Egyptian Journal of Biology, Volume 4, 2002, pp. 37-44
© Printed in Egypt. Modern Press, Cairo. Egyptian British Biological Society (EBB Soc)

Phenolic acid changes during *Orobanche* parasitism on faba bean and some other hosts

Somia S El-Akkad[1*], Esmat A Hassan[2] and Mohamed E Ali[2]

1. Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt
2. Botany Department, National Research Centre, Dokki, Egypt

ABSTRACT

The present work is intended to provide further information on broomrape parasitism based on phenolic acid changes in either the host plant(s) or in each of the host and the parasite in the host-parasite system. Detection of phenolic acids was carried out using high performance liquid chromatography (HPLC) in the host and broomrape tissues. This was carried out first in shoots and roots of faba bean before (33-day-old) and after (65-, 74-, 80-day-old) broomrape infestation. Shoots and roots of faba bean and other prospective hosts (tomato and eggplant), during parasitism, at full juvenility of broomrape just before blooming, were also scanned. Chlorogenic acid occurred predominantly as phenolic compounds in shoots and roots of the host plants. The occurrence of chlorogenic acid was unique to the roots of the faba bean plant at different stages of development of the parasite, as well as in the roots of other infected hosts. In shoots of the infected hosts, inconsistent variations were observed in the ratios of caffeic, ferulic, as well as ortho-, para- and metacoumaric acids. Detection of phenolic acids in stems of broomrape, parasitizing faba bean, indicated the occurrence of chlorogenic acid at different stages of growth of the parasite. In addition to chlorogenic acid, the results also revealed the existence of M-coumaric acid at the emergence stage, P-coumaric and ferulic acids at early blooming and caffeic acid at the late blooming stage. Qualitative changes were also observed in broomrape tissue according to the type of host (faba bean, chamomile, eggplant, and cabbage). On the other hand, chlorogenic acid was the only phenolic compound detected in the corm-like tubercle tissue of broomrape with different hosts. The possible interference of phenolic acids in tissues of both the hosts and broomrape was discussed.

KEYWORDS: Broomrape parasitism, Phenolic acids, HPLC, faba bean.

INTRODUCTION

Over 3000 species of flowering plants utilize a parasitic mode of nutrition (Stewart & Press 1990) and yet, basic information regarding their physiology and biochemistry is limited. Generally, a host plant challenged by a potentially invading organism responds with changes in the composition and physical properties of cell walls, and the biosynthesis of secondary metabolites that serve to isolate and limit the spread of the invading organism. These responses are collectively known as, hypersensitive reactions (Hopkins 1999). Some secondary metabolites associated with the hypersensitive reaction appear to constitute an early warning system sending signals to other cells and tissues to be prepared for resistance of secondary infection. Meanwhile, the plant responds to the initial infection by slowly developing a general immune capacity, a phenomenon known as systemic acquired resistance (SAR) (Ryals *et al.* 1996). The development of SAR is still poorly understood, but one component of the signaling pathway appears to be salicylic acid, which is known to be implicated in the immune strategies of plants (Durner *et al.* 1997). Hypersensitive reactions in the majority of cases include formation of phenylpropanoids as coumarines, lignin, suberin and cutin, flavonoids and tannins as well as simple phenols (Heldt 1997).

Broomrape (*Orobanche* spp.), belonging to the family Orobanchaceae, is a plant representing an obligate parasite with some plants. The haustorium, an organ that functions in attachment, penetration and solute transfer (Stewart & Press 1990) is a characteristic feature of all obligate parasitic plants. At the start of the process of parasitism, haustorial cells of *Orobanche* penetrate the host root tissues, eventually connecting the parasite to the vascular system of the host. This phase is a critical step in parasitism (Losner *et al.* 1994). Several studies revealed that phenolics can be involved in the resistance of the host as defense

* Address for Correspondence

compounds against broomrape (Jorrin *et al.* 1996).

Therefore, the aim of this study is to determine the variation in phenolic constituents of the host (healthy and infected) as well as associated broomrape parasite at different growth stages.

MATERIALS AND METHODS

Research was carried out in the experimental green house of Botany Department, National Research centre, Dokki, Cairo, Egypt. Samples were collected continuously from naturally infested fields in Nubaria (Alexandria- desert road) and El- Aiat (Giza, Egypt).

Subjects: Faba bean plants were infected with broomrape at [XX] days of age. HPLC was carried out on the shoots and roots at different ages: 33 (uninfected), 65, 74 and 80 days old. HPLC analysis was also carried out on broomrape itself, while infecting different hosts and at different growth stages.

Preparation of samples for HPLC analysis of phenolics: Detection of phenolic compounds was carried out in healthy (faba bean at 33 days old) and *Orobanche*- infected hosts (faba bean at 65, 74 and 80 days, tomato at 107 days and eggplant at 118 days) as well as in *Orobanche* itself. Samples were washed, cut into small pieces and preserved in 80 % absolute ethanol for not less than one month (cold extraction). The mixture of alcohol and sample pieces was homogenized and the extract solutions filtered through filter paper. A volume of each extract was filtered through 0.2 micron filter nylon membrane and preserved in dry vials for HPLC injection.

High performance liquid chromatography (HPLC): HPLC was carried out using a spectrophotometer 3200 variable wave length detector, const Metric 4100 quaternary gradient pump, LC talk version 2.03 software; thermo separation products, T sp, USA and econosil c 18, 5 microns 250 mm length, 4.6 mm internal diameter column (Attech Associates Inc, USA). Samples were extracted in 80% ethanol and filtered through a 0.2 micron filter membrane. Sample separation and identification were started by injection into a C_{18} column and eluted by acetonitrile and water (20: 80 v/v). Phenolic acids were detected at 254nm.

Preparation of authentic phenolic acid solutions: The authentic phenolic acid solutions were prepared by dissolving 0.1 g of each acid in 80% absolute ethanol. The acids were chlorogenic acid, caffeic acid, ferulic acid, Meta-coumaric, Ortho-coumaric and Para-coumaric acids (Sigma). A mixture of the six phenolic acids was prepared by transferring a fixed volume (1 ml) from each acid solution to a clean dry vial. The prepared authentic acids and their mixture were filtered through a 0.2 micron filter membrane and degassing was achieved by ultrasonic water bath. Twenty microns of each authentic phenolic acid and of their mixture were injected into the HPLC sample injector. Peaks were detected, recorded and identified on LC talk version 2.03 software as detailed in Christian (1990).

Identification of phenolic acids in plant extracts: a fixed volume (5ml) of each plant extract was degassed and twenty microns of each plant extract was analysed by HPLC, as for the authentic samples (see above). The identity of the phenolic acids was determined by comparison of the retention times of plant extract peaks with peaks of pure authentic samples.

RESULTS

I. Detection of phenolic acids in the host plants:

The HPLC data charts of faba bean at different ages (33 days (uninfected), and 65, 74 and 80 days after infection with broomrape; figure 1) indicate that the shoots contained coffeic, ferulic and chlorogenic acids except at the age of 33 days in where p-coumaric was shown in place of ferulic acid. The root tissue contained only chlorogenic acid at all ages.

The HPLC-analytical results in shoots and roots of faba bean (80 days), tomato (107 days) and eggplants (117 days) infected with broomrape are presented in figure 2. The data charts of both faba bean and tomato indicate the existence of chlorogenic, coffeic and ferlulic acids in shoots. Only chlorogenic acid was identified in roots.

In eggplant, chlorogenic acid was the only detected phenolic compound in the charts of both shoot and root extracts.

II. Detection of phenolic acids in the parasite

Detection of phenolic acids in broomrape at different stages of growth on faba bean: the life phases of broomrape were divided into underground stages (*I* & *II*), emergence stage (*III*), vegetative stage (*IV*) and different blooming stages (*V*1, *V*2 &*V*3), according to Hassan (1973 & 1996).

The results of HPLC analyses for the detection of phenolic acids (chlorogenic, caffeic, ferulic, ortho-, para- and metacoumaric acids) in broomrape at different stages of growth in faba bean are presented in figure 3. These results indicate the occurrence of chlorogenic acid as a unique phenolic compound in broomrape at the underground (*I* & *II*) and vegetative (*IV*) growth stages as well as at full blooming stage (*V*3). On the other hand, chlorogenic and m-coumaric acids were detected at the emergence stage (*III*), whereas chlorogenic, para-coumaric and ferulic acids were recorded at the blooming (bud) stage (*V*1). The blooming (unopened flowers) stage (*V*2) was characterized by the occurrence of chlorogenic and caffeic acids.

The HPLC analysis of the extracts of broomrape during vegetative growth, parasitizing faba bean, cabbage, chamomile and eggplant, are presented in figure 4. The results indicate that chlorogenic acid existed in the extracts of broomrape (at the vegetative growth stage) on faba bean, chamomile and eggplant. On the other hand, both chlorogenic and caffeic acids were recorded as traces in cabbage.

Chlorogenic, caffeic, ferulic, and ortho-, para- and meta-coumaric acids were also detected in the tubercular base structure of broomrape at blooming, parasitizing faba bean, pea and cauliflower. The results are shown in figure 5; chlorogenic acid was the only phenolic compound detected.

DISCUSSION

An understanding of the biochemical and concomitant mechanical events that underlay how a pathogen invades a susceptible host and how a resistant plant is able to defend successfully against infection is of extreme significance (Hardham 1992). This information is vital to exert sustainable control over plant diseases. During plant–pathogen interactions, initial contact can serve as an identifiable starting point at which a cascade of events takes place in both the plant and the pathogen where signals are exchanged. Main events, at the molecular level, include induction of certain genes with *de novo* synthesis of new proteins and/or repression of other genes, with disappearance of respective proteins (Truesdell & Dickman 1997). The occurrence and progression of such changes depend on whether that the host is resistant or susceptible and the invading organism is virulent or avirulent (Hughes 1996).

A number of strategies have been developed by plants to reduce the effect of invasion or limit the spread of the invading organism. The earliest response of the host is the activation of defense-related genes and corresponding synthesis of their products termed the "pathogenesis-related (PR) proteins". These include proteinase inhibitors that disarm proteolytic enzymes secreted by the pathogen (Ryan 1981), lytic enzymes that degrade the pathogen cell walls (Hopkins 1999), and activation of the genes that encode enzymes for the biosynthesis of isoflavonoids and other phytoalexins (van Etten *et al*. 1989), phenylpropanoids (Heldt 1997), and alkaloids (Aerts *et al*. 1996; Baldwin 1996). As a defense

mechanism, photosynthetic genes may be also repressed or retarded in order to minimize energy supplies (Criqui *et al*. 1992).

Dini *et al*. (1995) isolated several phenolic compounds from dried and powdered aerial parts of *Orobanche speciosa* (*O. crenara*) collected from Italy. The occurrence of phenolics was also recorded in extracts of the stem parasites *Cuscuta reflexa* and *C. platyloba* (Lofler *et al*. 1995).

It might be concluded that such phenylpropanoid metabolites can be assumed as existing normally at genetically determined levels either in the host root or the parasite as a means of routine mechanical support and natural defense. But, in the presence of broomrape, it is tentatively suggested that a signal is received and transduced to the host genome, resulting in a general up-regulation of genes encoding the phenylpropanoid pathway, thus providing the host cells with extra precursors of defensive compounds.

This study analysed phenolic constituents using HPLC in both host and broomrape tissues. Detection was done with reference to authentic samples of chlorogenic, caffeic, ferulic, and ortho-, para- and metacoumaric acids as well as mixtures of all. This was carried out first in shoots and roots of faba bean at different ages before (33 days old) and after (65, 74, and 80 days old) broomrape infection.

The most obvious characters underlying the phenolic patterns were the occurrence only of chlorogenic acid in faba bean roots at different stages of growth before and following progressive development of broomrape, whereas shoots, on the other hand, included chlorogenic acid as well as caffeic and ferulic acids. Chlorogenic acid was obviously predominant at all stages of growth of faba bean shoots (except in the 74-day-old plants), caffeic acid being the most abundant among the remainder of the phenolic acids. For an unknown reason, p-coumaric acid occurred in 65-day-old plants (instead of chlorogenic acid), then disappeared afterwards and was replaced by chlorogenic acid. The temporary occurrence of p-coumaric acid at this stage (65-day-old host) and concomitant disappearance of chlorogenic acid might be in alliance with corresponding establishment of broomrape with faba bean. In connected studies, differences in both quality and quantity of phenolic acids were recorded in roots and shoots of healthy and *Striga*-infested *Sorghum* cultivars (El-Hiweris 1987). Lyons *et al.* (1993), also recorded changes in phenylpropanoids in response to fungal infection. This first included accumulation of two caffeic acid esters, followed by subsequent addition of ferulic and para coumaric acids. The final step was the synthesis of lignin. Lehman *et al*. (1994) interpreted the effect of ferulic and paracoumaric acids on the bases of inhibition of leaf expansion of the host. It might be tentatively argued on the light of relatively recent research that plants under stress conditions (including infections) tend to retard photosynthetic processes in several ways including down-regulation of some photosynthetic proteins (Reinbothe *et al*. 1993).

The types of phenolic acids occurring in roots and shoots were also identified in tomato and eggplant as hosts other than faba bean and compared all together and compared with those of faba bean, at full juvenility of broomrape just before blooming. The occurrence of only chlorogenic acid in roots was further indicated in the three hosts. In shoots, however, there was some variation since caffeic and ferulic acids existed in both faba bean and tomato but were absent in eggplant. On the other hand, paracoumaric acid was present only in shoots of faba bean.

The above-mentioned phenolics were also detected at different stages of development in broomrape parasitizing faba bean. At all these stages, chlorogenic acid was recorded at relatively high levels, particularly during the blooming stages (V_1, V_2, V_3). This was accompanied with inconsistent occurrence of ferulic and coumaric (para and meta) acids. On the other hand, phenolic acids were compared in broomrape on different hosts. The results of HPLC analyses of phenolic acids also further confirmed the occurrence of chlorogenic acid as

a common and predominant phenolic compound in juvenile broomrape on different hosts other than faba bean. This phenolic compound was also unique in being the only one in the tubercular base of broomrape parasitizing either faba bean or other hosts. In this connection, Caboni *et al.* (1992) mentioned that chlorogenic acid can stunt root-promoted growth. Sahm *et al.* (1995) also observed elevated amounts of soluble phenolic compounds, particularly chlorogenic acid, in concomitance with stimulation of peroxidase, during tomato infection with *Cuscuta reflexa*.

It was evident that despite the exact mechanism of broomrape parasitism with different prospective hosts, the host-parasite system did not differ from each alone except in the formation of relatively higher amounts of derivatives at the lesion of penetration and connection. Phenolic acids appeared likely to occur either in different healthy hosts or in both individuals of the host-parasite system. Lignin is derived mainly from ferulic and paracoumaric acids with sinapic acid. It is covalently bound to cellulose in cell walls where lignified cell walls are compared to reinforced concrete (in which the cellulose fibers represent the xylem and lignin resembles concrete) (Heldt 1997). Suberin and cutin are also polymeric compounds of phenylpropanoids and fatty acids, cutin differing from suberin in having a relatively small proportion of phenylpropanoids (Anderson & Beardall 1991). In addition, tannins are also derived from phenylpropanoids, which shows that all the abovementioned compounds are produced from phenylalanine of the Shikimate pathway (Heldt 1997). In this respect, glyphosate which is a potent herbicide of broomrape blocks the enzyme 5-enolpyruvyl-shikimaye-3-phosphate synthase (EPSPS) that catalyzes a step in the synthesis of both phenylalanine and tyrosine (in addition to tryptophan) within the shikimate pathway (Hopkins 1999). This may confirm the extreme significance of this pathway for broomrape.

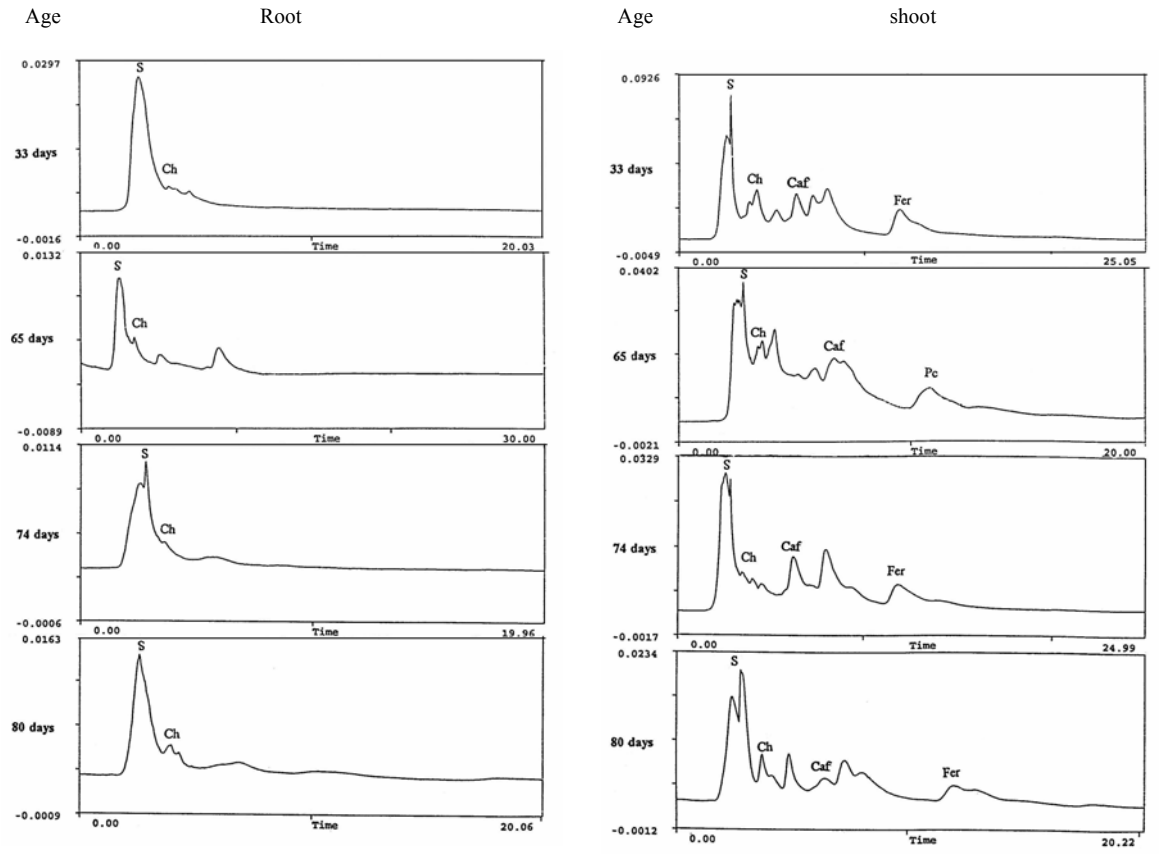

Figure 1: HPLC charts of root and shoot extracts of faba plants before (33 days) and after (65, 74 and 80 days) broomrape infection.

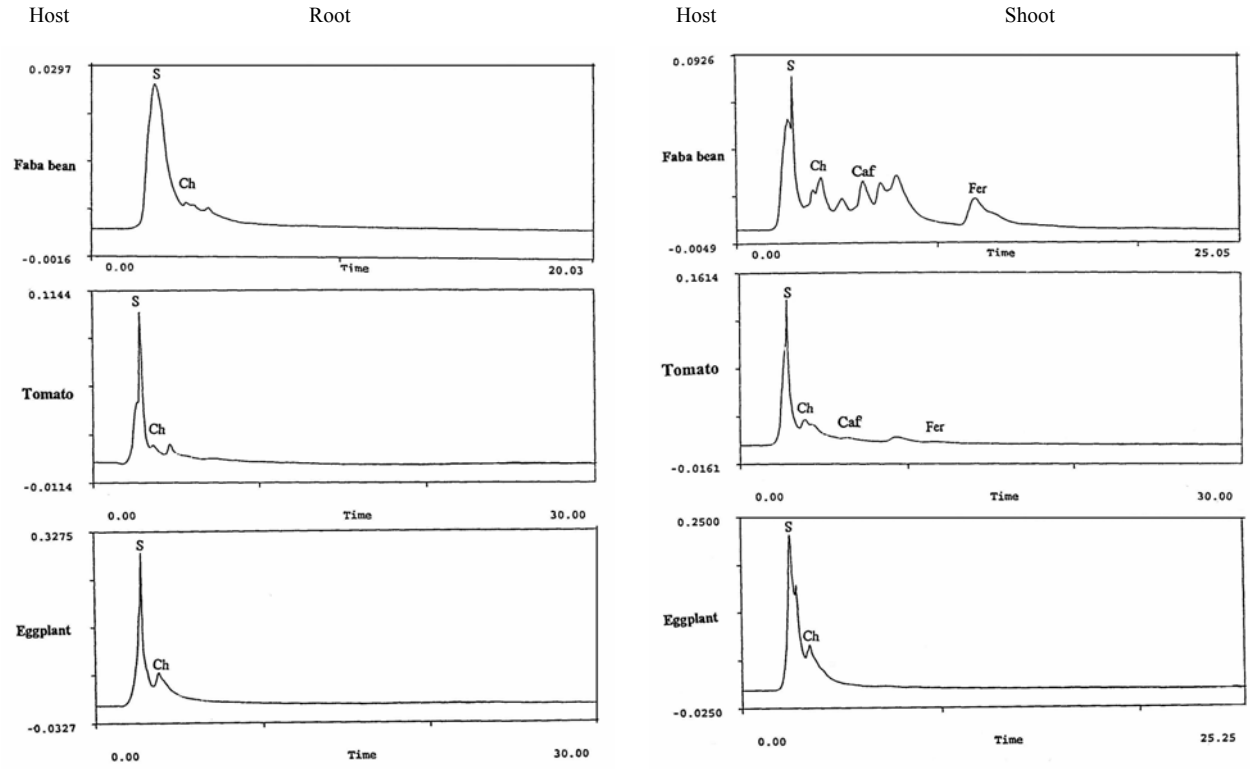

Figure 2: HPLC charts of the root and shoot extracts of broomrape-infected faba bean (80 days), tomato (107 days) and eggplant (118 days).

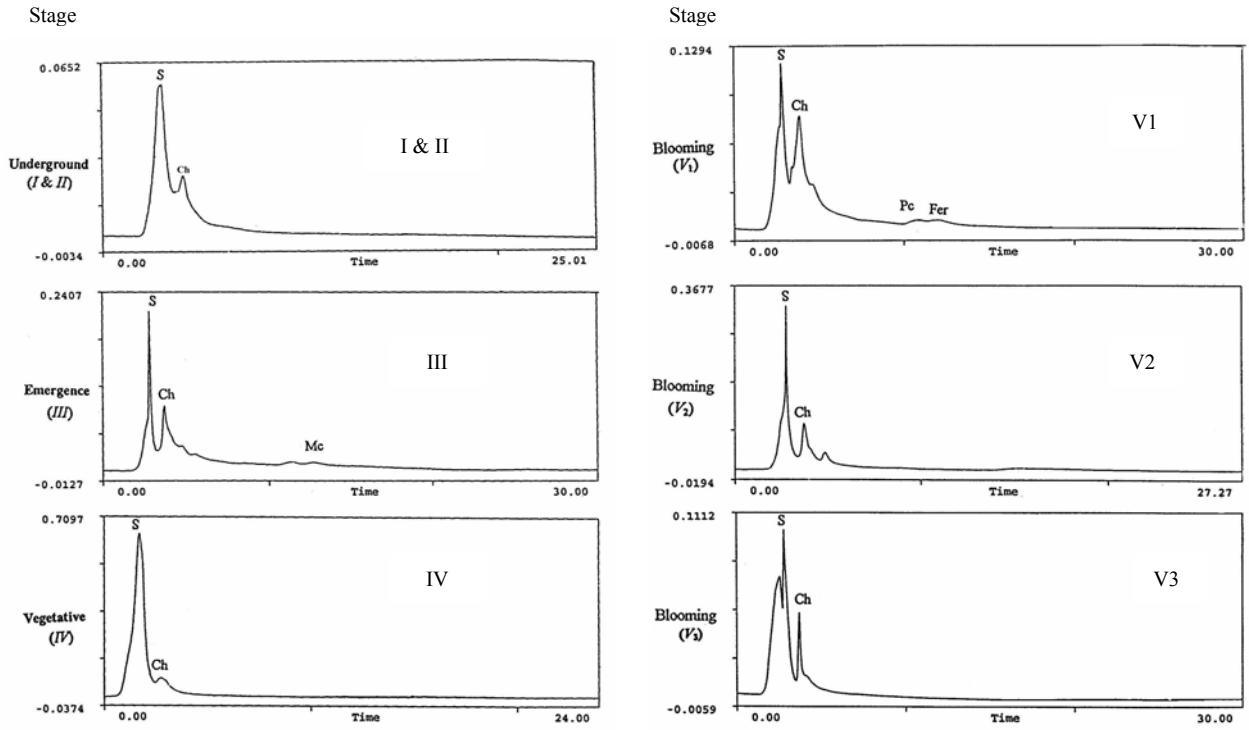

Figure 3: HPLC charts of the extracts of broomrape at different stages on faba bean: underground (I and II), emergence (III), vegetative growth (IV), blooming stages (V1, V2, V3).

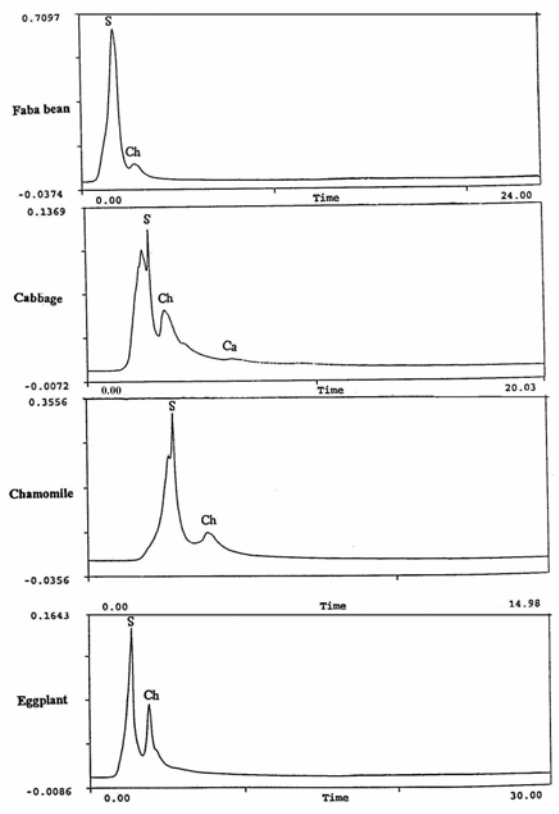

Figure 4: HPLC charts of the extracts of vegetative growth stage of broomrape on faba bean, cabbage, chamomile and eggplant.

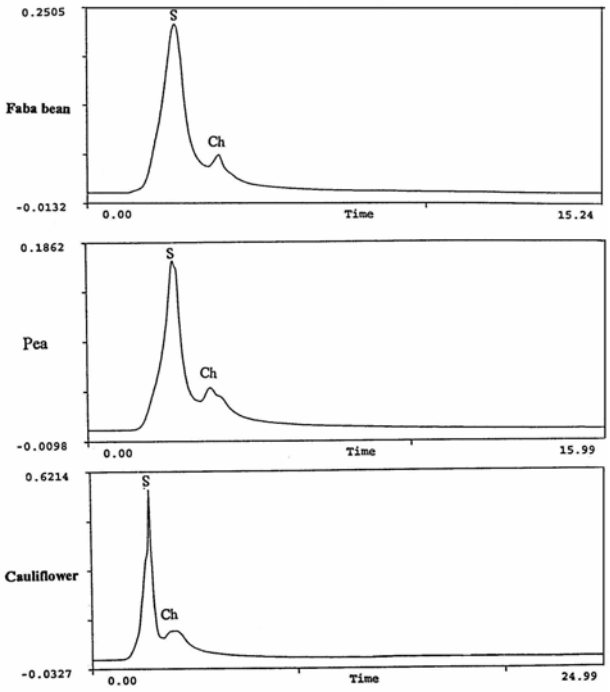

Figure 5: HPLC charts of the extracts of corm-like of blooming growth stage of broomrape on faba bean, pea and cauliflower.

REFERENCES

Aerts RJ, Schafer A, Hesse M, Baumann TW & Slusarensko A (1996) Signaling molecules and the synthesis of alkaloids in *Catharanlus roseus* seedlings. *Phytochemistry* 42: 417-422.

Anderson JW & Beardall M (1991) Molecular Activities of Plant Cell. P. C. Blackwell Scientific Publication, London, 348pp.

Baldwin IT (1996) Methyl jasmonate-induced nicotine production in *Nicotiana attenuata*: inducing defenses in the field without wounding. *Entomologia Experimentalis et Applicata* 80: 213-220.

Caboni E, Boumis G & Damiano C (1992) Effects of phenols, gibberellic acid and carbohydrate on the rooting of the apple rootstick M.9 Jork. *Agronomie* 12: 789-794.

Christian G (1990) HPLC tips and tricks. Oxford, UK, 608pp.

Criqui MC, Durr A, Parmentier Y, Marbach J, Fleck J & Jamet E (1992) How are photosynthetic genes repressed in freshly-isolated mesophyll protoplasts of *Nicotiana sylvestris*?. *Plant Physiology & Biochemistry* 30: 597-601.

Dini I, Iodice C & Ramundo E (1995) Phenolic metabolites from *Orobanche speciosa*. *Planta Medica* 61:389-390.

Durner J, Shah J & Klessig DF (1997) Salicylic acid and disease resistance in plant. *Trends in Plant Sci.* 2:266-274.

El-Hiweris SO (1987) Nature of resistance to *Striga hermonthica*(Del.) Benth parasitism in some *Sorghum vulgare* (Pers.) cultivars. Weed Research, Oxford, UK, 27: 305-311.

Hardham AR (1992) Cell biology of pathogenesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 43: 491-526.

Hassan EA (1973) *Orobanche* parasitism on *Vicia faba* : A case of correlative growth. PhD. Thesis, Faculty of Agriculture, Cairo University, pp. 29-37.

Hassan EA (1996) Developmental morphology of *Orobanche* parasitism of faba bean an approach to control. In: proceedings of Rehabilitation of faba bean. W Bertenbreiter & M Sadiki (eds.). Actes Edition, Rabat, Morocco, pp. 107-112.

Heldt HW (1997) Plant Biochemistry and Molecular Biology. Chapter 18, Phenylprpopanoids. Oxford University Press. Oxford, UK, pp. 377-393.

Hopkins WG (1999) Introduction to Plant Physiology. 2nd edition. John Wiley and Sons, Inc. NY, USA: 467-469.

Hughes MA (1996) Plant Molecular Genetics. Chapter 14. The molecular biology of disease and pest resistance-

plant pathogen interactions. Longman, UK, pp. 190-204
Jorrin J, De Ruck E, Serghini K, Perez De Luuque A, Munoz-Garcia J, Garcia-Torres L & Castejon- Munoz (1996) Biochemical Aspects of the parasitism of sunflower by *Orobanche*. In: Proceedings of the 6th International Parasitic Weed Symposium, MT Moreno, JI Cubero, D Berner, D Joel, LJ Musselman & C Parker (eds.). Cordoba, Spain, PP. 551-558.
Lehman ME, Blum U & Gerig TM (1994) Simultaneous effects of ferulic and p-coumaric acids on cucumber leaf expansion in split-root experiments. *Journal of Chemical Ecology* 20: 1773-1782.
Loffler C, Sahm A, Wray V, Czygan FC & Proksch P (1995) Soluble phenolic constituents from *Cuscuta reflexa* and *Cuscuta platyloba*. *Biochemical Systematics and Ecology* 23: 121-128.
Losner-Goshen D, Mayer AM, Ben-Hod G & Joel DM (1994) Host-parasite interaction: an immunocytochemical approach using antibodies for protein and pectic enzymes. In: Proceedings of the 3rd International Workshop on *Orobanche* and Related *Striga* Research. AH Pieterse, JAC Verkleij & SJ Ter-Borg (eds.). Amsterdam, Netherlands, pp. 248-254.
Lyons PC, Hipskind J, Vincent JR & Nicholson RL (1993) Phenylpropanoid dissemiation in maize resistant or susceptible to *Helminthosporium maydis*. *Maydica* 38: 175-181.
Reinbothe S, Reinbothe C & Parthier B (1993) Methyl jasmonate regulated transduction of nuclear-encoded chloroplast proteins in barley (*Hordeum vulgare* L. cv. Salome). *J. Biological Chemistry* 26: 10606-10611.
Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY & Hunt MD (1996) Systemic acquired resistance. *The Plant Cell* 8: 1809-1819.
Ryan CA (1981) Proteinase inhibitors. In: The Biochemistry of Plants: A Comprehensive Treatise. PK Stumpf & EE Conn (eds). Academic Press. New York, 6: 351-370.
Sahm A, Pfanz H, Grunsfelder M, Czygan FC & Proksch P (1995) Anatomy and phenyl propanoid metabolism in the incompatible interaction of *Lycopersicom esculentum* and *Cuscuta reflexa*. *Botanica Acta* 108: 358-364.
Stewart GR & Press MC (1990) The physiology and biochemistry of parasitic angiosperms. *Annual Review of Plant Physiology and Plant Molecular Biology* 41: 127-151.
Truesdell GM & Dickman MB (1997) Isolation of pathogen/stress-inducible cDNAs from alfalfa by mRNA differential display. *Plant Molecular Biology* 33: 737-743.
van Etten HD, Matthews DE & Matthews PS (1989) Phytoalexin detoxification: Importance for pathogenity and practical implications. *Annual Review Phytopathology* 27: 143-164.

الملخص العربى

تغيرات أحماض الفينول أثناء تطفل نبات الهالوك على نبات الفول وبعض العوائل النباتية الأخرى

سمية صلاح الدين العقاد[1] – عصمت عبد الفتاح حسن[2] - محمد السعيد على[2]

1- قسم النبات – كلية العلوم – جامعة عين شمس – القاهرة – مصر
2- قسم النبات – المركز القومى للبحوث – الدقى - مصر

تهدف هذه الدراسة إلى متابعة بعض نواتج الأنشطة الفسيولوجية سواء للعائل (الفول- الطماطم- الباذنجان) أو للطفيل المرتبط بالعائل (نبات الهالوك) وذلك بغية التوصل الى الآليات الدقيقة لمقاومة هذا الطفيل.

وقد تم تقدير الأحماض الفينولية كيفياً باستخدام جهاز الكروماتوجرافيا السائلة عالية الأداء (HPLC) فى كلٍّ من العائل والطفيل وقد أجريت هذه العملية أولاً فى أنسجة كل من المجموع الخضرى والجذرى لنبات الفول قبل الإصابة (عمر 33 يوما) وبعد الإصابة بالهالوك (أعمار 65 ، 74 ، 80 يوماً) . كما أجريت هذه التقديرات أيضاً على سبيل المقارنة فى نباتات الفول والطماطم والباذنجان المصابة بالهالوك فى مرحلة أقصى نمو خضرى للطفيل قبل إزهاره مباشرة. وقد أوضحت النتائج جميعها سمة واحدة مميزة وهى انفراد الجذور بوجود حمض الكلوروجينيك فقط، أما فى السوق فقد اتضح وجود نفس الحمض الفينولى بصورة سائدة غالباً ولكن إلى جانب أحماض فينولية أخرى هى الكافييك والفريوليك وأورثو وميتا وبارا كوماريك. وقد تراوح وجود أو غياب بعض هذه الأحماض الفينولية بالاضافة الى نسبها البينية باختلاف مراحل النمو فى نباتات الفول أو باختلاف نوع العائل من جهة أخرى .

وقد تمت متابعة وجود الأحماض الفينولية أيضاً فى الهالوك فى أنسجة الجزء العلوى المماثل للساق عند كافة مراحل نموه على نبات الفول وأوضحت النتائج وجود حمض ميتاكوماريك فى المرحلة الأولى التالية لإنبات بذور الطفيل وحمضى بارا كوماريك وفريوليك عند بداية إزهاره وحمض الكافييك فى أواخر مرحلة التزهير. كما سجلت أيضاً تغيرات كيفية لهذه المركبات فى أنسجة الطفيل باختلاف نبات العائل (فول أو كاموميل أو باذنجان أو كرنب) وقد اقتصرت الأحماض الفينولية على حمض الكلوروجينيك فقط فى الجزء القاعدى المتدرن للهالوك رغم اختلاف نوع العائل.