Kao and Michayluk (KM) Medium

Understanding Kao and Michayluk (KM) Medium: A Key Tool for Plant Tissue Culture

Tissue culture is a vital technique in modern plant biotechnology, enabling the clonal propagation of plants, breeding innovations, and the preservation of endangered species. Among the various tissue culture media developed, the **Kao and Michayluk (KM) Medium** is particularly well-regarded for its specialized use in somatic embryogenesis and the regeneration of protoplasts.

In this blog post, we'll explore what KM medium is, its applications in plant tissue culture, its unique role in aiding the regeneration of protoplasts, and the specific components that make up the formulation.

What is Kao and Michayluk (KM) Medium?

The **Kao and Michayluk medium**, first developed by K.K. Kao and M.R. Michayluk in 1975, was originally designed for the culture of plant protoplasts, which are cells that have had their cell walls removed. Protoplasts are fragile and require specialized conditions for survival and regeneration. KM medium provides a conducive environment that promotes both the growth and division of these cells, aiding in their eventual restoration to a full cellular state with a regenerated cell wall. This is crucial for genetic engineering applications, as protoplasts can take up genetic material and allow for the study and transfer of new traits into plants.

KM medium is also used in somatic embryogenesis, the process in which a plant or embryo is generated from somatic (non-

reproductive) cells. This process is widely used in plant breeding programs to develop new and improved varieties of plants with desirable traits such as disease resistance, stress tolerance, and enhanced nutritive value.

Key Applications of KM Medium

1. **Protoplast Culture and Regeneration**

   Protoplast regeneration is one of the standout applications of KM medium. When plant protoplasts are isolated and placed in the right culture conditions, they can reform their cell walls and differentiate into full plants. KM medium is specifically designed to provide the right balance of nutrients and phytohormones for this purpose.

2. **Somatic Embryogenesis**

   In addition to its utility in protoplast culture, KM medium successfully induces the differentiation of plant cells into embryos. This somatic embryogenesis is an essential process in the development of whole plants from tissue cultures. KM medium aids in the propagation of various plant species by helping to manipulate the developmental pathways and coax cells into forming embryos, which can then develop into complete plants.

3. **Micropropagation of Difficult-to-Culture Species**

   While KM medium is not as commonly used for routine micropropagation as other media like Murashige and Skoog (MS) medium, it excels with difficult-to-culture species, particularly those that require specific

nutrient balances for effective growth and regeneration. It’s ideal for advanced and delicate tissue culture tasks.

4. **Genetic Engineering Applications**
   Protoplast culture is a cornerstone technique in plant genetic engineering, and KM medium has been key to its success. Researchers use KM medium in the process of **protoplast fusion**, a step in the production of hybrid plants or genetically modified organisms. The medium allows scientists to perform cellular-level manipulations in a controlled environment that optimizes healing and reformation of the cell wall.

KM Medium Formulation (Per Liter)

Creating a properly balanced KM medium is essential for the success of plant cultures. Here’s a breakdown of the standard KM medium formulation on a per-liter basis:

Macronutrients (mg/l)

- **KNO_3:** 950
- **NH_4NO_3:** 165
- **KH_2PO_4:** 170
- **$MgSO_4.7H_2O$:** 185
- **$CaCl_2.2H_2O$:** 440

Micronutrients (mg/l)

- **KI:** 0.83
- **H_3BO_3:** 6.2
- **$MnSO_4.H_2O$:** 16.9
- **$ZnSO_4.7H_2O$:** 8.6
- **$Na_2MoO_4.2H_2O$:** 0.25
- **$CuSO_4.5H_2O$:** 0.025
- **$CoCl_2.6H_2O$:** 0.025

Iron Source (mg/l)

- **$FeSO_4.7H_2O$:** 27.8
- **Na_2-EDTA:** 37.3

Vitamins (mg/l)

- **Thiamine HCl:** 1
- **Pyridoxine HCl:** 1
- **Nicotinic acid:** 1
- **Glycine:** 2

Additional Organic Components (mg/l)

- **Myo-inositol:** 100
- **Glutamine:** 400

Carbohydrates (g/l)

- **Sucrose:** 20-30 (can vary depending on the species and application)

Phytohormones

While the base formulation of KM medium doesn’t inherently include phytohormones, different types of plant growth regulators (such as auxins and cytokinins) can be added as needed based on the culture’s specific requirements. For **protoplast culture**, 2,4-D (an auxin) or NAA (naphthaleneacetic acid) are frequently used, combined with BAP (benzylaminopurine) or kinetin (cytokinins) to induce shoot and root formation.

Gelling Agent

- **Agar:** 6-8 g/l (for solid medium)

Alternatively, a liquid KM medium can be used for suspension cultures.

pH

The medium pH is adjusted to **5.8** before sterilization, usually through autoclaving at 121°C for 20 minutes.

Conclusion

Kao and Michayluk (KM) medium plays an invaluable role in the fields of plant tissue culture and genetic engineering. With its carefully balanced formulation of macronutrients, micronutrients, and organic compounds, KM medium is ideal for promoting a range of plant development processes—especially the regeneration of protoplasts and somatic embryogenesis. While tissue culture media like Murashige and Skoog (MS) media are more widely used for general tissue culture purposes, KM media shines in specialized applications that involve delicate plant cells or advanced genetic modification techniques.

For researchers tackling complex plant science challenges, understanding and utilizing KM medium is essential to achieving successful outcomes in plant regeneration and propagation. Whether you're working on protoplast isolation, somatic embryogenesis, or something in between, KM medium offers the precise nutrient balance needed to turn isolated plant cells into fully formed plants.

So, the next time you need a reliable medium for these specialized tasks, turn to Kao and Michayluk (KM) medium—your plants (and lab) will thank you!

References

Kao, K. N., & Michayluk, M. R. (1975). "Nutritional requirements for growth of Vicia hajastana cells and protoplasts at a low population density in liquid media." *Planta*, 126(2), 105-110.