TRACHEARY FORMATION IN RICE CALLI

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Abstract: Calli induced from seedlings of rice (*Oryza sativa*) were subcultured in MS medium containing different tested plant growth hormones. The combination treatment of 2 ppm 2,4-dichlorophenoxyacetic acid and 1 ppm kinetin is most effective in production of tiny, white and hard calli, which contain 8% cells being tracheary elements in shapes of fork, nodule and band. Ultrastructural studies show the appearance of gradually degenerated organelles and unevenly wall-thickening occurring in differentiating tracheary elements. Mature tracheids contain only cell wall with distinct and unevenly thickened secondary wall, and partially remained primary wall.

INTRODUCTION

Tracheary formation is an excellent model for cell differentiation in plant (Roberts, 1976) and calli provide a good experimental system for studying tracheary differentiation (Aloni, 1980; Fukuda and Komamine, 1980). However, the effects of plant growth substances on the formation of tracheary element in calli vary with plant species, hormonal constituents and interaction, and external conditions (Minocha and Haperin, 1974; Thorpe and Gaspar, 1978). Our previous study indicated that the amount of tracheary elements was not relatedto plant regeneration from rice calli and its ratios in whole population of callus cell was quite low (Chen, 1980).

In the present study, the induction and pattern formation of tracheary elementsin rice calli will be studied.

MATERIALS AND METHODS

Plant materials

Sterilized seeds of *Oryza sativa* Tainan 5 were sowed on MS basal medium (Murashige and Skoog, 1962), containing 2 ppm 2,4-dichlorophenoxyacetic acid (2,4-D), and incubated in the dark at room temperature. After 2 weeks, calli in millimeters arised from seedlings were collected for further study.

Tracheary induction

Calli were subcultured in differentiation medium containing different hormonal compositions (Table 1) and incubated in the dark at 28°C. In each two-day interval, changes in morphology of calli were recorded. Cell count and ratio oftracheary elememt were measured by the method of Minocha and Halperin (1974).

Light microscopy

Calli were fixed in formalin-acetic acid-alcohol solution for 1 day. After being washed with 50% ehtanol, the calli were dehydrated in graded tertiary butanol series and embedded in Tissuemate (m.p. 62°C). Serial sections,

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10 μm in thickness, were collected, stained with safranin 0 and haematoxyline (Jensen,1962), and photographed by a Zeiss photomicroscope III.

Scanning electron microscopy

Paraffin sections collected from light microscopy of Materials and Methods (3) were deparaffined in xylene, infiltrated with three changes of absolute alcohol, dried in critical point apparatus, coated with gold in ion sputter, and finally observed under Hitachi S-520.

Transmission electron microscopy

Calli were collected and cut into 1 mm cubes. After 2 h fixed in 0.1 M cacodylate buffer (pH 7.0) containing 2.5% glutaraldehyde at 4°C, the cubes were washed three times in plain buffer, post-fixed in 1% osmium tetraoxide for 2 h, dehydrated in a graded ethanol series, infiltrated in Spurr's resin and polymerized at 70°C for 8 h. Sections in golden color were collected from ultramicrotomy, stained with ethanolic uranyl acetate and lead citrate, and finally examined in Hitachi H-600 at 75 KV.

RESULTS

Tracheary formation

Rice calli subcultured in MS medium containing different hormonal compositions showed distinct appearances of their growth. As shown in Table 1, several facts were worthly remained. In naphthaleneacetic acid (NAA) containing medium, most of calli turned into rooting (46%) and browning (44%) in ten days cultures and there were no white small calli observed in solitary or combinating treatment of NAA. The treatment of 2, 4-D, an effective calli inducer for rice seedlings, could keep calli in less differentiation within short peroid. However, the combinating treatment of 2 ppm 2,4-D and 1 ppm kinetin possessed greater potentiality for callus differentiation, e. g., rooting and formation of white, solid and tiny calli, which contained high percentage of tracheary elements in callus tissues.

Table 1. Effects of hormonal combination on the growth and differentiation ofrice calli*.

Hormonal combination!				10 days				20 days				28 days			
NAA	2,4-D	Kinetin	BA	RC	WC	YC	BC	RC	WC	YC	BC	RC	WC	YC	BC
(ppm)				(%)				(%)				(%)			
1				46	0	10	44	-	-	-	100	-	-	-	100
1		1		13	0	43	44	13	0	22	65	-	-	-	100
0.1		1		9	0	32	59	6	0	30	64	-	-	-	100
1			4	8	0	28	64	0	0	0	100	-	-	-	100
1			1	22	0	20	58	7	0	0	93	-	-	-	100
0.1			1	9	0	16	75	-	-	-	-	-	-	-	100
	2	1		7	10	73	10	25	10	54	11	47	10	30	13
	2			-	-	-	-	34	4	44	18	60	4	18	18

*RC, Rooting calli; WC, Whitish solid calli; YC, Yellowish calli; BC, Brownish calli.

!Basal medium (Murashige and Skoog, 1962).

Table 2. Effects of hormonal combination on tracheary differentiation.

Duration of subculture (days)	Hormonal combination* (ppm)				Tracheary differentiation (%)		
	NAA	2,4-D	Kinetin	BA	Yellowish calli	Whitish lid calli	Rooting calli
10	1				-	-	0.36
10	1		1		0.29	-	-
10	0.1		1		0.50	-	-
10	1			4	0.13	-	-
20		2			0.46	-	0.81
28		2	1		-	8.10	1.72

*Basal medium (Murashige and Skoog, 1962).

As shown in Table 2, ratios of tracheary element in yellowish calli were less than 1%. In rooting calli, the ratio of tracheary elements was also still lower toward 1.7%. However, white solid calli had a high content of tracheary elements and the ratio of tracheary elements was up to 8.1%. The following descriptions of tracheary formation based on calli grown on MS medium containing 2 ppm 2, 4-D and 1 ppm kinetin.

The growing regions of calli in differentiation medium could be originated from epidermal, subepidermal or subcortical cells (Fig. 1). Cells in growing region were small in size, dense in cytoplasmic staining and high in nucleocytoplasmic ratio (Figs. 1E, 1F, 2L, 2M). The earliest appearance of tracheary element was found in 13 dayd subcultured calli (Fig. 1C). Region of tracheary element extended from fast growing sites toward the central portion of calli (Figs. 1C,1D). In early xylogenesis of calli, tracheary elements were mixed with parenchymatous cells (Figs. 1C, 1M, 2C), and their orientation were diversified (Figs. 1M, 2D, 2H.). In old calli, tracheary tissues with multiple strands occupied the center of calli and mingled with thick-walled dead cells (Figs. 1B,1G, 1K, 3A). In adventitious root originated from calli also formed certain amount of tracheary elements in its central and basal area (Fig. 1B, arroheaded).

Secondary wall thickening in spiral, scalariform and intermediated forms were found in tracheary elements of calli (Figs. 2B, 2F, 2G, 2H). There was a distinct primary wall mediating between tracheary elements and adjacent parenchyma cells (Figs. 2D, 2H, 2K). However, partial hydrolysis of primary wall at the site without secondary wall thickening resulting the formation of large hole on cell wall between two adjacent tracheary elements was also observed (Figs. 2G, 2H).

Ultrastructural studies

In differentiating tracheids before the deposition of secondary wall had a numberous of plasmodesmata connected with their vicinity (Figs. 3A-D). Most of cell organelles show in normal appearance. In some cases, cisternae of endoplasmic reticulum aligned parallelly to the wall surface (Figs. 3A, 3C). In addition to the occurrence of membrane-bound ribosomes associated with endoplasmic reticulum, free ribosomes were present in cytoplasm. Moreover, sevaral large oil drops were also found in cytoplasm. It indicated that the cell became toward degeneration.

In the more advanced stage of differentiating cell, a large vacuole existed in the cell center and peripheral cytoplasmic contents extented along with cell wall (Figs. 3D, 3E). Swelling at the end of endoplamic reticulum was sometimes observed. Platids distinctly degenerated and were not easily recognized. However, mitochondria containing prominent crests or concentric membrane system still persisted after the formation of secondary wall

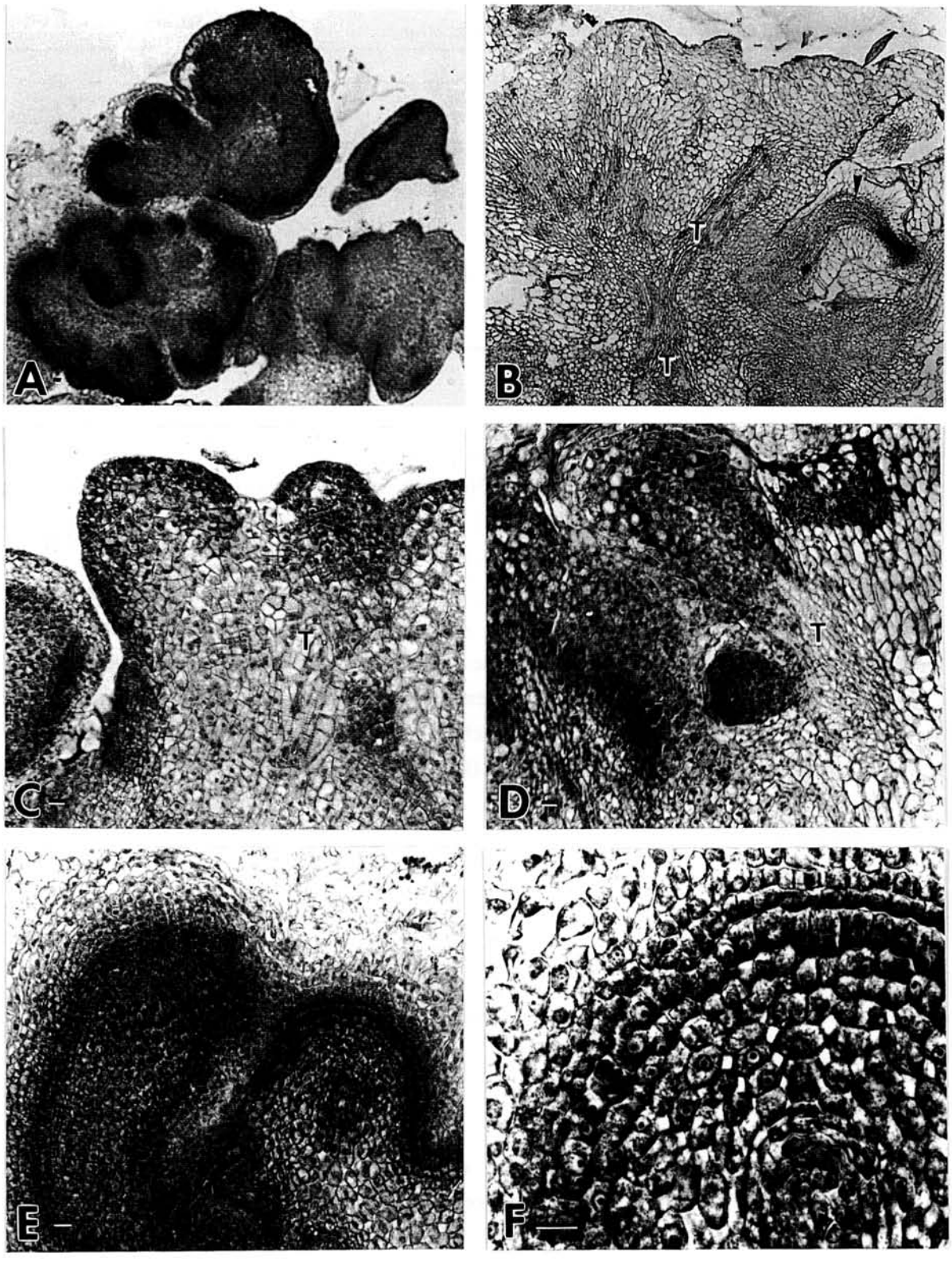

Fig. 1. Callus growth and distribution of tracheary elements in rice calli. A,cross section of 13-day-old culture. B, cross section of 29-day-old culture. C,distribution of tracheary elements in 13-day-old culture. D, distribution of tracheary elements in 29-day-old culture. E. new calli originated from inner layer of old callus. F. partial enlargement of E. G, tracheids in multiple and branched strands. H, tracheids in vascular nodule. J, tracheids and adjacent parenchyma. K, tracheids mixed with parenchymatous cells. L, tracheary nodule. M,trachary strands orientated in different direction. All bars in 10 um.

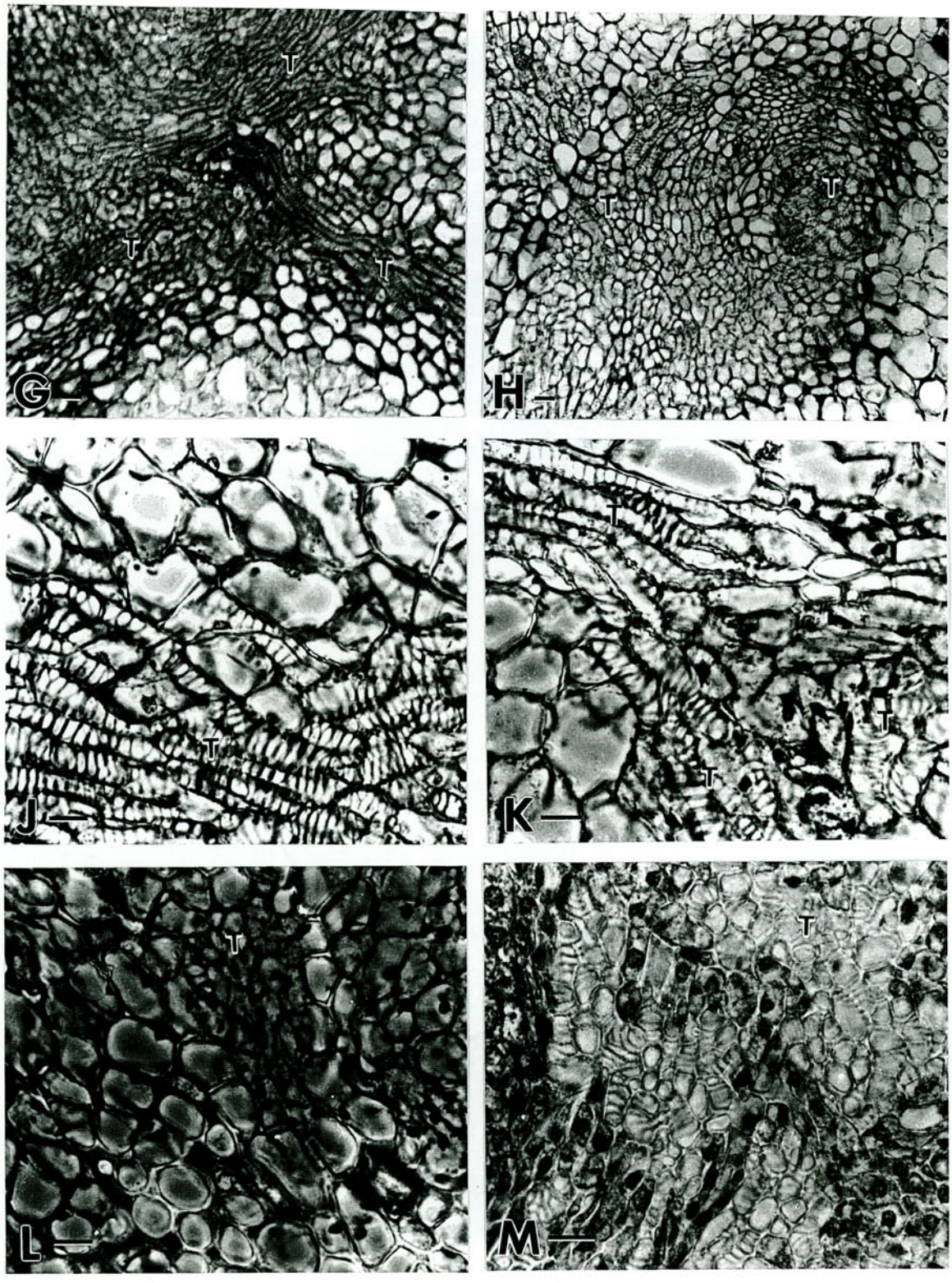

Fig. 2. SEM micrographs of tracheary elements in rice calli. A, tracheids inmultiple and branched strands. B, partial enlargement of A, showing secondary thickening. C, parenchymatous cells mingled with tracheids. D, ring form thickening in tracheids. E-F, tracheid in vascular nodule. G and H, scalariform tracheids (arrowed). H and J, tracheids (arrowed) and adjacent parenchymatous cells. K, partial enlargement of J. L, parenchyma cells far from tracheary strand. M, nucleus (arrowed) with prominent nucleolus (arrowhead) in parenchyma cell. All bars in 1 um.

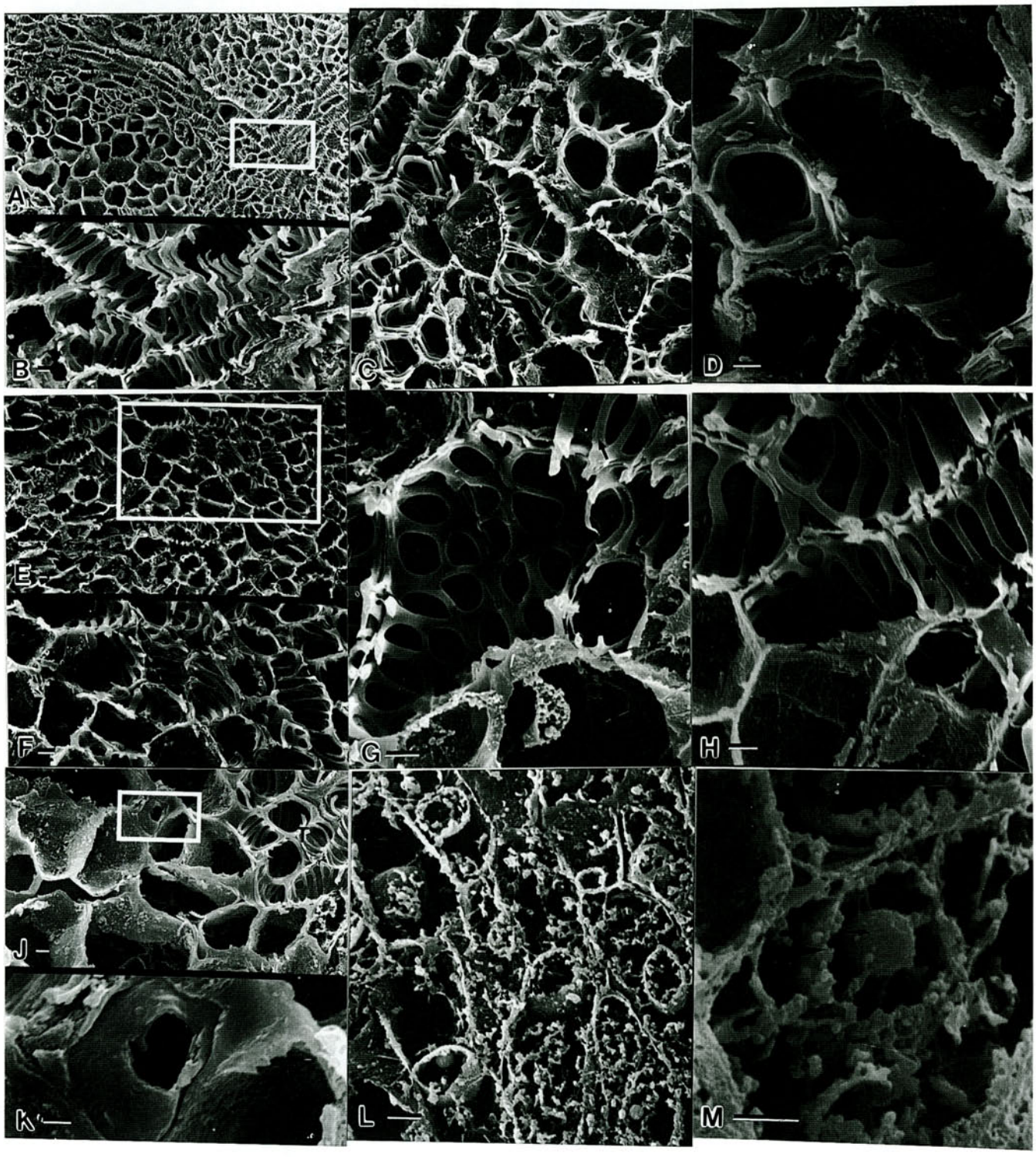

Fig. 3. Parenchyma cells and developed tracheids in rice callus. A, tracheids and near-by tissues. B, sieve element and phloem parenchyma. C, parenchyma cell containing large oil drops. D, Swelling endoplasmic reticulum in parehchyma cell adjacent to tracheids. E, well developed tracheid containing mitochondria and oil drops. F, scalariform tracheid. G, tracheids in ring form of wall thickening. H, loosen primary wall in tracheids. All bars in 1 um.

(Fig. 3E). Oil drops, which were common in the surrounding parenchymatous cells, were also found in late stage of differentiating tracheary elements (Fig. 3E). A few mitochondria also persisted intactly or partially disintegrated indicated that the breakdown of protoplasm was an active enzymatic process (Fig. 3E). Vesicles might contain lytic enzymes which participated in digesting process of cell content and unlignified cell wall. Plasma membrane could not be recognized and protoplasmic residues dispersed in cell lumen (Figs. 4E, 4F).

In mature tracheid, cell lumen was almost empty (Figs. 3G, 3H). The secondary thickening of tracheid remained attaching either to the wall of adjacent parenchamatous cell or to the rings of thickending on the adjacent tracheid and their primary wall persisted (Fig. 3G, arrowed). Partial swollen primary wall without secondary-wall apposition did show the appearance of loosen microfibrils (Fig. 3H, arrowheaded)

DISCUSSION

The treatment of 2, 4-D on rice calli not only maintained their growth, but also induced the formation of tracheary elements. The combination of 2, 4-D and kinetin could enhance the ratio of tracheary differentiation up to 8%. Similar results were reported in soybean calii (Fosket and Torrey, 1969). However, the combinating treatment of NAA, BA and kinetin often resulted in the rooting and browning of rice calli, which were contradicted to the facts observed inartichoke (Minocha and Halperin, 1976) and soybean (Fosket and Torrey, 1969).

Wetmore and Rier (1963) found that vascular nodule occurred in syringa calli treated with auxin and sucrose. Dalessandre and Roberts (1971) observed that different distribution of tracheary elements was related to the interaction of auxin and cytokinin. The explant of *Lactuca* formed central nodular vasculation in low concentration of 2, 4-D, whereas ring-shape vasculation in high concentration. In this study, all different patterns of tracheary distribution were originated from the combinating treatment of 2, 4-D and kinetin (Fig. 1).

Secondary wall thickening of tracheary elements in rice calli were spiral and scalariform. There were no reticulate or pit forms of wall thckening, appeared in dicots or other plants (Roberts, 1976). It indicated that wall patterns of tracheary elements were persisted in plant tissues, no matter of their occrurring in intact plants or cultured calli.

Deparaffined sections, observed under SEM, showed the disappearance of primary wall on the side wall without secondary thickening between two tracheary elements. Partial hydrolysis of primary wall regions could be explained by the loss of wall materials during specimen preparation. Primary wall did persist between parenchyma cells and tracheary elements in rice and other plants (Srivastava and Singh, 1972; Esau and Charvat, 1978). However, Srivastava and Singh (1972) indicated that the microfibril compactness of primary walls in parenchyma cell was different from that of tracheary elements, and the presence of some components in middle lamella protected the primary wall in parenchyma cell from the digestion of autolytic enzymes related from developing tracheids.The loosen arrangement of microfibrils and the digestion of wall materials on primary wall of tracheary elements had been observed in this study. Moreover, this partial wall digestion was common in the process of the perforation of end wall between two vessel elements (O'brien, 1970). In the latter case, no pectin film remained after perforation.

The degree of extension in secondary wall coil on the side wall reflected the time of maturation of tracheary elements (Esau and Charvat, 1978). The earlier mature tracheary elements contained losser helics than that of latter formed ones. In rice, however, the secondary wall on the two sides of common wall was mostly apposited by wall materials in opposite with one another, and the difference in spacing between spiral and scalariform tracheids in consecutive strands was evidently found.

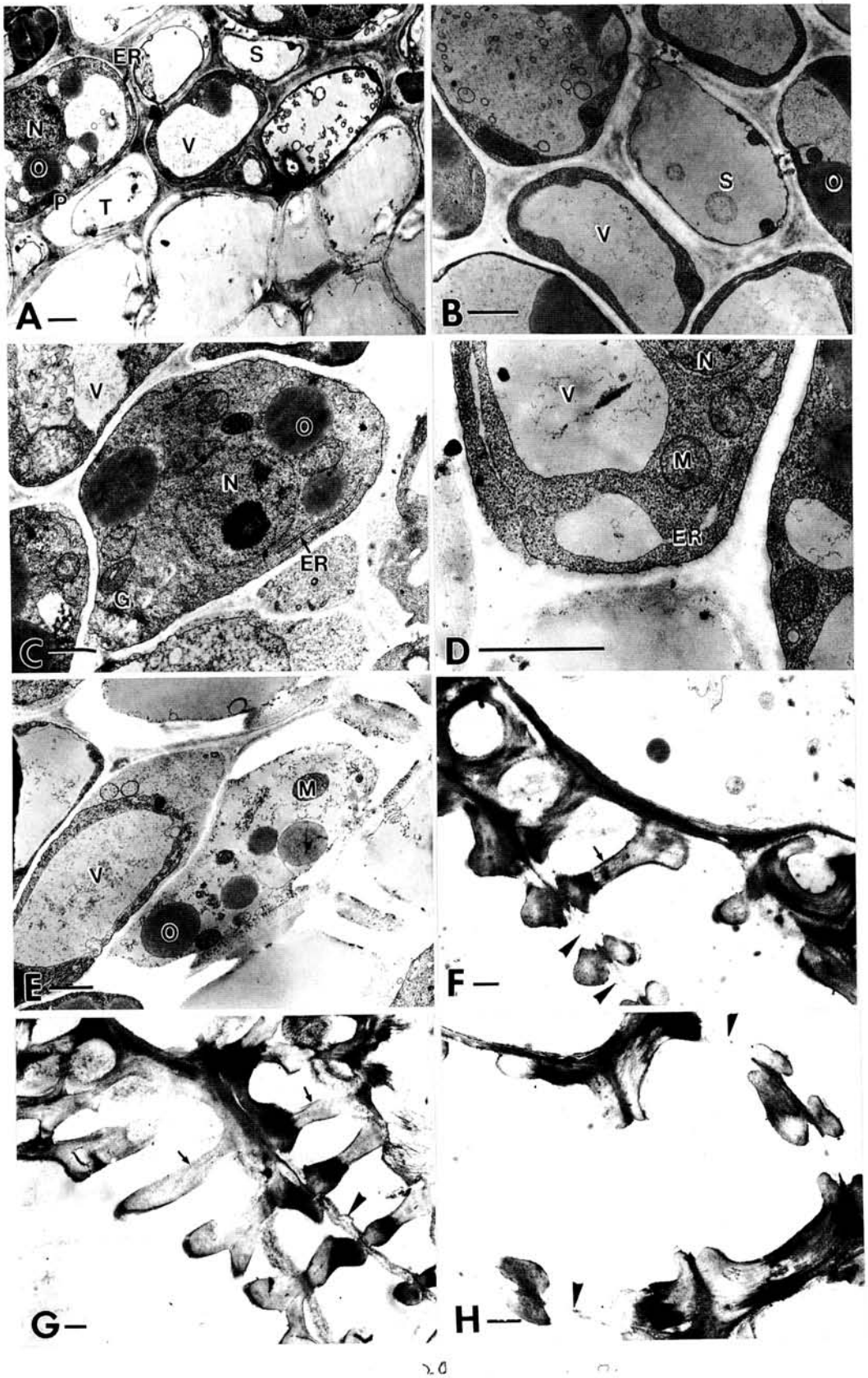

Fig. 4: Abbreviations

ER, endoplasmic reticulum; **G**, Golgi; **M**, mitochondrion; **N**, nucleus; **O**, oildroplet; **P**, plastid; **S,** sieve element; **T**, tracheary element; **V**, Vacuole;

During the development of tracheary elements, most organelles changed in strcuture, e. g., disappearance of plastids, swelling of endoplasmic reticulum, less dense of nuclear content and diminution of nucleolar size (Esau, et al.,1966; Esau and Charvat, 1978). Mitochondria found in all developmental stages of tracheary elements were similiar in their strcuture. Intact or partially-damaged mitochondria seen in empty cell lumen were observed in this study and other reports (Cronshaw and Bouch, 1965).

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水稻癒合組織的假導管形成

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源自水稻 (*Oryza sativa*) 幼苗癒合組織經繼代培養於含有 2ppm 2, 4-dichlorophenoxyacetic acid 和 1ppm kinetin 的 MS 培養基上，則會最有效地產生細小而質硬的白色癒合組織。此組織內含百分之八的假導管，分別以分枝狀、團狀及帶狀存在。超微結構顯示假導管細胞內分化過程中有胞器逐一退化及細胞壁的不均勻加厚現象，成熟的假導管內主含明顯及不均勻細胞壁加厚的次生細胞壁及一些初生細胞壁的遺跡。