B-15: Manipulation of culture conditions for optimization of micro-tuber production of potato (*Solanum tuberosum* L.)

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In vitro micro-tuber production is variety dependable and relies on complex physico-chemical and environmental factors. The chemical factors (i.e. the optimal chemical composition of the nutrient medium) play a significant role

in cost-effective micro-tuber production by reducing the unit cost per micro-tuber. A newly introduced or a commonly used individual chemical compound can act as a tuber assimilating promoter, but in combination with other compounds in the culture medium, it may inhibit the process. This study was, therefore, conducted to determine the optimum mictro-tuber production by manipulating the chemical composition of the culture medium (i.e. sucrose, 6-benzylaminopurine (BAP) and other commonly used growth promoting chemical factors of plant tissue culture media).

Stem segments with 2 internodes (cv. *Cardinal*) were used in designed experiments and incubated in glass tubes (25 x 150 mm) containing 15 ml of test medium. The number of replicates encountered in each treatment level was 10. Two sets of Complete Randomized Design Experiments (exp. 1 & 2) were employed to examine the combined effect of sucrose and BAP on micro-tuberization. In exp. 1 the treatments included 3 levels of sucrose (40.0, 60.0, and 80.0 g/l) and 4 levels of BAP (4.0, 6.0, 8.0, and 10.0 mg/l) arranged in a 3 x 4 incomplete factorial design. In exp. 2 the treatments included 4 levels of sucrose (60.0, 80.0, 100.0, and 120.0 g/l) and 4 levels of BAP (0, 2.0, 4.0, and 6.0 mg/l) arranged in a 4 x 4 complete factorial design.

The presumably selected physio-chemical and environmental condition of 80 g/l of sucrose, 4.0 mg/l of BAP, continuous darkness (exp. 3 - tested light regimes: continuous darkness, 3000 lux intensity of 12 h photoperiod) and 20°C of incubating temperature (exp. 4 - tested levels: 20 ± 2°C and 24 ± 2°C) were used in the Fractional Factor Experiment of second order (FFE $3^{11}//27$) to determine the effect of 11 growth promoting chemical factors (*Table 1*).

Table 1: Transition from natural factors to encoded variables in fractional factor experiment (FFE 3^{11}// 27)

Factors	*Factor*	*Unit designation* (0)	*Low* Level	*Medium Level*	*High Level*
MS* macro-nutrients	x_1	Strength	½	1	1½
MS micro-nutrients	x_2	Strength	½	1	1½
FeNa.EDTA	x_3	Strength	½	1	1½
Glycine	x_4	mg/l	1.0	3.0	5.0
Nicotinic acid	x_5	mg/l	1.0	3.0	5.0
HCl	x_6	mg/l	1.0	3.0	5.0
Thiamine-HCl	x_7	mg/l	1.0	3.0	5.0
Myo-inositol	x_8	mg/l	10.0	30.0	
Casein hydro-lysate	x_9	mg/l	30.0	50.0	70.0
Calcium panto-thenate	x_{10}	mg/l	1.0	3.0	5.0
Biotin	x_{11}	mg/l	1.0	3.0	5.0

* Murashige & Skoog (1962)

According to the given matrix of mathematical design (Maximov, 1980), 27 chemical combinations of treatments were prepared by incorporating the considered growth factors into the basal medium at 3 levels (low, medium, and high). The basal medium (80 g/l sucrose + 4.0 mg/l BAP) supplemented with 11 chemical factors at the medium level was treated as the control. Cross-sectional tuber area (nab-where n= 3.14, a= longer diameter/2, b= shorter diameter/2 of the micro-tuber, stolon length, number of micro-tubers per vessel, and fresh weight of the harvested micro-tubers were recorded after 2 months of incubation. ANOVA and DMRT (exp. 1 & 2) were computed by using SAS program (SAS Institute Inc., Cary NC). Means comparison of the temperature treatments and photoperiodic regimes (exp. 3 & 4) were tested by Student's t test ($p=0.05$). The significance of the regression coefficient in FFE was also tested at the probability level of 95%.

Use of homogeneous stem segments with 2 internodes taken from the 3 week old test tube plants allowed minimization of the number of replicates per experimental unit. Although 15 ml of culture medium cannot be recommended for cost effective micro-tuber production, the cultures were incubated in test tubes with one explant per tube in order to maximize the planning efficiency and precision. Most of the previously published papers, have been discussed by analysing the different morphological parameters of micro-tuberization such as (1) number of micro-tubers per explant/culture vessel/experimental unit; (2) micro-tuber fresh weight/dry weight; (3) micro-tuber diameter. However, the third parameter cannot be a desirable parameter to interpret the particular treatment effect, because, depending on the potato variety or especially the photoperiodic regime in which tuberization has taken place, the micro-tuber configuration may occur not only as marble shape, but also as elliptical or elongated tubers. Therefore,it would be more desirable to consider the cross-sectional tuber area (CSTA) by measuring 2 diameters of the harvested micro-tuber. In addition to that, in this study the stolon length was considered, for the first time, as an observational parameter to explain the initiating stage of micto-tuberization process. This would be a useful parameter to optimize the micro-tuber induction medium which can be re-irrigated by the micro-tuber assimilation medium at a later stage.

According to the analysed results of micro-tuber fresh weight and CSTA, within the tested range of sucrose x BAP combinations (exp.1), the best treatment combinaiton was 80 g/l sucrose x 4.0 mg/l BAP. The analysed results of exp. 2 showed that the increased levels of sucrose (more than 80/l in the incubation medium did not significantly promote either *in vitro* fresh weight or CSTA. Decreased levels or elimination of BAP had shown their significant different effects on the morphological parameters considered, and still 4.0 mg/l remained as the best level. Having taken the results of the 2 experiments into account, 80 g/l sucrose x 4.0 mg/l BAP was chosen as an optimum combination for cv. *Cardinal*.

Although the 12 h photoperiod light regime showed its significant promoting effect on CSTA (CI=5.15 $\pm$ 2.77), there was no significant effect on micro-tuber fresh weight (CI=0.023 $\pm$ 0.18), when compared with the continuous darkness of culture conditon. In the experiment (no. 4), where temperature treatments (20 and 24°C) were taken into consideration, there was no significant difference (CI=0.5 $\pm$ 0.9) in the number of micro-tubers/vessel at the probability level of 95%. Considering the overall aspects of the micro-

tuber production process, and especially the cost effective factor, the incubation of cultures under a continuous dark condition would be an advantage. However, disregarding the cost effective factor, 20°C treatment was selected to carry out the fractional factor experiment, in order to obtain the maximum number of tuberlets per explant.

The objective of the FFE was to attain an optimum micro-tuber production system using a relatively simple composition of nutrient medium, that does not contain excessive amounts of costly exogenous chemical compounds. The differences of the morphological parameters, which were considered after 2 months of incubation, were expected to respond only to the chemical treatment of the experiment. Referring to the given matrix of the experiment, 22 regression coefficients (11 linear regressions - B_1 to B_{11}, 11 quadratic regression - B^2_1 to B^2_{11}) were calculated for each parameter separately and the significance of the regression coefficient was tested ($p \geq 0.5$).

The regression equations for 4 morphological parameters were expressed as follow:

Micro-tuber fresh weight - A

$$Y_A = 0.1019 - 0.0094x^2_2 - 0.0161x_8$$

Micro-tuber cross sectional area - B

$$Y_A = 16.31 + 1.55x_3 + 1.33x_4 - 0.982x_7 - + 1.275x_{10}$$

Stolon length-C

$$Y_c = 19.35 - 3.01x_3 + 2.62x^2_3 + 5.05x_7 + 2.31x^2_8 + 2.22x_9 - 3.94x_{10}$$

Number of micro-tubers/explant - D

$$Y_D = 1.375 - 0.139x_3 + 0.111x_7 - O.125x_{10}$$

According to the 4 equations, it was clear that of the 11 factors considered, only 7 factors showed their positive or negative effect on *in vitro* tuberization. The other 4 factors did not show any effect within the tested range of concentrations.

It was interesting to notice that the inclusion of 3 factors into the culture medium, namely FeNa. EDTA, thiamine-HCl and calcium pantothenate, had shown their contrary effect on CSTA and number of micro-tubers per explant. Meanwhile,a low concentration of MS micro-nutrients and

myo-inositol in the culture medium would be beneficial for the micro-tuber fresh weight. But other exogenous chemical factors did not show either a positive or a negative effect within the tested range. In spite of that, there were 4 promoting and one retarding factors, which influenced the stolon length. This information would be helpful to determine the optimum chemical composition of the proposed induction medium and assimilation medium.

Considering the entire process of *in vitro* micro-tuber production and analysing the results of the experiments, 2 separate media can be recommended in designing successful micro-tuber production scheme for variety *Cardinal*: (1) Micro-tuber induction medium
(2) Micro-tuber assimilation medium