From Petri dishes to bioreactors – First experiences on optimization of Norway spruce SE-process for bioreactors

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The project Vegetative propagation of spruce – towards future plant production, carried out at Natural Resources Institute Finland (Luke), is focused on improving cost efficiency of Norway spruce (Picea abies (L.) Karst.) somatic embryogenesis (SE). Currently used SE-process at Luke is based on handwork which is time consuming and expensive. Using bioreactors for different steps of SE-process could reduce the amount of handwork and the cost of a single plant. So, what has to be taken into consideration when transferring SE-process based on petri dish culturing into bioreactors?

1. Selecting bioreactor type and model

There are many type of bioreactors differing in operating technologies and instrumentation; for example stirred tank, airlift and wave reactors. Temporary immersion system, TIS, is one of the most commonly used bioreactor type. There are several commercially available TIS models in different size (Fig 1), usually based on periodic wetting of the inoculum.

Fig.1 Example of commercially available TIS models. From left to right: Rita®, Plant Form and SETIS™.

2. Optimizing growth conditions and media for TIS bioreactor

When using TIS bioreactors growth conditions, nutrient availability, gas exchange and support frame for tissue has to be optimized. Immersion frequency and aeration time may vary between different steps on SE-process. Different support frames can be used depending on bioreactor model; foamed plastics, nets and meshes from different materials etc.

Liquid media gives tissue a better access to nutrients than solid media. That is why nutrient concentrations used in solid media could be too high in liquid media i.e. some optimization may be needed.

First experiences on Spruce SE-process optimization into bioreactor

Three TIS models, Rita®, Plant Form and SETIS™ (Fig1.), were tested. Rita® was too small for our propose and with SETIS™ we had handling problems which lead to contaminations. Plant Form was selected for further studies based on the size and sterility (Fig2.).

Optimization was done with six genotypes showing variation in their growth in bioreactors (Fig 3.). First we studied the optimal volume of nutrient solution. With more than 250 ml solution tissue was floating and moving around growing basket. With less than 180 ml solution tissues didn't get evenly nutrients

Fig.2 Plant Form bioreactors at use in Lukes SE-laboratory. From left to right: Proliferation experiment on Plant Form bioreactors. Norway Spruce maturated embryos inside bioreactor. Germination experiment on Plant Form bioreactors under LED-lights.

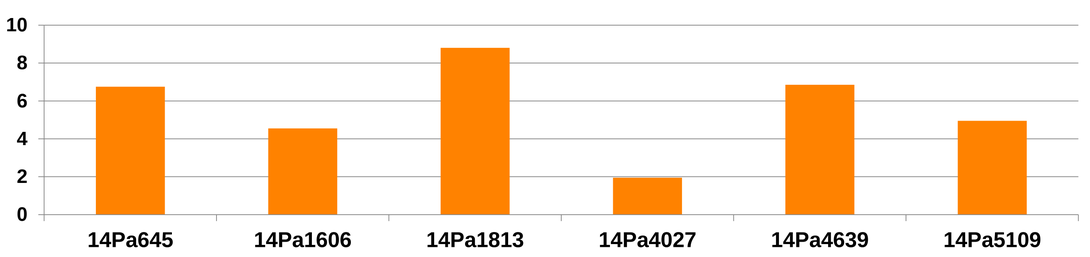

Fig.3. Variation in growth (increase of cell mass / unit time) of embryogenic spruce lines during proliferation step in bioreactors.

Best immersion time was two minutes but the frequency varies between SE-process steps. Aeration frequency needs to be connected to immersion times because aeration also helps the media flow back to bottom of bioreactor. Aeration is good to start few minutes after immersion ends and continue five to twenty minutes. Different pore sized filter paper and nets were tested as support frame. The most practical support frame for tissue was dense metallic net.

Finding the best liquid media for different steps in SE-process, especially for embryo maturation tuned out to be the most time consuming step in bioreactor process optimization and it is still an ongoing process

Leverage from the EU 2014–2020

European Union
European Regional Development Fund

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