Peculiarities using of biotechnological methods at various stages of vegetable crop breeding

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There was an enemy bombing of the village Selektsiyne, Kharkiv District, Kharkiv Region
on March 17, 2022

The aim of the biotechnological scientific work at the Institute Vegetable and Melons Growing of the National Academy of Agrarian Sciences are theoretically justified regularities of morphogenesis in the culture of in vitro isolated cells and tissues of vegetable plants and the development of methods for the accelerated creations of competitively capable, highly adaptive, new genotypes of vegetable plants and their identification by molecular and genetic methods

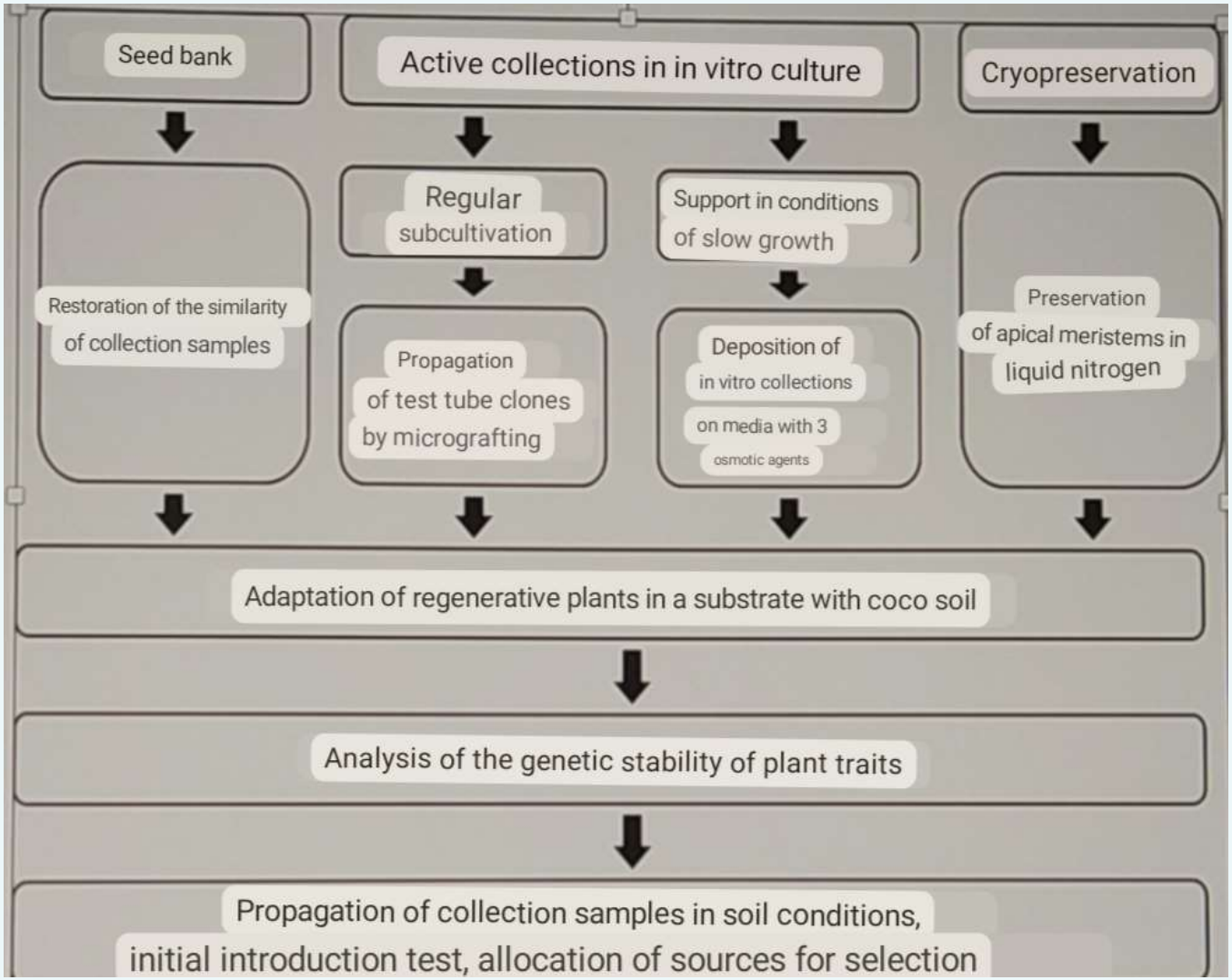


Fig. 1. The scheme of preservation biodiversity of vegetable plants using the methods of biotechnology

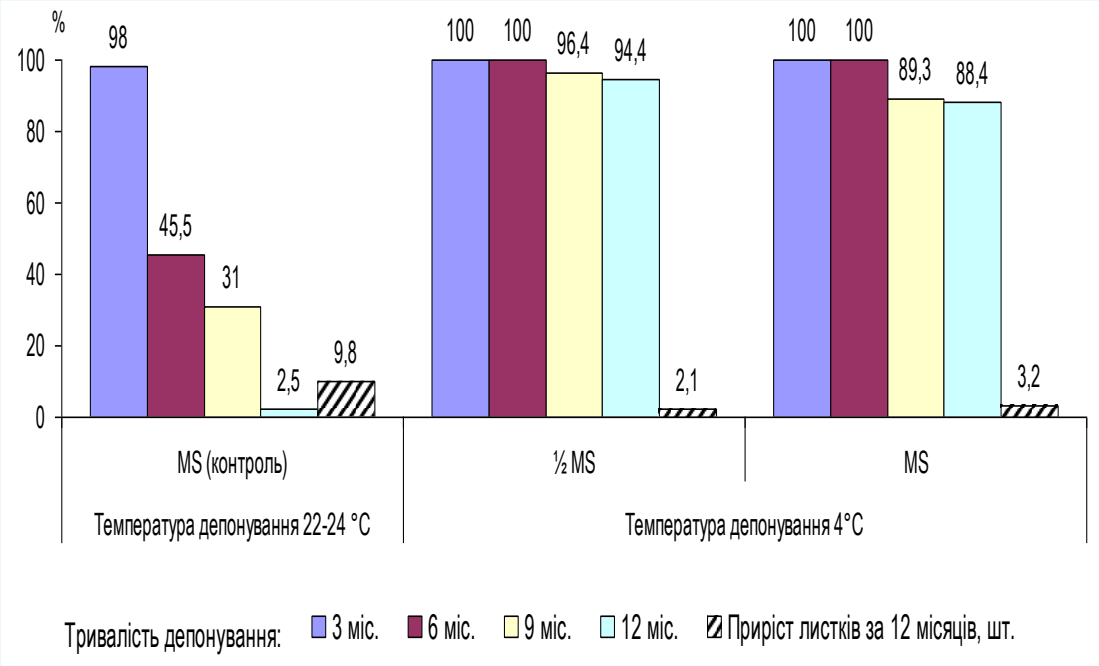


Fig. The influence of long-term storage conditions on the viability of test-tube plants of variety winter garlic Duches, %

The effect of cryoprotective substances on the viability and growth of apical meristems in spring garlic variety Manuilivskyi after 55 days of cultivation

Cryoprotective substance, container type	Number of viable meristems, %	Length, mm	
		shoots	roots
Without freezing and without treatment with cryoprotectants (absolute control)	100	54.3±4.1	19.5±3.9
15% aqueous solution of 1,2-PD (control 1)	100	41.0±3.8	10.8±2.7
1.2-PD-pk*	14.3±3.5	7.8±2.1	0
1.2-PD-ac**	44.0±5.3	36.1±4.5	7.5±2.5
PVS N (2M glycerol+1M sucrose+2.5M ethylene glycol on MS medium (control 2)	100	39.7±3.2	21.2±4.0
PVS N-pc*	37.5±5.1	7.7±1.9	0
PVS N-ac**	75.3±6.7	32.3±5.3	15.0±3.3
15% aqueous solution 1.3 BD (control 3)	100	48.0±4.8	17.5±4.2
1.3-BD-pk*	0	-	-
1.3-BD-ac**	0	-	-

Note. *pc - polyethylene containers;
**ac - aluminum thin-walled containers.

Cultivation of vegetable plants in vitro using LED lighting

Maintaining an in vitro collection of sweet potato plants

Adaptation to conditions in vivo

Plant cultivation in soil conditions

Propagation for a secondary introduction trial

The initial introduction test of a new vegetable crop (determining the terms and schemes of planting plants)

Scheme of the introduction a new vegetable crop of the Convolvulaceae family species *Ipomoea batatas L.* using in vitro technology

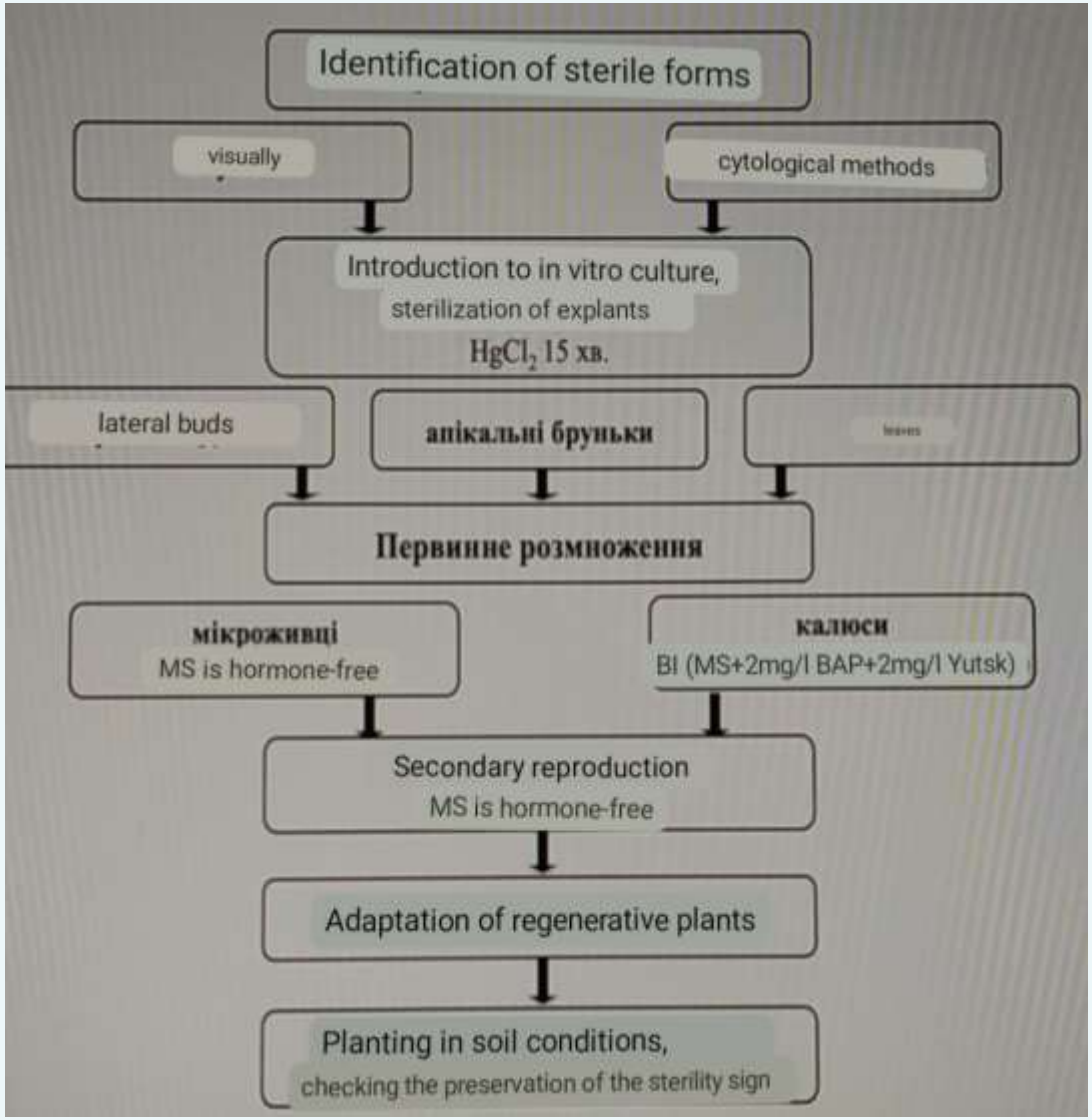


Fig. Scheme of a biotechnological method of sterile reproduction tomato forms

THE USE OF EXPERIMENTAL MUTAGENESIS AND IN VITRO CELL TECHNOLOGIES FOR CREATION AND PROPAGATION OF STERILE TOMATO FORMS

Processing by γ-rays tomato seeds

M_0

Identifying sterile plants

M_2

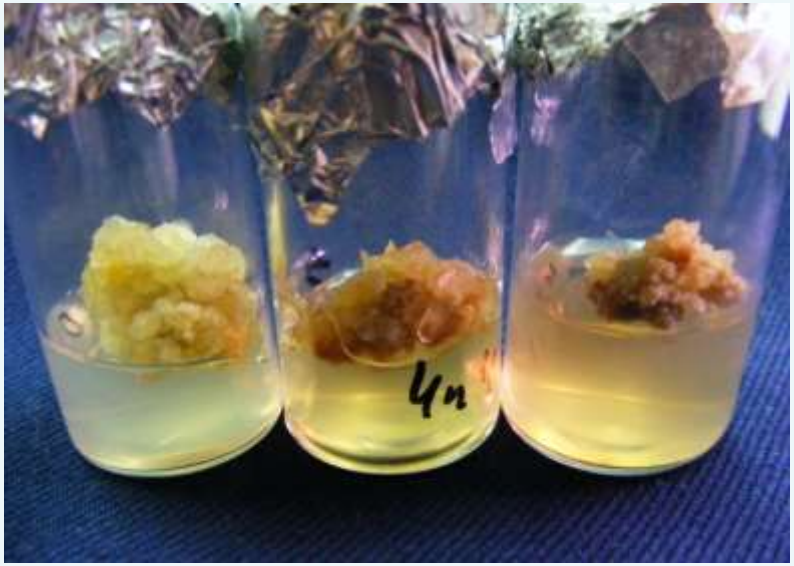
Breeding in vitro ms - genotypes

Adaptation of regenerant plants and sterility control

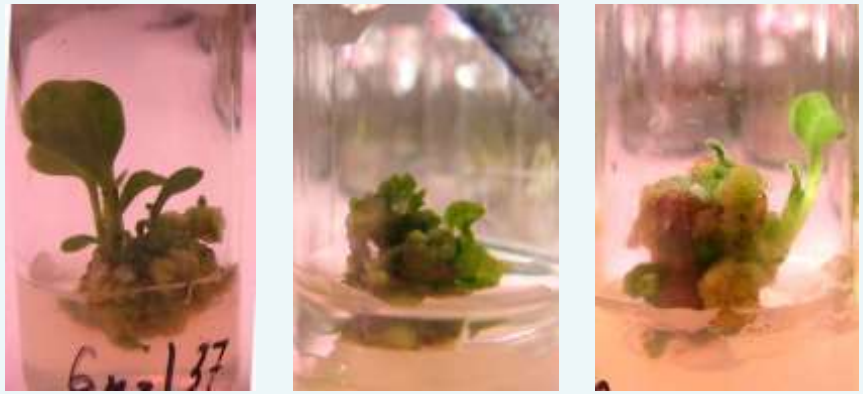
Hybridisation of sterile lines with fertile lines pollinators

CREATION OF RESISTANCE SOURCES FOR BREEDING OF VEGETABLE CROPS IN THE CULTURE OF ISOLATED CELLS AND TISSUES IN VITRO

Formation of organogenic shoots in tomato cotyledon explants at induction nutrient medium



Carrot calli of the D.c.306/1 genotype on media (from left to right): without black rot CF, with 30% CF, with 50% CF.



Cell breeding of eggplant used of culture filtrates from pathogens as selective agents

BIOTECHTOLOGICAL METHOD OF CREATING FUSARIUM RESISTENT SOURCES OF PLANTS MATERIAL TO BREEDING ENVIRONMENTS WITH CF

STAGE 1. Primary laboratory immunological elaluation of samples

STAGE 2. Screeing of resistans level in genotypes and selection to resisnant cell lines

ECCELERATION BREEDING PROCESS AT THE ACCOUNT OF MULTIPLE SELECTION IN VITRO AND IN VITRO CONDITIONS

STAGE 3 . Breeding and initial adaptation of microclones

STAGE 4 . Selective assessment of breeding material and selection of promising sources to resistance

- **bal 0 –** tissue development doesn't differ from cultivation on the control variant
- **bal 1 –** chlorotic tissues up to 25 % in growth callus are intence
- **bal 2 –** chlorotic tissues up to 50 %, growth callus are average
- **bal 3 –** chlorotic tissues up to 75 %, growth callus are despessed
- **bal 4 –** more that 75% chlorotic tissues, no growth callus

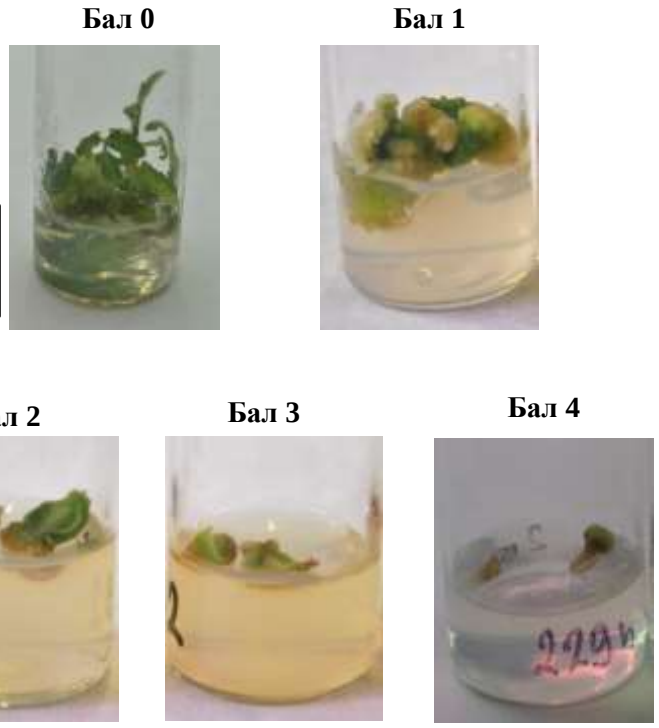


Fig. Scale for evaluating the degree of damage in tomato callus culture by a mixture of CF of pathogens Fusarium wilt in culture in vitro

The method of evaluation and breeding of sources for stress tolerant tomato plants at alternative technologies in vitro (utility model application U 2020 07598 dated 11/30/2020)

Stage 1.

Introduction of seeds of 10 varieties and hybrids into the sterile culture, created for different levels of tomato production intensity

Stage 2.

Assessment of influence to selective media on biometric indicators of test-tube plants





Abiotic resistance

Resistance to deficiency/excess of mineral nutrition elements of MS (1,5 x NH4NO+1,5 x KNO3+1,5 x CaCl2x2H2O+KH2PO4+MgSO4)

salt resistance (MS + 10 g/l NaCl)

drought resistance (MS + 0.05 g/l hydroxyproline)

Biotic resistance

Resistance to Alternaria (MS + 40% CF Alternaria spp.

Resistance to *Fusarium* (MS + 40 % CF *Fusarium* spp.

Stage 3.

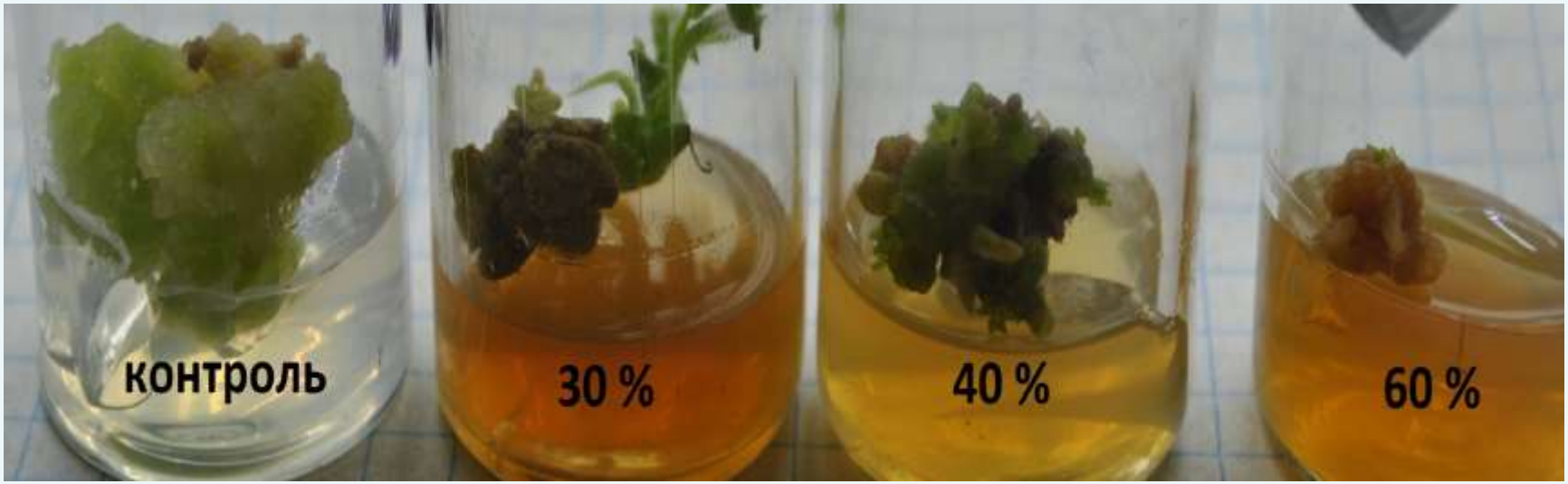
Determination of the peroxidase activity in regenerating plants after 3 weeks of culture on selective media

Stage 4.

Analysis of 71 coefficients of correlation in indicators on selective media in culture in vitro and establishing among them 11 significant at the 5% significance level

Development of regenerating plants of the Goldene koningin "Reine D'Or" tomato sample depending on the concentration of sodium chloride in nutrient medium

Development of regenerating plants of Goldene koningin "Reine D'Or" tomato sample depending on the concentration of hydroxyproline in nutrient medium



Callus development of investigated genotypes, depending on concentration CF Alternaria spp. in nutrient medium

Verification of the system of discriminant equations based on the developed model

Sample name	Group A	Group B	Group distribution
S. chilense, st.	7.83	-14.54	A
K-7311, st	7.05	14.44	B
Zulfiya F_1	2.48	21.09	B
Esmira F_1	5.78	29.65	B
Goldene koningin «Reine D'Or»	23.10	3.21	A
Potiron ecarlate	11.88	-14.25	A
Seven	-2.63	27.64	B
Dama	14.34	-3.81	A
T-5	8.14	-12.08	A
T-2	11.61	-6.59	A

The line T-5 was created for conditions in protected soil by the breeding method on a selective medium in vitro tissue culture. It is resistant to Fusarium wilt (score 7), has a high content of ascorbic acid in fruits (31.00 mg/%), the average weight of marketable fruit is 170 g.

According to results of the laboratory evaluation, the line T-5 was included in Group A and recommended for cultivating in organic vegetable growing technologies.

20.00.01.02.F “DEVELOPMENT OF THEORETICAL BASIS OF TOMATO ROOTSTOCK BREEDING USING MICROGRAFTING TECHNOLOGY”

The aim of researches are reveal the patterns morphogenesis of wild, semi-cultivated species and interspecies hybrids and, based on them, to develop methods of accelerated selection that will be comprehensively resistant to biotic and abiotic factors of the environment in tomato scion using the new technology of micrografting in vitro culture

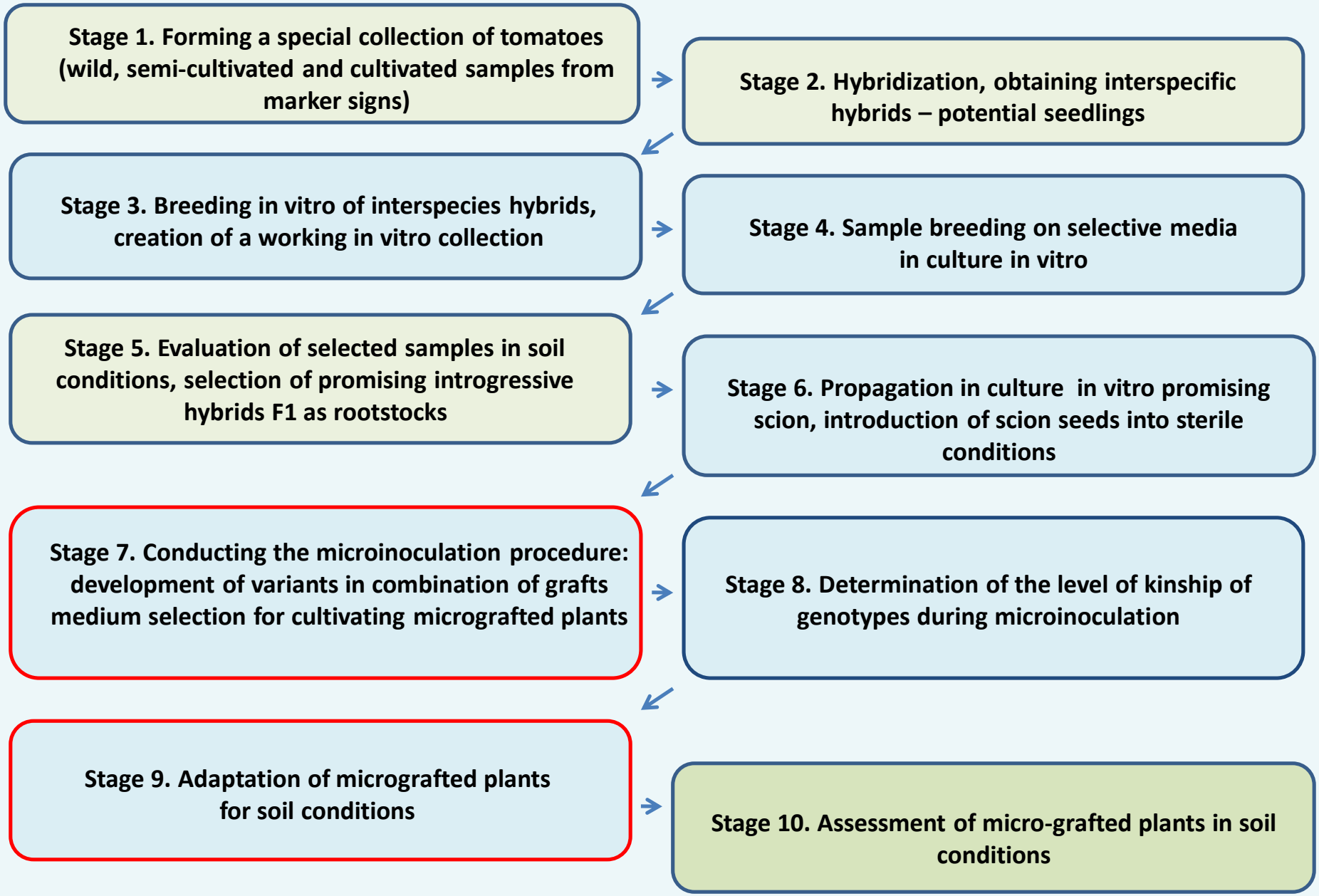


Fig. 1. The main stages of research for development in technology of micrografting tomato

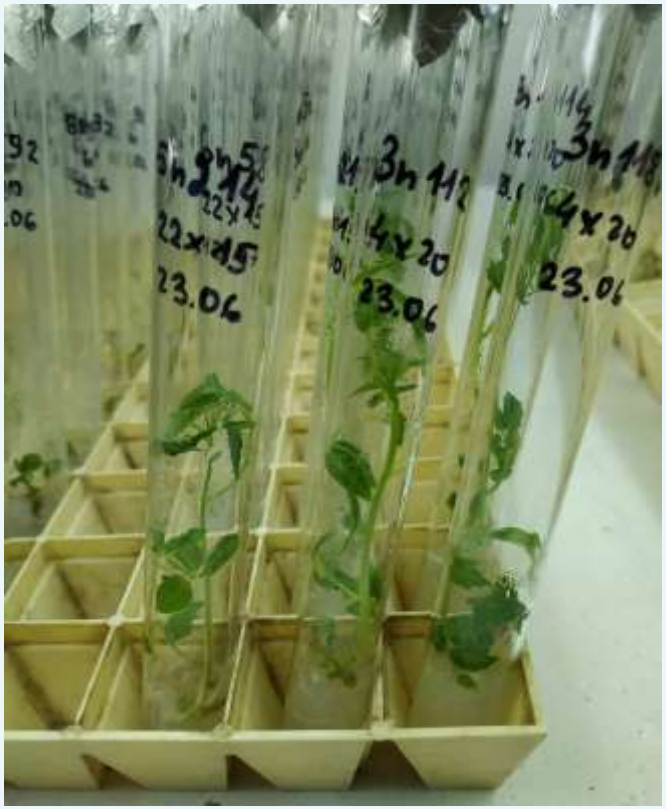
Development of techniques for overcoming postgamous incompatibility of the tomato Lycopersicon Tourn.

Fig. 5.1. Formation of normally developed (in the center) and abnormal regenerating plants of interspecies tomato hybrids in a hybrid combination L. esculentum Mill (Mo 500) / L. chilense

Fig. 5.2. In vitro tomato plants after 3 weeks of cultivation:
a – L. esculentum (Mo 638);
b – L. esculentum (Mo 638) / L. chilense;
c – L. esculentum (Mo 638) / S. pennellii Cor.

Fig. 5.4. Formation of embryos of interspecific hybrids:
a – globular stage of development;
b – heart-like stage of development;
c – an almost formed embryo

To determine the optimal conditions for short-term in vitro storage of a plants collection of Solanaceae family, 3 tomato samples were involved in the study: cultivated (line T-5) and wild (S. habrochaites) and an interspecific hybrid between them (BK-88). Microcuttings of these samples with a length of 10 mm were planted on nutrient media with the addition of sorbitol in concentrations of 20 g/l, 40 g/l, and 60 g/l. The control option is the hormone-free MS environment. Cultivation of regenerating plants on the indicated media was carried out for different periods: 1 month, 3 months, 6 months, 9 months, 12 months. Based on the results of the research of the working in vitro tomatoes collection, was established that in vitro wild species and hybrids based on them significantly exceeded the cultivated samples in terms of basic biometric indicators. In terms of the diameter of shootc, interspecific hybrids exceeded wild forms by an average of 1.5 times. They are more suitable for use as rootstocks for micrografting than wild forms.



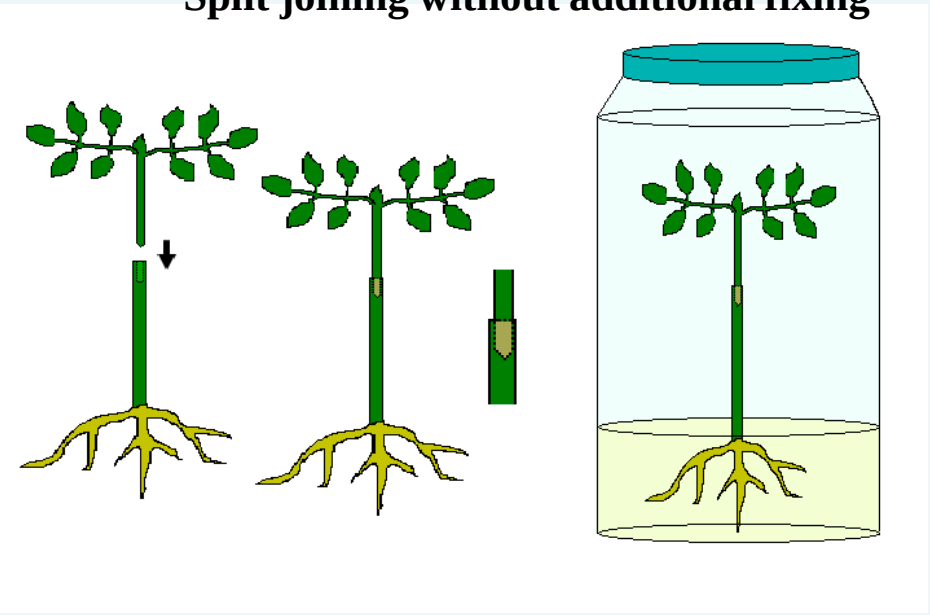
Fruits of a combination hybrid F_1 T-5 x *S. habrochaites* (д. 30)

Interspecies hybrid T-5 / S. pimpinellitomato folium

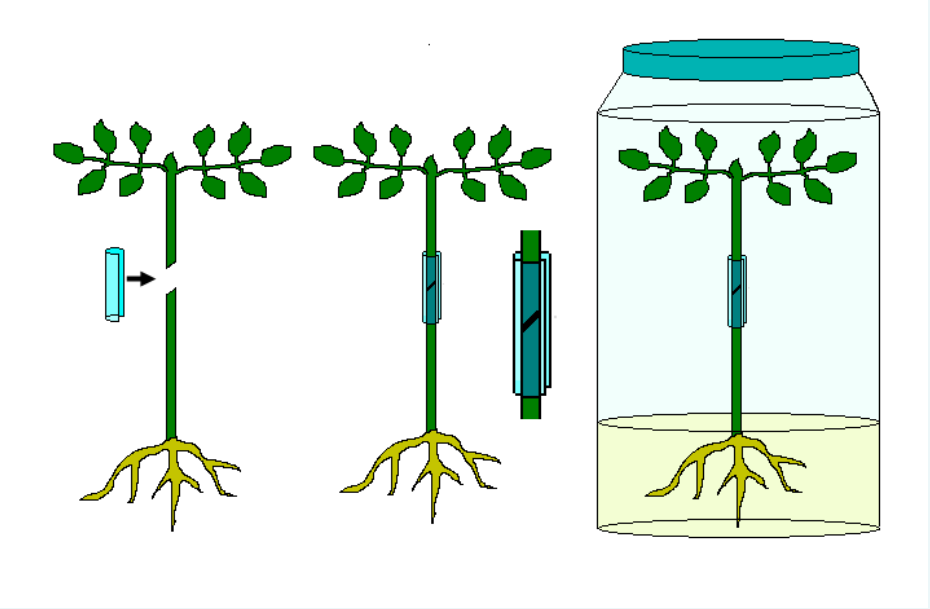
Experiment 1. To establish effective methods of joining grafts in in vitro culture

1) joint in a split without additional fixation;
2) fixing the scion in the joint using plastic tubes;
3) fixing the scion in the joint using aluminum foil;
4) joint in a split with additional fixation with aluminum foil;
5) fixation of grafts with laboratory Parafilm.

Split joining without additional fixing



Fixing the scion for joining use plastic tubes



Adaptation on MS medium with content a half of mineral salts with the addition of perlite without removing from the culture containers

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Н 34 Наукове обґрунтування ефективності методів біотехнології у селекції та насінництві овочевих культур: монографія; за ред. Т. В. Івченко. Київ: Аграрна наука, 2022. 200 с.

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У монографії обґрунтовано теоретичні аспекти і представлено результати практичного розв'язання проблеми інтенсифікації селекції та насінництва як традиційних, так і нішевих (яком, батат) овочевих культур за використання біотехнологічної ланки. Висвітлено питання використання методів ізольованих тканин для подолання таксономічних бар'єрів несумісності, створення джерел стійкості до некротрофних патогенів. Представлено сучасні експериментальні підходи до збереження генофонду овочевих культур *ex situ*, зокрема методи розмноження рослин у культурі *in vitro* й особливості використання мікросателітних ДНК-маркерів для генетичної ідентифікації та паспортизації генотипів.

Розраховано на наукових співробітників у галузі сільськогосподарської біотехнології, селекції та насінництва, здобувачів з підготовки третього рівня вищої освіти за спеціальністю 201 – агрономія, спеціалістів сільського господарства, які цікавляться сучасними біотехнологіями.

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Thanks for your attention !!!

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