Practical 2

Establishment of Axenic Plant Cultures as Explant Sources for *In Vitro* Experiments

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Objectives

1. To learn methods of plant material sterilization.
2. To gain experience in the establishment and maintenance of sterile cultures of seedlings and plants.
3. To establish sterile cultures of *Arabidopsis thaliana* and *Nicotiana sp.* as explant sources for future practicals.

Introduction

1. Sterile *in vitro* plant cultures are recommended as sources of explants to avoid repeated sterilization of plant material [2].
2. When seedling fragments are used as explants, seeds can be sterilized and germinated *in vitro*, providing axenic seedling cultures.
3. For explants taken from mature plant tissues, donor plants are typically not maintained in sterile culture, necessitating surface sterilization of the explant material [1].
4. An exception includes species from the *Solanaceae* family (e.g., tomato, tobacco), which are easily maintained in *in vitro* cultures through propagation by cuttings.
5. These cuttings are grown on Murashige and Skoog (MS) medium [3], commonly supplemented with 1% sucrose (MS10).
6. Plants from germinated *in vitro* seeds of the *Solanaceae* family can be propagated by cuttings, providing a continuous source of sterile explant material.

references

References

[1] A. C. Cassells. "Detection, identification, and control of bacterial contaminants of plant tissue cultures". In: *Methods in Molecular Biology*. Vol. 877. Springer, 2012, pp. 53–65.

[2] E. F. George, M. A. Hall, and G.-J. De Klerk. *Plant Propagation by Tissue Culture*. Vol. 1. Springer, 2008.

[3] T. Murashige and F. Skoog. "A revised medium for rapid growth and bio assays with tobacco tissue cultures". In: *Physiologia Plantarum* 15.3 (1962), pp. 473–497.