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EFFECT OF GROWTH REGULATORS ON COTYLEDONARY ARCHITECTURE OF *BRASSICA JUNCEA* L.

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Abstract

The major venation pattern in control as well as treated cotyledons of *Brassica juncea* conforms to Camptodromous type with festooned brochidodromous secondaries. The marginal ultimate venation is looped. The size of the areoles does not vary significantly. The cotyledonary area, size and number of tracheids vary in different treatments. In MOR 25 ppm treated cotyledons, the density of tracheids is maximum. The parenchymatous bundle sheath cells differentiated into tracheidal nodules are observed in GA 25 ppm. Miniature vessel elements without prominent secondary side wall thickening are observed in COL 25 ppm. The miniature vessel elements so far unreported at the vein endings are observed in DW, GA 50 ppm, MOR 25 ppm, 2,4-D 25, 50 ppm, MH 25 ppm and COL 25 ppm. The occurrence of miniature vessel elements at the vein endings is an unusual feature. It is evident that MCR, COL, GA, MH and 2,4-D do not affect major venation pattern and marginal ultimate venation, however, they affect cotyledonary area, size and number of tracheids as well as number of vein endings and vein termination number per areole.

Introduction

Although the leaf architecture has been extensively studied but the information regarding the cotyledonary architecture is very meagre. Hickey (1973) and Melville (1976) classified the leaf architecture of dicotyledonous and angiosperm leaves respectively. Gupta (1978) studied the cotyledon architecture in *Trifolium.* Recently, Murthy & Inamdar (1979) studied the effect of colchicine and morphactin on the cotyledonary venation of *Lycopersicon esculentum* var. *angurlata.* The effect of various growth regulators has been studied on the stomatal structure and ontogeny in *Brassica juncea* but not cotyledonary venation pattern (Rao & Inamdar, 1979). The present study describes the effect of growth regulators on the cotyledonary architecture of *Brassica juncea.*

Materials and Methods

Seeds of *B. juncea* sterilized in hydrogen peroxide and washed in glass distilled water were transferred to sterilized Petri dishes lined with sterilized filter paper. The seeds were grown under laboratory conditions using the following regulators as substrates: Distilled water (DW), GA (Gibberellic acid), MOR (Morphactin), 2,4-D (2,4-Dichlorophenoxy acetic acid), COL (Colchicine) and MH (Maleic hydrazide) in 25, 50 and 100 ppm concentration.

The cotyledons were cleared using the technique of Rao *et al.*, (1980). Photomicrographs were taken with the help of Carl Zeiss photomicroscope I using ORWO Np 15 film. Mean values of 50 observations showing the cotyledonary area in mm^2 size of tracheids in μm and number of tracheids per mm^2 in different concentrations of growth regulators are explained with the help of histograms.

Results

The shape of the cotyledon is bilobed ovate with depressed apex and obtuse base in both the treated and untreated plants. The cotyledons are more or less symmetrical. The margin entire, texture charataceous and glands are totally absent. The major venation pattern in both the treated and untreated cotyledons conforms to the pinnate camptodromous – festooned brochidodromous type of Hickey & Wolfe (1975).

i) Control (Distilled water)

The primary vein or midrib is the thickest vein and its thickness decreases gradually towards the apical region and gives off other degree veins on either side. The primary vein is moderate in thickness and its course is markedly curved. The secondary veins arise in alternate or subopposite fashion. Their number vary from 3 to 4. The secondary veins are strongly brochidodromous. They do not merge at the margin but upturn and join together in a series of prominent arches and forms brochidodromous type. The secondaries have a set of secondary loops outside the main brochidodromous type and form festooned brochidodormous type. The angle of divergence of 2^{o} veins on either side of the primary vein gradually increases towards the apex. The tertiaries are the next finest branches of secondary veins and their pattern is random reticulate. The next finer order of veins originating from the tertiaries and those of equal size are quaternaries. The marginal ultimate venation is looped. The vein endings are mostly simple or rarely once dichotomously branched. In the branched vein endings both the arms may be equal or unequal. The areoles are distinct with few vein endings. There is no relation between the size of an areole and the number of vein endings. The loop may be formed either by the union of veins or tracheids which are present at the vein endings (Fig. 1, C). Tracheids are either uniseriate or biseriate (Fig. 1, A, D) and their position may be juxtaposed (Fig. 1, A, D) or superimposed. Miniature vessel elements with two perforation plates (Fig. 1, B) is observed at the vein endings. All the major and minor veins are ensheathed by parenchymatous bundle sheath cells.

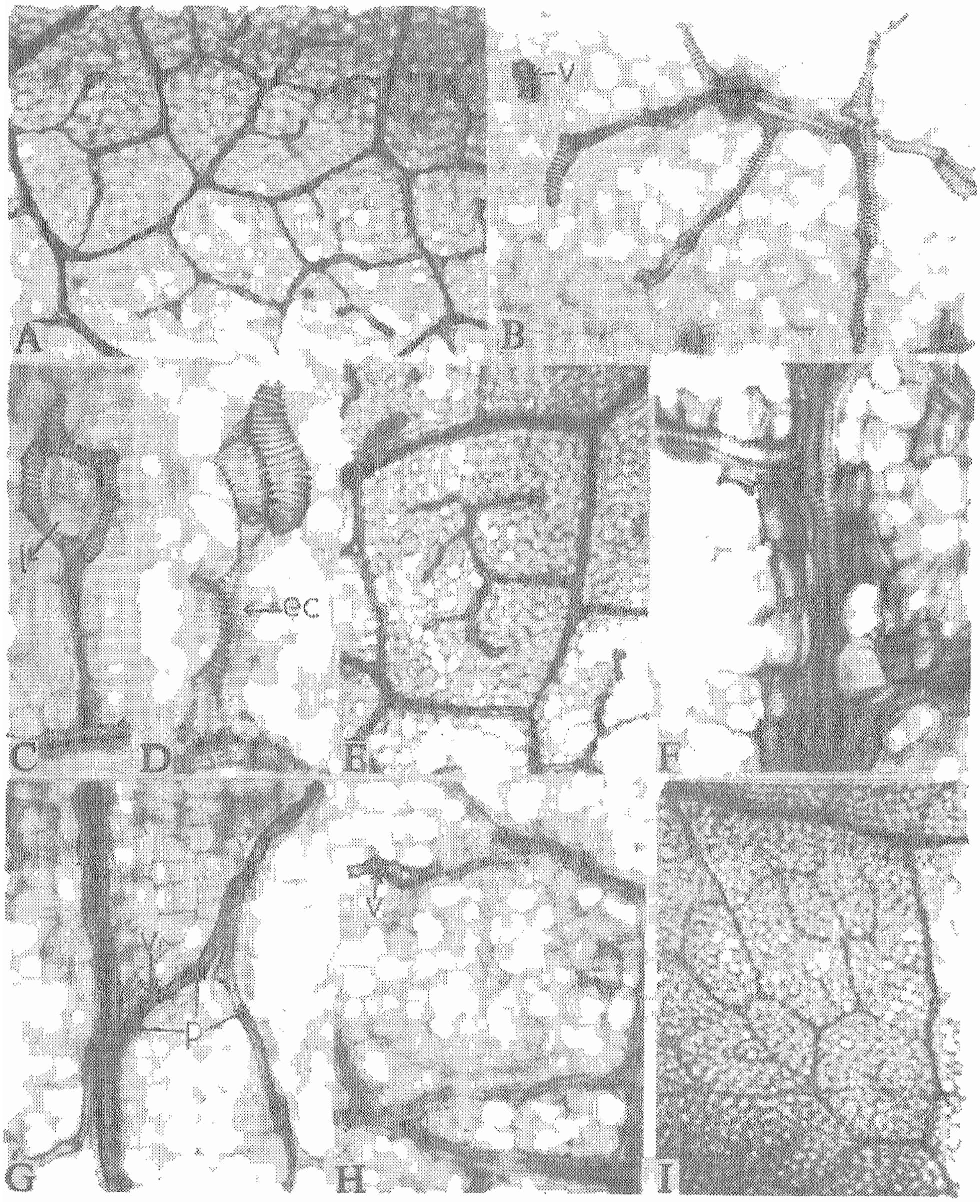

Fig. 1 Effect of growth regulators on cotyledonary architecture of *Brassica juncea* L. A-D. Distilled water X125, X285, X355, X570 E-F GA 25 ppm X115, X370 G-H. GA 50 ppm X255, X285 I. MH 25 ppm X115

ii) Morphactin (MOR)

The number of 2^{o} veins on either side of the midrib is 3 to 4 in all the concentrations. The density of tracheids increases enormously in MOR 25 ppm. Distribution of tracheids is uneven with greater density at apical region, on either side of the midrib and towards the margin rather than the basal region. Near the margin, groups of tracheids are present on either side of 3^{o} veins. Isolated tracheids are present only in 25 ppm (Fig. 2, G). Miniature vessel elements with two perforation plates are noticed at vein ending in 25 ppm (Fig. 2, F). In 50 ppm the tracheids are present mostly at the margin and the number is less as compared to 25 ppm. Tracheids are mostly elongated arranged in superimposed fashion (Fig. 2, E). Vein endings are simple or branched dichotomously. The thickness of all categories of veins increases in 100 ppm as compared to 25 and 50 ppm. Tracheids are distributed in the extreme tip region and margin. The vein endings are simple or rarely dichotomously branched. Simple ones are linear.

The increasing concentration of MOR inhibits the formation of tracheids and their size i.e. length (Figs. 3, 4).

iii) Gibberellic acid (GA)

The number of secondary veins varies from 4 to 5 in 25 ppm and 3 to 4 in 50 and 100 ppm. All the categories of veins are thickened in this treatment as compared to the control. The vein endings are either simple or branched. The branched ones divide once or twice dichotomously (Fig. 1, E). In 25 ppm accumulation of tracheids is more at the tip region. They are mostly isodiametric. The tracheids are present over the veins. The important feature is that the parenchymatous bundle sheath cells which are differentiated into tracheidal nodules run almost parallel to the primary vein (Fig. 1, F). Here, some of the sheath cells are converted into nodules and some remain as thin walled sheath cells only. In 50 and 100 ppm areoles are with or without vein endings; where the areoles are devoid of vein endings, loop formation is observed. The tracheids at the vein endings are uniseriate. Usually vessels occur only on the major veins (Fig. 1, G). but not on the minor veins and the vein endings. Miniature vessel elements contiguous with normal tracheids are observed in 50 ppm (Fig. 1, H). Isolated tracheids and extension cells are rarely observed. In 100 ppm one of the secondary veins after departure from the primary vein runs a small distance and divides into two. Both the branches run parallel to each other, upturn and form a brochidodromous arch like the other secondary veins.

The increasing concentration of GA increases the size of tracheids (Fig. 4) and cotyledonary area (Fig. 3) and inhibits the tracheid formation (Fig. 3), number of vein endings and vein termination number.

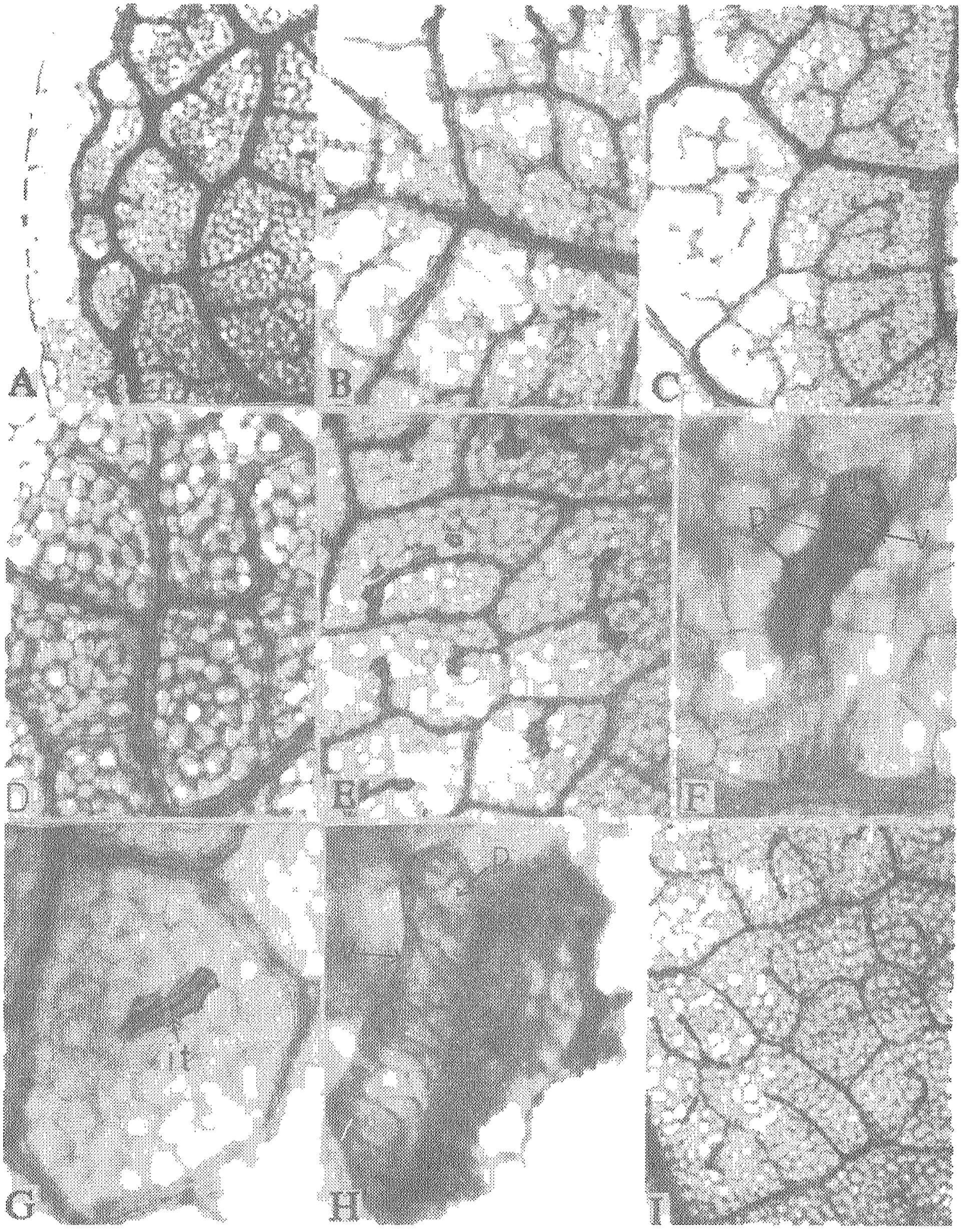

Fig. 2. Effect of growth regulators on cotyledonary architecture of *Brassica juncea* L. A. 2,4-D 50 ppm X120 B-C. 2,4-D 25 ppm X120 B-C. 2,4-D 25 ppm X120 D. 2,4-D 100 ppm X285 E-G. MOR 25 ppm X115, X570; X300 H-I COL 25 ppm X 1090, X115

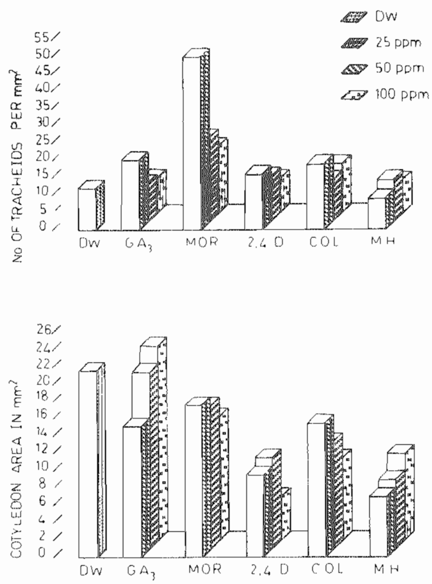

Fig. 3. Histograms showing the cotyledon area and number of tracheids per unit area in control and different concentrations of treatments.

iv) Colchicine (COL)

All the veins are thickened in this treatment. There is no clear cut demarcation between the 3^{o} and 4^{o} veins as they are equally thickened. Thickenings of major veins are not very prominent as in other treatments (Fig. 2, I). The vein endings are simple and mostly linear or curved. In 25 ppm, distribution of tracheids is more or less even. In the tracheids pit like structures are seen. Miniature vessel elements contiguous with a tracheid, without prominent secondary side wall reticulate thickening, are noticed in 25 ppm (Fig. 2, H). In 50 ppm the density of tracheids are less as compared to 25 ppm. The tracheids are mostly isodiametric and occur solitary or rarely in groups. Vein endings are also simple as in COL 25 ppm. In COL 100 ppm number of vein endings are more and vein termination numbers are less as compared to 25 and 50 ppm.

The increasing concentration inhibits the formation of tracheids (Fig. 3) and vein termination numbers. All the 3^{o} and 4^{o} veins are more or less equally thickened so much so that there is no clear cut demarcation between the 3^{o} and 4^{o} veins.

v) 2,4-Dichlorophenoxy acetic acid (2,4-D)

The number of 2^{o} veins ranges from 3 to 4 in 25 ppm and 4 to 5 in 50 and 100 ppm. All the categories of veins are thickened in 2,4-D 25 ppm (Fig. 2, A-C). The density of tracheids are more at the tip region (Fig. 2, B) where the areoles are devoid of vein endings, there loop formation is seen. Miniature vessel elements are observed in 25 and 50 ppm. In 50 ppm the distribution of tracheids is restricted to the margin and apical region of the cotyledon and are less as compared to 2,4-D 25 ppm. Tracheids are observed on either side of the minor veins. Tracheids are either solitary or in groups. Isolated tracheids are observed only in 50 ppm. In 100 ppm tracheids are observed mostly in groups at the vein endings. Tracheids are isodiametric. Bundle sheath cells which surrounded the veins are prominent (Fig. 2, D).

The increasing concentration of 2,4-D inhibits the formation of tracheids (Fig. 3) and their size (Fig. 4) and promotes vein termination number.

vi) Maleic hydrazide (MH)

The thickness of veins is normal. The secondaries range from 3 to 5 in 25 ppm and 3 to 4 in 50 and 100 ppm. Vein endings are simple or branched (Fig. 1, I). Loops are formed either by the union of tracheids or veins. In 25 ppm miniature vessel elements are observed. In 50 ppm the number of vein endings and vein termination number increases in contrast to 25 ppm. In 100 ppm number of vein endings is less and vein termination numbermore as compared to 25 ppm.

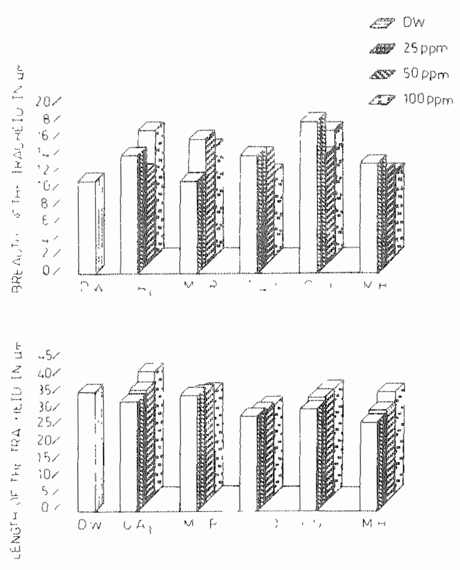

Fig. 4. Histograms showing the length and breadth of the tracheid in control and different concentrations of treatments.

The increasing concentration of MH inhibits the formation of number of vein endings and promotes the vein termination number and size i.e., length and of the tracheid (Fig. 4).

Discussion

Effect of growth regulators on the cotyledonary architecture is the subject of the present discussion. Jain & Mukerjee (1978) studied the influence of Chloroflurenol EMF 74050 on the leaf architecture of *Lycopersicon esculentum* Mill. The major venation is changed from camptodromous to campylodromous type and the thickness of veins and veinlets of all orders and bundle sheath increases considerably in treated plants. Vein endings become unbranched and absolute number of veinlets is reduced and there is accumulation of intramarginal tracheids. According to Murthy & Inamdar (1979) major venation pattern in both treated and untreated cotyledons remains unchanged in *Lycopersicon esculentum* var. *angurlata.* The increased concentration of Colchicine and Morphactin affects the average size of areoles per mm^2 as well as number and distribution of tracheids per areole and size of tracheids. Coin & Potzer (1933) pointed out that the soil moisture affects the vein density. However, which environmental factors are responsible for vein density is unknown. The major venation pattern remains unchanged in the treated cotyledons i.e., festooned brochidodromous type in *Brassica juncea* (Hickey & Wolfe, 1975). It is interesting to note that the major venation pattern in the cotyledons of *Brassica juncea* conforms to festooned brochidodromous, while that of mature foliage leaves to semicraspedodromous type. This reveals that the cotyledonary venation has no bearing on the foliar venation. In MOR treatment accumulation of tracheids is more in lower concentration than the higher. In GA veins are thickened. GA has also similar effect as far as the tracheid formation is concerned. Lower concentration of GA is favourable for formation of tracheids and vein endings and higher concentration for cotyledonary area and length and breadth of the tracheids. Differentiation of bundle sheath cells into tracheidal nodules is observed only in GA 25 ppm. It seems GA 25 ppm may favour the formation of tracheidal nodules. Kakkar & Paliwal (1972) also observed the differentiation of bundle sheath cells into tracheidal nodules in *Euphorbia.* The veins are thickened in COL also. The increasing concentration of COL inhibits the cotyledonary area, number of tracheids and promotes the length of the tracheids. Increasing concentration of 2,4-D inhibits the number of tracheids and length and breadth of tracheids and promotes the number of vein endings as well as vein termination number. while MH promotes the cotyledonary area and length of tracheids.

The vein endings are. simple or branched. Simple ones are linear or curved and branched ones divide once or twice dichotomously. Tracheids are lying over the veins or free in the areole. Extension cells are also observed. The number of tracheids per mm^2 is more in MOR 25 ppm as compared to all the treatments. It seems that MOR 25 ppm promotes the formation of tracheids.

Plymale & Wylie (1944) pointed the differences between the major and minor veins. According to them the major veins can be differentiated from the minor veins by the presence of secondary growth and xylem vessels and sieve tubes with sieve plates and companion cells. In contrast the minor veins showed no detectable cambial activity and had tracheids only in the xylem and phloem. Esau (1967) reported the presence of vessel elements in the minor veins of *Beta vulgaris* L. In a personal communication Cheadle pointed out that vessels are not unexpected even in small veins. Recently, Rao & Inamdar (in press) reported the presence of miniature vessel elements at the vein endings contiguous to the normal trachieds of *Brassica oleracea* var. *capitata* and *Sinapis alba.* During the course of present investigation the vessel elements at the vein endings are observed very rarely in control and occasionally in GA, MOR, 2,4-D, COL and MH. It may be noted that in COL treated cotyledons the vessel elements have weakly developed reticulate side wall thickening which may be due to the inhibitory effect of the growth substances. It may be true because of the inhibitory effect on the veins and tracheids that the thickenings are not properly developed in COL treated cotyledons. The vessel elements present at the vein endings are extremely small in size i.e., length (29 μm) and diameter (10 μm) and hence we prefer to call them as "miniature vessel elements". The occurrence of miniature vessel elements at the vein endings is an unusual feature.

Acknowledgements

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References

Coin, S.A. 1933. A comparison of leaf tissues of *Gaulussacia baccata* and *Vaccinium vacillans* grown under different conditions. *An. Midland Naturlist*, 14: 97-112.

Esau, K. 1967. Minor viens in *Beta* leaves: Structure related to function. *Proc. Amer. Physiol. Soc.*, 111: 219-233.

Gupta, M. 1978. Cotyledon architecture in Trifoliaceae. *Acta. Bot. Indica*, 6: 171-176.

Hickey, L.J. 1973. Classification of architecture of dicotyledonous leaves. *Amer. J. Bot.*, 60: 17-33.

Hickey, L.J. and J.A. Wolfe. 1975. The bases of angiosperms phylogeny: Vegetative morphology. *Ann. Misso. Bot. Gard.*, 62: 538-589.

Jain, V.K. and D. Mukherjee. 1978. Influence of chloroflurenol EMF 74050 on the leaf architecture of *Lycopersicon esculentum* Mill. *J. Ind. Bot. Soc.*, Abs. V-15.

Kakkar, L. and G.S. Paliwal. 1972. Studies on the leaf anatomy of *Euphorbia.* VI. The bundle sheath. in Puri, V. *et al.*, (eds.). *Biology of Land Plants.*

Melville, R. 1976. The terminology of leaf architecture. *Taxon,* 25: 549-561.

Murthy, G.S.R. and J.A. Inamdar. 1979. Effect of Colchicine and Morphactin on the cotyledonary venation of *Lycopersicon esculentum* var. *angurlata. Phyton,* 19: 337-347.

Plymale, E.L. and R.B. Wylie. 1944. The major veins of mesomorphic leaves. *Amer. J. Bot.,* 31: 99-105.

Rao, N.V. and J.A. Inamdar. 1979. Action of growth regulators on the cotyledonary and hypocotyledonary stomata of *Brassica juncea* L. in Periasamy, K. (ed.). *Histochemistry, Developmental and Structural Anatomy of Angiosperms. (A Symposium)* P & B Publications, Trichirapalli, pp. 220-228.

Rao, N.V. and J.A. Inamdar. 1984. Leaf architectural studies in the Brassicaceae. *Bot. Mag. Tokyo.* (in press).

Rao, V.S., K.N. Shenoy and J.A. Inamdar. 1980. Clearing and staining technique for leaf architectural studies. *Microscopica Acta.* 83: 307-310.