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Morphological, Physiological and Ecological Features of Vitrification in Plant Tissue Culture

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Abstract

This study interprets the characteristic features of vitrification in plant tissue culture. Vitrescence is physiological decrepitude that hovers morphological and ecological aspects at its back. The foremost determinants of hyperhydricity are both external and internal factors. These antecedents may be the imbalancing of compounds in culture media or other agents like light, temperature and water potential. Abnormal state of leaves, stem, disruption of vascular bundle, hyperhydricity of cells, loss of chlorophyll, adverse effect on cytoplasm and depletion of lignin in cell wall of micropropagated plants are some of the consequences of glassiness. Extreme level of temperature, humidity, low light intensity, high ammonium and chlorine in culture media, low agar and low level of cellulose with lignin, hyperhydric condition and imbalance in culture media are primary roots of vitrification. Passable check and balance of hormones, organic and inorganic compounds, supporting agents, atmospheric factors and proper exchange of gases in container with environment are capital remedies to reverse the hyperhydric malformation. The proper concernment of plant tissue culturists is highly wanted to eliminate this state during tissue culture. Further research is executing in this area of study.

Keywords: Translucency; Proliferation; Micropropagation; Hyperhydricity; Lignification

Introduction

Plant tissue culture is a culturing technique and vitrification refers to an abnormal state often take place in cultured plants. Vitrificartion (synonym; translucency vitrescence, glassiness, hyperhydric malformation) is a serious problem which affects usually shoot multiplication and culture vigour. The word vitrification has been derived from Latin word vitreum means glass via French word vitrifier. It is a physiological disorder that may have different aspects at the back. The vitrification may be due to imbalance in organic compounds, organic compounds temperature, light and water potential. But morphological physiological and ecological feature are amassed reported for its occurrence. The major signs of vitrification in plant tissue culture are hyperhydricity, lack of chlorophyll a and chlorophyll b, loss of lignification in cell wall of plant cell and the leaves may have large number of intercellular spaces than normal leaves. Abnormal growth of plant organs and high concentration of water in plant body characterize the hypertrophy and hyperhydricity respectively. In vitrification the leaves become translucent w and whirled and broad in size. The palisade mesophyll tissues are absent in abnormal vitrified leaves but spongy mesophyll tissues are intact in leaves vascular bundles also gets distorted by vitrification. The vessels and tracheids are defective in such micro propagated malfunctioned plants. The story behind these phenomena is that all sugar content of plant is converted into amino acids the concentration of cellulose and lignin falls down. As a result, the wall becomes less efficient and water begins to move into the cell. This all leads to hyperhydric malformation. Vitrification not only effects herbaceous but also the woody plants during vitro vegetative micropropagation [1-10].

The vitrified plant is more susceptible to infections plants with fewer stomata and vascular bundle and lack of grow cambium which is descending basipetally from leaves primordia are known to be victims of vitrification. The major factor involved in glassiness are total water potential of medium, availability of water, growth factors imbalancing and BAP (benzyl amino purine). Due to the loss of chlorophyll the organs of plants become less green in color. The vitrification is actually reversible process and it was firstly reported in 1960s. (Philips and Mathews, 1964s; Hacket and Anderson, 1967). Usually, if vitrification overcomes the tissue culturing, plants lose 60% of its production. The plant has poor growth of abnormal shoots, poor survival & low percentage of rooting. High concentration of growth regulators, NH4 and Cl in MS medium also accelerated vitrification. Relative concentration of ammonium is more critical in this manner. Abnormal photosynthesis and gas exchange are major consequence of vitrification. Figure 1 shows hyperhydrated vitreous leaves [11-15].

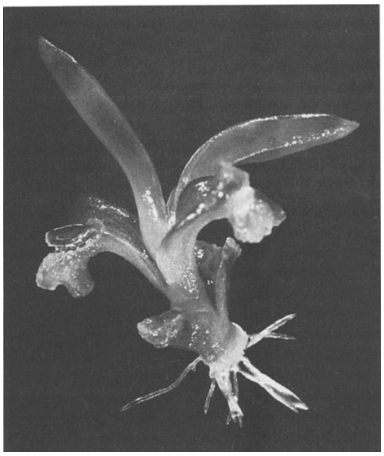

Figure 1: Carnation plant cultured in liquid medium showing hyperhydrated vitreous leaves (Shevchenko et al. 2020).

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Morphological and Physiological Aspects

Different morphological and physiological aspects are involved in causing vitrification. Actually vitrification is not accurred directly but its consequences are in steps. Firstly, leaves and then other organs and parts of micropropagated plants are affected [16-19].

Leaves as victim

The major effect on the leaves of vitrified plant is loss of its original coloration. They become shiny and translucid dark green. The leaves become glassy, leucious, pellucid and Hyperhydric in nature. Generally, two types of vitrification having observed in leaves. The succulent vitrification changes the normal leaves into thicker ones. And non-succulent vitrification makes the leaves whirled. The epiderm shows studded morphology and the stomata's raise eminent effect. The reduction of palisade mesophyll tissues is major sign of vitrified leaves. Lacunae of vitrified leaves is two to seven times larger than normal leaves. The glassiness of leaves with vitrification effect is due to low agar concentration and cellulose. Double size of leaf in this malformation is due to lack of differentiation and formation of large intercellular spaces [20,21].

Hyperhydricity of cell

Hyperhydricity is the high concentration of water in the cell. Loss of lignification in cell wall and increase in concentration of soluble phenol ultimately allows the water to enter in the cell. These condition at background hovers the wet of plant body [21-29].

Chlorophyll deficiency

The vitreous plants hold less chlorophyll and the rate of photosynthesis decreases 50%.

Effects on cytoplasm

The content of cytoplasm set of into less abundant and other organelles like chloroplasts and stomata resembles to amyloplast.

Shoot proliferation

Shoot number and production of biomes exerelated to high level due to access of cytokinin, auxiliary and adventitious buds rate increases. The activity of proliferation lowers down which in turn decreases the photosynthesis activity of stem. Two types of vitification have been seen in shoot passive and active vitrification. The vessels and tracheids are hypolignified [15-30].

Cell wall lignification

Cell wall is main gate to all internal activities. Slightly change in morphology of cell wall affects whole plant. If the amount of lignin shut down in cell wall the wall becomes less intact and it will allow all other factors to happen that causes vitrification.

Ecological Aspects

Ecological aspects comprise all the internal and external factors which influence the growth of culture and culture media.

Culture media environment

Quality of agar is highly effective of culturing of plants. The agar may have different effect on different species. Its means not only the quantity but also quality (brand) of agar also matters (Debergh, 1983). The culture media with less amount of agar has high possibility to get vitrified. Water is another factor of culture media which influence vitrification. The low amount of agar will increase the water concentration. The solidity of agar matters in this way. Even 1.1% increase in agar concentration can decrease vitrification. High level of cytokinin in the presence of low concentration of agar can cause vitrification. The balanced amount of agar and cytokinin is applicable only for particular concentrations. Imbalance of cytokinin and auxin causes vitrification due to defective xylem. The emission of gasses in closed container specially ethylene increases the chance of translucency. The chance of ethylene emission in solid culture media is high. Relative amount of ammonium ions in MS media and chlorine in high level mainly accelerate the vitrification. Low level of mignon and cellulose in culture media elevates the chance of glassiness [11]. If ammonium ions are large in concentration, the carbon and nitrogen ratio will decrease due to excess of nitrogen. Decrease in phenol concentration is parallel with carbon and nitrogen concentration. High calcium level reduces the vitrification in both herbaceous and woody plants. Figure 2 shows the reversible process of vitreous leaves of carnation into normal ones (Ho Thi Minh Thu et al. 2020).

Atmospheric factors

The exchange of external and internal atmosphere is crucial. The light and temperature from external atmosphere influence the tissue culturing. The quality, intensity and duration of light affect shoot growth and morphogenesis. Light regulates the leaves size. Any change in light regarding its quality and intensity causes vitrification. High light intensity the sign of normal tissue culture Lowering the intensity of light causes vitrification due to loss of Carbon dioxide. Humidity is another factor which put negative impact on culturing. In low humidity, possibility of vitrification is low. The balanced temperature is necessary for normal plant tissue culturing. High temperature causes glassiness of plants in micropropagation. Thus, not only internal but external atmosphere is effective in terms of vitrification. The table 1 shows the morphological and physiological changes in plant tissue culture related to vitrification [31].

Remedies

The best solution to vitrification is to increase the rate of shoot proliferation which in turn ultimately reduces the chance of translucency. This purpose, temperature, light and relative concentration of ammonium ions must be balanced. Agar and ammonium ions also used to eliminate vitrous condition in culture room. Due to these reasons the relative concentration of water and agar is controlled. High quantity of agar decreases the water availability

Figure 2: Conversion of vitrified leaves of carnation into normal due to 1% agar (Ho Thi Minh Thu et al. 2020).

Table 1: The effect of culture condition on morphological and physiological changes related to vitrification of micropropagated plants.

Sr.no	Media	Plant species	Morphological and physiological changes	References
1	Liquid or semi solid lower agar medium	*Dianthus caryophyllus*	• Abnormal mesophyll • reduced cuticular waxes • High ethylene • Vitreous shoots • Abnormal stomata	• Earle & Langhans (1975) • Sutter & Langhans (1979) • Mele et al. (1982) • Hakkart and virsluijs(1983) • Ziv et al. (1987 a, b)werker & Leshem (1987)
2	Liquid media	*Malus sp.*	• Vitreous shoots ,aerenchyma	Paques & Boxus (1987 b)
3	Semi-solid agar media	*Fragaria ananas*	• Changes in epicuticular waxes	Fabbri et al. (1986)
4	High agar and matrix potential	*Cynara scolymus*	• Decrease vitrification	Debergh et al. (1981)
5	Elevated sucrose	*Pyrus communis*	• Vitreous shoots	Orlikowska (1987)
6	High level of ammonia	*Prunus avium*	• Vitreous shoots	Riffaud & cornu (1981)

and thus the possibility of vitrification is reduced. Not only the concentration of water but cytokinin can also be balanced by increasing concentration of agar. Overall high level of carbohydrates in the medium, modified concentration of agar, changed light intensity and reduced humidity value within culture medium facilitate the reduction of vitrification. Another best solution to overcome this serious issue is to allow a better gas exchange (Hakkart and Versluijs, 1983). If agar is not available, it can also be replaced with pectin. The cold treatment in plant issue culture lowers the rate of vitrification in culture media. Anti-vitrifying complexes which composed of hydro soluble agar fraction is best remedy to reverse the effect of vitrification. Reinforcement of solid stationary medium, cobalt pretreatment and replacement of MS macronutrients by medium lacking chlorine ions are one of the useful reversible remedies in this regard.

Implication on Mass Propagation

In mass propagation remedies are applied by plant tissue culturists. Not always causes of defective conditions are poor acclimatization. But other factors are also involved in this regard. Insufficient wax deposition on cuticle of leaf can also cause withering. The implication on mass propagation can be done by setting balanced conditions in internal and external atmosphere. Majority, the rate of transpiration on and translocation must be promoted by tissue culturists. It will definitely help in reduction of vitrification rate.

Conclusion

Vitrification is not a restricted problem in plant tissue culture. The morphological, physiological & ecological aspects are the causes the glassiness but not every symptom in vitrification known to be specified to it. These signs can also be the result of other means. Further study is much more needed to fully uncover it's all aspects. But for plant tissue culturists hyperhydric malformation is sensitive concern to handle.

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