – P. 1549–1555.
5. *Рябушкин Н. А.* Специфика выделения ДНК из растительный объектов / *Н. А. Рябушкин, М. Е. Омашева, Н. Н. Галиакпаров* // Биотехнология. Теория и практика. – 2012. – № 2. – С. 9–26.
6. *Monaco M. K.* Gramene 2013: comparative plant genomics resources / [*M. K. Monaco, J. Stein, S. Naithana* et. al.] // Nucleic Acids Research. – 2013. – doi: 10.1093/nar/gkt1110.
7. *Gaut B. S.* DNA sequence evidence for the segmental allotetraploid origin of maize / *B. S. Gaut and J. F. Doebley* // Proc. Natl. Acad. Sci. – 1997. – Vol. 94. – P. 6801–6814.
8. Genome-wide analysis of WOX gene family in rice, sorghum, maize, *Arabidopsis* and Poplar / *X. Zhang, J. Zong, J. Liu* [et al.] // Journal of Integrative Plant Biology. – 2010. – Vol. 52, № 11. – P. 1016–1026.
9. *Chatterjee A.* Standartization of polymerase chain reaction for amplification of Gemini viral DNA / *A. Chatterjee and S. K. Sinha* // Journal of Bio-Protocols ISSN. – 2012. – Vol. 1, № 2. – P. 28–31.
10. *Сатарова Т. Н.* Кукуруза: биотехнологические и селекционные аспекты гаплоидии: [мо-ногр.] / *Т. Н. Сатарова, В. Ю. Черчель, А. В. Черенков*. – Днепропетровск: Новая идеоло-гия, 2013. – 552 с.
11. *Сиволап Ю. М.* Вариабельность и специфичность геномов сельскохозяйственных растений / *Ю. М. Сиволап, Н. Э. Кожухова, Р. Н. Календарь*. – Одесса: Астропринт, 2011. – 336 с.
12. *Zhang J.* Economical and rapid method for extracting cotton genomic DNA / *J. Zhang, J. D. Mc Stewart* // Journal of cotton science. – 2000. – Vol. 4. – P. 193–201.

13. Семена сельскохозяйственных культур. Методы определения всхожести: ГОСТ 12038-84. Введённый 01.07.86. – М., 1984. – 30 с.
14. *Атраментова Л. О.* Статистика для біологів: [підручник] / *Л. О. Атраментова, О. М. Утє-вська*. – Х.: Вид-во НТМТ, 2014. – 331 с.