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STUDIES OF ABIOTIC STRESS OF CATHARANTHUS ROSEUS

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ABSTRACT

Catharanthus roseus, popularly known as the Madagascar periwinkle, is a cornerstone of modern pharmacology due to its biosynthesis of the potent anti-cancer alkaloids vincristine and vinblastine. Despite their high value, these compounds are produced in extremely small quantities naturally, making extraction costly and inefficient. Furthermore, the plant's productivity is heavily dictated by abiotic stressors, such as salinity and thermal extremes, which fundamentally alter its metabolic output. This study investigates the application of in vitro micropropagation as a viable method for the effective cultivation and study of stress resilience in C. roseus.

We evaluated the effects of incremental salinity (50 mM to 200 mM NaCl) and storage temperatures (20°C and 4°C) on explant viability and morphological growth over a 21-day period. Results indicated that optimal conditions at 20°C yielded a 100% survival rate with vigorous shoot development. Conversely, while mild salinity (50 mM) acted as a metabolic stimulant enhancing defense responses, higher concentrations (150-200 mM) led to acute ionic toxicity and total growth inhibition. These findings highlight the critical role of maintaining optimal environmental parameters for the genetic preservation and pharmaceutical scale-up of this vital medicinal species.

KEYWORDS: Catharanthus roseus, abiotic stress, micropropagation, salinity, temperature stress, tissue culture, secondary metabolites.

INTRODUCTION

Plant biotechnology is an advanced field of science that involves the use of modern biological techniques to improve plants for human benefit. It combines principles of plant science,

genetics, and molecular biology to develop plants with better yield, improved quality, resistance to stress, and enhanced production of useful compounds. Techniques such as tissue culture, genetic engineering, and metabolic pathway manipulation are commonly used in plant biotechnology. (George et al., 2008).

One of the best examples to understand the importance of plant biotechnology is Catharanthus roseus, commonly known as Madagascar periwinkle. It is a well-known medicinal plant belonging to the family Apocynaceae. This plant has gained global importance because it produces valuable secondary metabolites, especially vincristine and vinblastine, which are widely used as anti-cancer drugs in chemotherapy. (El-Sayed and Verpoorte, 2007)

However, a major challenge with Catharanthus roseus is that these important compounds are produced in very small quantities naturally. This makes their extraction costly and inefficient. Additionally, the plant is sensitive to environmental conditions such as temperature, water availability, and soil salinity, which can affect both its growth and metabolite production.

Plant biotechnology offers effective solutions to these challenges. One of the most important techniques used is micropropagation, also known as plant tissue culture. In this method, small parts of the plant such as shoot tips or nodal segments are grown in a sterile nutrient medium under controlled laboratory conditions. This technique allows rapid multiplication of plants, ensuring the production of large numbers of disease-free and genetically uniform plants throughout the year. It also reduces dependence on seasonal growth and environmental factors. (Ali et al., 2006)

Plant biotechnology also focuses on genetic improvement. By studying the genes responsible for alkaloid production, scientists can develop improved plant varieties that produce higher amounts of desired compounds. Techniques like genetic engineering and molecular breeding can be used to enhance these traits. also plays a vital role in conservation of plant genetic resources. Through in vitro conservation and cryopreservation techniques, valuable plant species like Catharanthus roseus can be preserved for long periods without losing their genetic characteristics. This is especially important for medicinal plants that are at risk due to overexploitation or environmental stress. plant biotechnology helps in studying plant responses to abiotic stresses such as temperature and salinity. Understanding these responses allows researchers to develop stress-tolerant plant varieties and optimize cultivation practices. (El-Sayed and Verpoorte, 2007)

Catharanthus roseus, commonly known as Madagascar periwinkle, is a highly important medicinal plant belonging to the family Apocynaceae, widely distributed in tropical and

subtropical regions, including India. It is a small evergreen herb or subshrub characterized by glossy green leaves, a well-developed root system, milky latex, and attractive pink or white flowers. This plant has gained global significance due to its ability to produce a wide range of secondary metabolites, particularly monoterpenoid indole alkaloids, among which vincristine and vinblastine are of great importance as they are widely used as anti-cancer drugs in chemotherapy, while other compounds such as ajmalicine are used for treating hypertension. Because of its high medicinal value, there is an increasing demand for large-scale production and conservation of this plant. (Ali et al., 2006)

However, the natural production of these alkaloids is very low, making their extraction costly and inefficient. the growth and development of Catharanthus roseus are greatly influenced by environmental conditions such as drought, salinity, temperature extremes, and light intensity, which can negatively affect plant growth, reduce biomass, and disturb physiological processes like photosynthesis. Interestingly, mild levels of these abiotic stresses can act as stimulators and enhance the production of valuable secondary metabolites as part of the plant's defense mechanism, showing a balance between growth and metabolite production. (Zhao et al., 2005) To overcome these limitations and meet the increasing demand, plant biotechnology plays a crucial role by providing advanced techniques such as micropropagation for rapid multiplication of disease-free and genetically uniform plants, in vitro culture for controlled growth, metabolic engineering for improving alkaloid production, and cryopreservation for long-term conservation of germplasm. These approaches not only help in improving plant productivity but also ensure sustainable utilization and conservation of this valuable medicinal plant. Therefore, Catharanthus roseus serves as an excellent model for studying plant biotechnology, stress physiology, and secondary metabolite production, highlighting its immense importance in pharmaceutical, agricultural, and research fields as well. (George et al., 2008)

MATERIALS AND METHOD

This research investigated the effects of thermal and saline stress on the preservation and germination of C. roseus.

Plant Material and Sterilization Fresh nodes of Catharanthus roseus were obtained and initially sterilized to eliminate surface contaminants. Explants underwent thorough cleaning with running tap water and mild detergents (Tween 30), followed by surface sterilization using chemical agents like 70% ethanol and sodium hypochlorite within a laminar airflow chamber.

Media Preparation and Culture Explants were inoculated onto Murashige and Skoog (MS) medium. A 250 mL batch was prepared using 1.02 g MS basal salts, 7.5 g sucrose as a carbon source, 3 g agar as a solidifying agent, and 0.11 g $CaCl_2H_2O$ for cell wall stability. The pH was adjusted to 5.6 to 5.8 prior to sterilization.

MATERIALS:

Plant Source: *C*atharanthus roseus (Madagascar periwinkle) is primarily sourced from its native home of Madagascar, specifically along the dry southern and southwestern coasts. For scientific research, especially studies on abiotic stress, plants are obtained from a mix of wild-collected specimens, commercial plantations, and controlled laboratory cultures.

Chemicals: Abiotic stress in *Catharanthus roseus* triggers a significant shift in its chemical profile, primarily enhancing the production of Terpenoid Indole Alkaloids (TIAs) and protective osmoprotectants. This metabolic redirection is a defense mechanism that allows the plant to survive harsh conditions while increasing its pharmaceutical value.

Main Chemicals used in the Project: Sodium Chloride (NaCl): The primary chemical used to induce salinity stress.

Polyethylene Glycol (PEG 6000): A common osmoticum used to simulate drought/water stress by lowering the water potential of the growth medium.

Mannitol: Used as an alternative chemical for inducing osmotic stress.

Heavy Metals: Salts of metals like cadmium (Cd), zinc (Zn), and copper (Cu) are applied to study metal toxicity and its effect on alkaloid accumulation.

METHOD:

1. WATER STRESS MATERIALAND METHODOLOGY

1. Newly-formed nodal explants were collected from a plant near our college.

2. The explants were cut into 1 cm lengths and washed for 30 minutes in running tap water. They were then immersed for 1 minute in 70% ethanol, followed by 15 minutes in a sodium hypochlorite solution containing 2% active chlorine and two drops of the commercial detergent Pril.

3. The explants were then carefully washed three times with sterile distilled water. The MS basal medium (Murashige and Skoog, 1962) was made from sucrose (30 g/l) and agar (7 g/l) stock solutions.

4. The medium was adjusted to a pH of 5.7 0.05 before being placed into glass jars and autoclaved for 20 minutes at 121°C. The cultures were kept at 25°C with a photoperiod of 6 hours of light/8 hours of darkness and a light intensity of 100 mol m-2s -1.

5. Shot multiplication rate, shoot number per explant, and shoot length were measured in four-week-old cultures. To assess the consistency of the outcomes, the newly produced explants were subcultured every three weeks for five rounds.

6. The optimized medium and in vitro-derived explants. Water stress is induced in vitro. To generate various levels of water stress, the in vitro-derived nodal segments (0.5 cm) were moved to the optimum multiplication medium containing 1.5 mgl-1 BAP and 0.2 mgl-1 NAA, and supplemented with varying quantities of PEG 6000.

7. In the water stress experiment, three PEG concentrations were used: 0 (control), 3 percent (3 percent PEG), and 6 percent (6 percent PEG). Four explants were placed in each 250 mL culture jar.

8. The cultures were kept in the above-mentioned conditions of growth for 30 days. At the conclusion of the experiment, all regeneration, growth, and physiological features were measured.

2. MATERIALS AND METHODS FOR TEMPERATURE STRESS

1. Plant sample used for this experiment was Giloy, MS media, plant nutrients, and controlled environment.

2. Three phases of treatment were applied to the plant sample, usually at 170 w-m' (400-700 nm) with 12-hour photoperiods. They were preconditioned for 11 to 14 days at a consistent day-night temperature of 25 C.

3. The optimum temperature condition was set at 25 degree Celsius.

4. For several days, the temperature decreased to 10 degrees Celsius throughout the day and night, before returning to 25 degrees Celsius. And then the temperature increased to 50 degree Celsius.

5. Electron Microscopy samples were taken from the middle region of the nodes.

3. MATERIALAND METHODOLOGY FOR SALINITY STRESS

1. The following procedure was followed to introduce a sample of giloy in order to check the salinity stress condition.

2. The leaves were extracted from single nodal cuts after they were dissected. These nodes were surface sterilized for roughly 15 minutes in 0.524 percent NaOC1, then rinsed three times in sterile distilled water and blotted dry on sterile filter paper.

3. Murashige and Skoog (MS) basic inorganic salts (Murashige and Skoog, 1962) were used in the propagation medium, together with the following organic additives (mg l-): sucrose 50 000,myo-inositol 100, thiamine-HC1 2, anaphthalene acetic acid (NAA) 0.03, 6-benzylaminopurine (BAP) 0.30, and 8 g 1-1 D.

4. The media were adjusted to a pH of 5.6 before autoclaving.

5. These culture tubes were maintained under long days (16/8 h light/ dark schedule with 3000 lx fluorescent lights) and at 26 +_ 1 ° C.

6. In around 6 weeks, each plantlet had grown to the full length of the culture tube. A liquid culture approach was utilized after the cuttings were placed in the in-vitro media to speed up the proliferation rate. Plantlets were propagated in vitro in 250-cm 3 Erlenmeyer flasks with 25 ml liquid culture media (composition as described above without the agar). Gibberellic acid, 0.2 mg 1-1, was added to the liquid culture medium. Whole stem segments, ranging in length from five to eight nodes, were prepared. After that, the stem segments were inoculated into the flask and immersed in the liquid media. An orbital horizontal shaker improved nutrient distribution inside the flask for the plants.

7. The shoot flasks were then used to create in-vitro stem segments that were exposed to saline solutions. In-vitro saline media exposure In the three in-vitro tests, different concentrations of NaCL were utilized. The liquid culture solution with MS salts, organic salts, and laboratory grade NaCL salt at the necessary concentration made up each culture medium.

8. Before autoclaving, all solutions were adjusted to a pH of 5.6. In the Erlenmeyer flasks (two segments per flask), stem segments of identical size (five nodes) were put without the apical tip and roots and immersed in the saline solution. As previously mentioned, these plantlets were also kept at 26 °C for lengthy days (16 hours). Experiment 1 measured plantlet growth and survival 5 weeks after starting the saline treatment, while Experiments 2 and 3 measured it after 3 weeks.

Step 1 - Five very mild NaCL stress concentrations (0, 0.5, 1, 2, and 3 mM) were used on three media flasks.

Step 2 - To produce conditions of mild salt stress, eight concentrations of NaC1 were used in the

culture medium: 0, 0.5, 1, 2, 3, 4, 8, and 16 mM. Three samples were grown in these liquid saline culture media for about 4 weeks.

Step 3 - Five NaCL concentrations producing salt stress levels ranging from low to severe were used: 0, 8, 16, 51, and 102 mM.

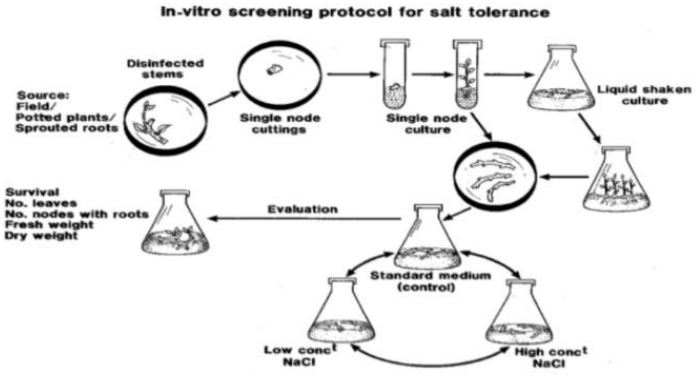


Fig 3. Process for Salinity stress Determination.

4. MATERIALAND METHODOLOGY FOR HEAVY METAL STRESS

1. PbCl2, CuCl2, CdCl2.H2O, and HgCl2 were among the heavy metal salts employed in this investigation. Lead concentrations in the stock solutions were 1.5, 2.0, and 2.5 mM; copper concentrations were 0.1, 0.2, and 0.3 mM; cadmium concentrations were 0.05, 0.06, and 0.08 mM; and mercury concentrations were 0.02, 0.04, and 0.06 mM.

2.Surface-sterilized for 2 minutes in 10-3 M HgCl2 rinsed in distilled water, and germinated between moist paper towels at 25°C in the dark for 3 days. The plants were then infected for 7-10 days on MS Basal media.

3. The samples were removed from the media after a 7-day growth period and placed in 500-ml jars (two seedlings per jar) containing 200 ml of the given heavy metal solution (Hoagland solution for control). Aluminum foil was used to cover the jars.

4. After 10 days of heavy metal treatment, the primary samples were used for pigment analyses.

RESULTS

Optimization of plant growth in the lab is an important step when you are working with plant tissue culture. This means you have to be very careful about things like temperature and light and what you feed the plants. For plants like Catharanthus roseus getting this right is crucial for getting lots of shoots and roots to grow.

Different plants are all a bit different so you have to figure out what works best for each one. This is why people do lots of experiments to find the mix of things like water and food and light.

One of the important things is the food you give the plants. Most people use something called Murashige and Skoog medium because it has all the nutrients. You also have to add sugar because the plantsre not very good at making their own food. The acidity of the food is also important. It has to be just right. The plants will not grow very well.

Plant growth regulators are also very important. These are like helpers that make the plants grow in the right way. Some of them help the plants make shoots and some help them make new roots. You have to get the balance right or the plants will not grow properly.

The bits of the plant you use to start the growth are also very important. Young bits of the plant are usually better than bits. You have to make sure they are very clean or the whole thing will get contaminated. The temperature and light in the lab are also important. Most plants grow best at around 25 degrees Celsius.

You also have to think about how you move the plants to a new pot and how long you leave them in the lab. If you do all these things right you can get lots of plants growing. This is really useful for things like making medicine and growing lots of plants for sale. Catharanthus roseus is an important plant, for making medicine so getting the optimization right is crucial.

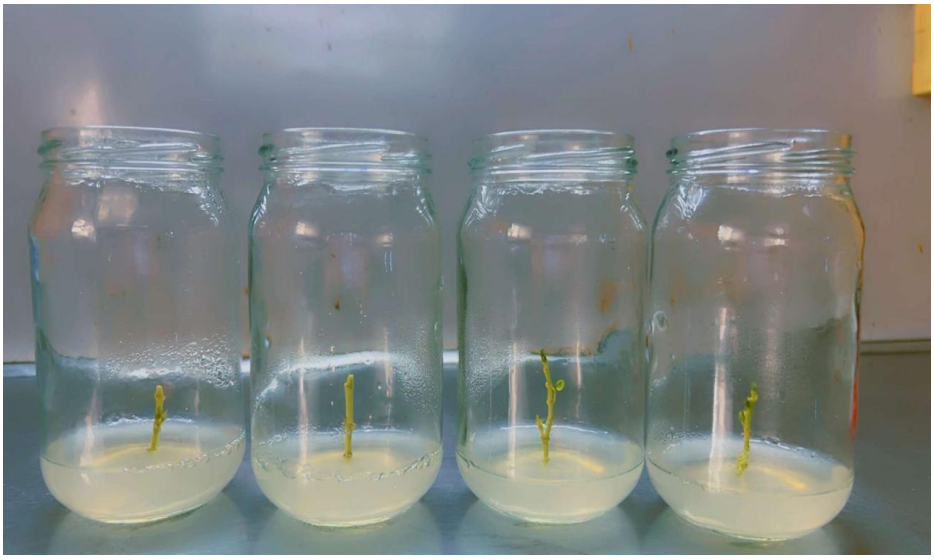


Figure 1: Microproprogation of catharanthus roses in vitro conditions using MS media

Abiotic stress such as salinity and temperature significantly affects the morphological and biochemical characteristics of Catharanthus roseus during in vitro culture. These stresses influence plant growth, development, and metabolic activities, leading to visible as well as internal changes in the plant tissues. (Hasanuzzaman et al., 2013)

A. Salinity Stress

Salinity stress is a deal for Catharanthus roseus. It changes how the plant looks and what is inside it. How much salinity affects Catharanthus roseus depends on how much sodium chloride in the special growth medium. In this study we used amounts of salinity to see how Catharanthus roseus would react.

Morphological Response at Different Salinity Levels

50 mM is a low level of salinity. At this level the little parts of the plant that we use to grow plants do really well and can handle the conditions. The shoots grow a lot. The tissues look healthy. This means that a little bit of stress can actually be good for Catharanthus roseus it helps the plant defend itself and grow better.

100 mM is a level of salinity. At this level the little parts of the plant can still grow,. Not as well as they would, without any salinity. The shoots and roots do not grow much as they would in normal conditions. The plants start to feel the stress. They can still survive and do their normal jobs, just not as well.

150 mM is a level of salinity. At this level the plant does not grow much and the shoots are shorter. The roots do not grow well either. The little parts of the plant start to show signs of stress, which means that the plant is not working properly.

200 mM is a high level of salinity. At this level the plant barely grows all and the little parts of the plant do very poorly. The tissues can become weak or damaged. The plant often does not survive for long. In cases the little parts of the plant die because there is just too much salt.

Figure 2 : In Vitro culture of Catharanthus roseus at 50mM of NaCl shows stimulated growth.

Figure 3: In Vitro culture of Catharanthus roseus at 100mM of NaCl shows osmotic.

Figure 4: In Vitro culture of Catharanthus roseus at 150mM of NaCl shows the culture exhibits acute salt toxicity characterised by severe growth retardation.

Figure 5: In Vitro culture of Catharanthus roseus at 200mM the NaCl shows level is lethal and severe contaminations can be observed.

B. Temperature Stress

Temperature stress affects plant growth a lot. It also impacts how plants work inside. We tested two temperatures, 20°C and 4°C on Catharanthus roseus plant pieces in this study.

Morphological Response at Different Temperature Conditions

20°C (Temperature)

At 20°C the plant pieces grew well. They looked healthy and strong. This temperature is good for growing Catharanthus roseus in a lab. The shoots. The callus formed properly. The cells. Grew into different parts. The tissues stayed green. Looked normal. This means the plants inside processes worked well. All the plant pieces survived at this temperature. This shows that the plants enzymes, nutrient uptake and photosynthesis worked properly. So 20°C is a condition for Catharanthus roseus to grow and regenerate.

4°C (Low Temperature Stress)

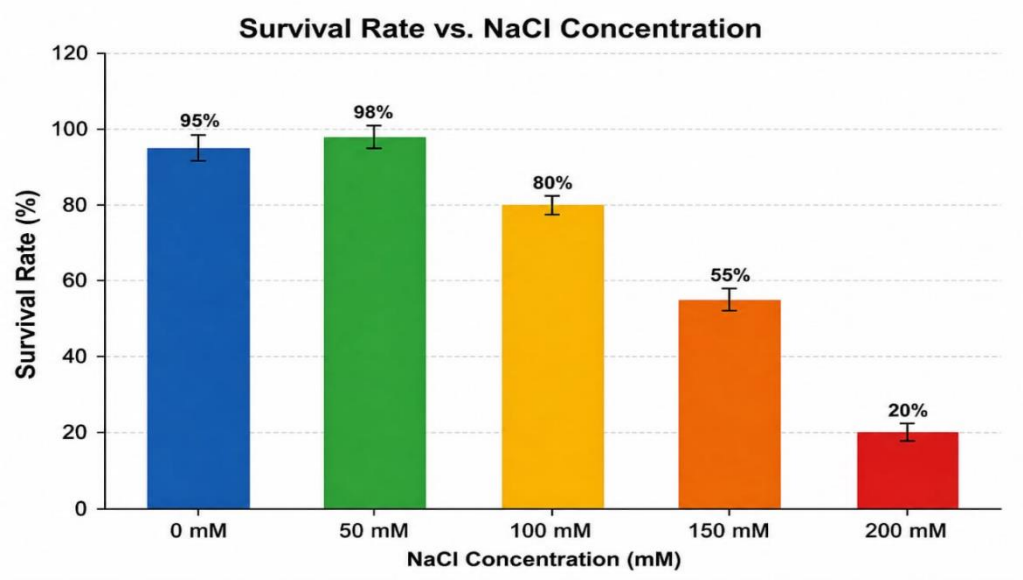
At 4°C the plant pieces did not grow all. The low temperature stopped the plant cells from working. The plant tissues looked damaged. They lost their shape. Did not develop properly. None of the plant pieces survived at this temperature. This low temperature might have slowed down the plants enzymes. It also reduced the plants metabolism. Affected its cell membranes. This stopped the plant from growing. Long exposure, to 4°C might have hurt the plant cells. This led to complete failure of Catharanthus roseus to regenerate.

Figure 6: In Vitro culture of Catharanthus roseus at 20º Celsius shows healthy growth of Catharanthus roses culture can be observed.

Figure 7: In Vitro culture of Catharanthus roseus at 20^{o} Celsius shows healthy branched tap root structure can be observed.

Figure 7 In Vitro culture of Catharanthus roseus at 4 o Celsius shows the culture enters the state of metabolic suspension and chilling stress which degrades the growth and slows down the metabolic activities

Figure 8: In Vitro culture of Catharanthus roseus at 4 o Celsius shows no root elongations.



Graph 1: The graph shows survival rate of catharanthus roses in different NaCl concentrations representing salinity stress.

CONCLUSION

The present study on Catharanthus roseus demonstrates the effectiveness of plant tissue culture techniques for its rapid propagation and improvement under controlled in vitro conditions. The use of Murashige and Skoog (MS) medium, along with proper aseptic techniques and optimized culture conditions, successfully supported explant growth, shoot multiplication, and root development, leading to the formation of healthy plantlets. This

confirms that micropropagation is a reliable and efficient method for large-scale production of this medicinally important plant.

The study also highlights the significant influence of abiotic stress factors, particularly salinity and temperature, on plant growth and development. The results clearly indicate that optimal temperature conditions (20°C) are highly favorable for regeneration, while low temperature (4°C) severely inhibits growth and leads to complete loss of survival. Similarly, salinity stress showed a concentration-dependent effect, where mild stress (50 mM) enhanced growth and adaptive responses, whereas higher concentrations (150 mM and 200 mM) caused severe growth inhibition and reduced survival.

These findings emphasize that while mild abiotic stress can act as a beneficial factor by stimulating plant defense mechanisms, excessive stress has detrimental effects on plant morphology and metabolic functions. Therefore, maintaining optimal environmental conditions is essential for successful plant growth and regeneration.

Table (1) The table is the representation of NaCl stress on catharanthus roses and it's survival rate and observation under different concentrations of NaCl.

Condition Type	Parameter	Survival Rate (%)	Contamination Rate (%)	Observation
NaCl Stress	0mM	Healthy cultures with minimal contamination		No survival; cold stress inhibitory
NaCl Stress	50mM	High survival; slight stimulatory effect		High survival; stimulatory effect
NaCl Stress	100mM	Moderate stress; some decline in viability		Moderate stress; decline in viability
NaCl Stress	150mM	Reduced survival; stress-induced susceptibility		Reduced survival; stress-induced susceptibility
NaCl Stress	200mM	Very low survival; high contamination observed		Very low survival; observed

Table (2) The table is the representation of temperature stress on catharanthus roses and it's survival rate and observation under different temperatures.

Condition Type	Parameter	Survival Rate (%)	Contamination Rate (%)	Observation
Temperature	20°C	100	5	Optimal condition; maximum regeneration
Temperature	4°C	0	10	No survival; cold stress completely inhibitory

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