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INVESTIGATIONS ON A METHODOLOGY OF TESTING RESISTANCE TO CHEMICAL STRESS OF HOP CULTURES *IN VITRO*

ŞTEFANIA GÂDEA, SUCIU T., MUNTEAN L., HENEGARIU O., LILIA MACOVEI

Abstract:

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The purpose of this research was to determine an adequate basic media in order to test the influence of some chemical stress factors (NaCl) on the resistance of hop neoplantules generated by minicuttings and cellular suspensions.

The basic media was Murashige-Skoog (1962) with addition of phytohormones (auxins and cytokinins) and the chemical factor of stress was NaCl in three concentration (0,3%, 0,5% and 1%). At 1% NaCl we noticed an inhibition in growth. We selected the most vigurous plants, V1 and V2, which were relayered and passed on the same basic media. The operation was repeated several times in order to obtain forms highly resistant to salinity.

Key words: basic media, auxins, cytokinins, minicuttings, neoplantules, salinity

Address: Universitatea de Ştiinţe Agricole şi Medicina Veterinară, Disciplina de Fiziologie vegetală, 3400, Cluj-Napoca, str. Mănăştur, 3, România

THE IMPORTANCE OF THE RESEARCH

This research is integrated into a wider theme regarding the resistance of some cultivated species to several chemical factors of stress (salinity, pH, herbicides a.s.o.) and producing of resistant forms.

The research aims at treating the following aspects:

- to determine an adequate basic media in order to test the influence of some chemical factors of stress (NaCl) on the resistance of hop neoplantules generated by minicuttings and celular suspensions;
- to identify proper sources to form appropriate calus;
- to obtain celular suspensions out of calus and to maintain the viability of the cells on environment of culture;
- to determine the time for treatment and the concentration of the chemical factors of stress.

THE MATERIAL AND THE METHOD

The vegetal material we used was minicuttings of hop ("Aroma") produced *in vitro* culture and calus cellular suspension of the same species.

The basic media was Murashige-Skoog (agar), or liquid, with organic compounds, sugar and hormones of growth (2,4-D, ANA and BAP), in different concentrations, depending on the material we used and the aim we had. This environment medium was sterilised for 20 minutes and a pressure of 1 atm (4).

The test tubes and the jars in which we inoculated the vegetal materials were passed on the space of growth in controlled conditions, for a photoperiod of 16 hours and the temperature was maintained at 22 ± 2^0C.

The chemical agent of stress we used was NaCl. Because we do not know exactly the doses and the effects of NaCl, we used three different concentrations (0,3%, 0,5%, 1%) and the duration of the treatment was of two hours. In order to show the effect of growth of the cells related to the effect of the chemical treatment, we also planned a witness test free of stress agent.

RESULTS AND DISCUSSIONS

The utilisation of chemical treatment for the cellular suspensions provided by the hop calus (1,6) generated a series of difficulties because it had a "mixed" aspect: it disociated with difficulty and showed remarkable differences in proliferation for it did not have the necessary viability to be used with high efficiency in succesive cultures.

The chemical treatments were easily put into practice with the minicuttings (2,3) and we tried to select proper cuttings in order to obtain resistant clones. We followed the speed of rooting (tab. 1.) and the growth of neoplants of hop (tab. 2.).

Tab. 1.

The effect of the NaCl concentration in basic media on the process of rooting of hop cuttings

Variants of concentration of NaCl (%)	The rate of rooting of hop cuttings		
	after 10 days	after 20 days	after 30 days
Mt	71.7	91.5	100.0
V_1 (0.3)	33.5	48.5	77.9
V_2 (0.5)	9.7	23.6	41.7
V_3 (1.0)	0	0	9.1

After we analysed the results we realized that the plants from the witness seed had a normal rooting. The plants which were treated behaved differently depending on the concentration of the chemical agent and the duration of treatment.

After 10 days we noticed remarkable differences between witness and variant. After 20 days the process of rooting was almost completed with the witness seed, but with V1 it was not even 50% and with V3 the process was totally stopped. After 30 days we noticed that from the total amount of treated plants 9,1% and 77,9% rooted. We

considered rooted also the plants presenting calus at the basis and very short roots, of 1-2 mm.

The highest viability appears with the cuttings in variant V1. The cuttings in variant V2 rooted in a proportion of 41,7% but both the growth of the roots and the aerial parts were inhibited. Even in the first 10 days dry and brown leaves began to appear and also a darkening of both the extremities of the cuttings. Out of the total amount of the cuttings we used in variant V3 only 9,1% rooted, forming few and short roots. The aerial part was highly affected, the leaves became dry in maximum 20 days; only the auxiliary gemma stayed green, grew extremly long and remained in the same stage during the experiment, even though they came out of rest.

After 30 days the most vigurous plants in V1 and V2 were relayered again on Murashige-Skoog normal environment and we made observations regarding the behaviour of the new neoplants. Unlike the witness, the process of calus and rhizom generation was on delay.

Tab.2.

The effect of the NaCl concentration of basic media on the growth of hop neoplants

Variant	Length (cm)	No. internodes	No. of branching	Plant mass (g/plant)
Mt.	5.8	6.0	2.0	0.460
V_1	4.1	4.0	1.0	0.316
V_2	2.2	3.0	0.0	0.180
V_3	0.5	2.0	0.0	0.104

In tab.2 we present the results regarding the effect of NaCl of basic media on the growth of hop neoplants. We notice a negative effect of NaCl on the growth of neoplants on all the variants. Thus, the length of plants is inferior, compared to the witness length, especially V2 and V3. It is also obvious the inhibitive effect of NaCl on the number of internodes, of branches and also mass plant. In order to produce plants resistant to salinity we chose the most vigurous plants from V1 and V2, we relayered them and passed on environment with more NaCl in order to select those with a special resistance.

CONCLUSIONS

Taking into account the preliminary results regarding the effect of NaCl from the basic media on the increasing the resistance to salinty of hop plants, we can draw the following conclusions:

- The best basic media for testing the effect of the chemical factors of stress on hop plants is Murashige-Skoog with addition of phytohormones.
- We used three variants of concentration of NaCl (0,3%, 0,5% and 1,0%) added to the basic media. In all cases we noticed an inhibition of the process of regeneration depending on concentration, but also a great variability in the growth process. The plants in variant V3 were not viable.
- The producing of cellular suspensions of hop is quite difficult because the calus is not friable enough.