Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/3554
DOI URL: http://dx.doi.org/10.21474/IJAR01/3554
ISSN NO. 2320-5407

RESEARCH ARTICLE

DIFFERENTIAL ACTIVITY OF FOUR SELECTED ENZYMES IN THE PISTILS AND POLLEN GRAINS OF TWO VARIETIES OF *HAMELIA PATENS* JACQ. (RUBIACEAE) FOLLOWING COMPATIBLE AND INCOMPATIBLE POLLINATION.

[*]Renjumol. R[1] and Radhamany P. M.[2].
Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram-695581.

Manuscript Info

Manuscript History

Received: 02 January 2017
Final Accepted: 03 February 2017
Published: March 2017

Key words:-
Hameliapatens,Peroxidase, Acid phosphatase, Polyphenol oxidase, Esterase,

Abstract

Hamelia patens Jacq.commonly called fire bush, is a fast growing, semi woody and evergreen perennial shrub of the family Rubiaceae. It is a native of central and southern Florida. It is chiefly grown for the showy bunch of beautiful flowers. In tropical America, local people make use of the extract of its leaves and stem, for curing diseases such as skin rashes, sores, insect sting and various fungal diseases of the skin because of its antibacterial and antifungal properties. In India this plant is particularly cultivated on a wider scale in home gardens as an ornamental plant. In *H. patens*, sexual reproduction is difficult due to self- incompatibility, while crossing between morphologically different plants of two accessions gives viable seeds. In the present study two varieties of *H. patens*, *H. patens* variety *patens* and *H. patens* variety *glabra*was chosen to study the activity of four enzymes, namely, peroxidase, acid phosphatase, polyphenol oxidase, esterase and total protein. Three developmental stages were identified for both the plants. Controlled pollinationswere carried out in the two plants. Stigma of two varieties were pollinated with its own pollen and also pollen from other variety. Crossed stigma showed higher activity of all the enzymes than self- pollinated and stigma from flowers before anthesis. The aim of the study was to understand if pollination with self and crossed pollen grains has any influence on the activity ofthe four selected enzymes in post pollinated pistils. The enhanced activity of these enzymes indicates their role mainly in the defence mechanism than anything to do with stigma receptivity. The results have been discussed in the light ofthe available literature.

Introduction:-

Hamelia patens Jacq.is commonly known as scarlet bush or firebush. It is a large perennial shrub or small tree. Also, the plants are used in folk medicine against a range of ailments.All parts of *H. patens* are used in natural medicine. A number of active compounds have been found in firebush, such as apigenin, ephedrine, flavanones, isomaruquine, isopteropodine, maruquine, narirutins, oxindole alkaloids, palmirine, pteropodine, rosmarinic acid, rumberine, rutin, seneciophylline, speciophylline, isopteropodine, stigmast-4-ene-3,6-dione and tannin (Cavanagh 1963, Duke 2007, Aquino et al1990, Ahmad et al2012&Paniaguaet al 2012) (−)-hameline, tetrahydroalstonine, aricine, uncarine F, stigmast-4-ene-3, 6-dione and 5,7,2-,5- -tetrahydroxyflavanone 7- rutiroside(Aquino1990, Ahmad et al 2012& and

Corresponding Author:- Renjumol. R.
Address:-Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram-695581.

Paniaguaet al 2012). The bark also contains significant amounts of tannins. Firebush contains crude protein and has *in vitro* digestibility (Ibrahim 1997).The plant possesses analgesic, antispetic, anti-inflammatory, febrifuge, refrigerant properties. Fruit of *H.patens*is edible(Irobi et al 1996) and the plant is used in herbal medicine to treat athlete's foot, skin lesions and rash, insect bites, nervous shock, inflammation, rheumatism, headache, asthma, and dysentery (Lachoriaet 1999 and Liogier 1999). Ethanolic extract of different parts like leaf, stem and root of *H. patens* shows anthelmintic and antimicrobial activity (Sapana 2012).It has been reported to exhibit that this plant possesses anti-diarrhoeal activity (Salud1996).It is also used for insect bites, menstrual disorders, uterine and ovarian affliction (Shrisha et al2011).Cytostatic and cytotoxic activity against tumor cell lines (Taylor et al2013 and wound healing activity (Sandhya et al 2011)with *H. patens*. Antifungal properties ofaqueous extracts of leaves, flowers and fruits of *H.patens*was also reported(Abubacker 2013). Antibacterial properties (Camporese 2003), vasorelaxant (Reyes &Chilpa 2004)and toxicity and antinociceptive effects of *H.patens* also studied (Castro 2015).

Development of fruits with healthy seeds is theculmination of the natural process of pollination andfertilization. Seeds are important for the plant forpropagation of the progeny and for a breeder to assessthe results of breeding while developing new cultivars.Louis and Radhamany(2009)and Louis et al (2012)reported RNase based gametophytic incompatibility in *H.patens*.Since legitimateand illegitimate pollinations are affected by alternations in the metabolic status of stigma and stylartissues (Dhaliwal1985), it would be imperative to study the changes in the enzyme activities of stigma-style tissues following compatible and incompatible pollination. Since the enzymes control biochemical reactions, and their synthesis is under the control of specific gene(s), any change in the activity of an enzyme would reflect in the pattern of gene expression and corresponding metabolic events in the cell. Hence, the enzymes can be used as tools to study the problem of self-incompatibility at the biochemical level. In the present investigation, changes in the activities of peroxidase, polyphenol oxidase, esterase and acid phosphatase along with total amount of protein have been studied in antherlobe and pistil of twovarieties of *H.Patens*;*Hamelia patens* variety *patens* and *Hamelia patens* variety *glabra*, in an attempt to understand the biochemical aspects of self-incompatibility in *Hamelia*.

Materials and methods:-

Two varieties of *Hamelia patens* Jacq.viz.*Hamelia patens* var. *patens*(Fig. 1)and *Hamelia patens* var. *glabra(*Fig.2)maintained in the Botanic garden, Department of Botany,University of Kerala, Thiruvananthapuram, and pistils from flowers of these two plants were used in the present investigation.

Controlled pollination:-

Emasculation and controlled pollinationswere carried out in both the varieties of *H. patens.* Early in the morning, a day before controlled pollination, mature flower buds were emasculated and bagged with butter paper bags at 'pre-balloon' stage, 18–24 h before pollination. For self-pollination, flower bud atthe 'balloon' stagewas pollinated by dusting the pollen grains from the same flower on the surface of the stigma and bagged. In the cross pollination, variety *patens* was considered as female plant and variety *glabra* considered as male plant. Following samples were collected for the test of isozymes.

1. Sample I-Self-pollinated pistil from the variety *patens*.
2. Sample II- Un-pollinated pistil fromthe variety *patens*.
3. Sample III-Self- pollinated pistil from the variety *glabra*.
4. Sample IV- Un-pollinated Pistil from the variety *glabra*.
5. Sample V-Pollinated pistil from the cross between variety *patens* x variety *glabra*.
6. Sample VI-Stamen from the variety *patens* after self pollination
7. Sample VII-Stamen from the variety *patens*before pollination
8. Stage VIII-Stamen from variety *glabra* after self pollination
9. Stage IX- Stamen from the variety *glabra*before pollination

Preparation of enzyme extract:-

Part of the pistil without ovary was collected and frozen in -40°C. Sample weighing about 450 mg were homogenized with 9 ml of cold 5% KCl (w/v) using a pre-chilled mortar and pestle. The homogenate was centrifuged at 0 ± 2°C at 10,000 rpm for 10 min. The clear supernatant was used directly for the assay of enzyme activities and estimation of protein(Jorgensen et al 1953, Ching et al 1987, Neog et al 2003 and McInnis et al 2006).

Assay of peroxidase:-
The activity of peroxidase was assayed according to Malik and Singh (1980). The assay mixture containing 2.5 ml of phosphate buffer (pH 6.5, 0.1 M), 0.2 ml ofdiluted enzyme extract and 0.1 ml of *o*-dianisidine (1 mg/ml methanol) was incubated at 28°C in a water bath for 2 min. The reaction was started by adding 0.2 ml of H_2O_2. The change in absorbency was recorded at 430 nm using a stopwatch at 30 s interval for 5 min. The enzyme activity was expressed in terms of the rate of increase of absorbance per hour per mg protein.

Assay of acid phosphatase:-
The reaction mixture contained 0.5 ml of substrate solution (50 mg *p*-nitrophenyl phosphate in 10 ml water + 25 ml of acetate buffer, 0.1 M, pH 4.8) and 0.1 ml of suitably diluted enzyme extract. The mixture was incubated at 35°C for 30 min. The reaction was stopped by adding 2.4 ml of 0.1 N NaOH and the absorbance was recorded at 410 nm. The enzyme activity was expressed in terms of micromoles of *p*-nitrophenol released/h/mg protein Malik and Singh (1980).

Assay of polyphenol oxidase:-
The polyphenol oxidase activity was measured as per the method of Sarvesh and Reddy (1988). The assay mixture (3 ml) contained 2.0 ml of 2 M carbonate–bicarbonate buffer (pH 10), 0.15 M of *o*-catechol and 0.2 ml of suitably diluted enzyme extract. The assay mixture was incubated for 2 min at 25°C. The reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The change in absorbency was recorded at 420 nm for 2 min. The enzyme activity was expressed in terms of micromoles of quinone formed/h/mg protein.

Assay of esterase:-
Esterase activity was assayed by napthol-acetate method(Sawhneyet al 1981).The assay mixture contained2.0 ml of 20 mM phosphate buffer of pH 10, 0.1 M of1-napthol acetate and 0.2 ml of suitably diluted enzymeextract. Change in absorbance was recorded at 420 nmfor 3 min and enzyme activity expressed in terms of micromoles of naphthol-acetate produced/h/mg protein. .

Total protein:-
The amount of protein was determined according to the standardmethod(Bradford 1976 andLowry et al1951). using Bovine serum albumin as standard.

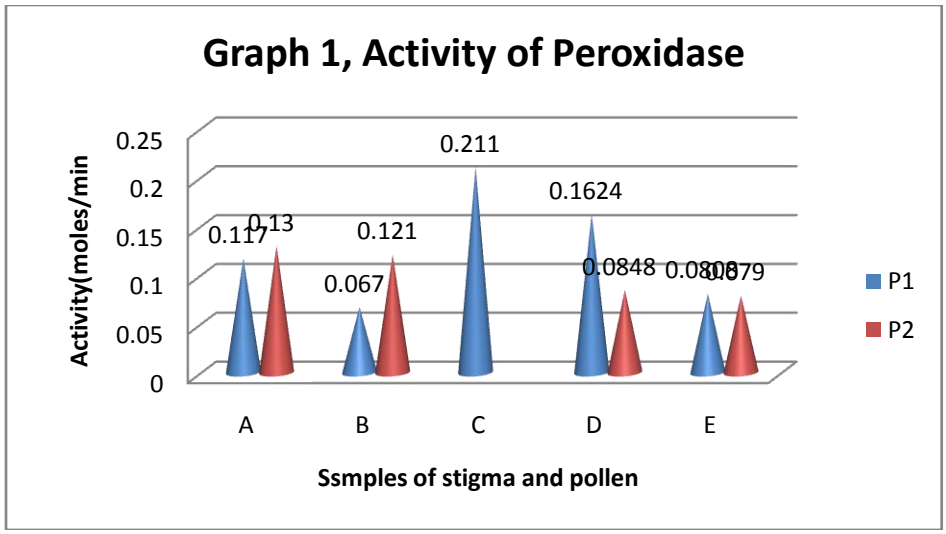
Statistical analysis:-
The data were collected from five sets of experiments and were subjected to analysis of standared deviation (Table-1 and Graph 1-5).

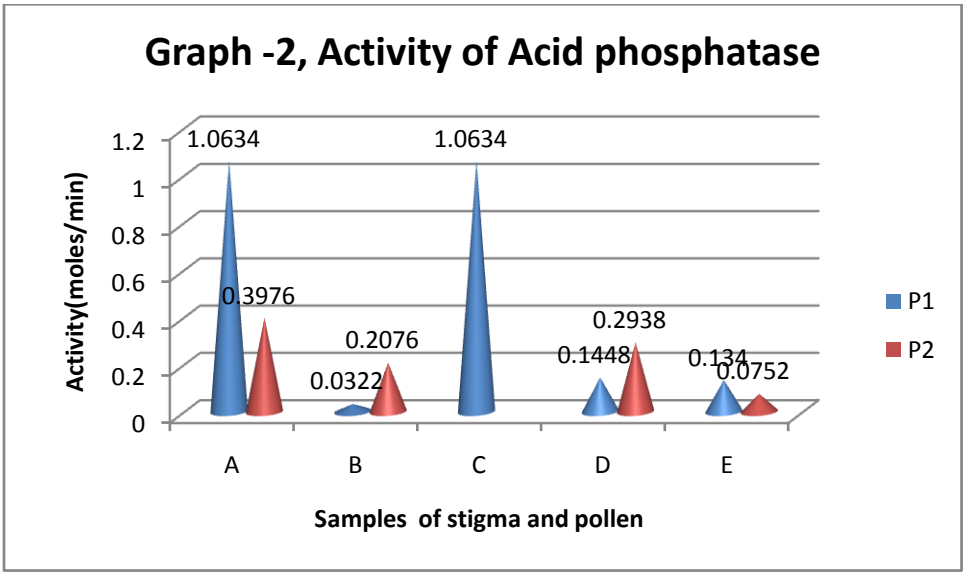
Results:-

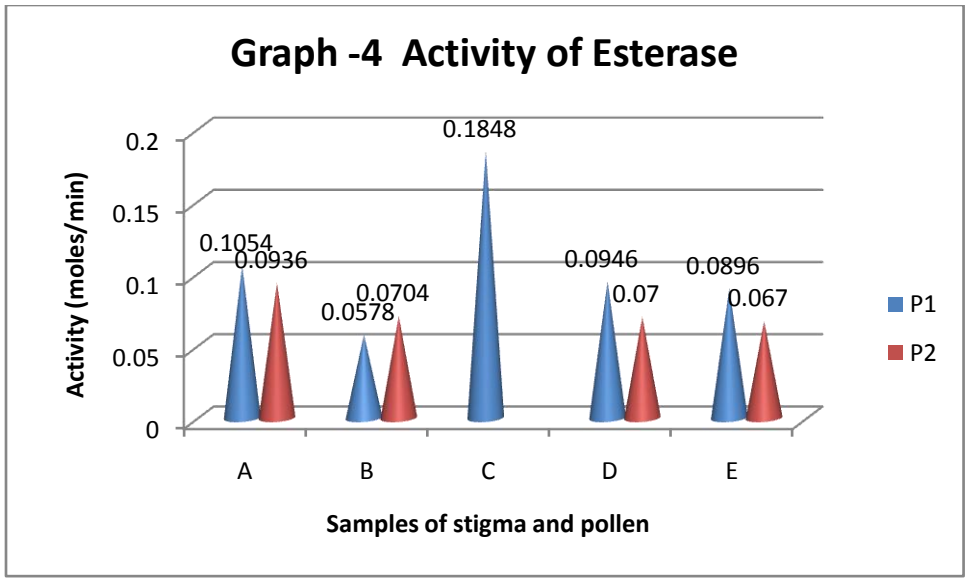
Table I:-Level of peroxidase, acid phosphatase, polyphenol oxidase and esterase on protein content after self and cross-pollination in two accessions of*H.patens*

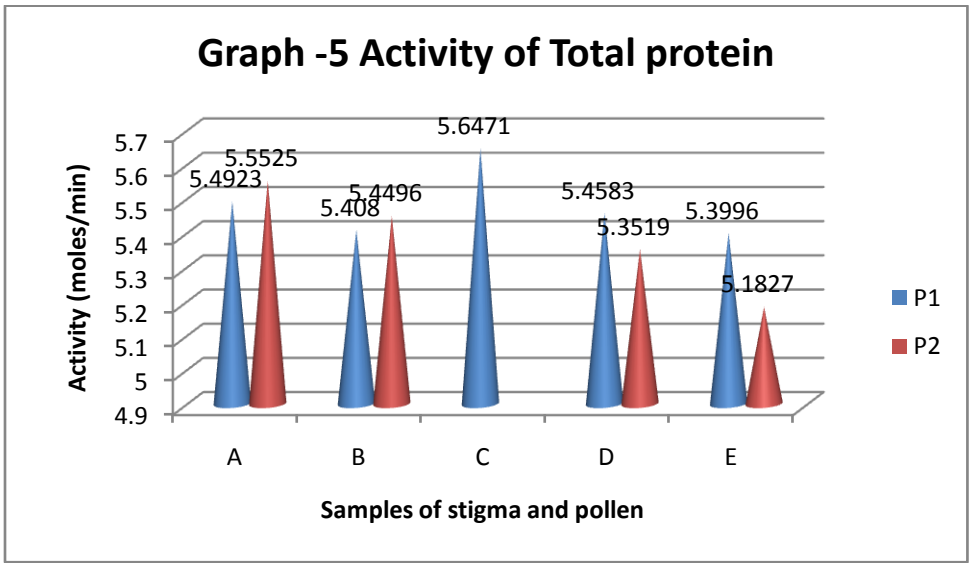
Sl. No	Samples	Peroxidase [h/mg(Mean±SD)]	Acid phosphatase [h/mg(Mean±SD)]	Polyphenol oxidase [h/mg(Mean±SD)]	Esterase [h/mg (Mean±SD)]	Total protein [Mean±SD]
1	Sample I	0.117±0.585	1.0634±0.0011	0.1358±0.0008	0.1054±0.0018	5.4923±0.0658
2	Sample II	0.067±0.0054	0.0322±0.00216	0.1072±0.0008	0.0578±0.0008	5.4080±0.1163
3	Sample III	0.130±0	0.3976±0.0011	0.1122±0.0008	0.0936±0.0020	5.5525±0.0903
4	Sample IV	0.121±0.0004	0.2076±0.0011	0.0952±0.0008	0.0704±0.0024	5.4496±0.0386
5	Sample V	0.211±0	1.0634± 0.0011	0.1882± 0.0008	0.1848± 0.0008	5.6471±0.0257
6	Sample VI	0.1624 ± 0.0005	0.1448 ± 0.0012	0.1648 ± 0.0008	0.0946 ± 0.0011	5.4583 ± 0.0332
7	Sample VII	0.0808 ± 0.0008	0.134 ± 0.0012	0.105 ± 0.001	0.0896 ± 0.0011	5.3996 ± 0.0856
8	Sample VIII	0.0848 ± 0.0008	0.2938 ± 0.002	0.1528 ± 0.0008	0.07 ± 0.0075	5.3519 ± 0.0385
9	Sample IX	0.079 ± 0.00125	0.0752 ± 0.0017	0.1126 ± 0.0005	0.067 ± 0.001	5.1827 ± 0.0336

Values are mean ±sd of six sets of samples


Graph 1, Activity of Peroxidase
Activity(moles/min
0.25
0.2
0.15
0.1
0.05
0
0.117
0.13
0.067
0.121
0.211
0.1624
0.0848
0.0808
0.079
A
B
C
D
E
Ssmples of stigma and pollen
P1
P2


Graph -2, Activity of Acid phosphatase
Activity(moles/min)
1.2
1
0.8
0.6
0.4
0.2
0
1.0634
0.3976
0.2076
0.0322
1.0634
0.2938
0.1448
0.134
0.0752
A
B
C
D
E
Samples of stigma and pollen
P1
P2





Graph 1-5:-Activity of the enzymes, peroxidase, acid phosphatase, polyphenol oxidase, esterase and total protein in different stages of stigma and anther(A-sample I and sample III, B-sample II and sample VI, C-sample V, D- sample VI and sample VIII, E-sample VII and sample IX)

Discussion:-

Comparative analysis of the activity of four isozymes namely peroxidase, acid phosphatase, polyphenol oxidase and esterase in *H.patens*var.*patens* and *H.patens*var.*glabra* were carried out to find out the differences in the metabolic activity of unpollinated,self-pollinated and cross-pollinated pistils of self-incompatible *H.patens*varieties in the present study. Peroxidase has been implicated as an indicator of stigma receptivity (Galen & Plowright 1987, Dupuis & Dumas 1990, Dafni &MotteMaues1998 and Stpiczynska2003) and mere adherence of pollen grains to stigma has been shown to increase peroxidase activity independent of the penetration of pollen tube (Galen & Plowright 1987, Bredemeijer 1982, Bredemeijer 1984, Bredemeyer&Blaas1975, Bredemeyer 1975, Bredemeyer 1979 and Linskens1969). In the present study, significantly higher activity of peroxidase was observed in cross-pollinated styles were noticed when compared to self-pollinated and unpollinated stigma in both the varieties studied. The mean value was found high in variety *glabra*(0.121), followed by variety *patens*(0.117). Peroxidase

enzyme which is known to be a defence induced enzyme(McInnis et al 2006, Cheong 2002, Delannoy 2003 and Do et al2003). It is possible that pollen grains landing on stigma set in signals which activate peroxidase as a defence strategy. The activity of this enzyme is modified by germinating pollen grains(Bredemeijer 1984). Activities of acid phosphatase showed a considerable variation between self and cross- pollinated combinations. Activity was significantly higher (1.1894) in the styles after cross- pollination when compared with the self andunpollinated stigmas. The highest enzyme activity was noticed in the variety *patens* (1.0634) than variety *glabra*(0.0322). The elevated levels of acid phosphatase in cross pollinated stigmaclearly indicate a role for this enzyme in pollen tube growth in the style. This enzyme is probably produced in response to the penetrating pollen tube for providing inorganic phosphates through hydrolysis of phosphate esters(Ching 1987). Activity of polyphenol oxidase is very low or negligible in stigmas after self-pollination and stigma before anthesis. In contrast, the crossed stigma showed increase in polyphenol oxidase activity over the selfed styles. The highest specific activity for this enzyme was recorded in the cross-pollinated samples of stigma (0.1882) and thehighest enzyme activity was noticed in the self-pollinated style of variety *patens* (0.1358) than variety *glabra*(0.1122). The consistently enhanced activity of polyphenol oxidase in the two plants is in line with its possible role in the defence mechanism (Mayer &Harel 1979, Bashan 1987 and Tyagi2000). In wet and dry stigmas of a wide range of plants the time of stigma receptivity has been correlated with esterase activity (Heslop-Harrison 1975, Heslop-Harrison1977, Kohn &Waser1985 and Lavithis&Bhalla 1995).A moderate level of esterase activity is maintained at all the stages of stigma. Esterase activity was higher in cross- pollinated style (0.1848) than other stages of stigma. Also it is noticed that esterase activity was very low in stigmas of two varieties. Higher activity of esteraseprobably could be a response to the germinating pollen and must be facilitating in the penetration of the pollen tube into the stigma by hydrolyzing the cutin layer (Heslop-Harrison 1975, Lavithis&Bhalla 1995 and Hiscock et al 2002).Estimation of total protein was found to be higher in all the stages but cross-pollinated styles shows higher amount (5.6471) than other stages of stigma.

Conclusion:-

The enzyme may directly act to prevent the growth of selfed pollen tubes or sometimes may remain absent to disturb the growth of the pollen tube (Nettancourt 1977). Higher level of peroxidase found incross-pollinated styles than in self-pollinated ones indicates its involvement in the regulationof pollen tube growth through styles. One cannot rule out the involvement of peroxidase,as a nonspecific factor of the self-incompatibility reaction, but it plays a significant part in this mechanism. There are reports that this enzymebrings about changes in structural glycoproteins, other enzymes, pectin or cellulose present in the pollen tube wall (Siegel &Galston 1955 and Stonier 1979).Higher level of acid phosphatase in self- than cross-pollination as observed in the presentstudy is in agreement with the earlier finding of Dhaliwal and Malik (1985) in stigma-style tissuesof*Brassica compestris*var. *Toria*. It can be inferred that the inhibition of pollen tubegrowth is due to some factor(s) that could cause the production of acid phosphatase inhigher quantity.This enzyme is probably produced in response to the penetrating pollen tube for providing inorganic phosphatases through hydrolysis of phosphate esters.

Elevated level of polyphenol oxidase in all the experimental samples following cross-pollinationsupports the complementary hypothesis and the earlier assumption of Mayer andHarel(1979) that the enzyme has the ability to produce quinone and selectively inhibit theactivities of some other enzymes. Though the results of the present study do not confirm any direct relationship between this enzyme and the manifestation of self-incompatibility, itis possible that polyphenol oxidase acts as complementary factor along with other elements like environment and genetic makeup of the plant. In wet and dry stigmas of a wide range of plants the time of receptivity has been correlated with esterase activity. A moderate level of esterase activity is maintained at all the stages, to maintain the receptivity of stigma. The elevated amount of esterase in cross- pollinated stigma could be facilitating in the penetration of the pollen tube in to the stigma by hydrolysing the cutin layer. Self-incompatibility mechanism in angiosperms is widespread and has receivedconsiderable attention in recent years. Self-incompatibility genes have been identified,characterized, cloned and transferred to self-compatible lines in several species (Hoopen 1998 and Brien 2002). From the present study, it may be concluded that significant changes in the activities ofcertain key enzymes occur in the stigma and style tissues of *Hamelia patens* followingcompatible and incompatible pollination. Variations in the enzyme activitiesas observed in the present study shows that the biochemical changes occur in the stigma styletissues affected by self-incompatibility mechanism that in turn affects the metabolicstatus of these tissues. As the synthesis of these enzymes is controlled by their correspondinggene(s), their quantitative changes may be due to the changes in the regulation of expressionof gene(s) and hence offer a basis to study the mechanism of self-incompatibilityat molecular level.

Acknowledgements:-
The authors are grateful to the University Grants Commission for the award of Rajiv Gandhi National Fellowship (RGNF) and to The Head, Department of Botany, University of Kerala for the facilities provided.

Fig. 1:-*Hamelia patens* var. *patens.*

Fig. 2:-*Hamelia patens* var. *glabra*

References:-
1. Abubacker.M N., Sathya, C., &Prabakaran, R. (2013).Invitro Antifungal potentials of *Hamelia patens* Jacq (Rubiaceae) Aqueous extracts of leaves, Flower and fruits. Biosciences Biotechnology Research Asia, vol 10(2), 699-704.
2. Ahmad, A., Pandurangan, A., Singh, N., &Ananad, P.(2012). A minireview on chemistryand biology of *Hamelia patens* (Rubiaceae).Pharmacog J, 4, 1–4.
3. Aquino, R., Ciavatta, M.L., De Simone, F., &Pizza, C.(1990). A flavone glycoside from*Hameliapatens*.*Phytochemistry*, 29, 2359–2360.
4. Bashan, Y., Okon, Y., &Henis, Y. (1987). Peroxidase,Polyphenol Oxidase and Phenol in Relation to Resistance Against*PseudomonasSyrigae*pv*Tomato*in Tomato Plants. *Can. J.Bot*, **65**, 366-372.
5. Bradford, M.M. (1976).A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.*Biochemistry*, 72, 248-254.
6. Bredemeijer, G M M. (1982). Mechanism of Peroxidase Isoenzyme Induction in Pollinated *NicotianaAlata*Styles.*Theor. Appl. Genet.* **62**, 305-309.
7. Bredemeijer, G M M. (1984). The Role of Pereoxidasesin Pistil-pollen Interactions. *Theor. Appl. Genet.* **68**, 193-206
8. Bredemeyer, G MM. (1975).The effect of peroxidase on pollen germination and pollen tube growth *in vitro*.*Pl. Cell Incompatibility Newslett*,**5**, 34–39.
9. Bredemeyer, G MM. (1979).The distribution of peroxidase isozymes, chlorogenic acid oxidase and glucose-6-phosphate dehydrogenase in transmitting tissue and cortex of *Nicotianaalata*styles.*Acta Bot. Neerl*, **28**,198–203.
10. Bredemeyer, G MM., &Blaas, J A. (1975).Possible role of a stylar peroxidase gradient in the rejection of incompatible growing pollen tubes.*Acta Bot. Neerl*, 24, 37–48.
11. Brien, M O., Kapfer, C., Major, G.,Laurin, M., Bertrand, C., Kondo, K., Kowyama, Y. &Matton, D.P. (2002). Molecularanalysis of the stylar expressed *Solanumchacoense*small asparagine-rich protein family related to theHT modifier of gametophytic self-incompatibility in *Nicotiana*. *Pl. J*, **32**, 985–996.
12. Camporese, A., Balick, M J., Arvigo, R., Esposito, R G., Morsellino, N., De Simone, F.,&Tubaro, A. (2003). Screening of anti-bacterial activity of medicinal plants fromBelize (Central America). J. Ethnopharmacol, 87, 103–107.

13. Castro, A J A., Ramos, S B., Morales, AH., Morales, J R Z., Chavez, M M G.,& Alvarez, C C. (2015). RevistaBrasileira de Farmacognosia, 25, 170-176.
14. Cavanagh, F. (1963). *Analytical Microbiology*, Academic Press Inc, New York,
15. Cheong, Y H., Chang, H S., Gupta, R., Wang, X., Zhu, T., & Luan, S. (2002). Transcriptional Profiling Reveals Novel Interactions between Wounding, Pathogen, Abiotic Stress, and Hormonal Responses in *Arabidopsis*.*PlantPhysiol*, **129**,661-677.
16. Ching, T M., Lin, T., & Metzger, R J. (1987). Purification and Properties of Acid Phosphatase from Plump and ShriveledSeeds of Triticale.*PlantPhysiol*, **84**, 789-795.
17. Dafni, A. &MotteMaues, M. (1998). A Rapid and Simple Procedure to Determine Stigma Receptivity.*Sex. Plant Reprod.***11**, 177-180.
18. Delannoy, E., Jalloul, A., Assigbetse, K., Marmey, P., Geiger, J.P., Lherminier, J., Daniel, J.F., Martinez, C. & Nicole, M. (2003). Activity of Class III Peroxidases in the Defense ofCotton to Bacterial Blight. *Molec. Plant Microbe Interactions*,**16**, 1030-1038.
19. Dhaliwal, A S.,& Malik, C P.,(1985). Enzyme changes in the stigma style tissue of *Brassica campestris*var. Toriafollowing compatible and incompatible pollinations. In *Recent advances in pollen research* (T. M. Varghese(ed.)), Allied Publisher Pvt Ltd, New Delhi, 15–23.
20. Do, H.M., Hong, J.K., Jung, H.W., Kim, S.H., Ham, J.H., &Hwang, B.K. (2003). Expression of Peroxidase-like Genes, H2O2 Production, and Peroxidase Activity during the
21. Duke, J. (2007). Dr. Duke's Phytochemical and Ethnobotanical Databases,19(2007).
22. Dupuis, I., & Dumas, C. (1990). Biochemical Markers of Female Receptivity in Maize (*Zea mays* L.) Assessed Using *inVitro*Fertilization.*Plant Sci.* **70**, 11-20.
23. Galen, C.,& Plowright, R.C. (1987). Testing Accuracy of using Peroxidase Activity to Indicate Stigma Receptivity.*Can. J. Bot,***65**, 107-111.
24. Heslop-Harrison, J. (1975). Incompatibility and Pollen-stigma Interaction.*Annu. Rev. Plant. Physiol*,**26**, 403-425.
25. Heslop-Harrison, Y., &Shivanna, K R. (1977). The Receptive Surface of the Angiosperm Stigma.*Ann. Bot*, **41**, 1233-1258.
26. Hiscock, S J., Bown, D., Gurr, SJ.,& Dickinson, H G. (2002).Serine Esterases are Required for Pollen Tube Penetration of the Stigma in *Brassica*. *Sex. Plant Reprod.* **15**, 65-74, 2002.
27. Hoopen, R.,Harbord.,Maes,R.M.,Nanninga, N., &Robbins, T P. (1998). The self-incompatibility (S) locus in*Petunia hybrid* is located on chromosome III in a region, syntenic for the Solanaceae.*Pl. J*, **16**, 729–734.
28. Hypersensitive Response to *XanthomonasCampestris*v.*Vesicatoria*in*Capsicum Annuum*.*Molec.Plant MicrobeInteractions*.**16**, 196-205.
29. Ibrahim, M B.(1997). Antimicrobial effects of extract leaf, stem and root bark of *Anogeissusleiocarpus* on *Staphylococusaureaus, Streptococcus pyrogenes, Escherichia coli* and *Proteus vulgaris. J. Pharma,Devpt*. 2, 20-30.
30. Irobi, O N., Young, M. & Anderson, W.A. (1996). Antimicrobial activity of Annatto (*Bixaorellana*) extract. *International Journal of Pharmacy*, 4, 87-90.
31. Jorgensen, E., &Vejleby, K A.,(1953). New polyphenol oxidase test.*Physiol. Plant*, 6, 533-537.
32. Kohn, J R., &Waser, N M. (1985). The Effect of*DelphiniumNelsonii*on Seed Set in *IpomopsisAggregata*, a Competitor for Hummingbird Pollination.*Am. J. Bot,***72**,1144-1148.
33. Lachoria, L., Jain, P.C. & Agarwal, S.C. (1999). Activity of some plant extracts against dermatophytes. *Hindustan Antibiot. Bull*, 41, 17-21.
34. Lavithis, M., &Bhalla, P L., (1995). Esterases in Pollen and Stigma of *Brassica*.*Sex. Plant Reprod.* **8**, 289-298.
35. Linskens, H F., Havez,R.,Linder, R.,Salden,M., Randoux,A., Laniez,D., &Coustant,D. (1969).Etude des glycanne-hydrolases au cours de la croissance du pollen chez *Petunia hybrid* auto incompatibillity.*C.R. Acad.Sci*. (Paris), 1857–1885.
36. Liogier, H A, (1999). Plantasmedicinales de Puerto Rico y del Caribe.Iberoamericana de Ediciones, Inc. San Juan, PR., 566.
37. Louis, CF., Raj, RSN.,&Radhamany,PM.(2012).Identification and characterisation of incompatibility factors in *Hamelia patens* Jacq.(Rubiaceae).*J HorticSci&Biotech*, 87,531-538.
38. Louis, CF.,&Radhamany, P.M.(2009). Pollen-pistil interactions in *Hamelia patens* Jacq.(Rubiaceae). *J Indian Bot Soc*,**88,** 92-97.
39. Lowry, O H., Roserbrough, N J., Farr, A L.,& Randall, R J. (1951). Protein measurement with folin-phenol reagent.*J. Biol. Chem.*, 193, 256–275.
40. Malik,C. P.,& Singh, M B. (1980). *Plant enzymology and histoenzymology*, Kalyani Publishers., 431.

41. Mayer, A M., &Harel, E H. (1979). Polyphenol Oxidase in Plants.*Phytochemistry*,**18**, 193-215.
42. McInnis, S M., Emery, D C., Desikan, R P., Hancock, J T., &Hiscock, S J. (2006). "The Role of Stigma Peroxidases in Flowering Plants: Insights from further Characterization of a
43. Neog, B., &Singh, I D. (2003).Study of floral biology of some cultivated tea (*Camellia* spp.) growing in the upper Assam region of the northeast India. *J. Pl. Crops*, 1, 50-52.
44. Nettancourt, D. (1977). *Incompatibility in angiosperms*, Springer-Verlag, 230.
45. Paniagua-Vega, D., Cerda-García-Rojas, C.M., Ponce-Noyola, T.,& Ramos-Valdivia, A.C. (2012). A new monoterpenoidoxindole alkaloid from *Hameliapatens*micropropagated plantlets. *Nat. Prod. Commun*, 7, 1441–1444.
46. Reyes-Chilpa, R., Rivera, J., Oropeza, M., Mendoza, P., Amekraz, B., Jankowski, C.,&Campos, M., (2004). Methanol extracts of Hamelia patens containing oxindolealkaloids relax KCl-induced contraction in rat myometrium. Biol. Pharm. Bull, 27, 1617–1620.
47. Salud, P G., Miguel A., Zavala, S., Rosario Vargas, S., Edgar Hernandez, Z., Rosa, M.,& Perez, G. (1996).Antidiarrhocal activity of *Hamelia patens* Mehanol extract in rats and mice. *Phytotherapy Research,* **10**, 686-688.
48. Sandhya, S., SaiKumar, P., Vinod, K. R., David Banji., & Kumar, K. (2011). Plants as potent antidiabetic and wound healing agents - A review.*Hygeia J. D. Med*, **3**, 11-19.
49. Sapana, K., Parul, S., Tribhuwan, S., &Rekha, V. (2012). Anthelmintic and antimicrobial activity of *Hamelia patens* Jacq.(Rubiaceae).*International Journal of Natural Products Research*,**1**, 54-56.
50. Sarvesh, A., &Reddy, T P. (1988). Peroxidase, polyphenol oxidase, acid phosphatase and alkaline inorganic pyrophosphataseactivities during leaf senescence in varieties of castor (*Ricinuscommunis*L.).*Indian J. ExplBiol***.**26, 133–136.
51. Sawhney, S., Basra, A S. &Kohli, R K. (1981). Enzyme activity and electrophoretic patternofisoenzymes of peroxidase, esterase and alkaline and acid phosphatase in relation to flowering in *Amaranthusviridis* L. - Quantitative SD Plant.*Biol. Plant*, 23, 335-341.
52. Shrisha, D L., Raveesha, K A., &Nagabhushan.(2011). Bioprospecting of selected medicinal plants for antibacterial activity against some pathogenic bacteria.*Journal of Medicinal Plants Research*, **5**, 4087-4093.
53. Siegel, S M., &Galston, A W. (1955). Peroxide genesis in plant tissues and its relation to indole acetic acid distribution. *Arch. Biochem. Biophys*, 54, 102.
54. Stigma-specific Peroxidase (SSP) from *SenecioSqualidus*(Asteraceae)." *J. Exp. Bot.* **57**, 1835-1846.
55. Stonier, T.,Stasinos, S. and Murthy Reddy K.B.S.(1979): The masking peroxidase-catalysed oxidation of IAA in*Vigna*. *Phytochemistry*.,18, 25–28.
56. Stpiczynska, M. (2003). Stigma Receptivity during the Life Span of *PlatantheraChloranthа*CusterRchb) Flowers.*Acta Biol. Cracovia Series Bot.* **45**, 37-41.
57. Taylor, P., Arsenak, M., Abad, M. J., Fernandez, A., Milano, B., Gonto, R., Ruiz, M. C., Fraile, S., Taylor, S., Estrada, O., &Michelangeli, F. (2013).Screening of Venezuelan medicinal plant extracts for cytostatic and cytotoxic activity against Tumor cell lines. *Phytotherapy Research*, **27**, 530-539.
58. Tyagi, M., Kayastha, A M., & Sinha, B. (2000). The Role of Peroxidase and Polyphenol Oxidase Isozymes in Wheat Resistance to *AlternariaTriticina.Biol. Plant*,**43**, 559-562.