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NUTRITION OF PLANT CELLS AND ORGANS IN VITRO*

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SUMMARY

Assessment of the nutritional requirements of cultured plant cells and organs can be simplified by considering separately the inorganic salts, the organic constituents, and the natural complexes of undefined composition.

There appears to be a high degree of constancy in the salt requirements of diverse plants and their organs. The same salt provisions also appear to be satisfactory in the pursuit of a variety of research objectives. The salt requirement usually can be determined by comparing the morphogenic responses of a plant culture to some of the major formulae.

In constrast, the requirements with respect to the organic constituents are not so constant. Some organic compounds serve growth regulatory functions. Close attention should be paid to the hormonal substances, especially auxin and cytokinin. Assessment of hormonal requirements should consider the kinds and interactions of the hormones. Two other organic constituents are generally essential to plant cell and organ cultures: a sugar and the vitamin, thiamin. It is important only that adequate amounts of these two substances are provided. Sucrose has been satisfactory as the sugar in most cases.

Natural complexes of undefined composition should be included in culture media only as a last resort. The form in which the medium is provided and the culture conditions with respect to light and temperature may also influence nutritional requirements.

A great variety of nutrient formulations has been used in the culture of plant cells and organs. This variety reflects the diversity of plant genera and plant parts which have been cultured, as well as the variety of research objectives which have been pursued by many investigators. It is routine in plant culture first to develop the nutrient medium and then to establish other conditions best suited to specific needs. Widespread success with diverse genera and plant parts, and for a variety of purposes, is not expected by simply applying an existing set of cultural conditions.

The assessment of nutritional requirements of cultured plant cells and organs can be simplified by considering separately the three major classes of components: (a) inorganic salts, (b) organic constituents, and (c) natural complexes of undefined composition. Consistency in requirements among diverse genera, cell types, and research applications has been rather characteristic of the inorganic salts. Thus, a few major salt mixtures have been adequate to satisfy the needs of all plant cell and organ cultures.

In contrast, the organic compositions have varied extensively. This is expected, inasmuch as the biosynthetic rates of many of the compounds are variable among plants and their organs and tissues, and because many of the substances serve growth regulatory functions.

The natural complexes of undefined composition, the third category of substances, should be used only as a last resort. Every possible effort should be made to develop a chemically defined medium. If successful culture is not achieved even after all definable substances known to be physiologically active have been included in optimal amounts, then the addition of natural complexes should be considered.

Inorganic salts. The inorganic salt mixtures of White (1), Heller (2) and Murashige and Skoog (3) are reproduced in Table 1 because they have

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TABLE 1

INORGANIC SALT COMPOSITIONS OF THE THREE MAJOR PLANT CULTURE FORMULAE

Salt	White (1)	Heller (2)	Murashige and Skoog (3)
	mg/l		
NH_4NO_3			1650.0
KNO_3	80.0		1900.0
$NaNO_3$		600.0	
$Ca(NO_3)_2 \cdot 4H_2O$	300.0		
$CaCl_2 \cdot 2H_2O$		75.0	440.0
KCl	65.0	750.0	
KH_2PO_4			170.0
$NaH_2PO_4 \cdot H_2O$	16.5	125.0	
Na_2SO_4	200.0		
$MgSO_4 \cdot 7H_2O$	720.0	250.0	370.0
$MnSO_4 \cdot 4H_2O$	7.0	0.1	22.3
$Fe_2(SO_4)_3$	2.5		
$FeCl_3 \cdot 6H_2O$		1.0	
$FeSO_4 \cdot 7H_2O$			27.8
$Na_2 \cdot EDTA$			37.3
$ZnSO_4 \cdot 7H_2O$	3.0	1.0	8.6
H_3BO_3	1.5	1.0	6.2
KI	0.75	0.01	0.83
$CuSO_4 \cdot 5H_2O$		0.03	0.025
$Na_2MoO_4 \cdot 2H_2O$			0.25
$CoCl_2 \cdot 6H_2O$			0.025
$AlCl_3$		0.03	
$NiCl_2 \cdot 6H_2O$		0.03	

been used most extensively in plant cultures and have served as bases for further modifications. Each mixture was developed using specific test cultures, e.g. White used tomato root cultures, and Murashige and Skoog used tobacco callus.

Whereas consistency has been characteristic of the salt requirements, it is nevertheless advisable that at least a comparison is made of the major salt formulations for each plant culture in question. Indeed, substantial differences in growth and developmental effects can be expected among them. Usually the difference is evident in the rate of growth of cultures. Figs. 1 and 2 illustrate the difference between two salt formulations in supporting growth of tomato root and citrus embryo cultures. In rare instances, the choice of salt formulation may spell the difference between success and failure of a culture.

If it is necessary to refine the salt requirements for a particular case, the experimenter should recognize the many ionic interactions which could influence specific nutrient requirements. He should also consider the ionic form in which an element is provided, e.g. nitrogen as NH_4^+ or NO_3^-. The response by primary cultures to salts is sometimes influenced by the nutritional status of the plant or organ which is used as source of explants. Toxic effects in a high salt medium should be anticipated when explants are obtained shortly after the source plants have been fertilized heavily.

Organic constituents. Diversity has been characteristic of the organic substances utilized in plant culture media. This diversity reflects the variations in requirements which are encountered in working with many plant genera,

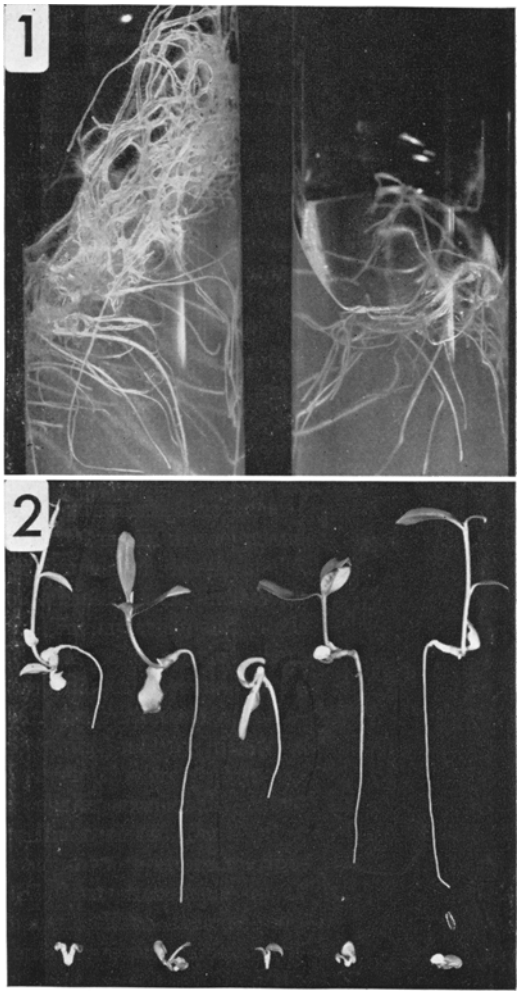

FIG. 1. Growth of excised tomato roots in response to salt formulations of Murashige and Skoog (*left*) and White (*right*).

FIG. 2. Citrus embryos cultured on media containing Murashige and Skoog (*upper row*) and White (*lower row*) inorganic salts.

organs, tissues, and research objectives. Nevertheless, it is possible to present a minimal list of those substances which have been demonstrated consistently to be essential or beneficial to plant cultures. This list is shown in Table 2.

TABLE 2

ORGANIC CONSTITUENTS MOST FREQUENTLY INCLUDED IN PLANT CULTURE MEDIA

Category	Substance Used	Concentration
		mg/l
Carbohydrate	Sucrose	20,000–30,000
Hormones:		
Auxin	IAA, NAA, or 2,4-D	0.1–3.0
Cytokinin	Kinetin or benzyladenine	0.01–30.0
Gibberellin	GA_3	0.01–10.0
Vitamins	Thiamin·HCl	0.1–30.0
	myo-Inositol	100
	Nicotinic acid	0.1–5.0
	Pyridoxine·HCl	0.1–5.0
Amino acid-amides	L-Aspartic acid-asparagine	5–500
	L-Glutamic acid-glutamine	5–500
	L-Arginine·HCl	5–500
	L-Tyrosine	100
Purines-pyrimidines	Adenine or adenine sulfate	40–160

While the reasons have not been disclosed experimentally, sucrose has been the most effective carbohydrate in plant culture media. It is usually provided in a concentration of 2 to 3%. Other oligosaccharides, as well as some polysaccharides, have an effectiveness approaching that of sucrose, but have rarely been superior.

Perhaps the most critical organic substances are the hormones. The major hormonal substances are auxin and cytokinin. Gibberellin has been shown to be beneficial to callus and organ cultures of certain plants; it is not generally essential. The evaluation of hormonal requirements should consider kinds as well as interactions. For example, the auxins differ markedly in their effectiveness in stimulating callus growth and in influencing organized development. As a growth stimulant, the most potent auxin has been 2,4-dichlorophenoxyacetic acid (2,4-D), followed by 1-naphthaleneacetic acid (NAA), 3-indoleacetic acid (IAA) being the weakest. However, 2,4-D strongly represses organized development, including embryogenesis and shoot initiation. NAA is less suppressive of shoot initi-

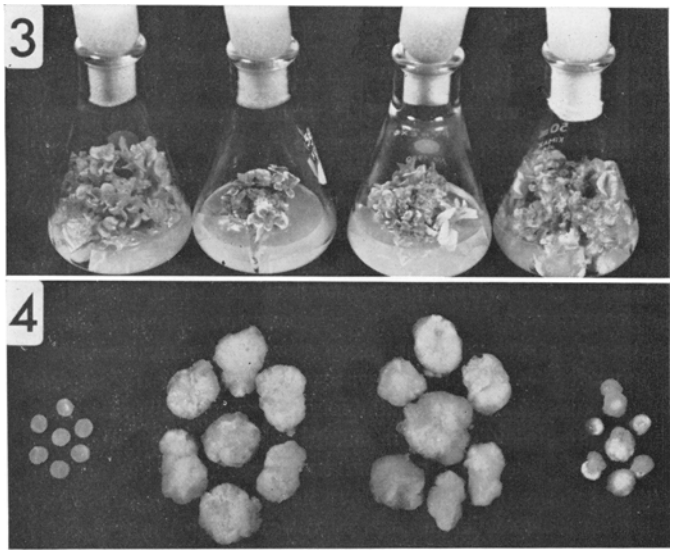

FIG. 3. Influence of deficiency of thiamin on morphogenesis in tobacco callus culture. Cultures on ends, 0.1 mg per liter thiamin·HCl; two central cultures, no thiamin in medium. Note repressed development and chlorotic leaves in thiamin-deficient cultures.

FIG. 4. Growth in vitro of citron albedo sections as influenced by medium addenda of orange juice. *Left to right:* 0, 3, 10, and 30% by volume juice. Basal medium contained Murashige and Skoog's salts, 5% sucrose, the growth regulators 2,4-D and kinetin, and the vitamins thiamin·HCl, *myo*-inositol, nicotinic acid, and pyridoxine·HCl in optimal amounts.

ation than 2,4-D, whereas the auxin IAA shows no adverse organogenetic effects. Indeed, in studies of organogenesis the preferred auxin is IAA.

The more widely employed cytokinins are 6-furfurylaminopurine (kinetin) and 6-benzylaminopurine (benzyladenine). Neither of these occurs naturally nor are they as potent as the naturally occurring cytokinins. Nevertheless, their use is necessitated by the scarcity and virtually prohibitive cost of the native substances. Kinetin and benzyladenine are about equally effective in most cultures.

TABLE 3

SOME NATURAL COMPLEXES AS NUTRIENT MEDIUM INGREDIENTS

Complex	Suggested Concentration
Coconut endosperm	10–30% (by volume)
Malt extract (commercial preparation)	500 mg/l
Yeast extract	100–10,000 mg/l
Tomato juice	5–10% (by volume)
Orange juice	3–30% (by volume)
Protein hydrolysates	500–5000 mg/l

One of the most interesting interactions among biologically active substances has been that involving auxin and cytokinin. The optimal concentration of one in a nutrient medium is dependant on the concentration of the other. Moreover, where organized development is manifested, the type of organ formed is controlled by the relative amounts in the medium of the two hormones. A relatively high concentration of auxin combined with a low concentration of cytokinin results in root initiation; the reverse relationship leads to shoot formation.

Many vitamins have been included in plant culture media. Extensive lists can be found elsewhere, e.g. Butenko (4), Gautheret (5), and White (6). However, only one, thiamin, has been shown to be generally essential or otherwise beneficial. Fig. 3 shows thiamin deficiency symptoms in tobacco cultures. Note the depressed organ

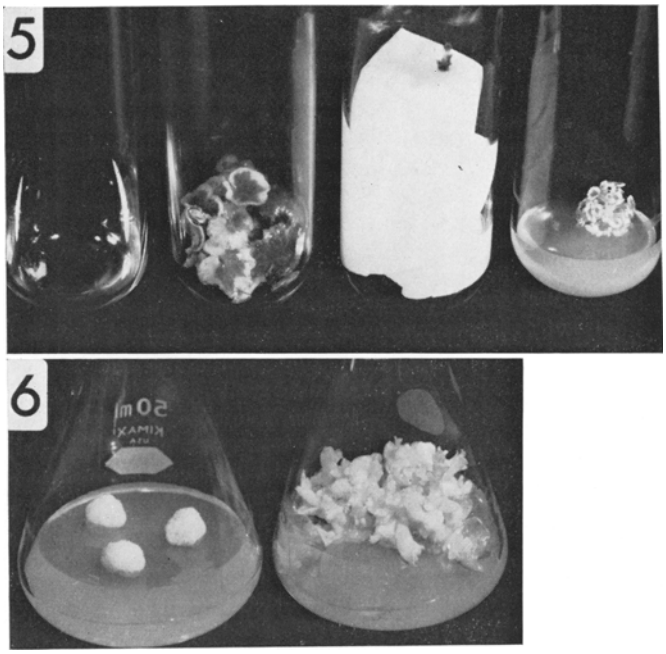

FIG. 5. Growth and development in vitro of strawberry begonia shoot tips as influenced by physical form of nutrient medium. *Left to right:* liquid formulation, unagitated; liquid, with cultures rotated on drum at 1 rpm; liquid, with filter-paper wick support; and 0.8% Bacto-agar gel. Medium composition and volume are the same in each case. Note best development in liquid cultures agitated gently.

FIG. 6. Tobacco callus cultured in constant darkness on nutrient agar containing 2 mg per liter each of kinetin and IAA. Medium of culture on right contained 100 mg per liter L-tyrosine, 160 mg per liter adenine sulfate, and 340 mg per liter $NaH_2PO_4 \cdot H_2O$.

initiation and chlorotic foliage. The usual concentration range of thiamin is 0.1 to 1.0 mg per liter, expressed as the hydrochloride. Some plant cultures, e.g. lemon callus, show maximal growth at 10 mg per liter thiamin·HCl; still others, e.g. gerbera cultures, grow best at 30 mg per liter.

Other vitamins which have been employed in plant cultures are inositol, nicotinic acid, and pyridoxine. Their requirements have not been shown to be critical in most cases, although stimulations of cell and organ cultures have been obtained with them.

Certain plant cultures have benefited from the inclusion in the nutrient medium of amino acids or their amides. Significant examples are aspartic acid-asparagine, arginine, glutamic acid-glutamine, and tyrosine. Lists of other amino acids employed in plant culture media can be found elsewhere (4–6). It should be noted that only the L-isomers are physiologically active. The D-forms could have negative effects. It is also important to recognize antagonisms among some of the amino acids, and tests with these compounds should include combined actions.

Other organic nitrogenous substances, especially purine and pyrimidine derivatives, also have been shown to be helpful when plant cultures are employed in morphogenetic investigations. Adenine has long been known to promote shoot initiation and therefore should be included in experiments concerned with organogenesis.

Natural complexes. Perhaps we will remain unable to establish cultures of certain plants or tissues and organs in a chemically definable medium until additional growth-regulating substances have been identified. While awaiting their identification, and as last resort, it may be necessary to include certain natural complexes of undefined composition. Complexes employed frequently are coconut and other endosperm fluids, protein hydrolysates, yeast and malt extracts, and various fruit pulp or juices (Table 3). Fig. 4 shows the effect of orange juice on the growth of citron callus. This tissue has not been cultivable in a chemically defined medium containing 2,4-D, kinetin, gibberellin, and diverse amino acids and vitamins. Its successful culture has required an addition of 3 to 10% by volume of orange juice.

Other considerations. Frequently the physical form in which a medium is provided may be critical. For example, shoot initiation in tobacco callus occurs best when the tissue is cultured on the surface of an agar gel medium. In contrast, strawberry begonia cultures produce a maximal number of shoots when the tissue is submerged in a gently rotated liquid medium (Fig. 5). Experience in this laboratory with other plant genera has shown clearly that various physical qualities of the nutrient medium can influence the nutritional requirements significantly, perhaps through an effect on the availability and rate of assimilation of nutrients.

Still other factors which may determine specific nutritional requirements of plant cultures are the light and temperature characteristics of the in vitro environment. In Fig. 6 are shown cultures of tobacco callus grown in constant darkness on agar media containing 2 mg per liter each of kinetin and IAA. The medium of the culture on the right contained additional supplements of $NaH_2PO_4 \cdot H_2O$ (340 mg per liter), adenine sulfate (160 mg per liter), and L-tyrosine (100 mg per liter). In spite of the relatively high kinetin-IAA ratio, no shoots were initiated in cultures grown in constant darkness unless the additional supplements were provided. An analogous influence by temperature on the nutritional requirements of plant cell and organ cultures is expected, although this has not been specifically explored.

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