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REGULATORS OF POLLEN GERMINATION AND TUBE GROWTH OF DIFFERENT PARTS OF *CATHARANTHUS ROSEUS* L.

SAMINA RAUF AND M. ISHAQ KHAN

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Alcoholic extract of vegetative and reproductive parts of *Catharanthus roseus* was tested for substances responsible for germination and growth of pollen tube of the same plant. Several promoters and inhibitors of pollen germination and tube growth were detected in extracts of anthers, stigma, style and ovary. In the ovary extract no pollen tube growth promoter was found. The major differences in the quality of the growth regulators depended on the type of tissue tested. Extracts of leaf, young fruit, mature fruits and seeds contained compounds which inhibited the pollen germination and tube growth of *C. roseus*. The Rf value of the inhibitors differed according to the tissue used. No promoter was found in any of these extracts.

Introduction

Germination of pollen requires sufficient supply of moisture, inorganic elements and sugar as a source of energy. There are experimental evidences that natural growth promoting susbstances control the growth of pollen tube (Leopold, 1964; Setia *et al.*, 1985). These substances include promoters and inhibitors of tube growth (Iwanami, 1957; Miki, 1961; Brewbaker & Majumder, 1961; Malik & Ahluwalia, 1985).

Six classes of plant growth regulators (Auxins, Gibberellins, Kinins, Brassins, Ethylene and Inhibitors) are known to occur in pollens of different plants (Malik & Ahluwalia 1985). Increased growth of pollen tube was observed by Brink (1924) as a result of mixing crushed stigma, ovary or the extract of raw potato with the culture medium. Martin (1969, 1970) and Martin & Telek (1971) have found that the principal component of stigma exudate and secretions are phenolic substances and lipids. Phenolic substances were found as glycosides or esters. Khan & Jahan (1988) found three inhibitors of seed germination from the anthers of *Bombax ceiba*, one of which was phenolic in nature. In the present study an attempt was made to localize the site of regulators of pollen germination and tube growth and quantify these substances present in the vegetative and reproductive parts of *Catharanthus roseus*.

Materials and Methods

White flowers of *C. roseus* growing luxuriantly in the nursery of the Department of Botany, University of Karachi was used. At anthesis 20 flowers were collected and their

Table 1. Germination of pollen and pollen tube growth of *Catharanthus roseus* in the presence of alcoholic extract of various parts of the same plant.

Flower parts used	% pollen Ger. after 60 min.	% of Control	Pollen tube growth (µm) after 90 min.	% of Control
Control	80.60 ± 4.40	100	432.50 ± 23.45	100
Leaf	13.30 ± 0.66	16.50	183.60 ± 25.38	42.45
Calyx	72.00 ± 6.26	89.10	125.10 ± 13.80	28.92
Corolla	40.80	50.62	52.80 ± 8.15	12.21
Corolla tube	25.33 ± 4.66	31.43	50.70 ± 8.15	11.72
Anther	95.33 ± 0.76	118.27	465.40 ± 17.97	107.61
Stigma	96.82 ± 1.15	120.12	585.20 ± 17.73	135.31
Style	93.83 ± 2.48	116.41	591.80 ± 19.98	136.32
Ovary	95.19 ± 2.40	118.05	227.50 ± 13.46	52.60
Young fruit	54.18 ± 5.03	67.22	223.42 ± 15.27	51.66
Fruit coat	59.30 ± 8.36	72.57	161.80 ± 14.49	37.41
Seeds	58.18 ± 6.43	72.18	225.50 ± 12.80	52.14

calyx, corolla, corolla tube, anthers, stigmas, styles and ovaries were pooled and extracted in 10 ml of 80% ethanol for 24 h at 15°C. Similarly 500 mg fresh weight of leaf, young fruit, mature fruit coat and seeds were also extracted in 10 ml of 80% ethanol. After 24 h of extraction, ethanol was filtered, dried at room temperature and redissolved in 1 ml of pollen growth medium containing 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2$ $2H_2O$, 200 ppm $MgSO_4$. $7H_2O$, 100 ppm KNO_3 dissolved in 20% sucrose.

Pollen grains of *C. roseus* were picked up on a brush and spread in 0.01 ml of pollen growth medium kept on a microscopic slide. The slide placed in a Petri dish having a wet filter paper lining was tightly covered and incubated at 32 ± 2°C. After 60 min of incubation pollen germination and after 90 min tube growth were recorded under a binocular at a magnification of 40 x.

Paper chromatographic sepration of all the extracts were carried out employing Whatman filter paper No. 1 which was prewashed with 80% ethanol and dried. Alcholic extract of *C. roseus* was loaded in seperate chromatographic papers (22cm x 5 cm) and ascending chromatography performed using Isopropanol: Ammonia: Water (10:1:1 v/v) as a solvent. After development, the chromatogram was cut into ten equal strips (Rf 0.1-1.0) together with one control strip of the same size. Each strip was further cut into small pieces and kept in vials together with 1 ml of the growth medium. After 60 min., of extraction, 0.01 ml extract from these vials were taken out and used for pollen germination and tube growth study.

Results

All the reproductive parts of flower showed the presence of promoters affecting pollen germination and tube growth of *C. roseus* except ovary which showed pollen tube growth inhibitors only (Table 1). Paper chromatographic separation of these extracts indicated the presence of several promoters and inhibitors of pollen germination and tube growth (Figs 1 & 2). An analysis of nonreproductive parts of the flower i.e., calyx, corolla and corolla tube showed the presence of inhibitors of pollen germination and tube growth only (Table 1). Paper chromatographic separation of these extracts also revealed several inhibitors of pollen germination (Fig. 1) and tube growth (Fig. 2). No growth promoting substance was detected in any of the extracts of non-reproductive parts of the flower. Similarly alcoholic extracts of leaf, young fruit, mature fruit coat and seeds showed the presence of inhibitors of pollen germination and tube growth (Table 1). However, paper chromatographic separation of these extracts revealed the presence of several germination and growth inhibiting zones (Figs. 3 & 4). The Rf value of the inhibitors differed according to the tissue used. Inhibitors of pollen tube growth was found to be greater in these plant parts similar to that of the non-reproductive parts of the flower than the inhibitors of pollen germination.

Discussion

The present study showed the presence of a number of germination and growth promoting substances in the reproductive parts of *C. roseus* flowers which may regulate the pollination mechanism in this plant. However no promoter of pollen germination and pollen tube was found in the leaves, seeds and fruits. The presence of pollen germiation factor (PGF) in all the vegetative and reproductive parts have been reported by Brewbaker & Kwack (1963). In anther extract of *C. roseus* two pollen germination promoters and one tube growth promoter was detected. This finding is similar to that of Brink (1924), Brewbaker & Majumder (1961) and Brewbaker & Kwack (1963) while Hodgkin & Lyon (1983) did not find any such promoters in the extract of *Brassica oleracea* anthers.

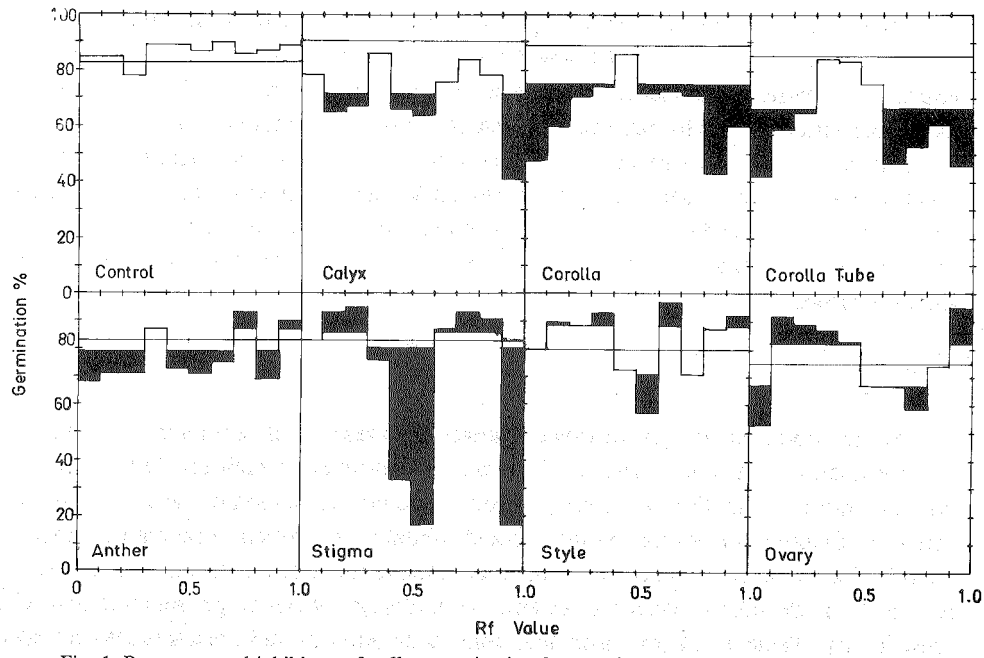

Fig. 1. Promoters and inhibitors of pollen germination from various parts of *Catharanthus roseus* flowers. Dark areas are significantly different (P 0.05) from the control.

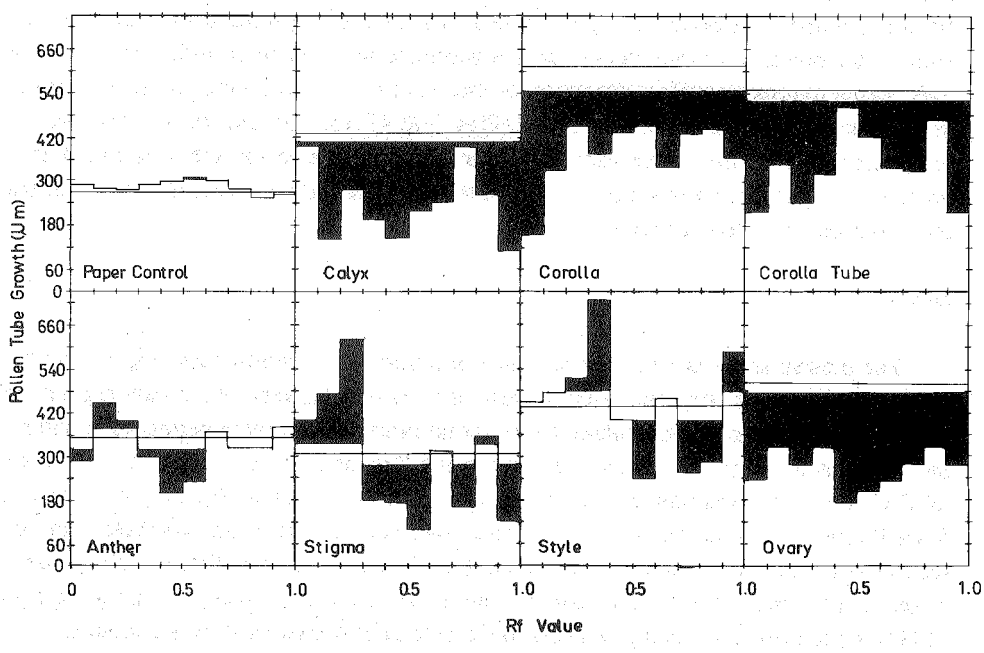

Fig. 2. Promoters and inhibitors of pollen tube growth from various parts of *Catharanthus roseus* flowers. Dark areas are significantly different (P 0.05) from the control.

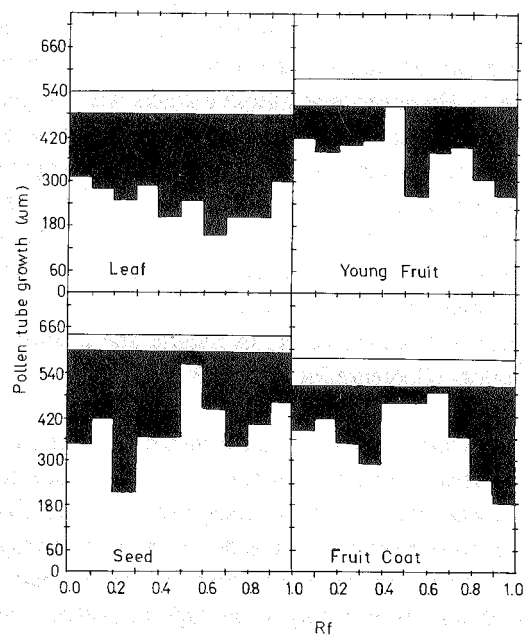

Fig. 3. Paper chromatographic separation of inhibitors of *Catharanthus roseus* pollen germination from various parts of the same plant. Dark areas are significantly different (P 0.05) from the control.

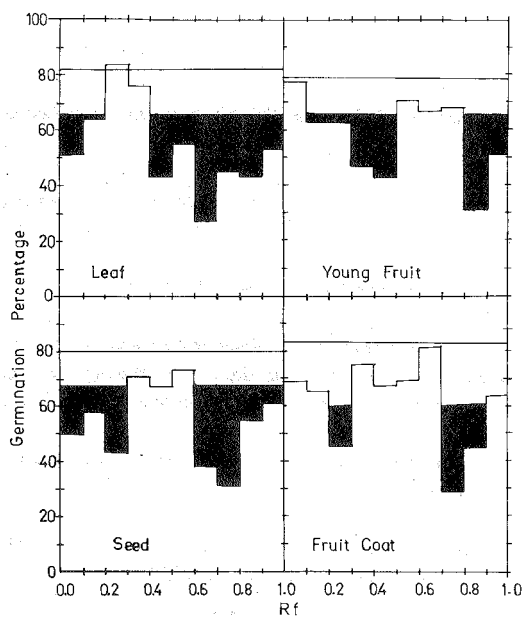

Fig. 4. Paper chromatographic separation of inhibitors of *Catharanthus roseus* pollen tube growth from various parts of the same plant. Dark areas are significantly different (P 0.05) from the control.

Results obtained in the present study show that stigma and style of *C. roseus* contain only the promoters of pollen germination and tube growth as is evident from the quantitative estimation (Table 1). However, when these extracts were subjected to paper chromatographic separation distinct promotory and inhibitory zones were obtained. This may suggest the low quantity of inhibitors in stigma and style as compared to the promoters so that the pollen tube which penetrate the stigma surface may grow through the style to the ovary without being inhibited significantly. However, when the pollen tube enters the ovary its growth is immediately checked presumably due to the presence of high quantity of inhibitors of pollen tube growth (Table 1 & Fig. 2). It is interesting to note that stigma extract was quite different from the others as regards the quality of pollen germination and tube growth inhibitors (Compare Fig. 1 with Fig. 2).

The chemical nature and biological significance of stigmatic extract was worked out by Martin (1969). He tested ten different species from various families through paper chromatography and found phenolic compounds occuring as glycosides. From this study he also concluded that these phenolic glycosides in the stigma may interact with growth substances to control pollen germination and tube growth and may account for the specificity of stigmas which may permit only a few type of pollen germination on their surface. The chemical nature of promoters and inhibitors of pollen germination and tube growth of *C. roseus* needs investigation in order to ascertain the relationship between the two in controlling the germination and growth of pollen.

References

Brewbaker, J.L. and B.H. Kwack. 1963. The essential role of calcium in pollen germination and tube growth. *Amer. J. Bot.*, 50: 859-865.

Brewbaker, J.L. and S.K. Majumder. 1961. Cultural studies of the pollen population effect. *Amer. J. Bot.*, 48: 457-464.

Brink, R.A. 1924. The physiology of pollen. *Amer. J. Bot.*, 11: 471-436.

Hodgkin, T. and C.D. Lyon. 1983. Germination of *Lilium* and *Petunia* pollens on TLC plates and their inhibition by extract from *Brassica oleracea* tissues. In: *Pollen: Biology and implication for p[lant breeding (Eds.)*. David L. Mucahy & Ercole Ottviane. Elsevier Sci. Pub. Co. Int. USA.

Iwanami, Y. 1957. Physiological researches of pollen. XIII. Growth inhibition of the pollen tube of *Camellia japonica*. *Bot. Maz.* (Tokyo) 70: 144-149.

Khan, M.I. and B.Jahan. 1988. Allelopathic potential of senesced anthers of *Bombax ceiba* L., inhibiting germination and growth of lettuce seeds. *Pak. J. Bot.*, 20: 205-212.

Leopold, A.C. 1964. *Plant growth and development*. McGraw-Hill Book Company, New York.

Malik, C.P. and K. Ahluwalia. 1985. Growth regulator contents in maturing pollen grains of *Kigelia pinnata*. In: *Recent advances in pollen research*. (*Ed.*). T.M. Varghese. Allied Publishers Private Limited, New Delhi pp. 43-45.

Martin, F.W. 1969. Compounds from the stigmas of ten species. *Amer. J. Bot.*, 56: 1023-1027.

Martin, F.W. 1970. The stigmatic exudate of *Strelitzia*. *Phyton* (Buenous Aires) 27: 47-53.

Martin, F.W. and L. Telek. 1971. The stigmatic secretions of the sweet potato. *Amer. J. Bot.*, 58: 317-322.

Miki, M. 1961. Pollen germination and pollen tube growth in the presence of pistil slices *in vitro*. *Mem. Coll. Sci. Univ. Kyoto Ser.* B. 28: 375-388.

Setia, N., G.S. Mangat and C.P. Malik. 1985. Effect of growth regulators and antimetabolites on pollen germination and tube elongation in *Cicer arietinum*. In: *Recent advances in pollen research* (Ed). T.M. Varghese. pp. 63-68. Allied Publishers Private Limited, New Delhi.

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