OBSERVATIONS

Effect of NaCl on germination and seedling growth

Sodium chloride salinity considerably reduced the germination and seedling growth of rice. The rate and final percentage of germination decreased with increase in concentration of NaCl (Table 1). Growth rate of embryo axis also decreased in all tested levels of NaCl (Table 1). At 1M NaCl, although the extension growth of embryo axis was reduced to 61 percent of the control, final percentage germination was 100 percent. At 0.15, 0.2 and 0.25M concentrations, the percentage germination after 120h was only 88, 56 and 44 respectively. The growth of embryo axis at 0.15, 0.2 and 0.25M NaCl levels was reduced to 53, 30 and 19 percent respectively of the control value. Since 0.15M NaCl inhibited the seedling growth by around 50 percent (Table 1) this concentration was employed in all subsequent experiments.

Administration of GA_3 (10ppm) significantly increased the growth of shoot and root systems of seedlings under saline conditions (Fig. 2A, Table 2). It also enhanced the percentage germination of seeds under the influence of salt (Table 4). The dry weight and extension growth of the axis of GA_3 treated salt stressed seedlings were 33 and 38 percent more than that of salt stressed ones (Table 2). However, the dry weight accumulation of GA_3 treated salt stressed seedlings were much less compared to the control (Table 2). GA_3 also increased the extension growth of rice in non-saline conditions (Table 2). Extension growth was 22 percent more in GA_3 treated as compared to control after a period of 120h.

Among the various concentrations of putrescine tested 10^{-5} M brought about maximum stimulation of growth of control and salt treated seedlings (Fig. 1B). Hence, this concentration was employed in the subsequent experiments. Putrescine application enhanced the rate of radicle emergence and growth of axis (Table 3,4) in both the absence and presence of NaCl (Fig. 2B). The final percentage germination of seed in 0.15M NaCl treatment was increased from 72 to 91 percent as a result of putrescine application (Table 4). The same concentration of putrescine brought about an increase of about 17 percent in growth of embryo axis under saline conditions in comparison with the salt control (Table 2).

GA_3 and putrescine treatment even in the absence of salt stress resulted in a greater loss of endosperm dry weight and an increase in the accumulation of dry matter of embryo axis compared to the control (Table 2,3). Endosperm of salt-stressed seeds showed a higher dry weight, about 38.5 percent more than water control. On the other hand, the dry matter accumulation of axis had been reduced to about 48.5 percent of the control in presence of salt (Table 2). Compared to the salt control 69 and 45 percent increase was observed in the dry matter content of GA_3 and putrescine exposed salt stressed embryos respectively. Similarly there was a considerable decrease in the dry weight of endosperm (about 23 percent in GA_3 treated and 20 percent less in putrescine treated). However, seedling emerged from GA_3 treated seeds showed better growth than putrescine treated ones under saline as well as non-saline conditions (Table 2,3).

Fig. 1 A : Effect of GA_3 on seedling growth of rice (after 120h of germination).

a. Control, treated with
b. 2.5 ppm,
c. 5 ppm,
d. 7.5 ppm,
e. 10 ppm GA_3

B : Effect of putrescine on seedling growth of rice (after 120h of germination).

a. Control, treated with
b. 10^{-6} M,
c. 10^{-5} M,
d. 10^{-4} M,
e. 10^{-3} M putrescine.

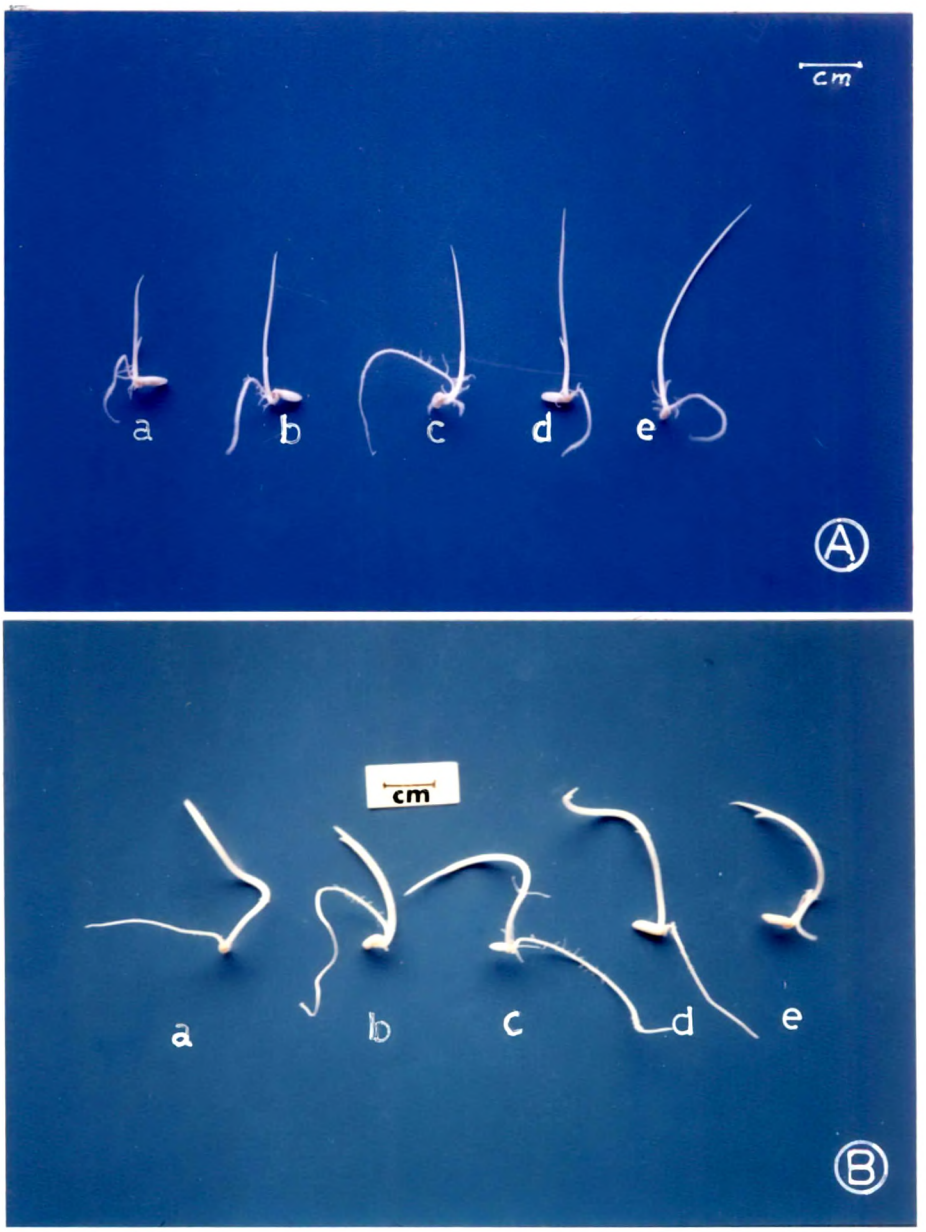

FIG. 1

Fig 2 A · Effect of NaCl (0.15M) and GA_3 (10 ppm) on seedling growth of rice (after 120h of germination).
a Control, treated with
b 0.15M NaCl,
c 10 ppm GA_3,
d. 0.15M NaCl containing 10 ppm GA_3

B : Effect of NaCl (0.15M) and putrescine (10^{-5}M) on seedling growth of rice (after 120h of germination)
a Control, treated with
b 0 15M NaCl,
c. 10^{-5}M putrescine,
d 0 15M NaCl containing 10^{-5} M putrescine.

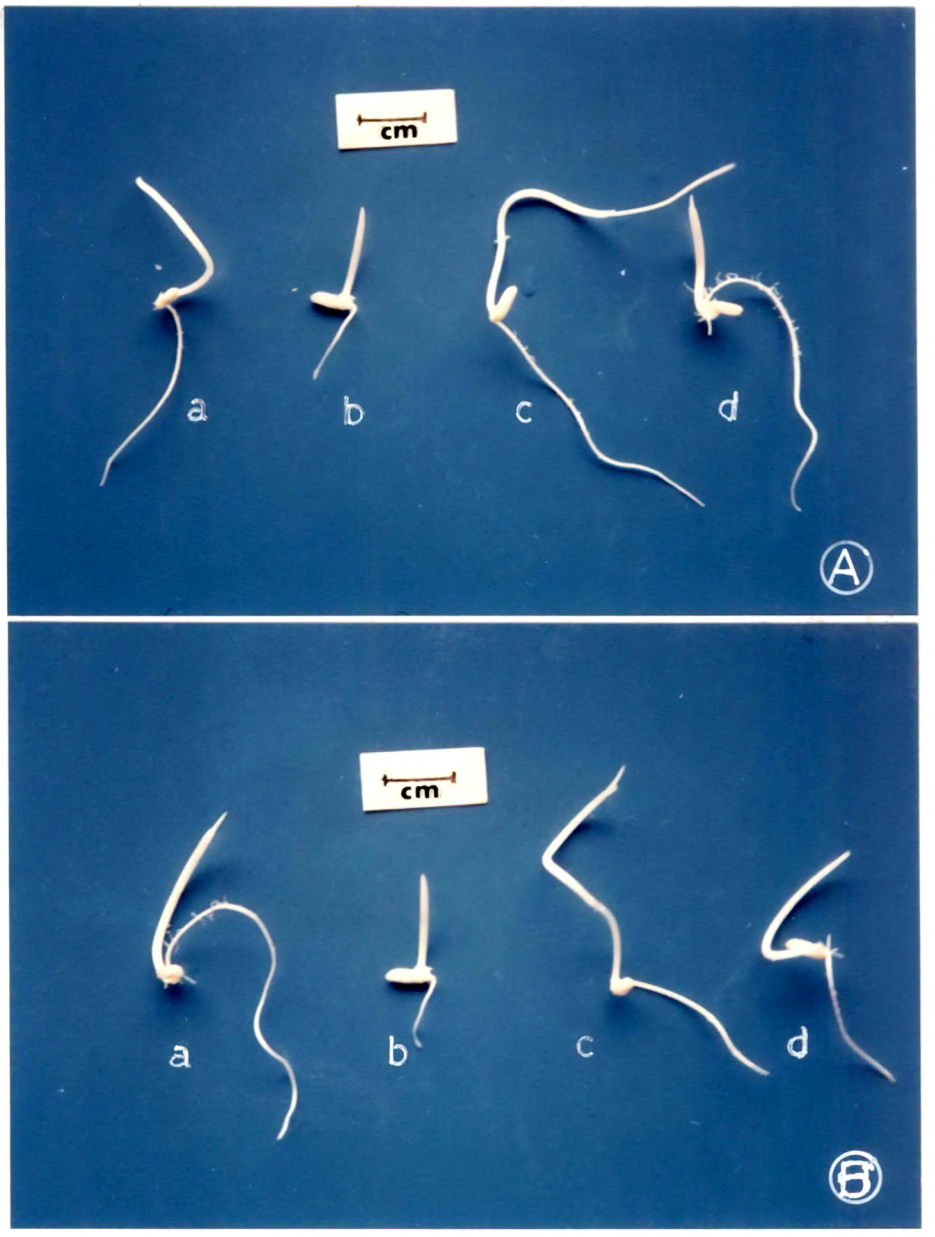

FIG. 2

Structure of rice grain

The grain or fruit (caryopsis) of rice is the ripened ovary containing the matured ovule It is closely invested by the fertile lemma and palae called the husk or hull. The fruit contains a new plant in miniature and is well provided with food reserves to sustain the embryonic plant till it is well established. The seed coat (testa) is the product of integuments, the perisperm is derived from the nucellus, the endosperm is produced as a result of fusion between one male generative nucleus and two polar nuclei and the embryo is a result of fertilization of the ovum by a male gamete In addition, the extra ovular tissue, especially the ovary wall (pericap) becomes closely associated with the seed during its formation

The husk of rice is highly silicious hence ideal for SEM studies The following observations were made on the basis of scanning of the husk near the germ region

Near the base of the husk lie two small glumes which are similar in size and shape Palae is slightly smaller than lemma and it is similar in structure and shape (Fig. 3) The outer epidermis of husk has wavy margins due to uneven deposition of silica (Fig 4B,C). These irregularly undulated tangential walls of the abaxial epidermis, gives husk a comb shaped appearence (Fig 4A). The epidermal cells are with numerous simple projections called papillae (Fig 4C,D). The papillae are arranged in parallel rows (Fig. 4C). At regular intervals trichomes of 50 to 55 μm length are observed (Fig 4B) The epidermis is characterised by the presence of

Fig. 3 : Rice caryopsis X128.
a and b. glumes c palae d. lemma

FIG. 3

Fig. 4 A-F : Husk of rice under SEM

A The outer surface of the husk at low magnification showing comb shaped appearance of epidermis (arrowhead). X500.

B A close view of epidermal surface. X900.

C Epidermal surface showing stomata (arrows), papillae and trichome. X2000.

D A magnified view of epidermal papillae. X2000.

E Husk of a seed after NaCl (0.15M) exposure. Note the thick deposition on the epidermis (arrowhead). X900

F. Magnified view of 'E' showing thick deposition on papillae (arrowhead). X2000.

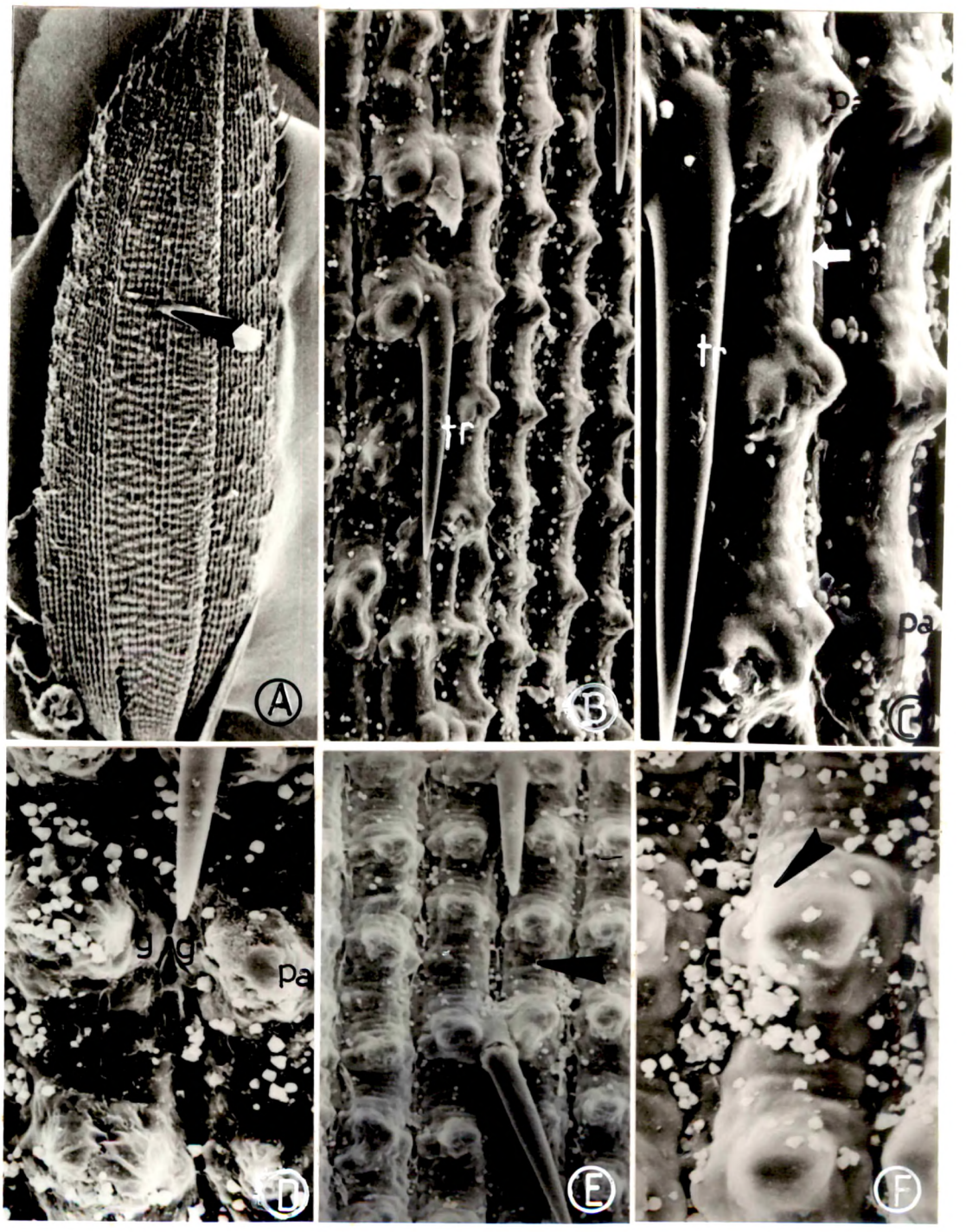

FIG.4

Fig. 5 : Longitudinal section of rice grain (adapted from Grist - "Rice") showing the scan line of EDX-analysis.

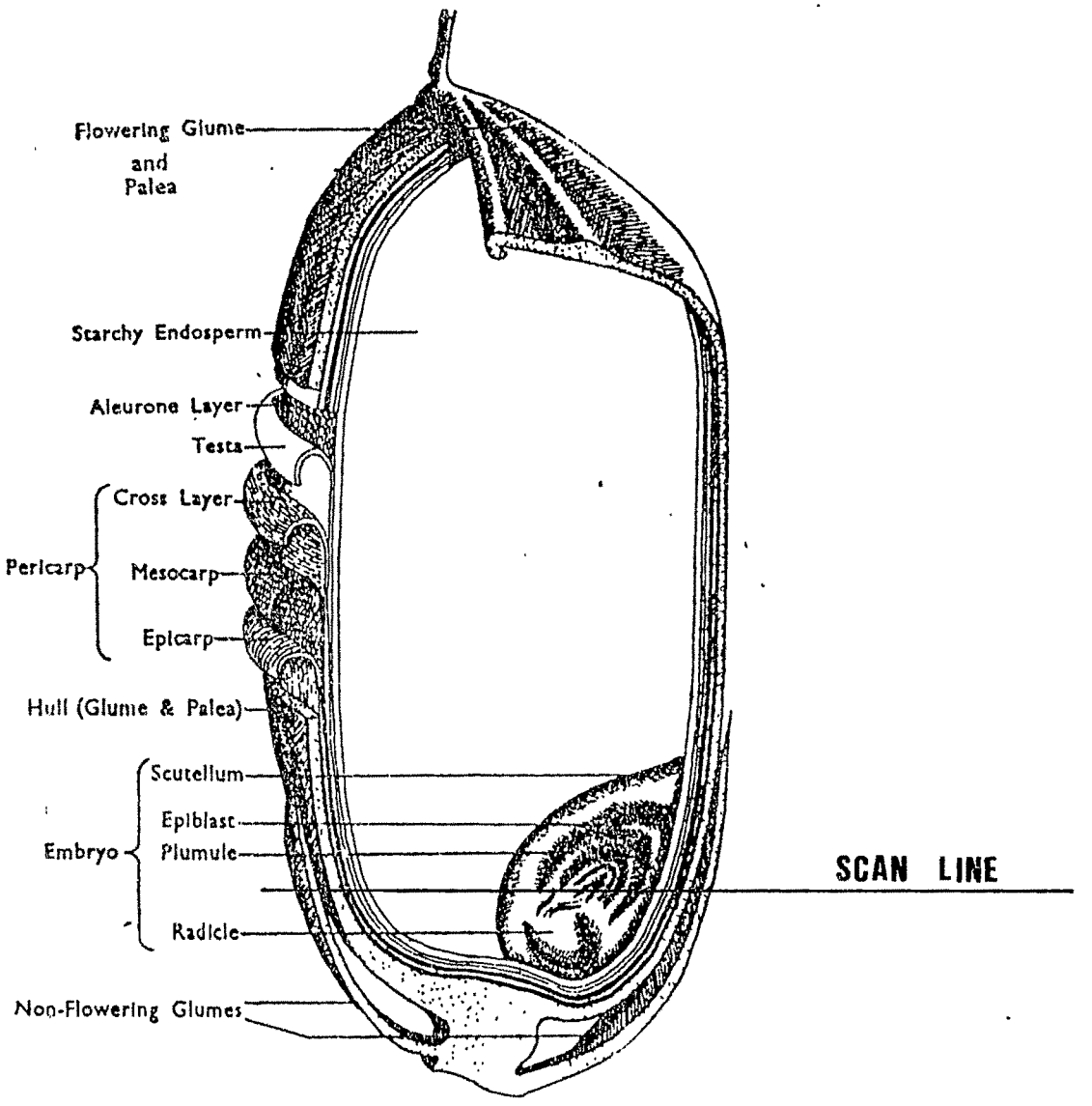
Flowering Glume and Palea
Starchy Endosperm
Aleurone Layer
Testa
Pericarp
Cross Layer
Mesocarp
Epicarp
Hull (Glume & Palea)
Embryo
Scutellum
Epiblast
Plumule
Radicle
Non-Flowering Glumes
SCAN LINE

FIG. 5

numerous stomata distributed throughout (Fig. 4C). Eventhough their distribution is more on the ridges, they are also present on the papillae (Fig. 4C). The deposition of silica is more on the papillae and trichomes compared to other region of the husk (Fig. 4D).

The husk of NaCl exposed seeds had a thick deposition all over its surface (Fig. 4E). The granules and papillae had a thick coating over them masking the stomata (Fig. 4F).

Energy dispersive X-ray microanalysis

Elemental analysis of the longitudinal section of the germinated rice seed along with the husk was done. It was carried out in the initial stages of germintaion but a comparatively good spectra was obtained only after 120 h of germination.

The husk epidermis of rice has high deposition of silica. As the aleurone layers are very close to the husk, a very high peak of silicon (Si) was observed in the X-ray spectra of aleurone tissue of all treatments. The silicon peak was also superimposed in the spectra of other regions. The irregular surface remained a limiting factor while scanning the areas, especially of endosperm. Later on, relatively flat areas were selected and the spectra were obtained.

Three tissue regions i.e, scutellum, endosperm and aleurone were scanned for the detection of elements. In the non-stressed seeds, a clear peak each of K^+, Ca^{2+} and S was obtained in all the scanned regions (Figs. 6,7,8). Mg^{2+} also was

traced except in the aleurone region (Fig. 8). The total mean peak to background numbers (Table 5) give a rough estimate of how much of the elements K^+, Ca^{2+}, Mg^{2+} and S combined were present in the different tissues of control seeds. (However, this percentage of total mean peak to background numbers should not be confused for the actual percent of the elements in the tissues). Ca^{2+} content was more in the aleurone while, K^+, and S did not show much difference in aleurone and scutellum (Table 5). Mg^{2+} peak was not distinguishable in the aleurone but as compared to other elements it showed high percentage in the endosperm (Fig. 8, Table 5).

The EDX analysis of the tissue regions of NaCl exposed seeds did not show sharp peaks of K^+, Ca^{2+}, Mg^{2+} or S (Fig. 9). A high percentage of total mean to background number of Na^+ and Cl^- was obtained in the aleurone tissue followed by scutellum and endosperm (Table 6).

Accumulation of Na^+ in the tissue regions was considerably limited by the treatment of GA_3 or putrescine (Figs. 11 to 15). At the end of 120 h the levels of Na^+ in the scutellum, aleurone and endosperm tissues of GA_3 treated salinized seeds were 19, 26 and 9 percent respectively less than that of salt control (Table 8). Putrescine treatment of salinized seeds contained much less Na^+ (21,26 and 31 percent respectively) than, GA_3 treated salt stressed ones (Table 8,10). Similarly, the analysis of Cl^- indicates that Cl^- accumulation is sensitive to both GA_3 and putrescine. A reduction in the level of Cl^- to the tune of 45,44 and 22 percent was

noticed in the scutellum, aleurone and endosperm of putrescine treated salinized seeds compared to salt control. GA_3 administration also reduced the accumulation of Cl^- in the scutellum, aleurone and endosperm tissues to 33,47 and 16 percent respectively.

Spectra of K^+, Ca^{2+}, Mg^{2+}, and S were obtained in the salt stressed seeds on application of GA_3 or putrescine (Table 8,10). But their percentage of peak to background number could not exceed the control except in the level of Mg^{+2} in the scutellum and endosperm of GA_3 treated salinized seeds (Table 8). The Mg^{2+} peak was not observed in any of the aleurone tissue except in case of control (Table 5). The calcium percentage in the putrescine exposed scutellum and aleurone cells of salinized cells showed a decrease of 37 and 16 percent respectively compared to the control (Table 10). Similarly in case of GA_3 treated salinized seeds also, there was a reduction of 37 and 27 percent (Table 8). Application of GA_3 showed traces of Fe in all the scanned regions (Table 7) of non-salinized seeds but not in salinized ones. It could also bring about an increase in the peak to background number of S in the aleurone and endosperm of non-stressed seeds (Table 7). GA_3 brought about a better result in elevating the peaks of Mg^{2+}, K^+ and S compared to putrescine in NaCl stressed (Table 8,10) as well as in non-stressed (Table 7,9) conditions.

Table 1 : Effect of NaCl salinity on germination and seedling growth of rice (after 120 h of incubation). Each value is the average of 50 separate measurements.

NaCl Concentration (M)	% germination	Fresh weight of embryo axis (mg)	Dry weight of embryo axis (mg)	Dry weight % compared to the control value	Length (root tip to coleoptile tip) (mm)
0.0	100 ± 0.0	14.5 ± 1.3	16.5 ± 0.06	100	50 ± 1.1
0.5	Nil	-	-	-	-
0.4	11.1± 0.1	No significant growth			2 ± 0.02
0.3	26 ± 1.6	17 ± 0.03	0.2 ± 0.004	1.2	5 ± 0.08
0.25	40 ± 1.3	28 ± 0.6	2.2 ± 0.03	13.3	12 ± 0.35
0.2	46 ± 2.3	60 ± 1.6	6.7 ± 0.4	40.3	18 ± 0.5
0.15	72 ± 2.2	65 ± 0.2	8.0 ± 0.09	48.4	21 ± 0.3
0.1	100± 0.0	98 ± 0.1	11.1 ± 0.01	67.3	39 ± 0.5

Table 2 : Effect of GA_3 and NaCl on average dryweight of endosperm and embryo axis, root and shoot length of rice seedlings (after 120h of germination). Each value is the average of 50 separate measurements.

Treatment	Dry weight of endosperm (mg)	Dry weight of embryo axis (mg)	Root length (mm)	Shoot length (mm)
Control	14.3 ± 0.02	16.5 ± 0.01	35 ± 0.82	15 ± 0.4
NaCl (0.15 M)	19.7 ± 0.05	8 ± 0.02	13 ± 0.23	8 ± 0.1
GA_3 (10 ppm)	11.5 ± 0.03	22 ± 0.01	50 ± 0.5	21 ± 0.6
GA_3 (10 ppm) + NaCl (0.15 M)	15.1 ± 0.01	13.5 ± 0.005	28 ± 0.02	16 ± 0.3

Table 3 : Effect of putrescine and NaCl on endosperm and embryo axis, root and shoot length of rice seedling (after 120 h of germination). Each value is the average of 50 separate measurements.

Treatment	Dry weight of endosperm (mg)	Dry weight of embryo (mg)	Root length (mm)	Shoot length (mm)
Control	14.3 ± 0.02	16.5 ± 0.01	35 ± 0.82	15 ± 0.4
NaCl (0.15 M)	19.73 ± 0.05	8 ± 0.02	13 ± 0.23	8 ± 0.1
Putrescine (10^{-5} M)	12.2 ± 0.07	18.7 ± 0.03	39 ± 0.4	18 ± 0.5
Putrescine (10^{-5} M) + NaCl (0.15M)	15.7 ± 0.03	11.6 ±0.01	20 ± 0.2	14 ± 0.08

Table 4: Effect of NaCl, GA_3 and putrescine on germination of rice. Each value is the average of 4 replicates (25 seeds each).

Treatment	Duration of germination (h) 24	48	72	96	120
Control	0.0	72 ± 1.5	95 ± 2.1	100 ± 0.0	100 ± 0.0
NaCl (0.15M)	0.0	41 ± 1.6	48 ± 2.8	60 ± 1.5	72 ± 1.2
GA_3 (10 ppm)	0.0	96 ± 2.6	100 ± 0.0	100 ± 0.0	100 ± 0.0
GA_3 (10 ppm) +NaCl(0.15M)	0.0	58 ± 3.0	78 ± 2.1	90 ± 1.8	94 ± 0.9
Putrescine (10^{-5}M)	0.0	92 ± 3.1	100 ± 0.0	100 ± 0.0	100 ± 0.0
Putrescine (10^{-5}M) +NaCl(0.15M)	0.0	56 ± 2.3	77 ± 1.7	86 ± 2.1	91 ± 2.3

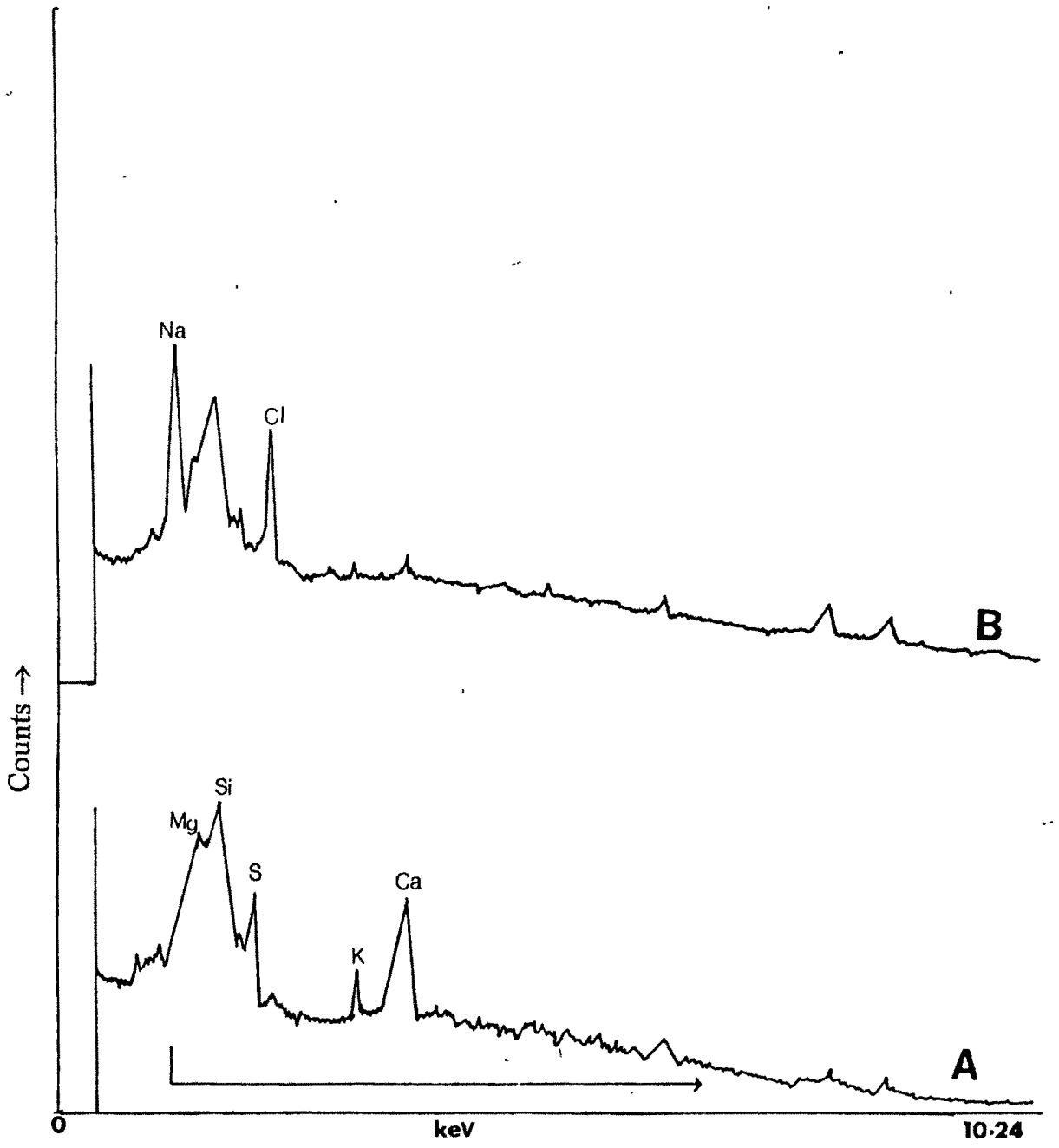

Fig. 6. Energy dispersive X-ray spectra of rice scutellum (after 120h of incubation) under A.non-stressed B.NaCl (0.15M) stressed conditions.

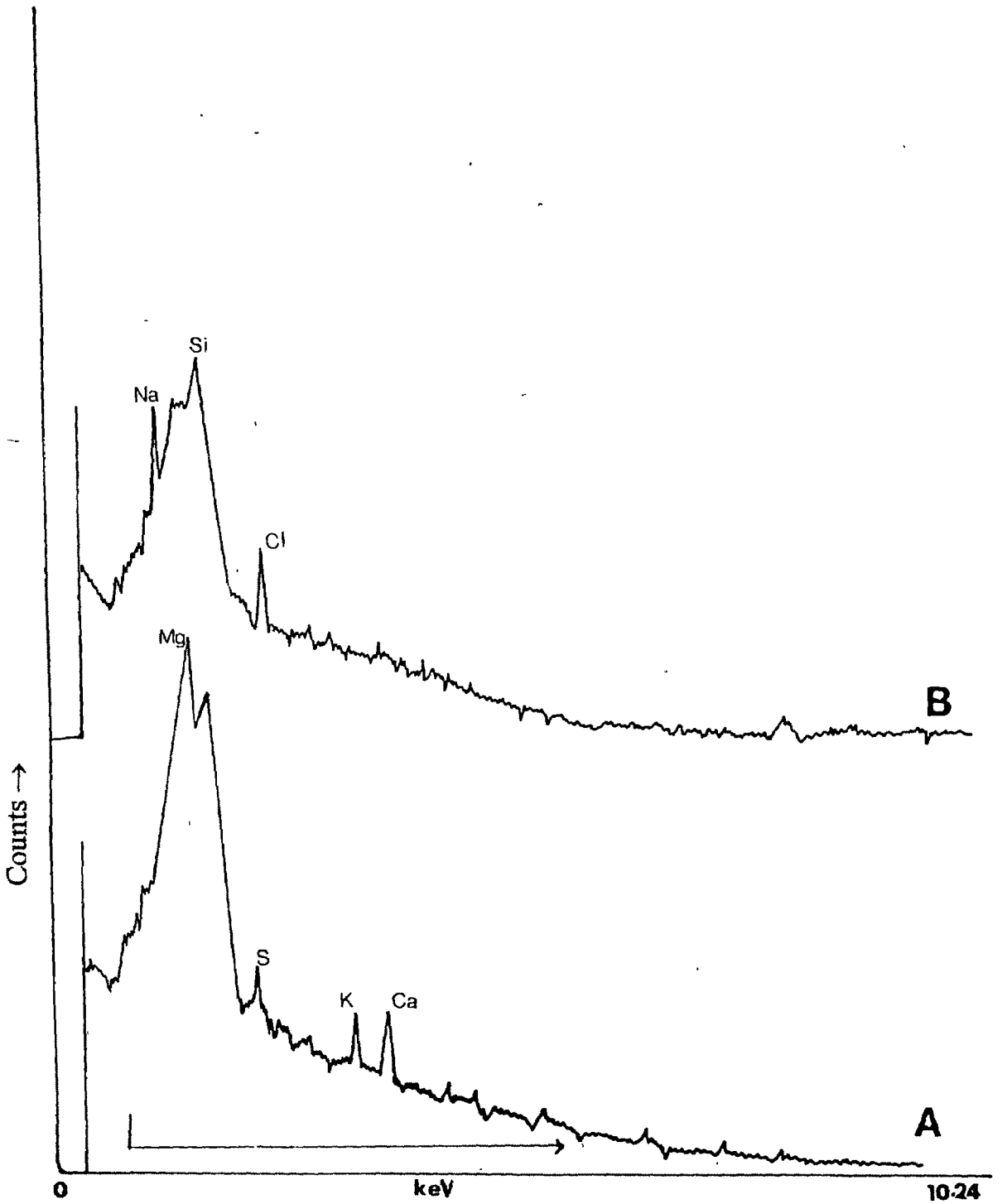

Fig. 7. Energy dispersive X-ray spectra of rice endosperm tissue (after 120h of incubation)under A.non-stressed B.NaCl (0.15M) stressed conditions.

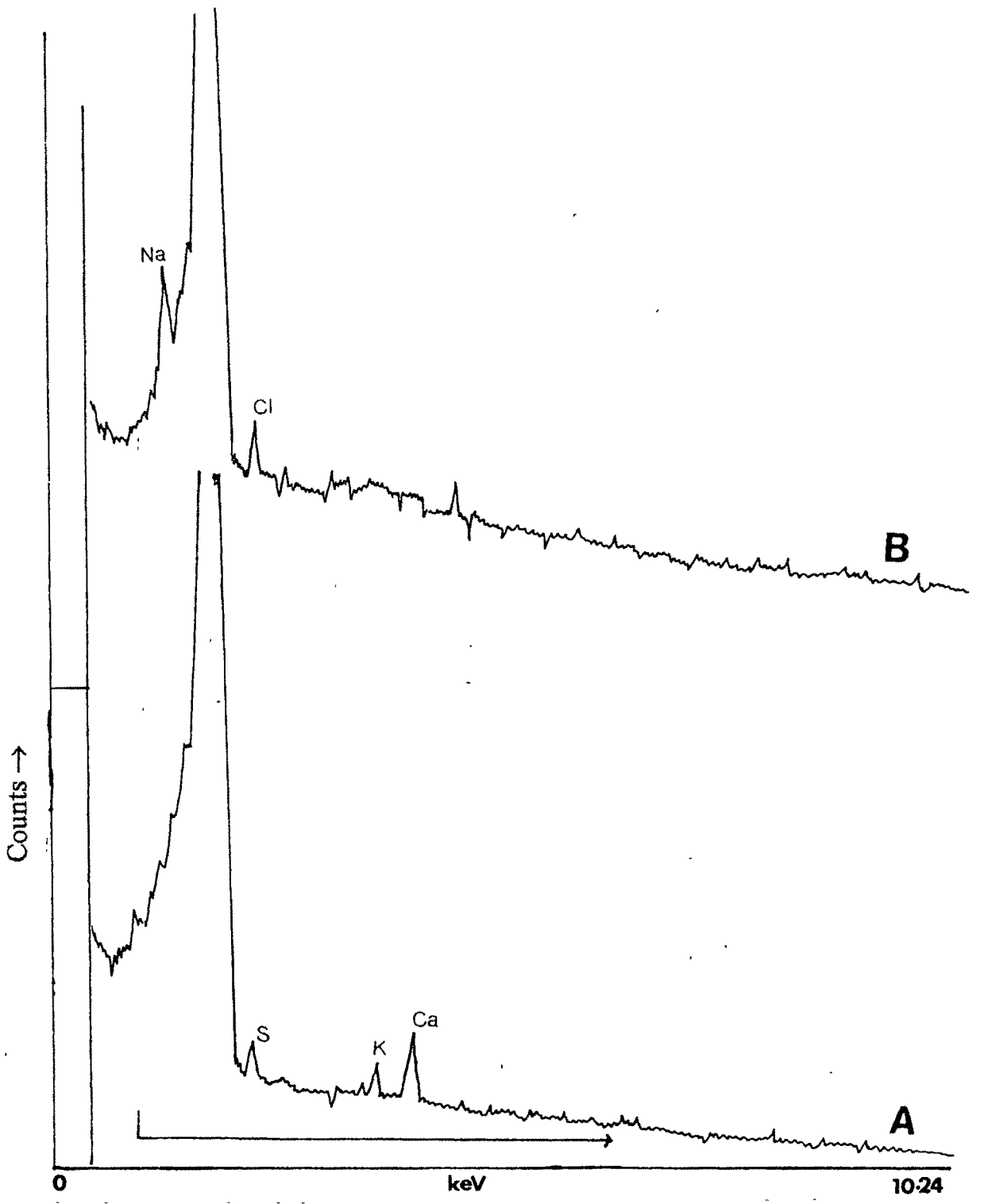

Fig. 8. Energy dispersive X-ray spectra of rice aleurone tissue (after 120h of incubation) under A.non-stressed B.NaCl (0.15M) stressed conditions.

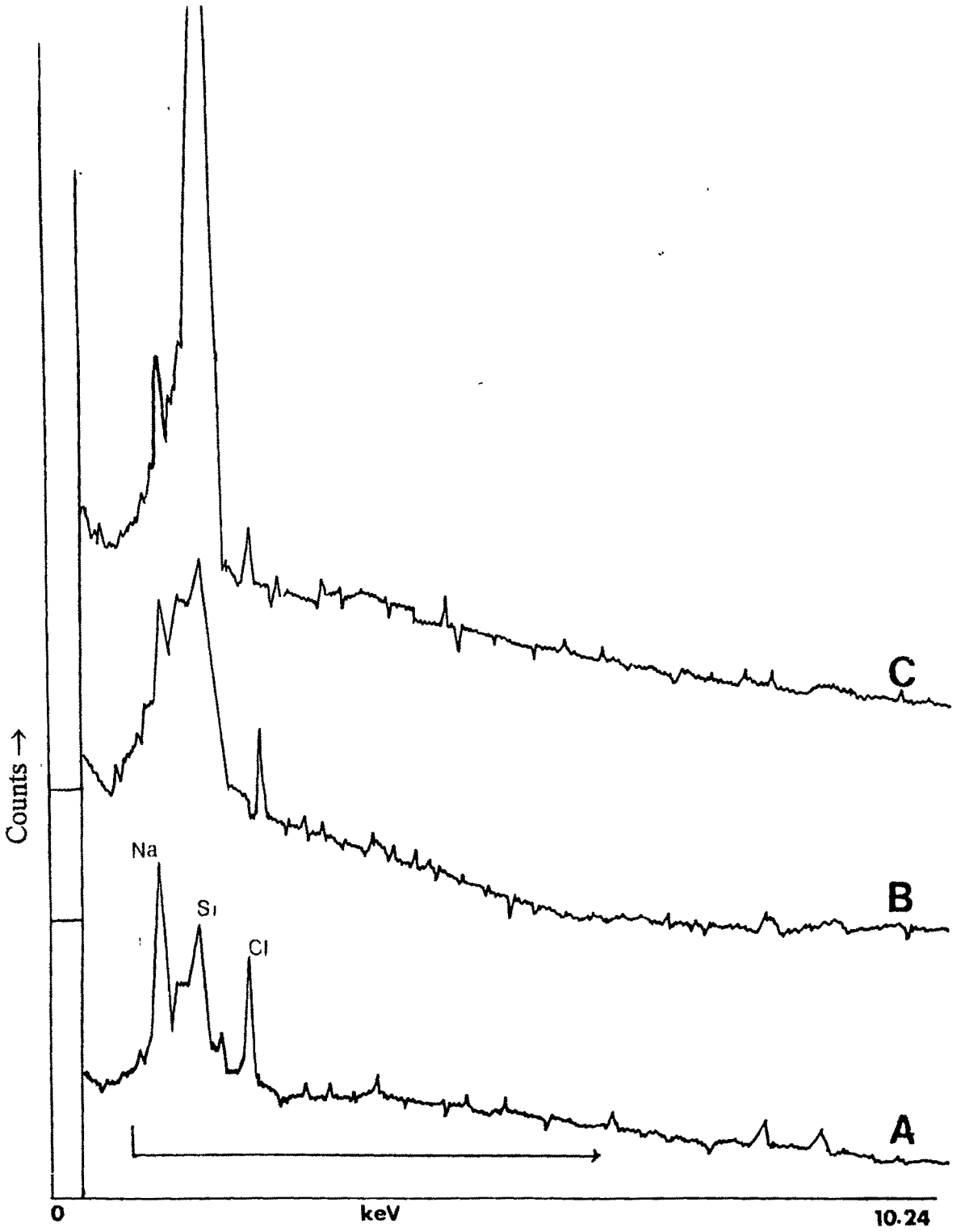

Fig. 9. Energy dispersive X-ray spectra of rice seed (after 120h of incubation)under NaCl (0.15M) stressed condition. A.Scutellum B.Endosperm C.Aleurone tissues.

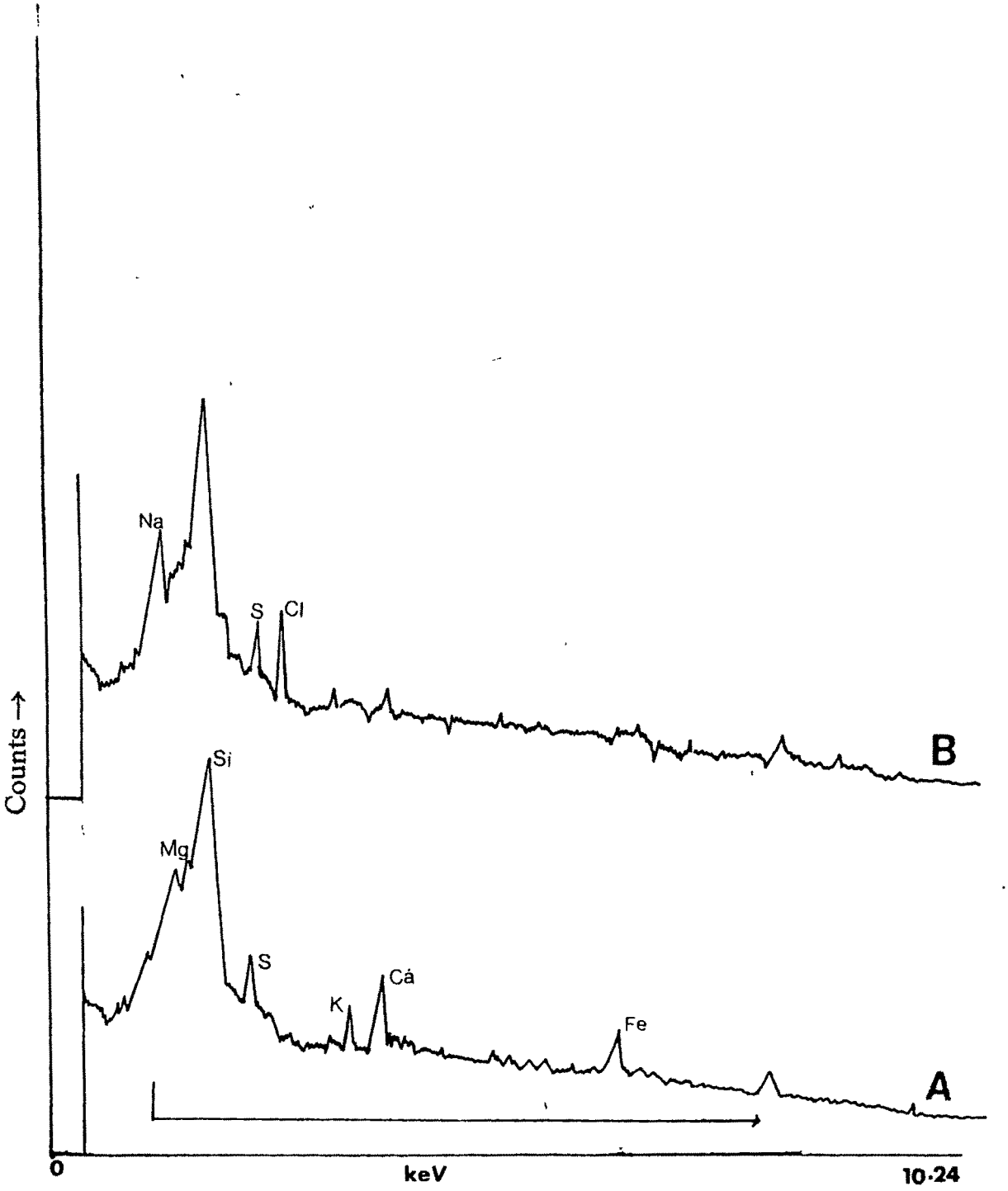

Fig. 10. Energy dispersive X-ray spectra of rice scutellum (after 120h of incubation) exposed to A.GA_3 (10ppm) B.NaCl (0.15M) containing GA_3 (10ppm).

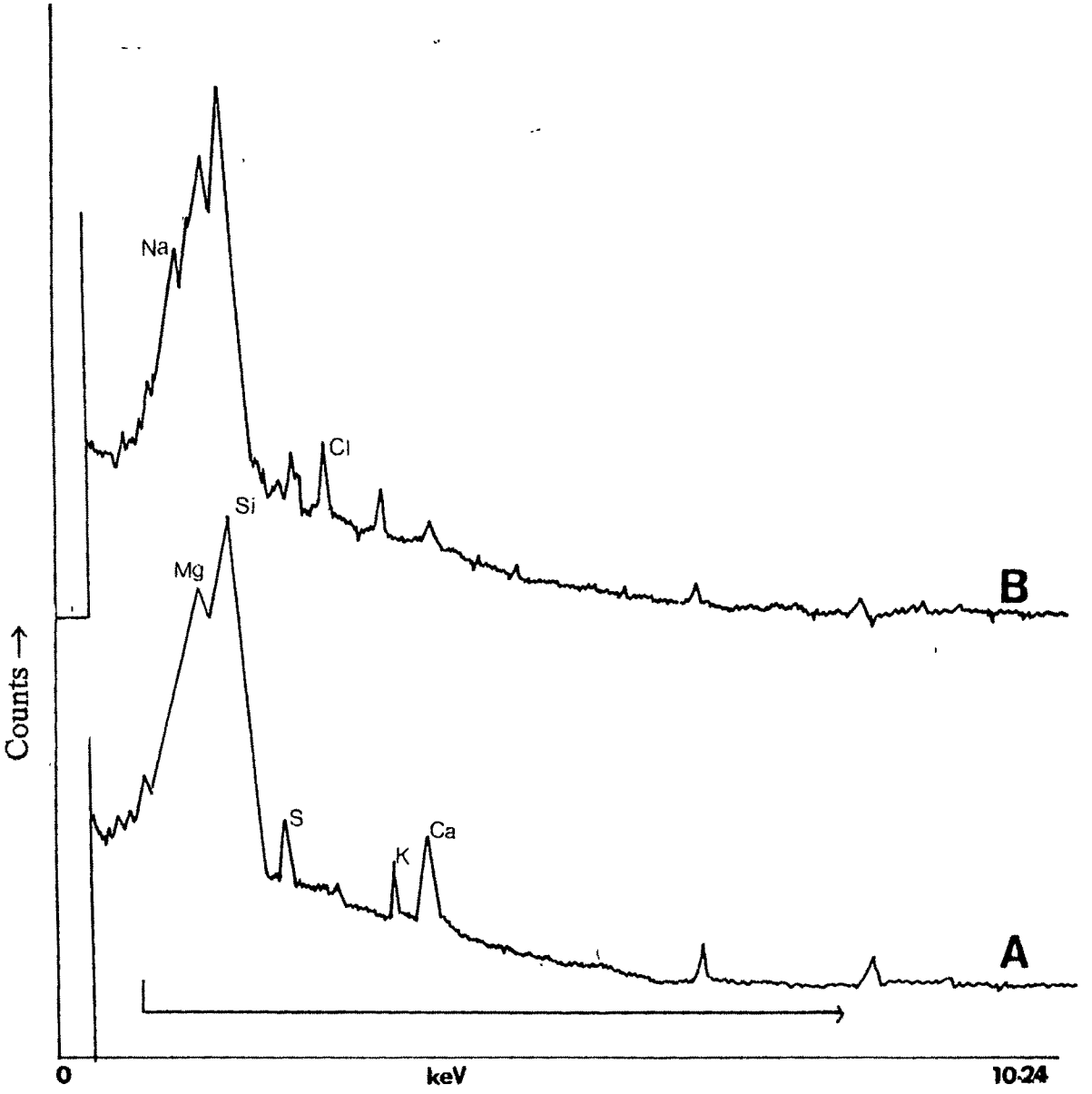

Fig. 11. Energy dispersive X-ray spectra of rice endosperm (after 120h of incubation)exposed to A.GA_3 (10ppm) B.NaCl (0.15M) containing GA_3 (10ppm).

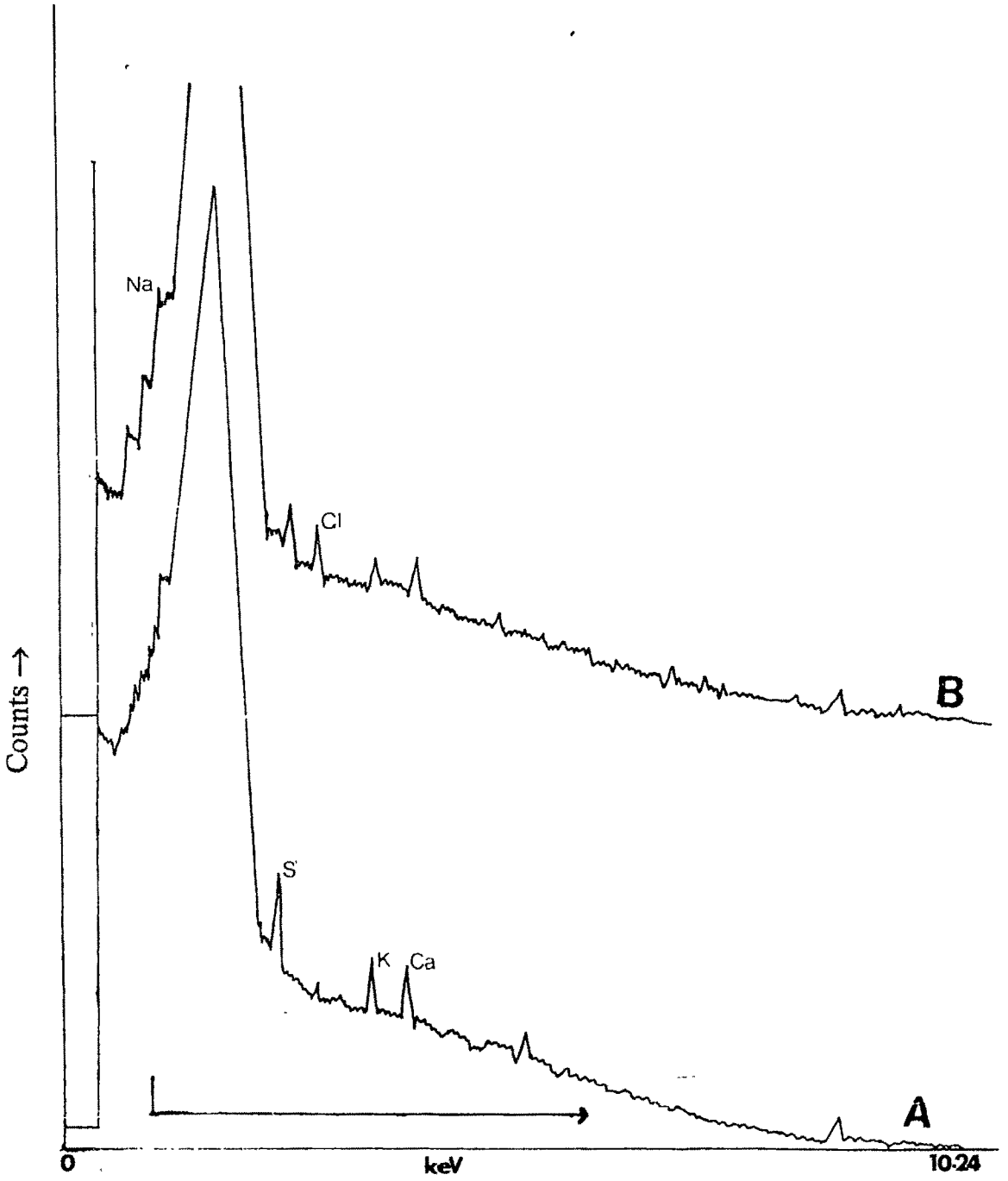

Fig. 12. Energy dispersive X-ray spectra of rice aleurone tissue (after 120h of incubation) exposed to A.GA_3 (10ppm) B.NaCl (0.15M) containing GA_3 (10ppm).

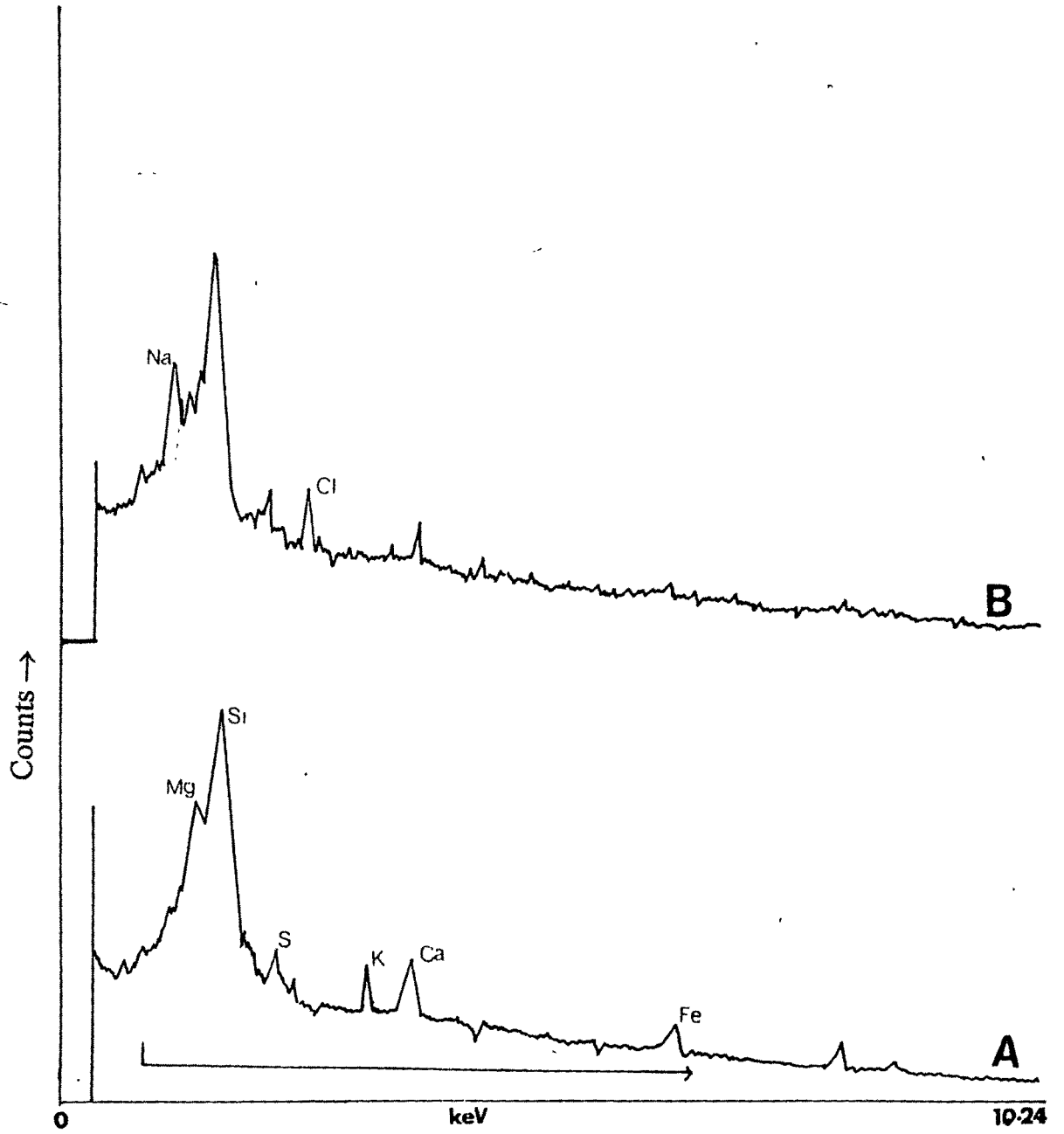

Fig. 13. Energy dispersive X-ray spectra of rice scutellum tissue (after 120h of incubation) exposed to A.putrescine (10^{-5} M) B.NaCl (0.15M) containing putrescine (10^{-5} M).

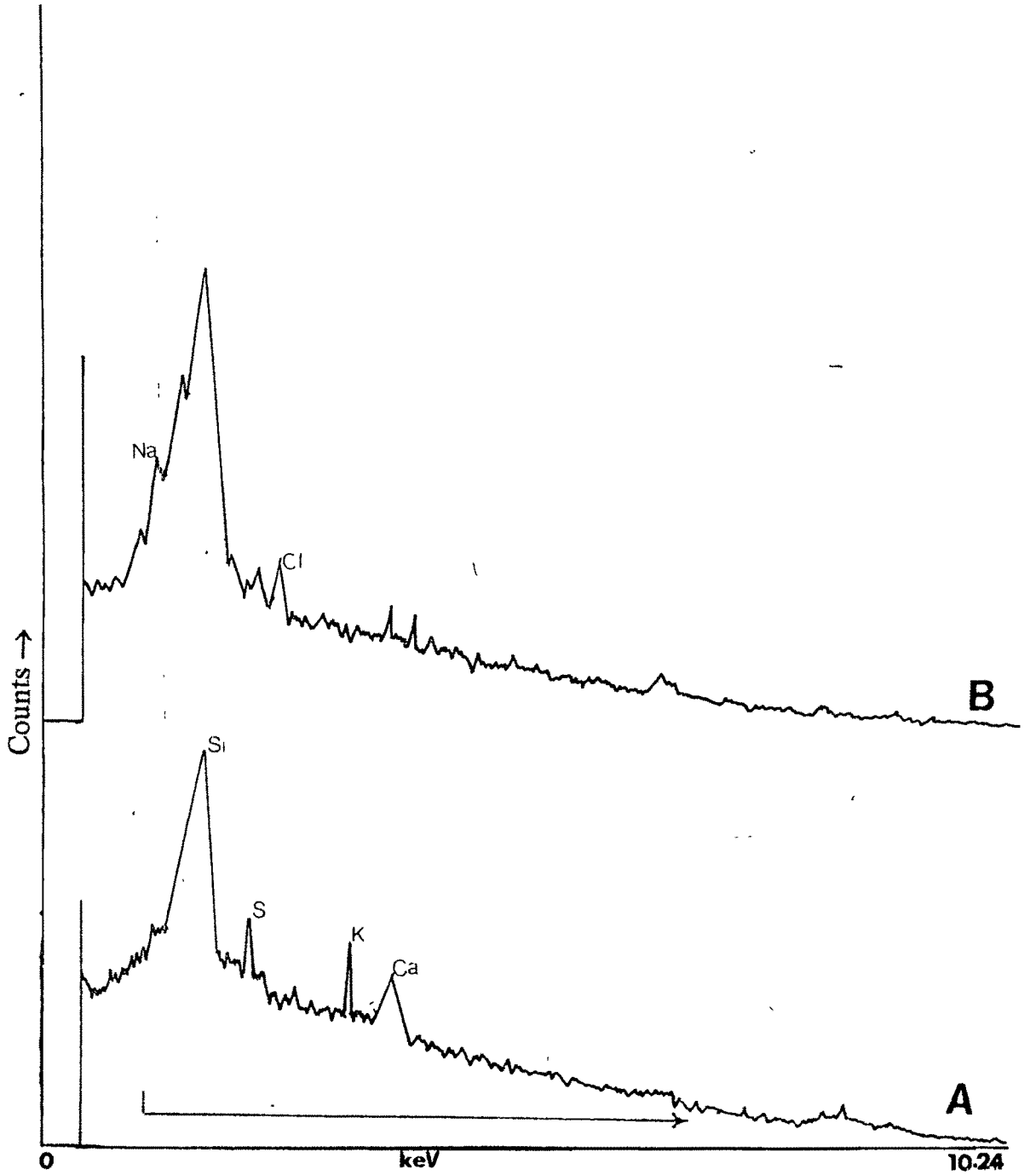

Fig. 14. Energy dispersive X-ray spectra of rice endosperm tissue (after 120h of incubation) exposed to A.putrescine (10^{-5} M) B.NaCl (0.15M) containing putrescine (10^{-5} M).

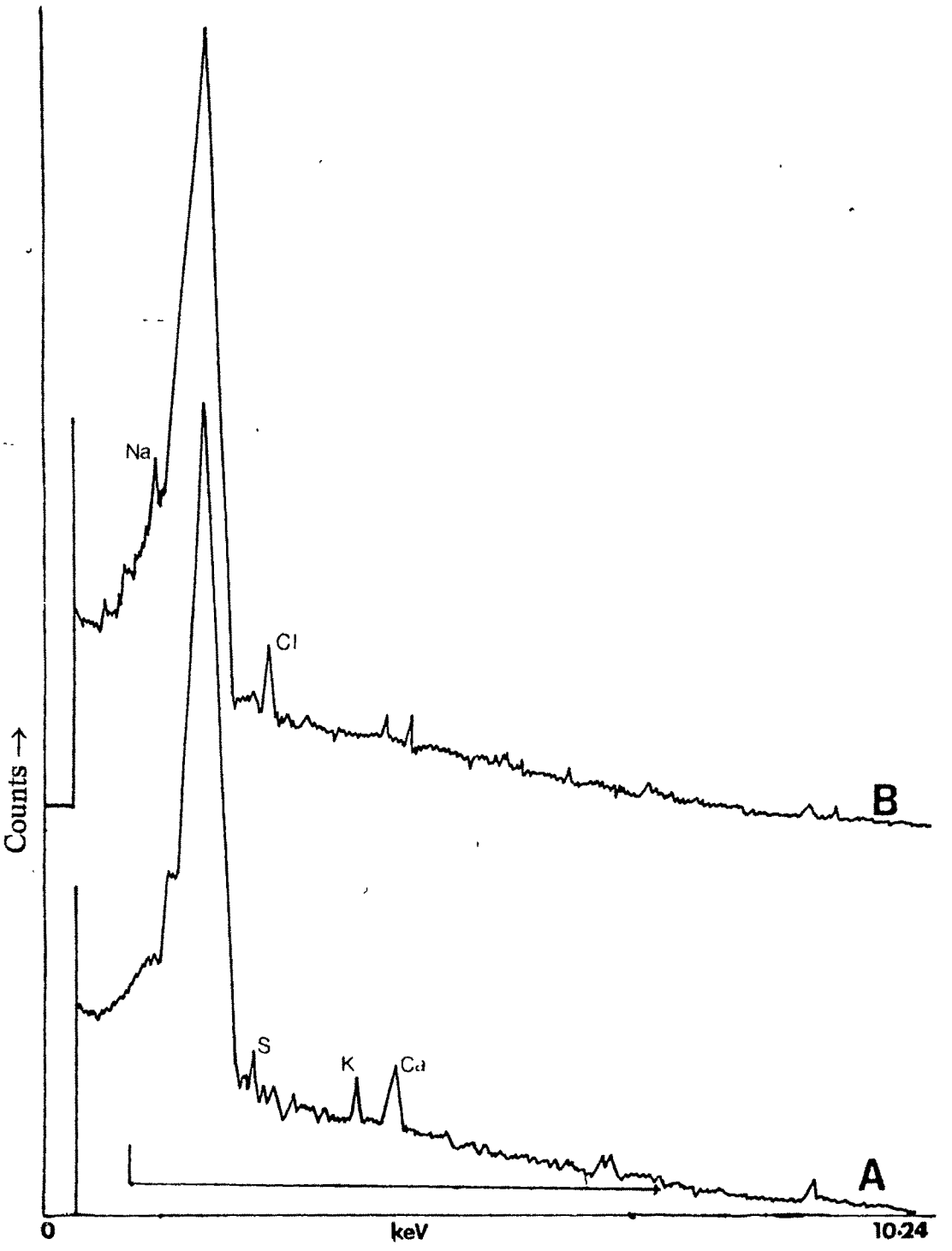

Fig. 15. Energy dispersive X-ray spectra of rice aleurone tissue (after 120h of incubation) exposed to A.putrescine (10^{-5} M) B.NaCl (0.15M) containing putrescine (10^{-5} M).

Table 5 : The total mean peak to background numbers and percentages of the elements in the various tissue regions of rice germinated for a period of 120h at non-stressed conditions.

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
Cl^-	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
K^+	Scutellum	24	16
	Aleurone	28	18.7
	Endosperm	17	11.3
Ca^{2+}	Scutellum	45	30
	Aleurone	95	63.3
	Endosperm	-	-
Mg^{2+}	Scutellum	35	23.3
	Aleurone	28	18.7
	Endosperm	24	16
S	Scutellum	36	24
	Aleurone	35	23.3
	Endosperm	22	14.7

Table 6 : The total mean peak to background numbers and percentages of the elements in the various tissue regions of rice germinated for a period of 120h at NaCl (0.15M) stressed condition.

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	54	36
	Aleurone	74	49.3
	Endosperm	43	28.7
Cl^-	Scutellum	40	26.7
	Aleurone	45	30
	Endosperm	32	20
K^+	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
Ca^{2+}	Scutellum	-	-
	Aleurone	34	22.7
	Endosperm	-	-
Mg^{2+}	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
S	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-

Table 7 : The total mean peak to background numbers and percentages of the elements in the various tissue regions of rice seeds germinated for a period of 120h under the influence of GA_3 (10 ppm).

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
Cl^-	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
K^+	Scutellum	27	18
	growth Aleurone	30	20
	Endosperm	30	20
Ca^{2+}	Scutellum	31	20.6
	Aleurone	24	16
	Endosperm	34	22.7
Mg^{2+}	Scutellum	50	33.3
	Aleurone	-	-
	Endosperm	71	47.3
S	Scutellum	34	22.7
	Aleurone	44	29.3
	Endosperm	37	24.7
Fe	Scutellum	21	14
	Aleurone	18	12
	Endosperm	17	11.3

Table 8 : The total mean peak to background numbers and percentages of the elements in the various tissue regions in rice seeds germinated for a period of 120h in NaCl (0.15M) solution containing GA_3 (10 ppm).

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	44	29.3
	Aleurone	68	45.3
	Endosperm	56	37.3
Cl^-	Scutellum	27	18
	Aleurone	34	22.7
	Endosperm	27	18
K^+	Scutellum	20	13.3
	Aleurone	24	16
	Endosperm	19	12.7
Ca^{2+}	Scutellum	16	10.7
	Aleurone	25	16.7
	Endosperm	14	9.3
Mg^{2+}	Scutellum	41	27.3
	Aleurone	-	-
	Endosperm	7	46.7
S	Scutellum	34	22.7
	Aleurone	33	22
	Endosperm	24	16

Table 9 : The total mean peak to background numbers and percentages of the elements in the various tissue regions in rice seeds germinated for a period of 120h under the influence of putrescine (10^{-5} M).

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
Cl^-	Scutellum	-	-
	Aleurone	-	-
	Endosperm	-	-
K^+	Scutellum	21	14
	Aleurone	22	14.7
	Endosperm	33	22
Ca^{2+}	Scutellum	32	14.7
	Aleurone	24	16
	Endosperm	28	18.7
Mg^{2+}	Scutellum	47	31.3
	Aleurone	-	-
	Endosperm	-	-
S	Scutellum	24	16
	Aleurone	26	17.3
	Endosperm	37	24.7

Table 10 : The total mean peak to background numbers and percentages of the elements in the various tissue regions in rice seeds germinated for a period of 120h in NaCl (0.15M) solution containing putrescine (10^{-5} m).

Element	Tissue region	Mean peak to background number (mm)	% of peak background number
Na^+	Scutellum	43	28.6
	Aleurone	55	36.6
	Endosperm	30	20
Cl^-	Scutellum	22	14.6
	Aleurone	25	16.7
	Endosperm	25	10.7
K^+	Scutellum	16	10.7
	Aleurone	15	10
	Endosperm	17	11.3
Ca^{2+}	Scutellum	17	11.3
	Aleurone	15	10
	Endosperm	16	10.7
Mg^{2+}	Scutellum	38	25.3
	Aleurone	-	-
	Endosperm	-	-
S	Scutellum	23	15.3
	Aleurone	-	-
	Endosperm	-	-

Histochemical studies

Seeds incubated for 24h were taken as the representative tissue during the early phase of germination. The section of seeds showed following features.

The outer covering of the seed is pressed to form flat layers. Inner to this lies the endosperm which is made up of parenchyma cells filled with starch granules (compound) and protein bodies (Figs. 16A, 17A). The outer layer of endosperm is made up of rectangular cells called aleurone cells rich in protein bodies and lipid globules (Figs. 17A, 18A). The number of aleurone layers varied in different regions of the rice grain. Near the germ they are of single layer and away from that the number varied from 2 to 4.

The outline of the aleurone cells of seeds incubated for 24h were highly irregular (Fig. 16A). The aleurone cells showed the presence of scattered cytoplasmic polysaccharides which took pink stain when treated with Schiff's reagent (Fig. 16B). Their cell walls also took dark stain. The starch grains in the endsoperm were large, overlapping and seen close to the aleurone (Fig. 16A,B). CBB stained the cytoplasm of aleurone cells positively. The entire cytoplasm took the stain indicating the presence of numerous protein rich bodies (Fig. 17A). The cytoplasm was dense and not vacuolated. Endosperm of these seeds also contained number of protein bodies (Fig. 20A). The protein content was more towards periphery than central endosperm, as a result the entire subaleurone region turned blue which appeared like overstaining (Fig. 20A). Lipid globules were also

localized in the aleurone. But they were absent in the endosperm (Figs. 18A, 21A).

Many changes were observed in the histochemistry of aleurone cells and endosperm of seeds after 120h of germination. The aleurone cells became turgid and their outline became more regular (Fig. 16C). The polysaccharide contents in the cytoplasm disappeared (Fig. 16C). The cell walls of aleurone cells became thin. The protein mass in the aleurone cells were very much shrunk (Fig. 20B). The aleurone cells were also characterised by the presence of persistent vacuoles within them (Figs. 17B, 20B). Few lipid stained bodies were found in the aleurone cells after 120h of germination. As the Sudan Black B got accumulated in the vacuoles of plastic sections of aleurone tissue, an accurate interpretation was also difficult (Figs. 18B, 21B). The starch granules in the subaleurone region were less concentrated towards the aleurone layers and their size was considerably reduced (Figs. 16B, 19A). Similarly the protein content in the endsoperm was also seen to a smaller extent compared to that of seeds incubated for 24h (Figs. 17B, 20B).

Unlike the control seeds, more starch and protein bodies were localized in the endosperm of seeds exposed to NaCl for 120h. The aleurone cells were not turgid and showed irregular cell wall like that of the seeds incubated for 24h (Fig. 16D). The cytoplasmic polysaccharide contents remained undigested even after 120h of germination (Figs. 16D, 19B). Vacuolation had started in the aleurone cells, but their size was small compared to the control (Fig. 17C). The protein

bodies were also more in the aleurone cells (Fig. 20C). The starch grains in the subaleurone region were as large as to the those of 24h germinated seeds but their concentration was slightly less (Figs. 16D, 19B). More proteins were also localised in the endosperm compared to the control (Figs. 17C, 20C). The presence of lipid globules in the aleurone were not clearly distinguishable (Figs. 18C, 21C).

The aleurone cells and endosperm appeared much clear in the GA_3 treated rice seed sections compared to the control and salt exposed. No cytoplasmic polysaccharides were traced in the GA_3 exposed stressed and non-stressed seeds (Figs. 16E, F). The aleurone cells were characterised by big vacuoles, scanty protoplasm with few protein bodies (Fig. 17D). Eventhough the GA_3 treated NaCl stressed seeds showed more protein bodies in the endosperm and aleurone compared to that of GA_3 treated control seeds, they were much reduced as compared to the salt control (Figs. 17D, E) The parenchyma cells of the sub-aleurone region were very clear due to the digestion of starch grains in GA_3 treated seeds (Fig. 16E). GA_3 also increased the digestion of starch grains (Fig. 16F) and protein bodies in the endosperm (Fig. 17E) of seeds under the influence of salt. There was no significant change in lipid concentration in the aleurone of GA_3 treated salinized and non-salinized seeds (Fig. 18D, E).

Exposure to putrescine also brought about results similar to that of GA_3 treated rice seeds in hydrolysis of starch, protein and lipids (Figs. 19C, 20D, 21D).

Fig. 16 A-F : Longitudinal sections of rice seed passing through aleurone and outer endosperm stained for total polysaccharides.

A : Control after 24h of incubation. X 500.
B : Magnified aleurone cells of control. X 1250.
C,D,E,F : Control, NaCl, GA3 and NaCl containing GA3 treated after 120 h of germination respectively. X 500.

(p - pericap ; ac - aleurone cell ; sg - starch grain ; en - endosperm ; sa - subaleurone layer. white arrow indicates cytoplasmic polysaccharides, white arrow head indicates starch grains, black arrow indicates cell wall).

From Fig. 16 to 37, the concentration of NaCl, GA_3 and putrescine is 0.15M, 10 ppm and 10^{-5} M respectively wherever mentioned.

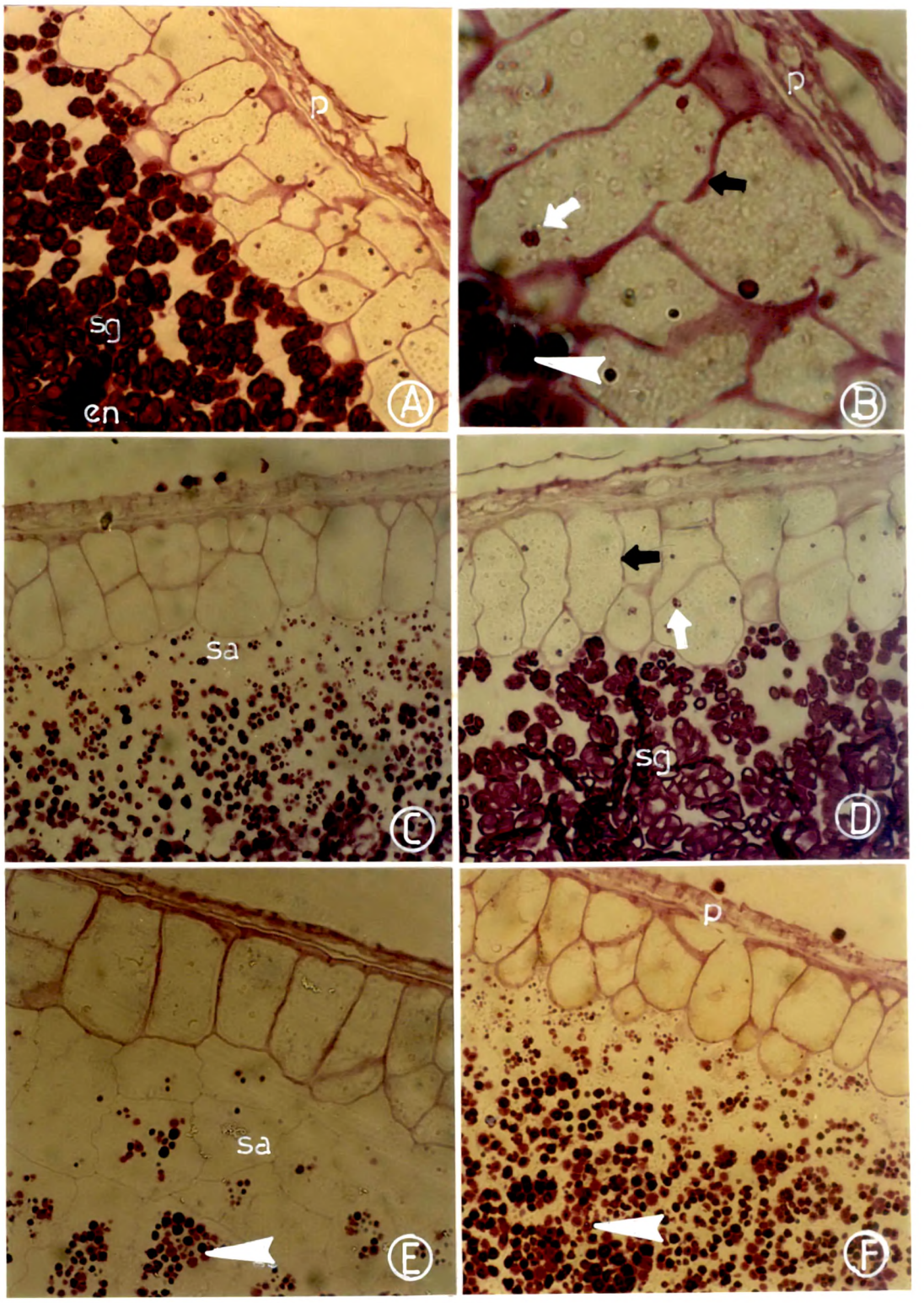

FIG.16

Fig. 17 A-E · Longitudinal sections of rice seed passing through aleurone and endosperm stained for proteins

A Control after 24 h of incubation. X 1250.

B,C,D,E · Control, NaCl, GA_3 and NaCl containing GA_3 treated after 120h of germination respectively. X 500.

(p - pericap ; ac - aleurone cell ; v - vacuole ; n - nucleus , en - endosperm. white arrow indicates protein bodies in endosperm, black arrow indicates protein bodies in the aleurone cell)

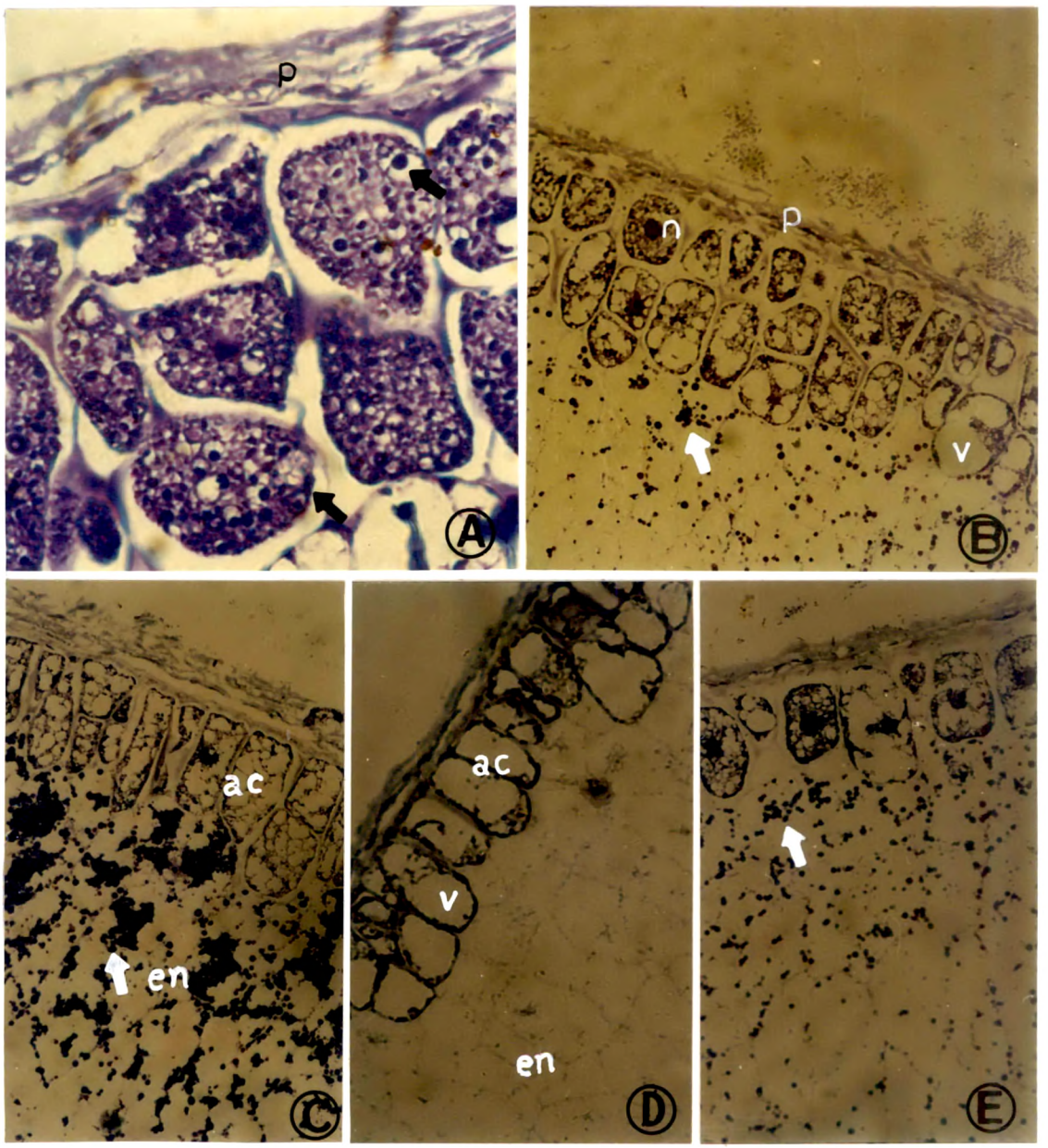

FIG.17

Fig. 18 A-E : Longitudinal sections of rice seed passing through aleurone and endosperm stained for lipids.

A Control after 24 h of incubation X 1250.

B,C,D,E : Control, NaCl, GA_3 and NaCl containing GA_3 treated after 120h of germination receptively. X 500

(sa -sub aleurone; white arrow indicates lipid globules, ac - aleurone cell, p - pericarp, v - vacuole) .

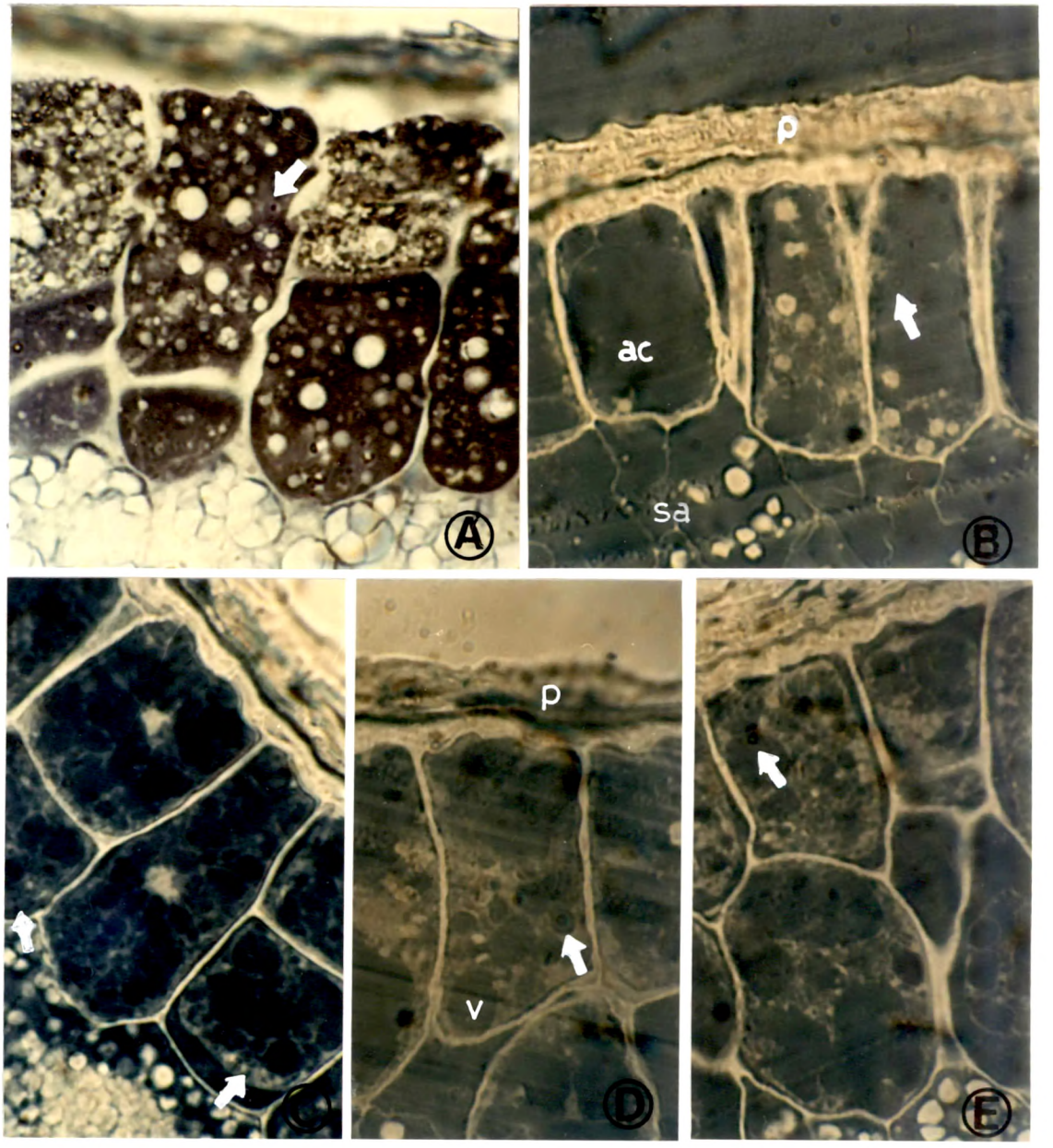

FIG.18

Fig 19 A-D : Longitudinal sections of rice seed passing through aleurone and endosperm stained for total polysaccharides

A Control X 500.

B,C,D . treated with NaCl, putrescine, NaCl containing putrescine respectively. X 500

(p - pericap ; sa - subaleurone ; ac - aleurone cell , sg - starch grains). Note the small starch grains in the subaleurone region (thick arrow), wavy margin of cell wall (thin arrow), cytoplasmic polysaccharides (arrow head)

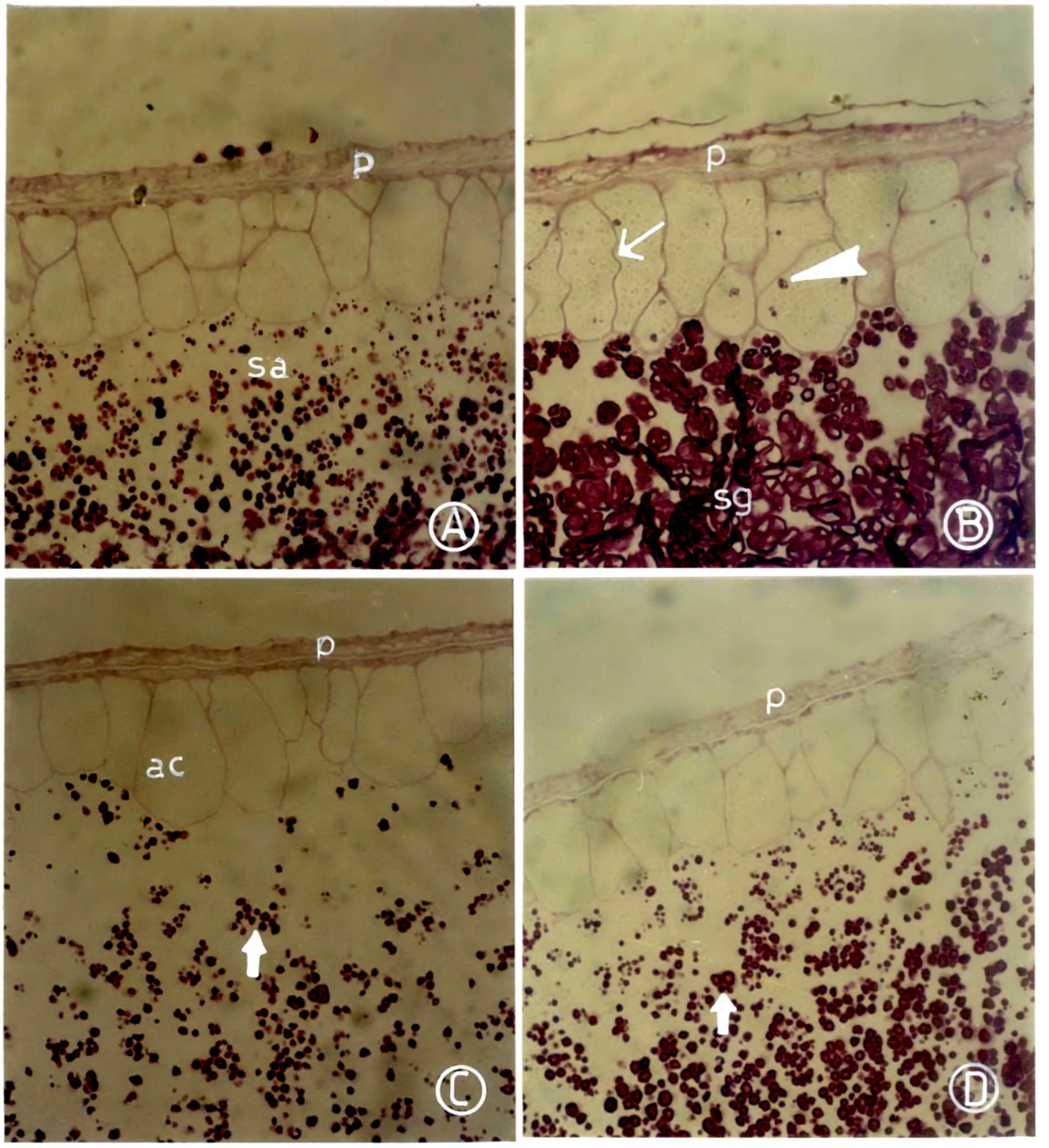

FIG. 19

Fig. 20 A-E : Longitudinal sections of rice seed passing through aleurone and endosperm stained for proteins.

A : Control after 24h of incubation. X 500.
B,C,D,E : Control, NaCl, putrescine NaCl containing putrescine respectively. X 1250.

Arrow indicates protein bodies
(p - pericap ; ac - aleurone cell ; en - endosperm, n - nucleus ; v - vacuole)

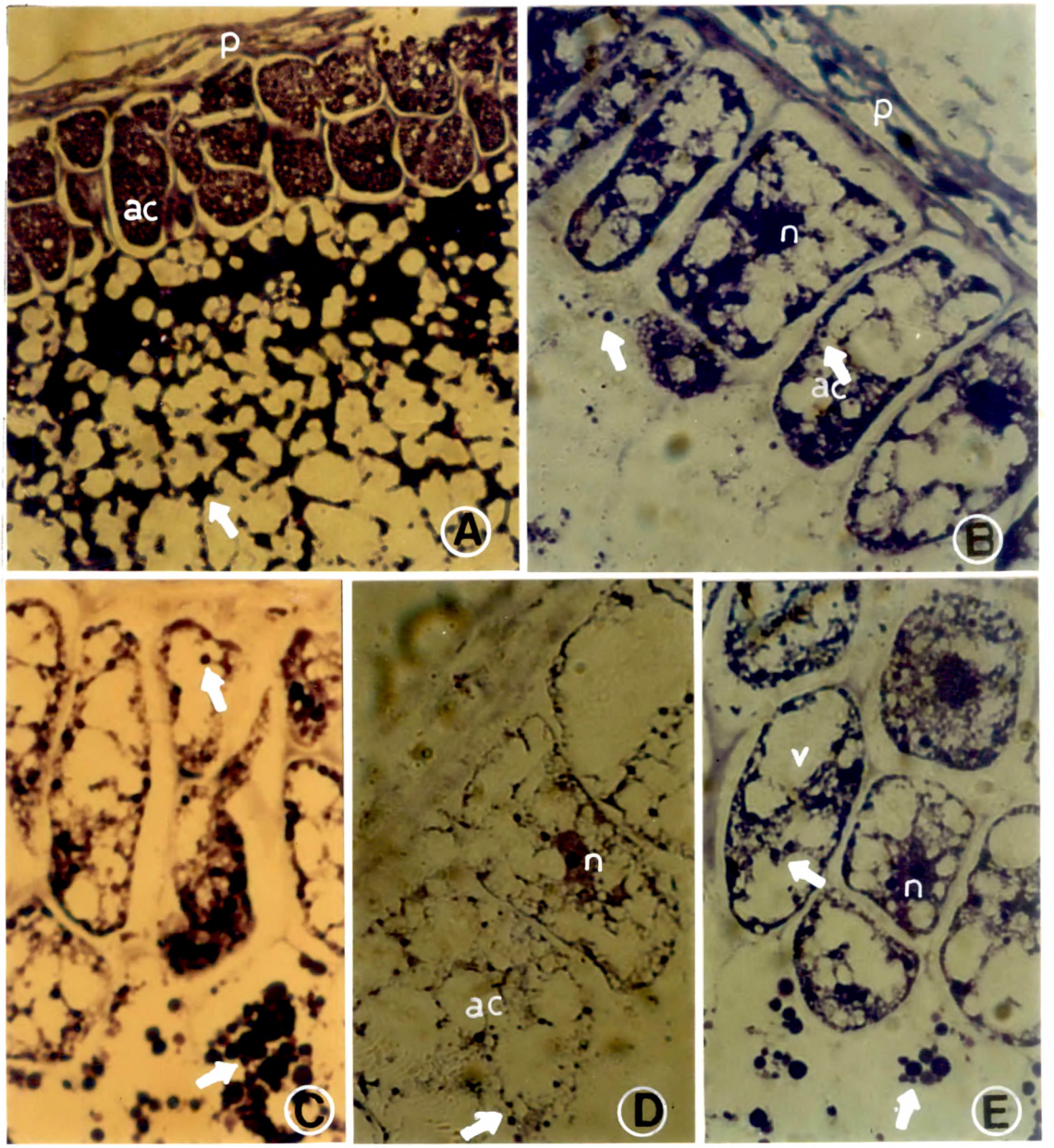

FIG. 20

Fig, 21 A-E : Longitudinal sections of rice seed stained for lipids.

A : Control after 24h of incubation
B,C,D,E : Control, NaCl, putrescine, NaCl containing putrescine respectively. X 500.

(p - pericap, ac - aleurone cell ; en - endosperm ; Arrow indicates protein bodies, arrow head indicates starch grains)

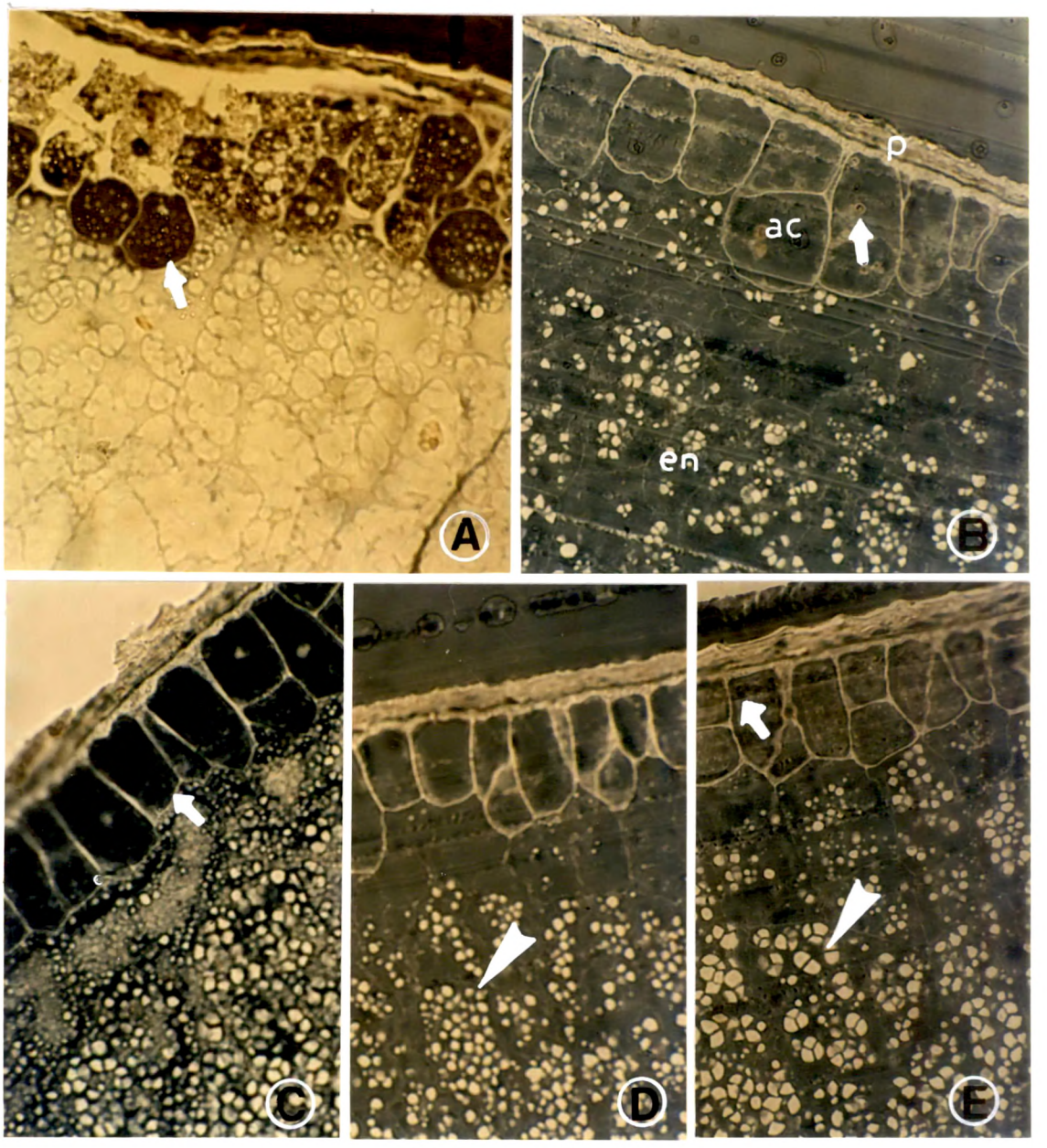

FIG. 21

Administration of putrescine in salt treated seeds led to better hydrolysis of reserve food material than salt control Aleurone cytoplasm was free from polysaccharides (Fig. 19D) Vacuolation was better and endospermic protein contents were also less compared to the salt treated seeds (Fig 20E, B) There was no much differenc in the lipid content (Fig 21E) However, putrescine as well as GA_3 treatments failed to bring about better hydrolysis of starch and protein in NaCl exposed seeds compared to that of non-stressed seeds

Localization of succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G6PDH) in the aleurone cells

The activity of SDH and G6PDH was detected by the localization of formazan production in the aleurone cells The reaction product (formazan) was deposited in the form of granules in the cells The granules appeared dark blue in case of SDH and bluish black in case of G6PDH At times, where the activity was more, the whole cell took stain (Fig 23E) It was observed that in most of the cells deposition was throughout (Fig. 22D, E) but in some cases it was more on the periphery compared to the centre (Figs 22B, 25C).

The aleurone peels of non-stressed showed high activity of SDH compared to that of G6PDH (Figs 22B, 23B, 24B, 25B). Compared to the aleurone cells of seeds germinated under non-saline conditions (Fig. 22B) a low activity of SDH was observed in the seeds germinated under the influence of NaCl (Fig 22C) But these cells showed a better activity of G6PDH (Fig 23C)

Fig. 22 A-E . Aleurone cells of 120h germinated rice seeds treated for succinate dehydrogenase reaction.

A : Control without reaction product

B,C,D,E : seeds germinated in water, NaCl, NaCl containing GA_3 and GA_3 respectively. X 1250.

Arrow indicates enzyme localization

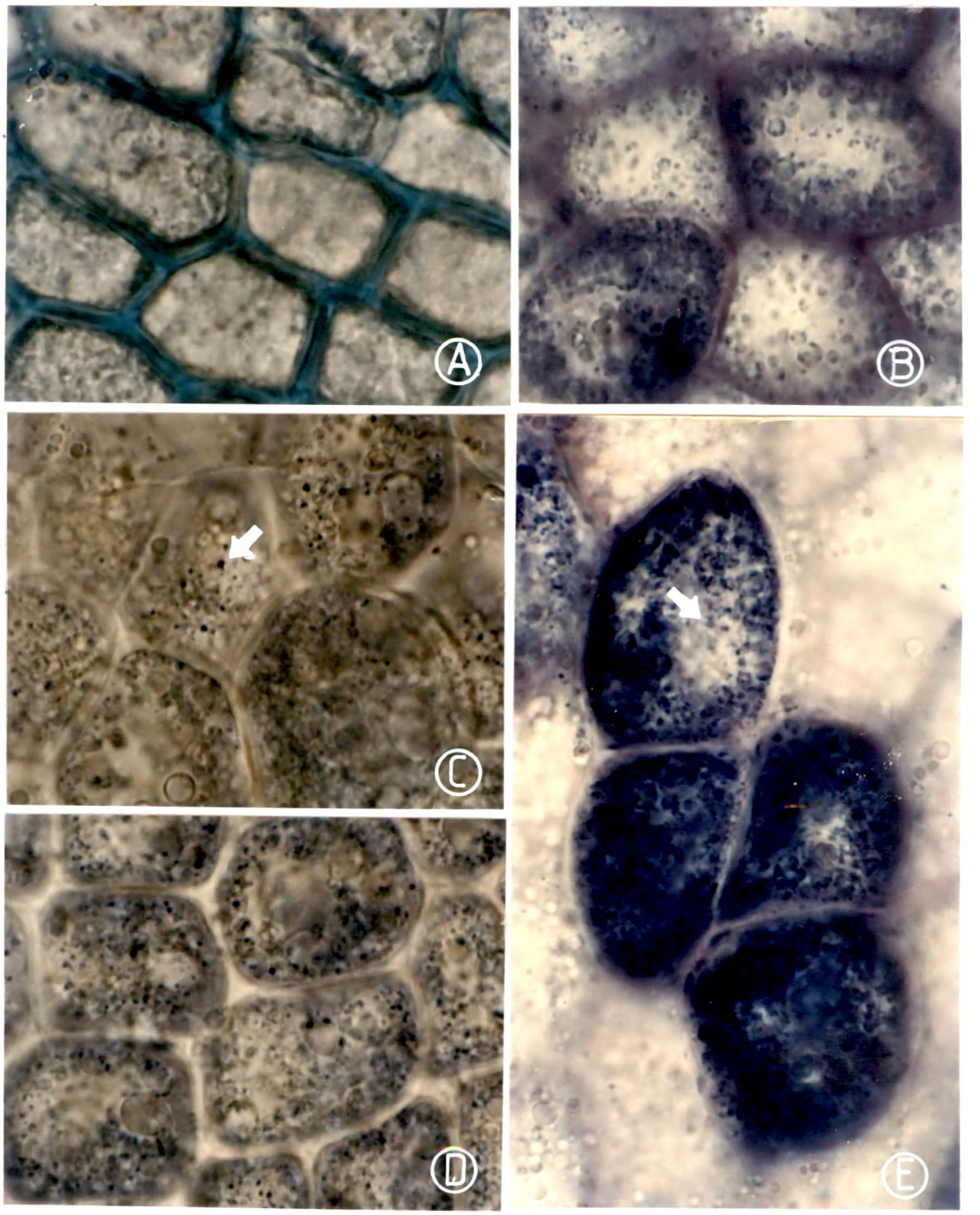

FIG. 22

Fig 23 A-E · Aleurone cells of 120h germinated rice seeds tested for glucose-6-phosphate dehydrogenase reaction.

A · Control without reaction product

B,C,D,E Seeds germinated in water, NaCl, GA_3 and NaCl containing GA_3 solution respectively. X 1250

Arrow indicates enzyme localization

Fig 24 A-E : Aleurone cells of 120h germinated rice seeds tested for succinate dehydrogenase reaction

A Control without reaction product

B,C,D,E Seeds germinated in water, NaCl, NaCl containing putrescine and putrescine respectively X 1250.

Arrow indicates enzyme localization

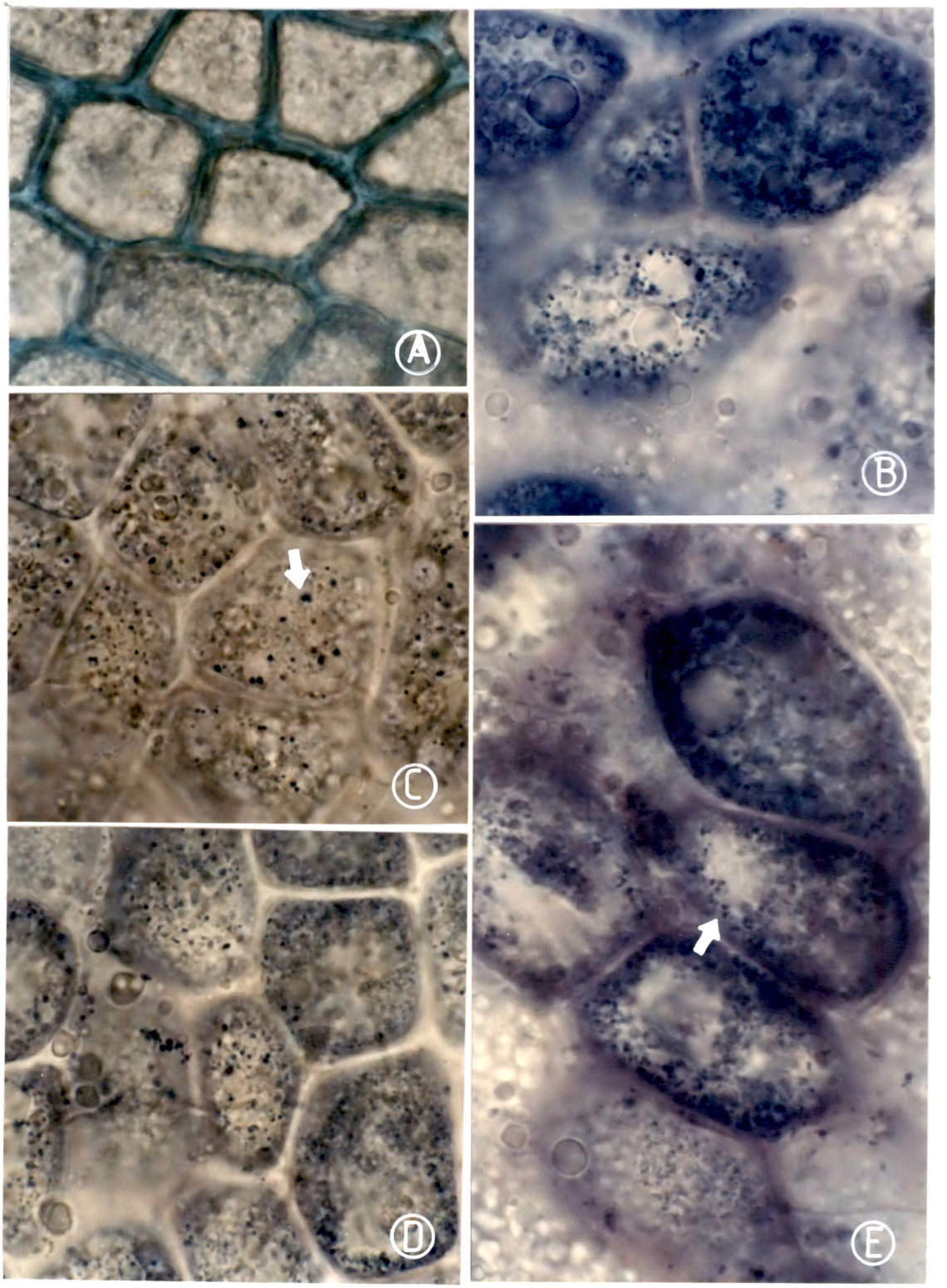

FIG. 24

Fig 25 A-E · Aleurone cells of 120h germinated rice seed tested for glucose-6-phosphate dehydrogenase reaction

A · Control without reaction product
B,C,D,E seeds germinated in water, NaCl, NaCl, containing putrescine and putrescine respectively X 1250.

Arrow indicates enzyme localization

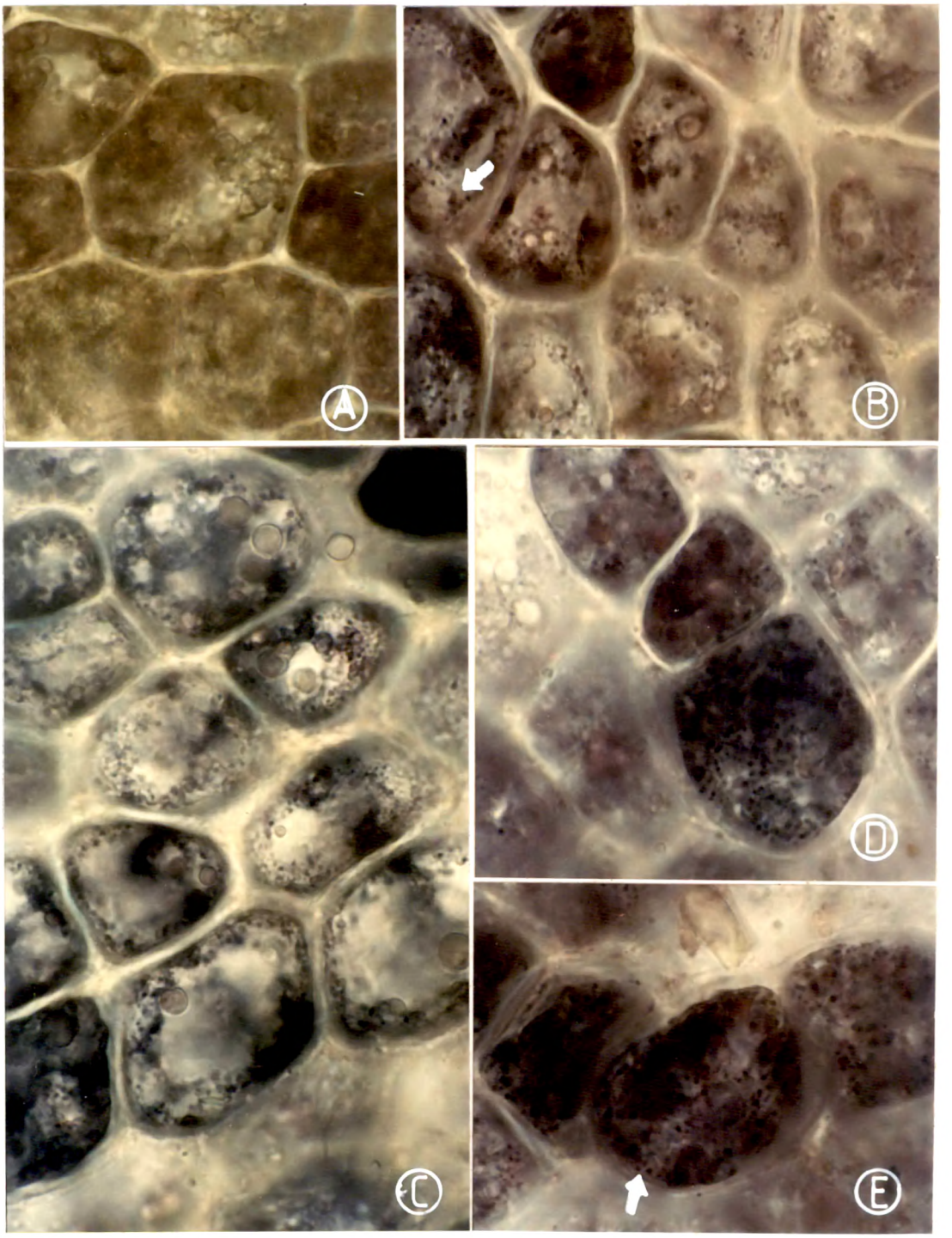

FIG. 25

An enhanced activity of SDH was observed in the aleurone cells under the influence of GA_3 (Fig. 22E). The enzyme activity in cells of seeds germinated in presence of salt was also stimulated by GA_3 (Fig. 22D).

A very low activity of G6PDH was observed in the cells of aleurone layer of seeds germinated for 120h (Fig. 23B). However, exposure to NaCl led to a significant increase in the activity of enzyme (Fig. 23D). Incorporation of GA_3 in the saline as well as in non-saline germination media showed a slight increase in the activity of G6PDH (Fig. 23D, E).

Exposure of seeds to putrescine increased the SDH activity in aleurone cells (Fig. 24E). The activity of SDH showed a slight increase in salt stressed cells under the influence of putrescine (Fig. 24D) compared to salt control (Fig. 24C).

Addition of putrescine in saline germination medium increased the activity of G6PDH compared salt control (Fig. 25C,E). A slight rise in activity of G6PDH was also observed in the aleurone cells of putrescine treated seeds under non-stressed conditions (Fig. 25D).

Ultrastructural studies

The ungerminated or 24h water imbibed rice seeds could not be sectioned less than 1μm thickness. The central endosperm of the seeds, even after 120h, remained extremely hard for sectioning. Despite these limitations the results

obtained provided a firm basis to understand the structural changes in the aleurone cells and surrounding regions under the influence of NaCl, GA_3 and putrescine.

Structure of the 120h germinated rice caryopsis

The seed coat : The endosperm and germ of rice is surrounded by 3 distinct layers of tissue, the pericap, seed coat and nucellus. The pericarp is about 3.5 to 4μm thick and consists of several crushed cells (Fig. 26A). Inner to this lies a pressed layer of cells, the seed coat with thick cuticle on its inner side (Fig. 26A,B). Touching the seed coat cuticle is the 0.5 to 0.8 μm thick cuticle of crushed nucellar cells (Fig. 26A,B,C). The attachment between these two cuticles is very weak and they often became separated while sectioning and handling of tissue.

Aleurone cells: Inner to the nucellus is the aleurone layer (Fig. 27D,E). It completely surrounds the grain and is tightly bound to the scutellum as well as starchy endosperm cells. The aleurone cells are cuboid or rectangular in shape (Fig. 27A,F). The lateral walls between the aleurone cells have many plasmodesmata (Fig. 26D,E) and where the cell wall separating aleurone cell from endosperm cells contains no plasmodesmata (Fig. 26F).

The cytoplasm of the aleurone cells of 120h germinated control seeds had highly vacuolated cytoplasm with a few aleurone grains (Fig. 27A). They appeared to increase in their volume and lost their spherical appearance. Aleurone

grains at different stages of digestion were observed in the cells (Figs. 26E, 27A). Their diameter varied from 2.2 to 4.5μm. The partially digested aleurone grains were surrounded by a number of spherosomes. The spherosomes showed a distinct pattern of arrangement around the periphery of the grain (Fig. 28A). The number of spherosomes were more towards the plasma membrane (Fig. 28C). The spherosomes were spherical and had diameter varying from 0.2 to 0.4μm.

As the aleurone grains, spherosomes along with the vacuoles were tightly packed within the cytoplasm, little space was left for other cytoplasmic organelles. Within the electron dense small space, mitochondria, plastids and microbodies were observed (Fig. 28B,C,E). Occasionally, rough endoplasmic reticulum was observed in the peripheral cytoplasm of the cells (Fig. 28D,F). The vesicles associated with endoplasmic reticulum or dictyosomes could not be traced Ribosomes were also found free in the cytoplasm (Fig. 28B). The nucleus had a nucleolus with dense nucleoplasm (Fig. 28E).

Subaleurone region: The subaleurone region was characterised by the occurrence of large compound starch grains and protein bodies (Figs. 29A,B, 30A). The cell walls separating the cells were very thin compared to the lateral walls of aleurone cells (Fig. 29E). The number of starch grains was less compared to that of protein bodies at the subaleurone region (Fig. 29E). The dark bands in the starch granules were a result of swelling and subsequent breaking of starch in

the section (Fig. 29A). Protein bodies were spherical and membrane bound. They appeared in 3 distinct morphological forms. Large spherical bodies (Ls) with a dense centre surrounded by concentric rings measuring 1.4 to 2μm in diameter (Fig. 29B), small spherical bodies (Ss) with no dense centre surrounded by concentric rings with a diameter of 0.5 to 0.75μm (Fig. 29E) and crystaline spherical bodies (Cs) with wavy margin measuring 0.4 to 0.5μm in diameter (Fig. 29B,D).

Ultrastructure of rice aleurone cells treated with NaCl for 120h

The aleurone cells of seeds germinated in NaCl solution for 120h had a dense cytoplasm with many vacuoles (Fig. 27B). The cytoplasmic organelles were in abundance. Aleurone grains were many in number and they were either in undigested state or in the beginning stage of digestion (Fig. 30B, D). Their size was small compared to those of control (Figs. 30B, 28A). As most of the aleurone grains were in undigested state, their structure was very clear. They had transparent areas called globoid cavities (gc) in which the phytin globoids (pg) were present (Fig. 30A). Phytin is the insoluble mixture of potassium, magnesium and calcium salt of myoinositol storage form of phosphate and macronutrient mineral elements in seeds. The globoids generally did not fill the cavities and the remaining space of the globoid cavity was empty (Fig. 30A). But in some cases the globoids filled the cavities completely (Fig. 30B). Usually, the aleurone grain

contained one globoid, but occasionally two (Figs. 30A,B). The size of the globoids varied from 0.5 to 3.0μm in diameter.

The ground substance of the aleurone grain also contained protein carbohydrate bodies (pcb) (Figs. 30C,D). They were finely granular in appearence and memberane bound (Fig. 30D). The peripheral spherosomes were less and not well organised as in case of control (Figs. 30B, 28A). A well defined nucleus was seen in the centre of aleurone cell (Fig. 27B). The chromatin in the nucleus appeared condensed in the aleurone cell of NaCl treated seeds (Fig. 30C).

Conspicuous changes were also seen in the cytoplasm involving mitochondria, endoplasmic reticulum and plasma membrane. In the cytoplasm a higher density of mitochondria was observed (Figs. 30C, 31A). The cristae were undistinguishable. Besides these, some mitochondria with vacuolated matrix were also observed (Fig. 31B,D). The endoplasmic reticulum elements were elongated, frequently dilated and fragmented (Fig. 30D). Ribosomes were seen free in the cytoplasm and also attached to the ER (Fig. 30C,D). Certain invaginations of the plasmalemma and small vesicles which seemed to have invaginated into the vacuoles of aleurone cells were seen (Fig. 32A,B). Intravacuolar vesicles or inclusions were often found in the cells (Fig. 32C,D). Other than these, needle shaped crystals with a size of about 0.36 μm were observed in the vacuoles (Fig. 33A,B,C). These crystals appeared in bundles or scattered (Fig. 33A,B). These

crystals were also found overlaying on other organelles which may be due to their displacement from the vacuole while sectioning (Fig. 33D).

Ultrastructure of the rice aleurone cells treated with GA_3 for 120h

Aleurone cells exposed to GA_3 for 120h expanded considerably (Fig 27C). GA_3 brought about certain changes in the cell wall structure. The cell walls were thin compared to that of aleurone cells exposed to other treatments (Fig. 27C). The cell wall lost its fibrillar appearance and was highly digested (Fig. 34B).

The extension and fusion of aleurone grain membranes led to the formation of large vacuolar systems . Vacuoles derived from aleurone grains filled almost the entire cell. They developed a large central vacuole although few small vacuoles were still present in the peripheral cytoplasm (Fig. 27C). The central vacuole was seperated from the cytoplasm by a tonoplast (Fig. 34A).

The erosion of cell walls and scanty cytoplasm affected the interpretation of the status of cell organelles. The quality of organelles was considerably reduced in the microscopic field. The membranes and the matrix of aleurone grains had become fused totally. No intact aleurone grain was observed. The phytin globoids were almost digested (Fig. 27C). The protein bodies showed an increase in their volume and were undergoing the process of digestion (Fig. 34E). The number of spherosomes along the cell membrane became less frequent particularly compared to the control (Figs. 34B).

In the pockets of cytoplasm mitcochondria, plastids and ER were located (Fig. 34A,C,D). Mitochondria were observed near the cell wall (Fig. 34B). No structural changes was observed in the mitochondria and plastids. Free ribosomes were not prominent. The stacks of rough ER cisternae were clearly distinguishable (Fig. 34D).

Ultrastructure of the aleurone cells of GA_3 treated salinized seeds

Application of GA_3 stimulated vacuole formation by digestion of aleurone grains in the salt treated seeds. These aleurone cells had more vacoules compared to the salt treated cells (Fig. 27D,B). Most of the aleurone grains observed were in the process of digestion and the spherosomes were getting oriented around the aleurone grains (Fig. 35A,B). It was observed that small vacuoles were developing from the plasma membrane into the cell (Fig. 35C). The cell wall and other organelles did not show any structural change (Fig. 35B,D). Endosplasmic reticulum were conspicous. The frequency of ribosomes and mitochondria was reduced.

Ultrastructure of aleurone cells of putrescine treated seeds

At ultrastructural level, aleurone cells of seeds germinated for 120h under the influence of putrescine were characterised by the appearance of large electron translucent vacuoles (Fig. 27E). There were 3 to 5 large vacuoles pushing the cytoplasm towards the periphery unlike a central large vacuole of aleurone cells

from seeds germinated in presence of GA_3 (Fig. 27E,C). The cell wall did not show any change compared to the control. Protoplasm was intact and the aleurone grains were few in the cytoplasm and were seen towards the periphery of the cell (Figs. 27E, 36D). The few observed phytin globules and the protein carbohydrate bodies were in the digestion process (Fig. 36A). The ground cytoplasm of cells possessed few rough ER in stacks close to the cell periphery but they were generally absent in other regions (Fig. 36C). Spherosomes and microbodies were also present in these vacuolated cells like those of the control cells, but few in number (Fig. 36B,C).

Ultrastructure of aleurone cells of salinized seeds treated with putrescine

Like GA_3, putrescine also stimulated the digestion of aleurone grains to form vacuoles in NaCl exposed aleurone cells (Fig. 27F). Few phytin globules were still in the undigested form (Fig. 37A,C). RER stacks were generally absent and appeared in the form of single short strands (Fig. 37A). Mitochondria formed groups at many electron dense areas and were seen in the close vicinity of aleurone grains (Fig. 37B). There was no significant change in the structure of other cell organelles or membranes (Fig. 37D).

Fig. 26 A-F : Aleurone cell wall of rice germinated for 120h.

A : External (ex) and internal surface (in) of outer cell wall. X 18,400.

B : Internal surface of outer cell wall. X 18,400. Arrow indicates junction between seed coat and nucellus

C : Cell wall showing the junction (arrow) between seed coat and nucellus. X 33,600.

D and E : Adjacent aleurone cells showing lateral cell wall. X 18,400; 25,600. Arrow indicates plasmodesmata.

F : Aleurone cell and subaleurone cell. X 18,400

(p - pericap ; sc - seed coat ; scc - seed coat cuticle ; nu - nucellus cuticle ; lcw - lateral cell wall ; sa - subaluerone ; pb - protein body, nuc - nucellus).

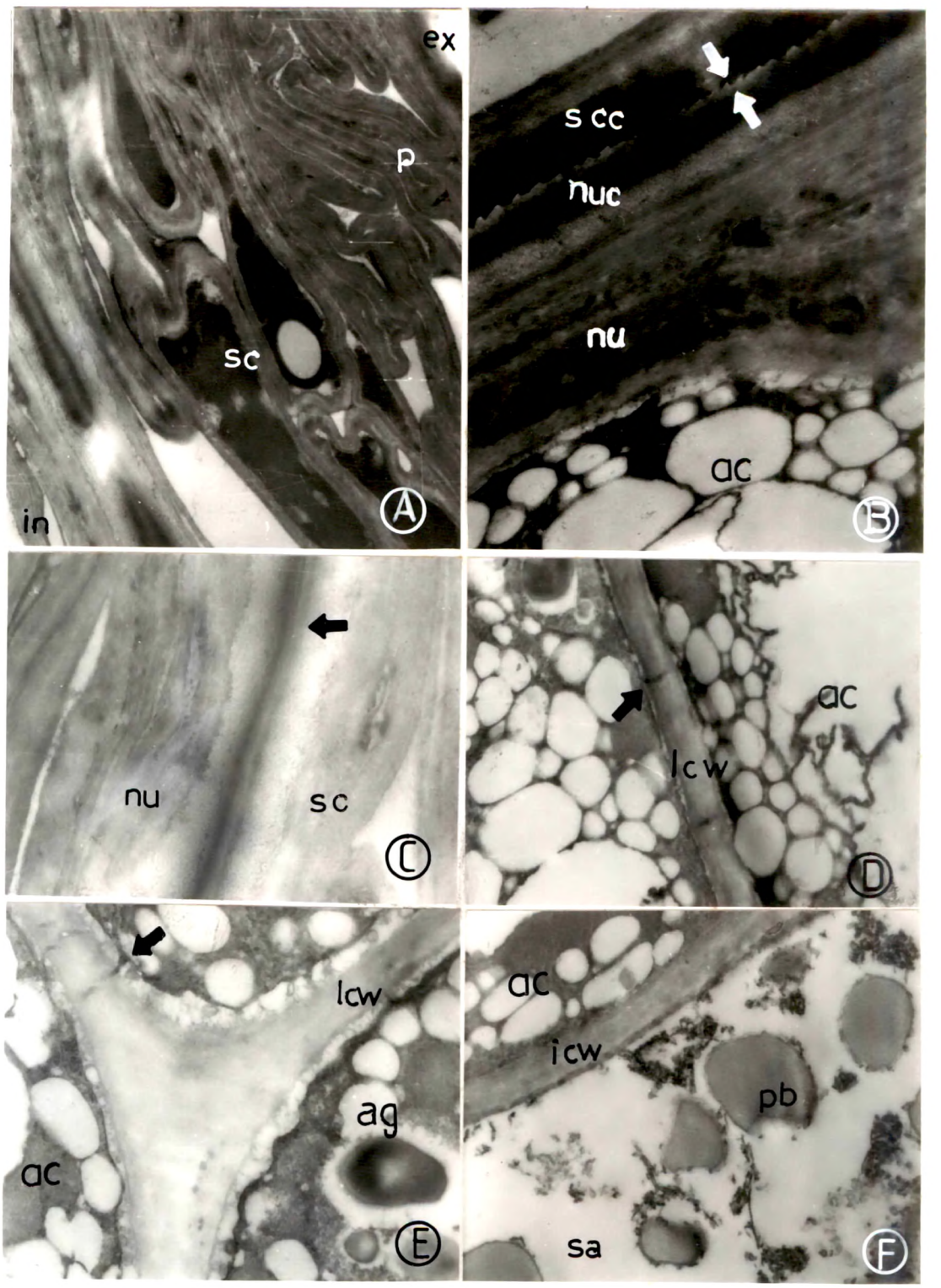

FIG. 26

Fig. 27 A-F · Ultrastructure of aleurone cell of rice after 120h of germination A Control Seeds germinated in B NaCl C. GA_3 D NaCl containing GA_3 E. putrescine F. NaCl containing putrescine. X 3480.

(ag - aleurone grain ; v - vacuole ; n - nucleus arrow indicates nucleolus , Arrow head indicates aleurone grain).

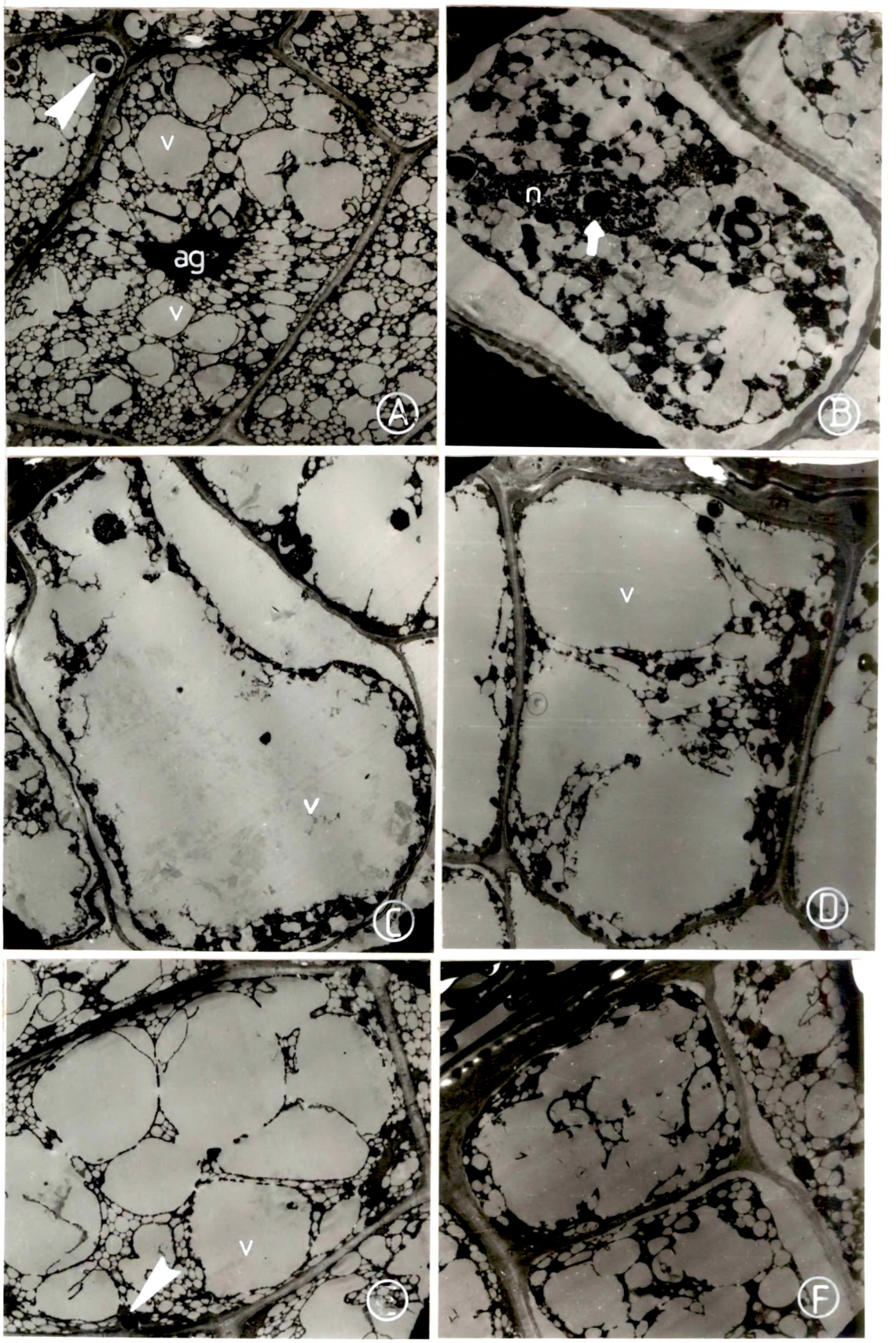

FIG. 27

Fig. 28 A-E : Ultrastructure of the aleurone cells of control rice after 120h of germination.

A : Digesting aleurone grain (ag) surrounded by spherosomes (s) X 13,600.

B Aleurone cell showing mitochondria (m), rough endoplasmic reticulum (rer) and ribosomes (arrow). X 25,200.

C Plastids (pl) in the cell peripheral cytoplasm. X 33,600

D and F . rough endoplasmic reticulum (rer) and microbodies (mb). X 46,00

E : Aleurone cell with a nucleus (n) and nucleuolus (nu) X 46,000

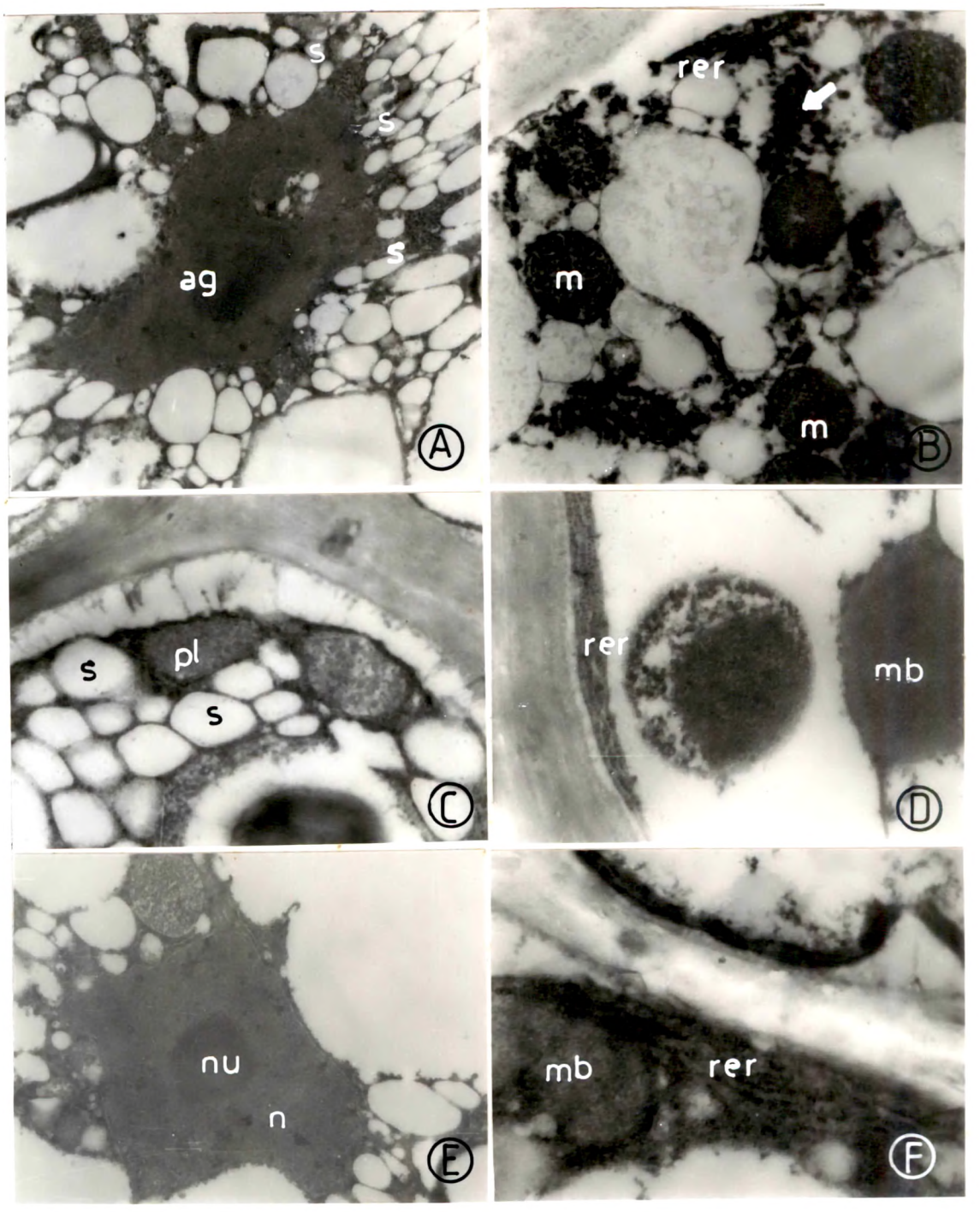

FIG. 28

Fig 29 A-E Endosperm of the control rice after 120h of germination.

A : starch grains (sg). Arrow indicates the wrinkled margin X 7800.
B,D,E . Protein bodies B,D. X 25200 ; E. X 10,600
C : Starch grains as seen under SEM. X 9000

(Ls - large spherical bodies ; Cs - crystalline spherical bodies ; Ss - Small spherical bodies).

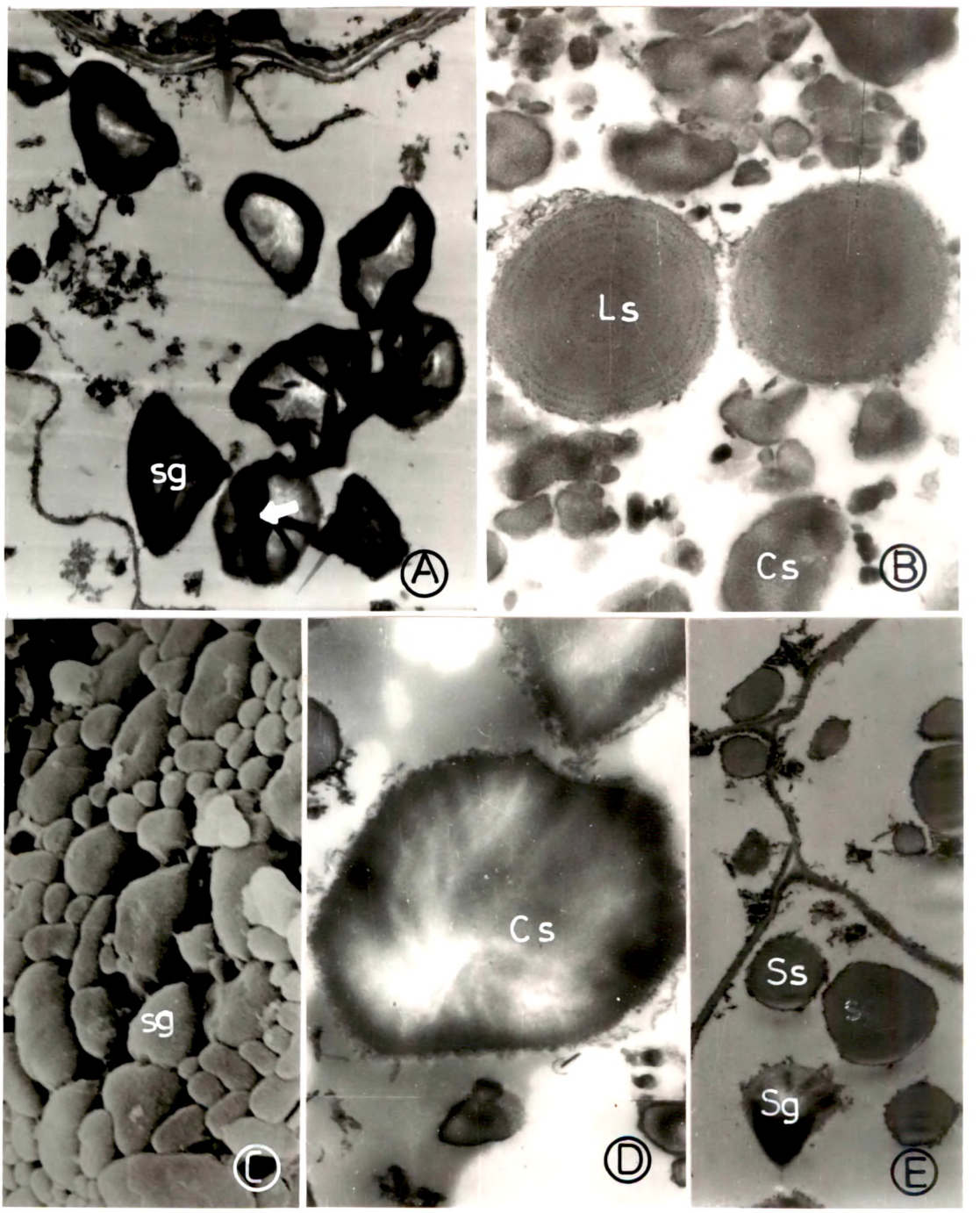

FIG. 29

Fig. 30 A-D : Ultrastructure of aleurone cells of rice exposed to NaCl for 120h

A Globoid cavity (gc) containing phytin globule (pg)
B : Aleurone grain containing two globoids X 33,600.
C : Nucleus with a nucleolus X 18,400
Note the condensed chromatin (arrow) and free ribosomes (arrow head)
D : Phytin globule and associated protein carbohydrate body (pcb) X 62,000. Note short strands of rough endoplasmic reticulum.

(ag - aleurone grain, rer - rough endoplasmic reticulum)

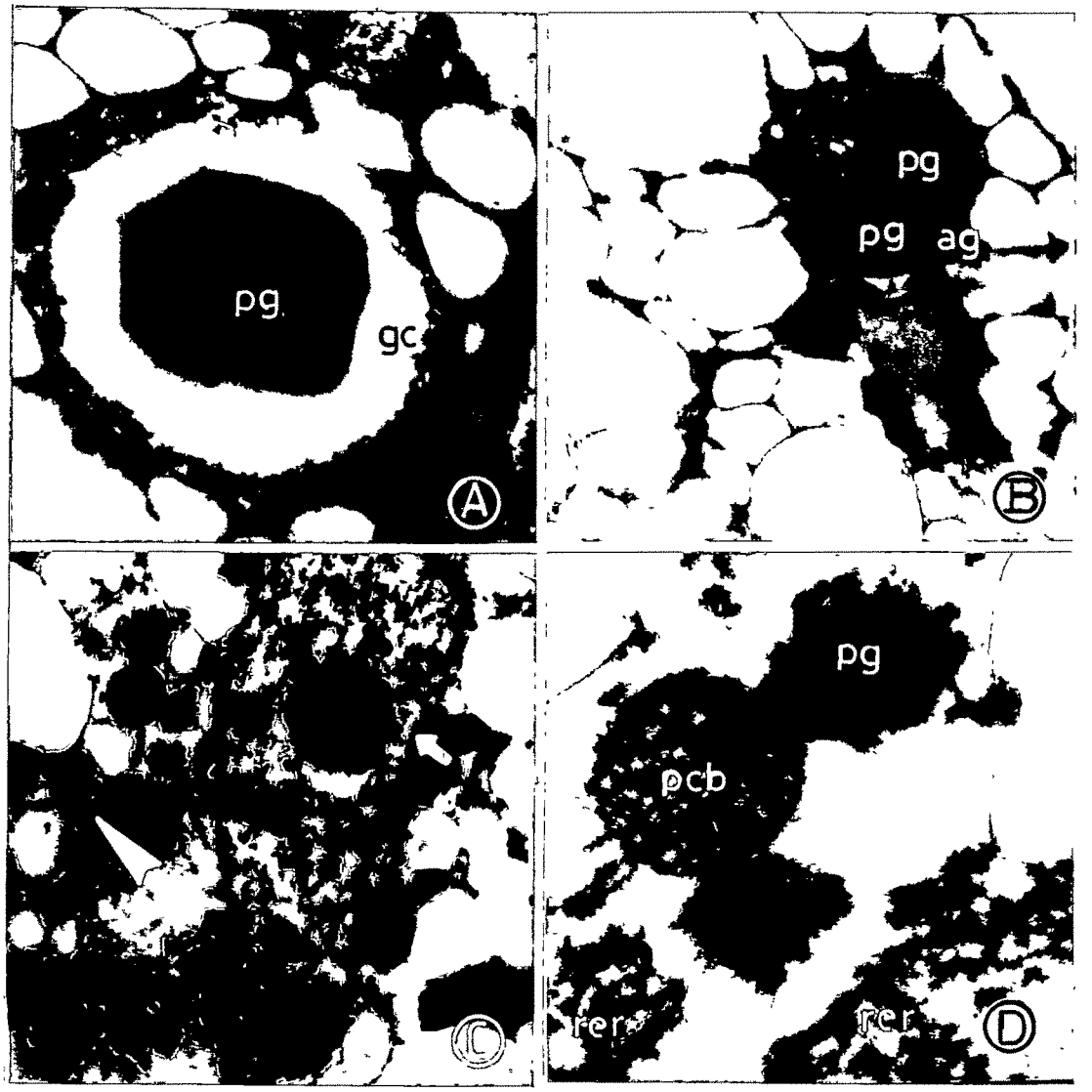

FIG. 30

Fig. 31 A-D : Strands of cytoplasm in the aleurone of rice exposed to NaCl for 120h showing

A . Normal mitochondria (m). X 33,600.
B,D : Vacuolated mitochondria (arrow) X 46,000.
C : Rough endoplasmic reticulum (rer) and plastid (pl) X 62,000

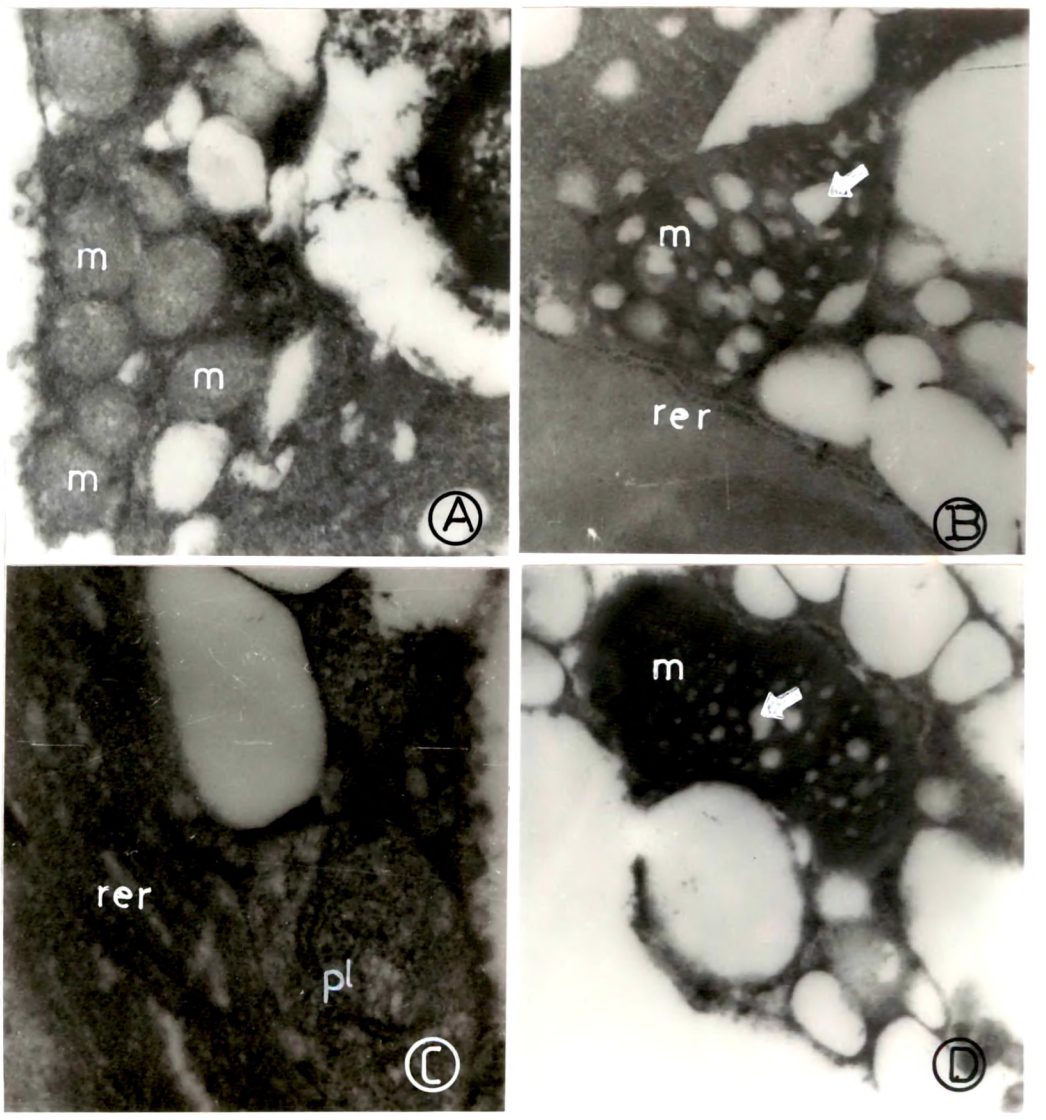

FIG. 31

Fig. 32 A-D : Invaginations of plasma membrane of aleurone cell of salt exposed rice for 120h.

A : Invagination of membrane into the vacuole. X 25,200.

B : Blebbing from the cell membrane. X 33,600.

C-D : Pinocytic vesicles releasing in to the vacuole. Arrow indicates blebbing. X 10,600 ; X 25,200.

(ag - aleurone grain ; s - spherosomes)

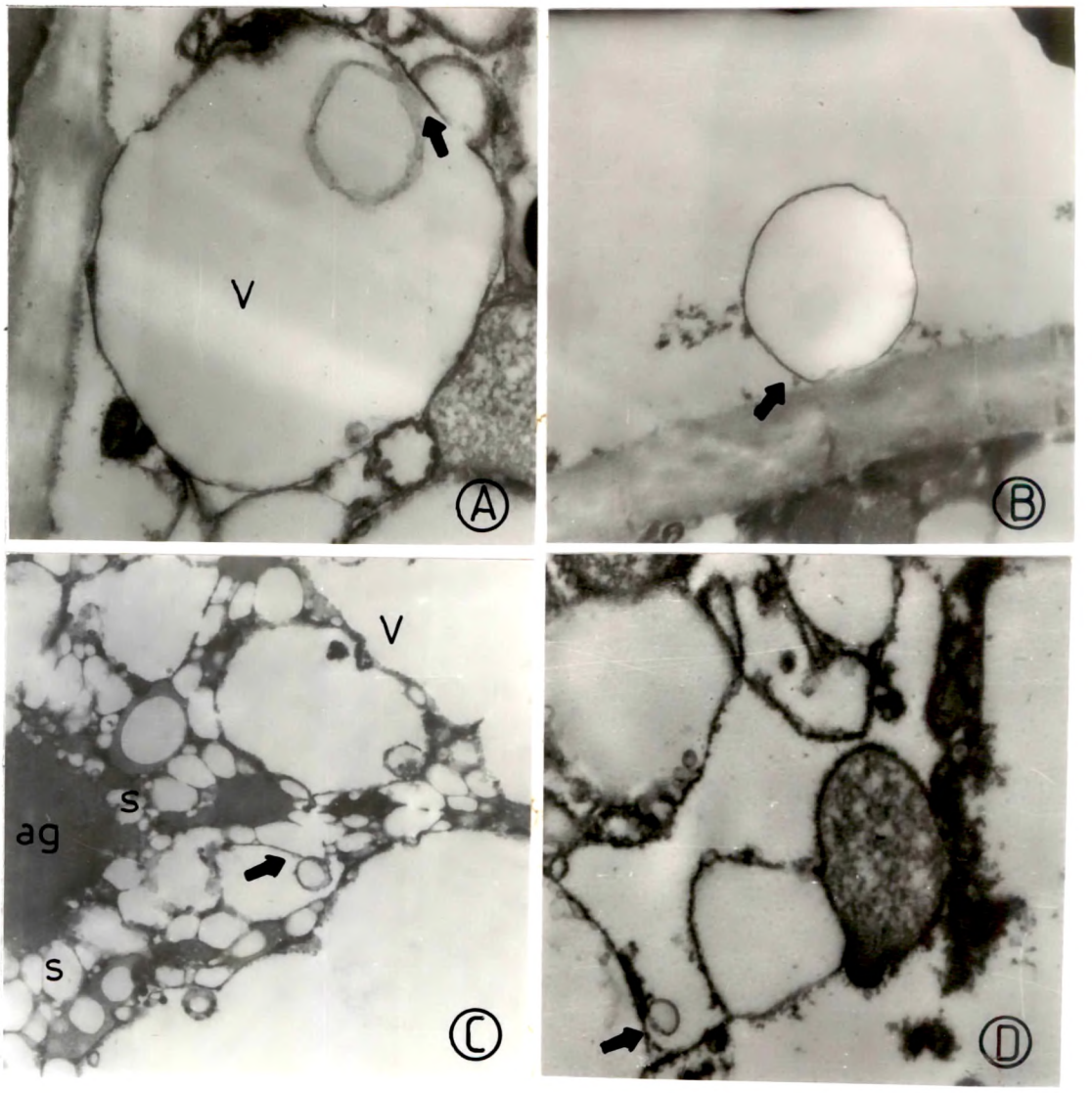

FIG. 32

Fig 33 A-D Crystals in the vacuole and other regions of aleurone cytoplasm of NaCl exposed rice seed for 120h

Arrow head shows crystals
X 13,600; X 62,000 , X 46,000 and X 33,600 respectively.

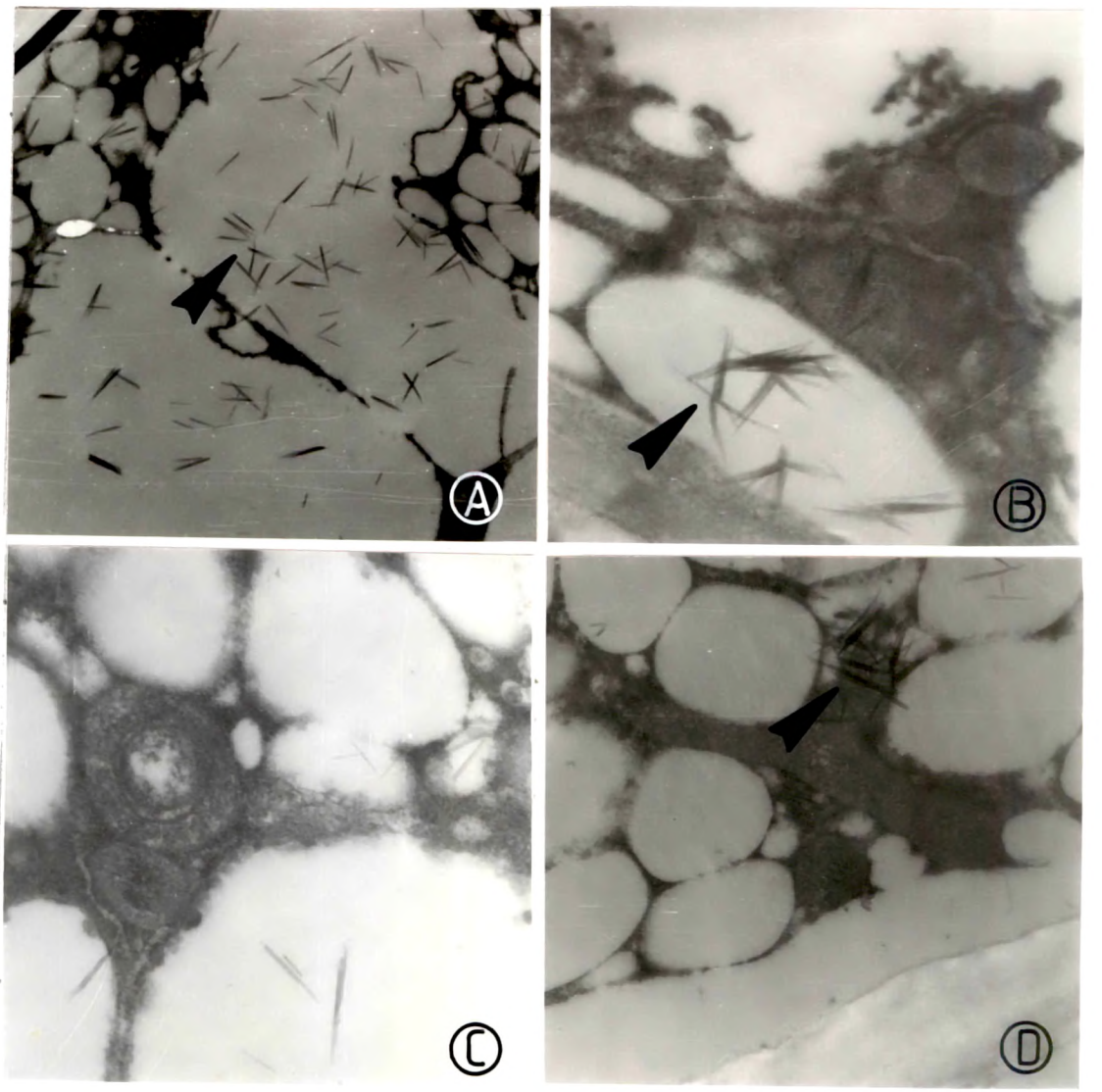

FIG. 33

Fig. 34 A-E . Aleurone cytoplasm of GA_3 incubated rice for 120h.

A : Large central vacuole (v) with peripheral cytoplasm X 13,600
B : Thin cell wall (cw). X 25,200.
C : Long profiles of rough endoplasmic reticulum (rer) and mitochondria (m). X 62,000.
D : Group of mitochondria . X 25,200
E : Protein carbohydrate body (pcb) undergoing digestion. X 25,200.

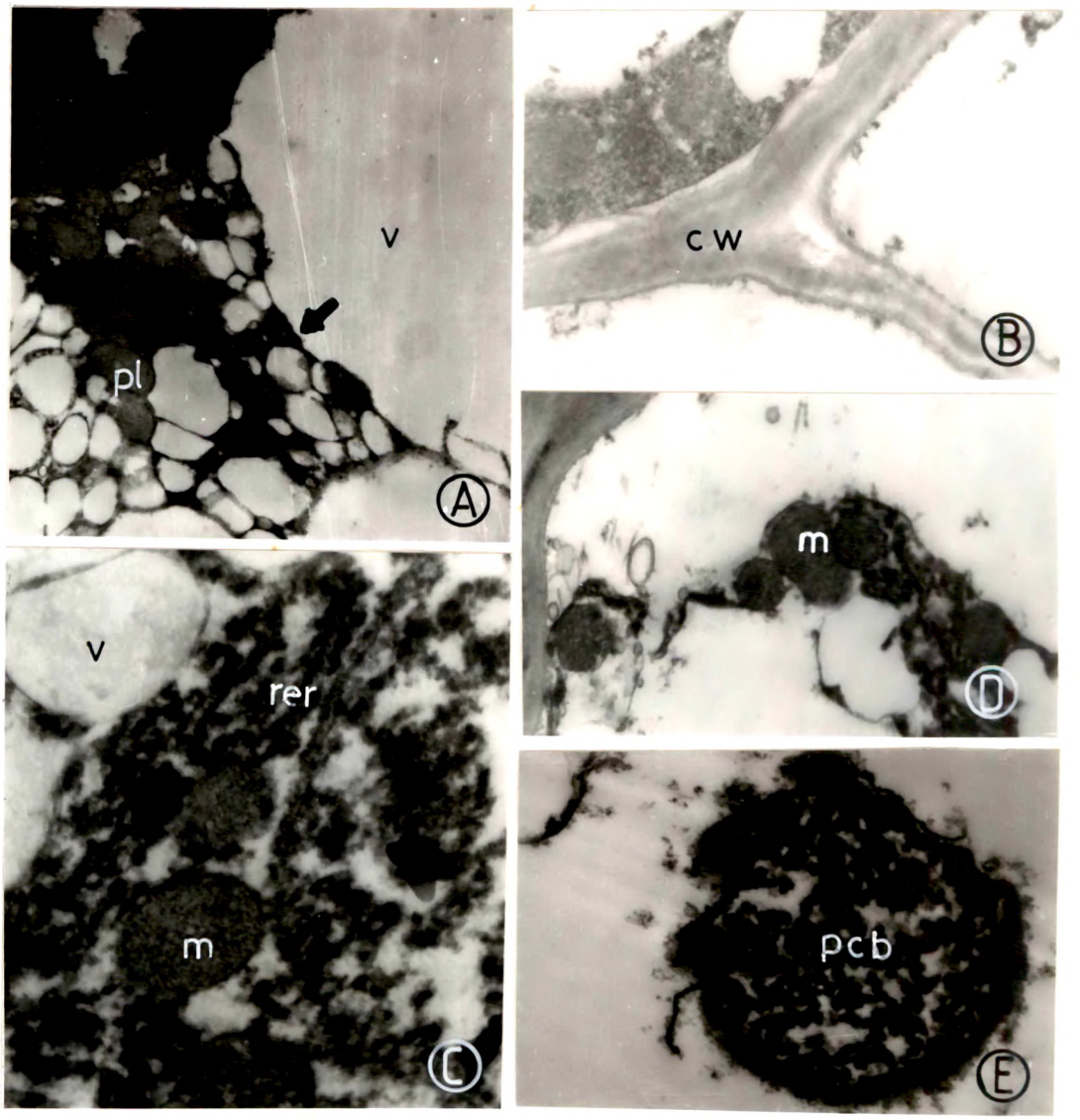

FIG. 34

Fig. 35 A-D : Aleurone cytoplasm of rice incubated in NaCl containing GA_3.

A : phytin globule (pg) and protein carbohydrate body (pcb) undergoing digestion. X 46,000.

B : Portion of cytoplasm showing digesting aleurone grains surrounded by spherosomes (arrow). X 13,600

C . Pinocytic vesicles (arrow heads) releasing into the cell. X 33,600

D . plastid (pl). X 36,000

(cw - cell wall ; ag - aleurone grains)

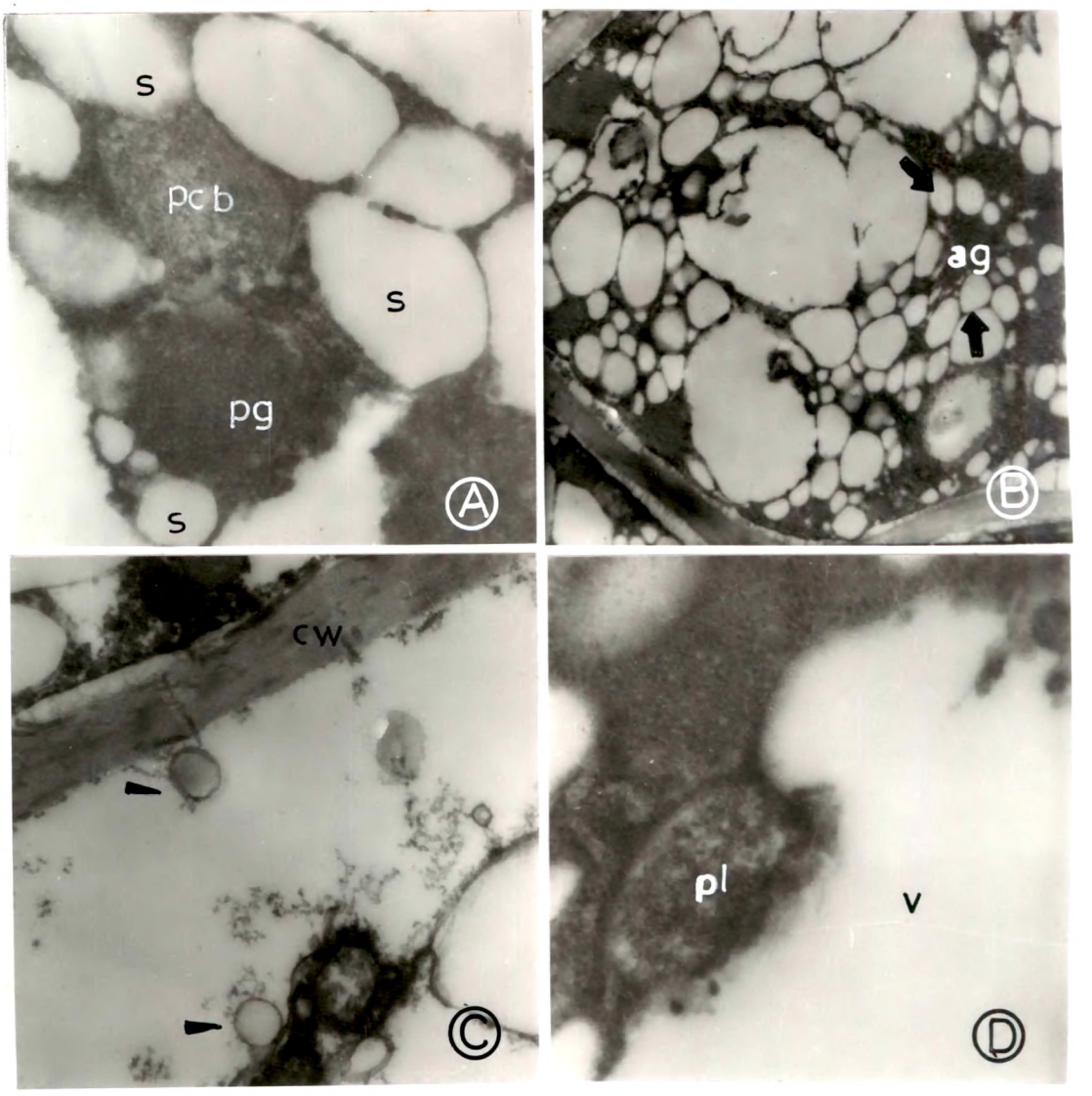

FIG. 35

Fig. 36 A-D : Aleurone cytoplasm of rice germinated in putrescine solution.

A,B Aleurone grains (ag) showing different stages of digestion X 62,000; X 46,000.

C Micro bodies (mb), rough endoplasmic reticulum (rer) near the periphery. X 62,000.

D Peripheral scanty cytoplasm of the aleurone cell (ac). X 10600

(pcb - protein carbohydrate body ; pg - phytin globule ;
s - spherosomes ; cw - cell wall ; v - vacuole).

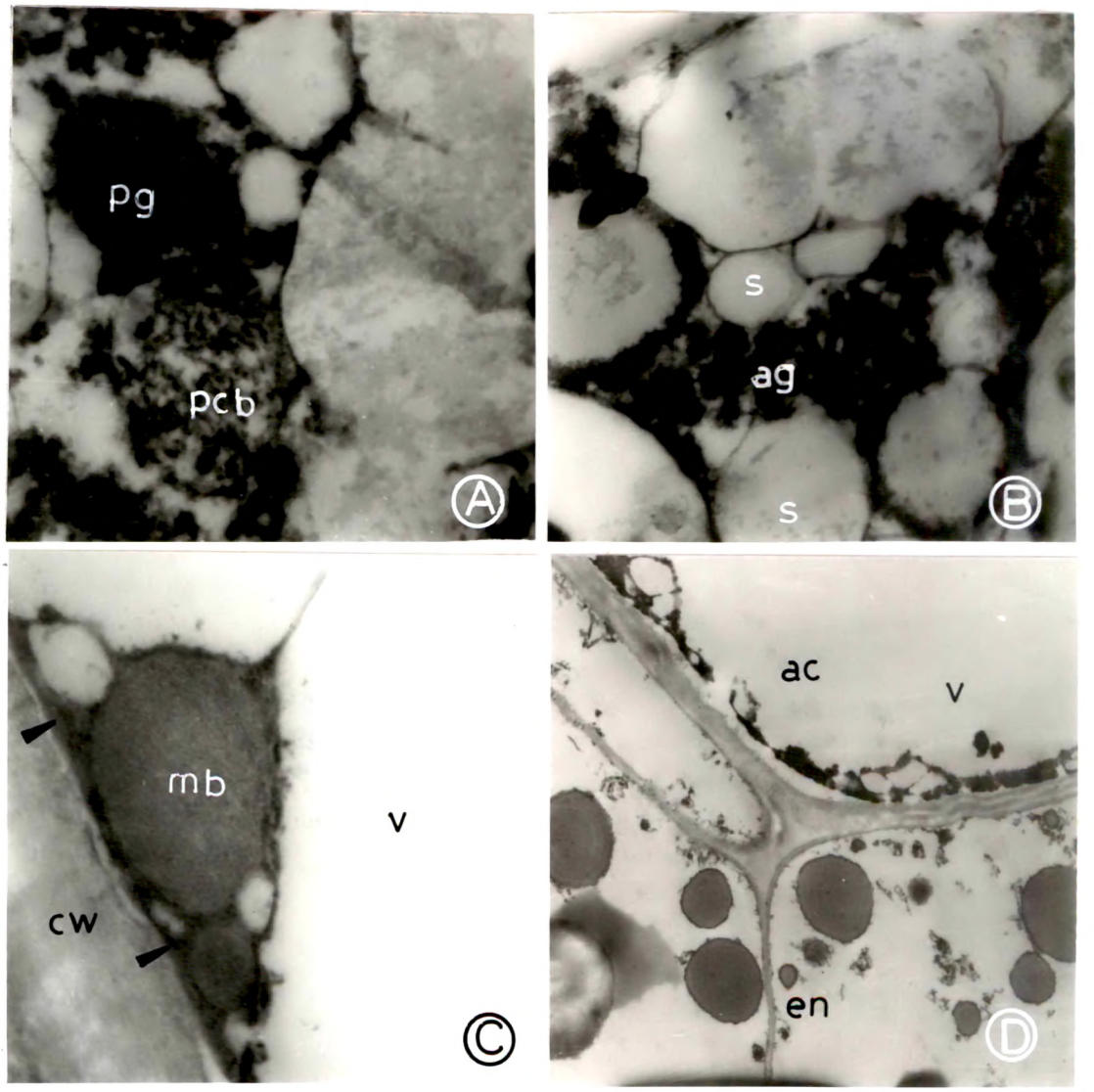

FIG. 36

Fig. 37 A-D : Aleurone cytoplasm of rice germinated in NaCl containing putrescine partially digested

A : Aleurone grain with short fragments of rough endoplasmic reticulum (rer). X 62,000.

B : Group of mitchondria (m). X 25,200.

C : Aleurone grain with intact phytin globules (pg). X 33,600.

D : plastid (pl). X 62,000.

(pcb - protein carbohydrate body ; s - spherosome ; v - vacuole)

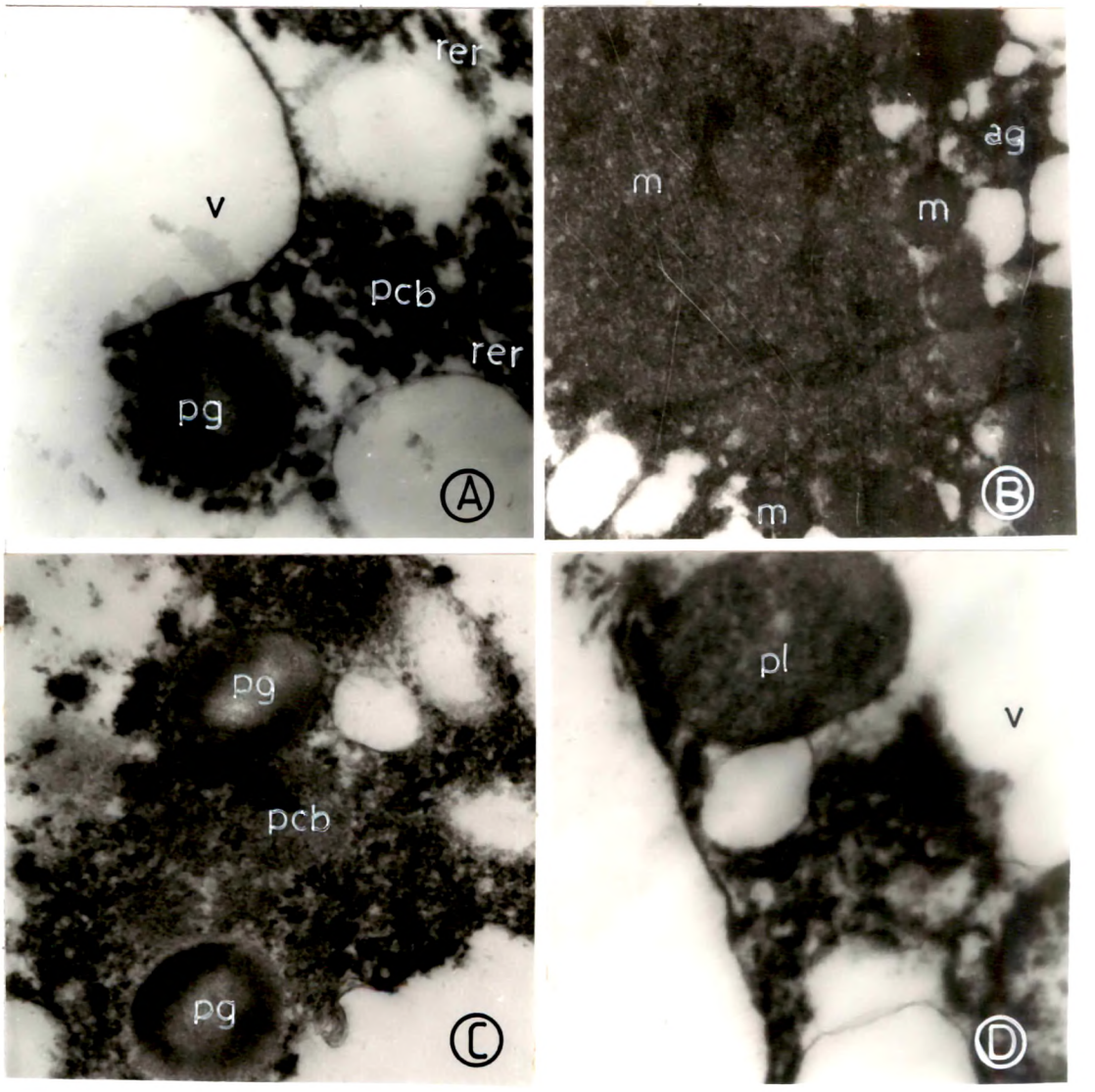

FIG. 37