PGR-Free (Plant Growth Regulator-Free) Medium

PGR-Free Medium in Plant Tissue Culture: A Path to Natural Growth

In the world of plant tissue culture, the choice of medium plays a crucial role in ensuring the success of in vitro plant propagation. One medium that has been gaining traction due to its minimalistic nature is **PGR-Free (Plant Growth Regulator-Free) Medium**. As its name suggests, this medium contains no plant growth regulators (PGRs), making it ideal for specific applications in plant tissue culture. In this blog post, we'll dive into what PGR-Free Medium is, what it's used for, and how you can prepare it on a per liter basis.

What is PGR-Free Medium?

PGR-Free Medium is a type of culture medium designed to facilitate plant growth and development in vitro without the addition of external plant growth regulators like auxins, cytokinins, or other hormones. PGRs are compounds that regulate various aspects of plant growth, such as cell division, elongation, and differentiation, but sometimes these chemicals are unnecessary or even undesirable, depending on the application.

PGR-Free Medium relies on the plant's inherent endogenous hormones to drive growth and morphogenesis. This medium is often used to promote *natural, unaltered growth* or to study the intrinsic growth patterns of plants in the absence of

external stimulation. It’s commonly applied in scenarios where:

- **Plant acclimatization is critical**, such as in seed germination studies or transplanting of plantlets.
- *Minimal interference with plant development is desired*, such as in genetic or molecular studies where external PGRs might skew results.
- *Hardwood species*, or those that are naturally capable of producing their own sufficient levels of hormones, can grow without the need for exogenous hormonal supplementation.

Applications of PGR-Free Medium

PGR-Free Medium finds practical use in several different areas of plant tissue culture, including:

1. **Seed Germination:**
   Seeds often contain enough intrinsic hormones to initiate germination. A PGR-Free Medium allows for natural development without hormonal interference, making it easier to observe the inherent behavior of the plant during its early growth stages.

2. **Plantlet Regeneration:**
   Once a plantlet reaches a certain developmental stage, PGRs are often no longer necessary for continued growth. Plantlets will thrive by relying on their endogenous hormone production. The use of PGR-Free Medium is a gentle way to transition these plants towards eventual

acclimatization outside of the tissue culture environment.

3. **Root Induction:**
   At an advanced stage, sometimes exogenous rooting hormones, such as auxins, are considered unnecessary. Some plants can initiate root development on their own. A PGR-Free Medium provides the right nutrient environment without hormone supplementation.

4. **Micropropagation of Hardwood or Woody Plants:**
   Certain hardwood (woody) species, such as oak, walnut, and chestnut, are often cultured in PGR-Free Medium. These species inherently possess strong self-regenerative capabilities, minimizing the need for added PGRs.

5. **Molecular and Genetic Studies:**
   Research applications that involve the genetic manipulation of plants (such as transgenic studies) sometimes require the complete exclusion of exogenous growth regulators to avoid any interference with gene expression studies or phenotypic observations.

6. **Avoiding Hormone-Induced Stress:**
   Some plants are sensitive to overexposure to PGRs, particularly when prolonged exposure could lead to abnormalities like vitrification (hyperhydricity) or callus formation. A PGR-Free Medium mitigates hormone-related stressors and abnormalities.

Formulation of PGR-Free Medium (Per Liter Basis)

Now that you're aware of the significance of PGR-Free Medium, let's delve into a simple and standard formulation of such a medium. One of the most commonly used basal mediums in plant tissue culture is **Murashige and Skoog (MS) Medium**, which can easily be adapted to a PGR-Free format.

Here's a basic formulation to make 1 liter of PGR-Free Medium:

Ingredients:

- **Macronutrients:**
    - NH_4NO_3 (Ammonium Nitrate): 1650 mg
    - KNO_3 (Potassium Nitrate): 1900 mg
    - $MgSO_4.7H_2O$ (Magnesium Sulfate): 370 mg
    - KH_2PO_4 (Potassium Phosphate Monobasic): 170 mg
    - $CaCl_2.2H_2O$ (Calcium Chloride): 440 mg
- **Micronutrients:**
    - H_3BO_3 (Boric Acid): 6.2 mg
    - $MnSO_4.H_2O$ (Manganese Sulfate): 16.9 mg
    - $ZnSO_4.7H_2O$ (Zinc Sulfate): 8.6 mg
    - KI (Potassium Iodide): 0.83 mg

- Na2MoO4.2H2O (Sodium Molybdate): 0.25 mg
- CuSO4.5H2O (Copper Sulfate): 0.025 mg
- CoCl2.6H2O (Cobalt Chloride): 0.025 mg

- **Iron Source:**
  - FeSO4.7H2O (Ferrous Sulfate): 27.8 mg
  - Na2-EDTA (Disodium Ethylenediaminetetraacetate): 37.3 mg

- **Vitamins:**
  - Thiamine-HCl (Vitamin B1): 0.1 mg
  - Pyridoxine-HCl (Vitamin B6): 0.5 mg
  - Nicotinic Acid (Niacin): 0.5 mg
  - Myo-Inositol: 100 mg

- **Carbon Source:**
  - Sucrose: 30,000 mg (3%)

- **Gelling Agent:**
  - Agar (if solid medium is desired): 6 to 8 g/L

- **pH Adjustment:**

- Adjust the pH to approximately 5.7 using KOH or HCl.

Preparing the Medium:

1. Dissolve all the macronutrients, micronutrients, and vitamins in 900 ml of distilled water.
2. Add sucrose and stir until completely dissolved.
3. Adjust the pH to 5.7 using KOH or HCl while stirring.
4. Add the required amount of agar if you are preparing a solid medium.
5. Bring the final volume up to 1 liter with sterile distilled water.
6. Dispense the medium into culture vessels, usually around 20-30 ml per vessel.
7. Sterilize the medium by autoclaving at 121°C for 15-20 minutes.

Note: Always ensure that your laboratory practices and instruments are sterile to avoid contamination.

Conclusion

PGR-Free Medium serves as an indispensable tool in plant tissue culture, enabling more natural plant growth without the influence of externally applied hormones. While it may not be suitable for all scenarios (such as when a rapid multiplication of shoots is required), it shines in

applications that prioritize the plant’s inherent growth patterns, such as seed germination, recovery from transformation, or propagation of species that can manage growth with their own hormonal balance. By eliminating plant growth regulators from the equation, you introduce fewer variables into the system, which can simplify protocols and minimize physiological stress on the plant.

If you’re delving into plant culture or research and looking for a medium that encourages natural growth free from external PGR influence, then a PGR-Free Medium could be the ideal solution.