Low Salt MS Medium (LS)

Understanding Low Salt MS Medium (LS) and Its Applications in Plant Tissue Culture

Plant tissue culture is an indispensable technique in modern plant biology, biotechnology, and horticulture. It involves growing plant cells, tissues, or organs on a nutritionally rich synthetic medium under sterile, controlled conditions. The composition of the growth medium plays a pivotal role in determining the success of tissue culture experiments. Among several types of media available, the Murashige and Skoog (MS) medium is perhaps the most commonly used due to its versatility across plant species and tissue types.

However, the regular MS medium provides a relatively high salt concentration, which can sometimes be unsuitable for certain plant species or experimental requirements. For these cases, a modified version known as **Low Salt MS Medium (LS Medium)** is used. In this blog post, we'll explore what Low Salt MS Medium is, what it is used for, and provide the essential recipe needed for its preparation.

What is Low Salt (LS) MS Medium?

Low Salt MS Medium (commonly abbreviated as LS) is a modified form of the standard Murashige and Skoog medium, where the

concentration of salts, particularly the macronutrients, is reduced. While MS medium is rich in salts and nutrients to support robust growth, high salt concentrations can sometimes lead to osmotic stress in sensitive plants or tissues, leading to growth inhibition, poor differentiation, or even necrosis.

LS medium is developed to meet the needs of more salt-sensitive strains, genotypes, or species. Plants that are adapted to less nutrient-intense environments, or certain recalcitrant species that are difficult to grow under regular MS conditions, may benefit from LS medium. By lowering the salt concentration, one can mitigate excessive ionic strength and osmotic pressure, making it better suited for these cultures.

Applications of LS Medium:

- **Micropropagation of Salt-Sensitive Plants**: Some species or varieties are highly sensitive to high salt loads and can exhibit stunted growth or callus formation on regular MS. LS medium offers a more balanced nutrient concentration for these plants.
- **Embryo Culture**: Embryos from seeds may be sensitive to high osmotic potential in regular MS medium, as it could lead to dehydration. Low salt formulations like LS provide a more conducive environment for embryo rescue and development.
- **Rooting Medium**: While standard MS medium is often used for the multiplication phase, LS medium is sometimes implemented in the rooting stages of plant tissue culture. Lower salt concentrations can induce better root initiation, particularly in some hard-to-root species.

- **Research**: For specific research in plant physiology or development, particularly studies that involve stress acclimatization (such as studies on osmotic or ionic balance), the reduced salt content in LS medium offers an ideal experimental alternative.

LS Medium Formulation (Per Liter)

The basic recipe for LS medium builds upon the same nutrients as the MS medium but with modified salt contents to reduce the overall salt concentration.

Major Components of LS Medium:

1. Macronutrients (Reduced Concentration)

- Ammonium Nitrate (NH_4NO_3) – 825 mg/L
- Potassium Nitrate (KNO_3) – 950 mg/L
- Calcium Chloride ($CaCl_2 \cdot 2H_2O$) – 220 mg/L
- Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$) – 185 mg/L
- Potassium Phosphate (KH_2PO_4) – 85 mg/L

2. Micronutrients

- Boric Acid (H_3BO_3) – 6.2 mg/L
- Manganese Sulfate ($MnSO_4 \cdot H_2O$) – 16.9 mg/L
- Zinc Sulfate ($ZnSO_4 \cdot 7H_2O$) – 8.6 mg/L

- Potassium Iodide (KI) – 0.83 mg/L
- Sodium Molybdate ($Na_2MoO_4 \cdot 2H_2O$) – 0.25 mg/L
- Cobalt Chloride ($CoCl_2 \cdot 6H_2O$) – 0.025 mg/L
- Copper Sulfate ($CuSO_4 \cdot 5H_2O$) – 0.025 mg/L

3. Iron Chelates

- Ferric Sodium EDTA – 36.7 mg/L

4. Vitamins

- Thiamine-HCl (Vitamin B1) – 0.1 mg/L
- Pyridoxine-HCl (Vitamin B6) – 0.5 mg/L
- Nicotinic Acid – 0.5 mg/L

5. Carbon Source

- Sucrose – 30,000 mg/L (3%)

6. Gelling Agent (Optional for Solid Media)

- Agar – 7,000 mg/L

Additionally, the pH of LS medium is typically adjusted to 5.7–5.8 before autoclaving.

Variability in LS Medium

Depending on the specific needs of the tissue culture system or the plant species, the concentration of the macronutrient salts could vary slightly from the standard LS formulation. Sugar concentration may be adjusted, and different plant growth regulators (such as auxins or cytokinins) can be added separately, based on the experimental objectives.

How to Prepare LS Medium?

1. **Weigh the Salts**: Carefully weigh out each salt (macronutrients, micronutrients, and iron). It is usually best practice to prepare stock solutions of the micronutrient and stock vitamins for easier, more precise measurements.
2. **Mix**: Dissolve the salts in distilled or deionized water under stirring.
3. **Add Sucrose**: Add the sucrose to the medium and ensure it is completely dissolved.
4. **pH Adjustment**: Use a pH meter to check the medium's pH. If the pH is too low (acidic), use a small amount of 1 N KOH to raise the pH. If it is too high, add a small amount of 1 N HCl to lower the pH.
5. **Agar Addition (Optional)**: If you are preparing solid medium, add agar, and warm the solution gently to dissolve (if the solution is for liquid medium, omit this step).
6. **Autoclave**: Sterilize the medium using an autoclave at 121°C for 15-20 minutes.

Conclusion

Low Salt MS Medium (LS) is an essential tool in plant tissue culture when dealing with species or tissues that require a reduced ionic strength in their growing environment. By decreasing the salt content of the medium, LS enables more delicate tissues or species to grow and differentiate without being overwhelmed by osmotic stress. Whether you’re working on micropropagation, embryo culture, or specific research on plant stress physiology, LS can offer a balanced alternative to the robust, high-nutrient MS medium.

Being mindful of the critical role that medium composition plays in tissue culture success, the use of LS medium allows for adaptability and fine-tuning to achieve optimal plant growth and development in sensitive species.