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ORIGINAL ARTICLES

PAL Gene Activity and Total phenolic Compounds in some Members of Lamiaceae

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ABSTRACT

Phenylalanine ammonia – Lyase (PAL) catalyzes the biosynthesis of total phenolic compounds (TPC) in family lamiaceae (formally known as labiatae). Increases in (TPC) contant in plant tissue in vitro coincided with increases in PAL activity. The activity of the gene responsible for the production of the PAL enzyme in four plants from family lamiaceae was investigated to better understand the accumulation of (TPC). The expression of the PAL gene was relatively high in *Lavendula angustifolia L. Mentha spicata L.*, and *Ocimum basilicum L.* and low in *Thymus vulgaris L.*, when comparing PAL gene activity with (TPC) production in plants tissues, it was found that the plant producing the highest phenolic compounds (Lavendula) had the highest PAL gene expression as evidenced by band intensity, while (Thymus) , which had very low PAL gene expression, had the lowest phenolic compound production.

Key words: gene expression , medicinal plant, *Mentha spicata L., lavendula angustifolia L., Ocimum basilicum L.* and *Thymus vulgaris L.*,

Introduction

Research has confirmed that plant phenolic compounds have antioxidant, medicinal and antimicrobial properties. Some of the important phenolic compounds are the derivatives of the phenyl propanoid pathway (Perry, Shetty K, 1999).

Plants, including herbs and spices, have many phyto chemicals which are potential sources of natural antioxidants, e.g . phenolic compounds, flavonoids, tannins and phenolic acid (Dawidowicz *et al*., 2006). These compounds have antioxidant, anti-inflammatory and anticancer activities (Lee *et al*., 2004). Phenolic compounds are also thought to be capable of regenerating endogenous α – tocopherol in the phosphilipid bilayer of lipoprotein particles back to its active antioxidant form (Rice – Evans *et al*., 1996).

Ocimum basilicum L., Thymus vulgaris L., Mentha spicata L. and Lavendula angustifolia L. are members of the family Lamiaceae (formally known as Labiatae).

Ocimum basilicum L.(sweet basil) is a popular herb and is well known for its diversity as a source of essential oils, its flavor and delicacy as a spice, and for its beauty and fragrance as an ornamental plant. Sweet basil is especially desired and used in excess by the perfume, pharmaceutical, and food industries because of its natural aroma and flavor.(Stary and Jirazek, 1985).

Thymus vulgaris L. (Thyme or oregano) is one of the most commercially important medicinal plants. It is rich in volatile oils producing a characteristic aromatic scent. The part used medicinally is the essential oil, which is extracted from the leaves and flowering tops. It is reputed to have many other putic actions including: carminative, antimicrobial, antiseptic, expectorant, diaphoretic actions, and detergent as well as for culinary (in food production) as flavors and perfumery industry (Boon and Smith, 1999).

Menth spicata (Mint) is a perennial plants with oil glands mostly on the under surface of the leaves. Mint can be used for several medicinal purposes such as aromatic reviving, calmative enteric, gaseous carminative, flatulence of stomach, added to water for drink and to laxative medicin drug to prevent colic, other uses such as better menting the flavor, in tooth paste and medicine drug are mentioned in several purposes.

Lavendula angustifolia L. (Lavender) is one of the most widely used, versatile herbs known today. Both the flowers of the plant and the essential oils derived from the lavender plants can be used for therapeutic uses. Lavender is commonly used for anxiety, depression, mental exhaustion, insomnia, scrapes and wounds, digestive problems, headaches, skin problems. In addition to this, Lavender can be used to treat exhaustion, heat exposure, fevers, aches and pains, over-exertion, jet lag, rashes, sprains, sunburn, sunstroke, bruises and burns, it can also be used as a disinfectant and insect repellant. Lavender is an antiseptic, natural antibiotic, sedative, detoxiflier . (Stary and Jirazek, 1985)

All the previonely mentioned members of family lamiaceae contain in their tissues phenolic compounds which are used as antioxidants and have antimicrobial effect

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The enzyme phenylalanine ammonia – lyase (PAL) catalyzes the initial step of the phenylpropanoid pathway leading to the formation of 4 – coumaroyl – CoA (De – Ek namkun and Ellis, 1987; Woodrow and Berry, 1988).

As an enzyme, the activity of PAL , which is known to increase in plants under stress , has an inverse relationship with the activity of rubisco (ribulose – 1,5 – bisphosphate carboxylase / oxygenase) , the enzyme responsible for fixation of CO_2 in both C_3 and C_4 plant (Buchanan *et al*., 2002) . Thus , as the plant comes under stress , PAL enzyme activity increases in plant tissue, causing a reduction in rubisco gene activity (Ishida *et al*., 1997). An inverse relationship exists between the amount of rubisco enzyme and PAL , meaning that as the PAL enzyme activity increases in the plant tissues, a reduction in rubisco gene activity frequently recognized as a stress enzyme ,controlling photosynthesis occurs. Because PAL enzyme activity is linked to the synthesis of TPC ,this study investigated the occumulation of total phenolic compounds in four plants from family lamiaceae to determine whether PAL activity was a predictor of TPC accumulation .

The main aim of this study was to compare the concentration of total phenolic compound between the four lamiaceae members. Also , to compare between the concentration of total phenolic compound and the PAL gene expression in each plants. And this was done to identify the best plant in phenolic production also to have a better understanding about the relation between PAL gene activity and phenolic compound production .

Materials and Methods

This work was done in the tissue culture lab- Horticulture Department- faculty of Agriculture- Alexandria University- Egypt.

Plant materials:

The plants used in this investigation were *ocimum basilicum L., Thymus vulgaris L., Mentha spicata L. and Lavendula angustifolia L.* planted in 25 Cm pots were grown in the experimental station, faculty of Agriculture, Alexandria University.

Determination of total phenolic content:

The free bound phenolic contents in plants were determined using folin- ciocalteu's method (AOAC, 2000). The free and bound phenolic extracts were diluted to an appropriate concentration. The diluted solution (0.5 ml) was then oxidized with folin- ciocalteu reagent (0.5 ml) and then neutralized with saturated 1 ml of 25% (w/v) sodium carbonate solution. The volume was adjusted to 10ml with distilled water, then thoroughly mixed and allowed to stand for 45min at ambient temperature. The solution was centrifuged for 5min at 4000g and the absorbance of the clear supernatants was measured at 725nm using a spectrophotometer (UNICO 3200.) A standard calibratin was prepared using ferulic acid (0, 20, 40, 60, 80 and 100 mg/ml) and the content of total phenolics in each extract was calculated and expressed as milligrams of ferulicacid equivalent (FAE) per gram of the sample. Total phenolic contents in plant extracts was expressed in gallic acid equivalents (GAE) and was calculated using the following equation:

$C = cV/m$ (Nuno Rainha *et al*., 2011).

Where C is the total content of phenolic compounds , mg GAE /g dry extract , c the concentration of gallic acid obtained from the calibration curve , mg / ml , V the volume of extract ml and m is the weight of extract , g.

DNA extraction:

Total DNA was extracted from the plants using gene jet TM plant genomic DNA purification Mini Kit # K0791, # K0792 from fermentas

100mg of young leaves were taken from each plant and thoroughly washed with water then ethanol to remove dust and other contaminants, milled under liquied nitrogen. The DNA was extracted using Fermentas plant tissue DNA purification kit. The DNA was quantified spectrophotometrically at 260nm and DNA existace was tested using electrophoresis on 1.2% agarose gels. DNA was stored at – 20 for further work

For the PCR reaction the forward primer used for the PAL gene was GGG TCT CTC TAC CAG GTG TTA T and the revers primer sequence was GAT CAC GTC CTT CAT TAC GAC C. each of forward and the revers primers were used to amplify about 350 bp of the cDNA (El- Naggar and Read , 2010). The forward and revers primers were designed using the gene bank (ncbi. Org).

Master Mix (Dream Taq ^{TM} green PCR Master Mix (2x) containing (DNA polymerase+ optimized green buffer + $MgCl_2$ and dNTPs.)

The PCR amplification was performed with initial denaturation at 94 °c for 3 min, 35 cycles of denaturation 94°c for 30 sec, annealing at 55 °c for 30 sec extension at 72°c for 30 sec, and a final extension at 72°c for 5 min

and storage at 4°c. PCR amplifed product were subjected to electrophorasis in a 1.7% agarose gel containing Ethidiumbromide in (1x) TBE buffer at 65 volts. for 1 hr using cleaver submarine electrophorasis unit. Gene ruler 100 bp plus DNA ladder from Fermentas was used to identify the DNA amplification

Results and Discussion

Data were subject to analysis of variance (ANOVA) using SAS program , SAS Institute (SAS Institute, 2002).

The results indicated that there were significant difference between concentrations of total phenolic compounds in the four species as there was significant difference between the amount of total phenolic compound in *Lavendula angustifalia* .and the amount of total phenolic compound in *Ocimum basilicum.,* and *Thymus vulgaris*. Also there was a significant difference between the amount of total phenolic acid in *Mentha spicata.,* and the amount of total phenolic compound in *Thymus vulgarisl.*

In the other hand there was not significant difference between the amount of total phenolic compound in Lavendula angustifalia. and *Mentha spicata*., , also no significant difference between the amount of total phenolic compound between *Mentha spicate*, and *Ocimum basilicum.,*

The highest concentration of total phenolic compounds was in *Lavendula angustifalia*., followed by Mentha spicata., and *Ocimum basilicum.*, while the lowest concentration of phenolic compounds was found in *Thymus vulgaris* .,Tab. (1)

Using PCR & gel electrophorasis it was found that the bands are found between 300 and 400 bp. Which is consistent with the 350 bp. application designed using the gene bank (ncbi. Org)

By using PCR and using the primer of the pal gene [GGG TCT CTC TAC CAG GTG TTA T] it was found that the gene expression of *Lavendula angustifalia.* gives the highest gene expression of the pal gene followed by *Mentha spicata.,* and *Ocimum basilicum.,* and the gene expression of the *Thymus vulgaris*. gives the lowest gene expression of the pal gene.

Table 1: Total phenolic compounds in leaves.

The plants	Leaf extract TPC (mg g^{-1} fresh wt. of leaves)
Lavendula angustifalia.	13.400 a
Mentha spicata.,	11.678 ab
Ocimum basilicum.,	11.368 b
Thymus vulgaris.	5.445 c
L.S.D	1.835

Note : Means with the same letter in the same column are not significantly different . p≤0.05 : L.S.D
L.S.D : least significant difference at 0.05 probability

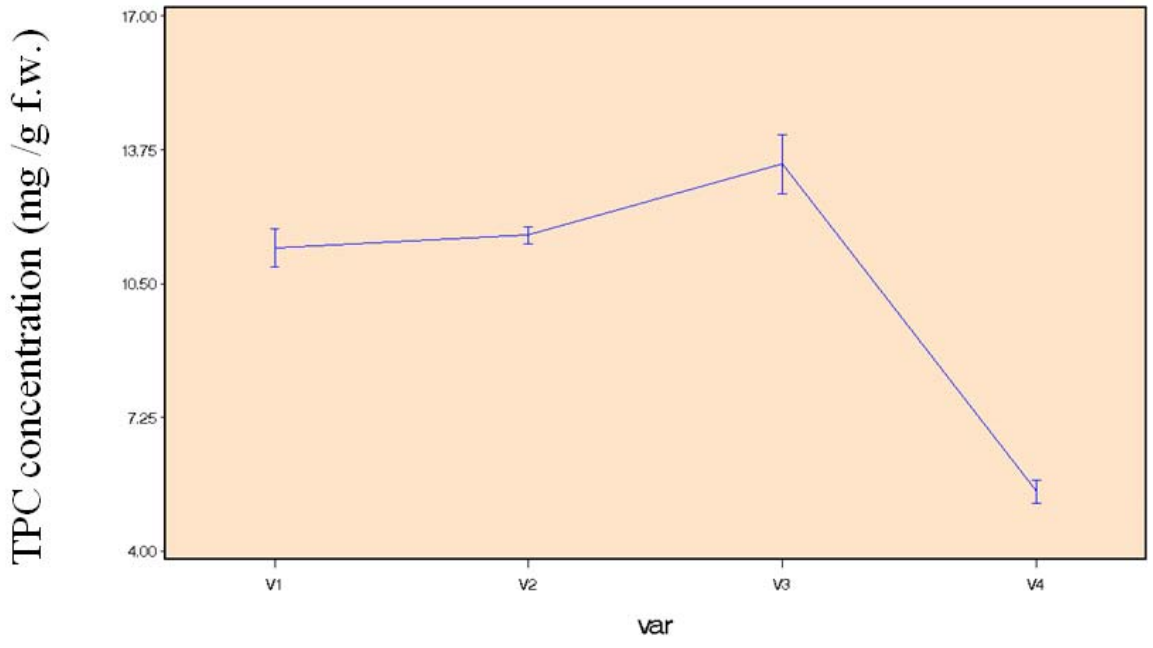

Fig. 1: Total phenolic compounds in:
V1 : *Ocimum basilicum.* V2 : *Mentha spicata*
V3 : *Lavendula angustifalia.* V4 : *Thymus vulgaris*.

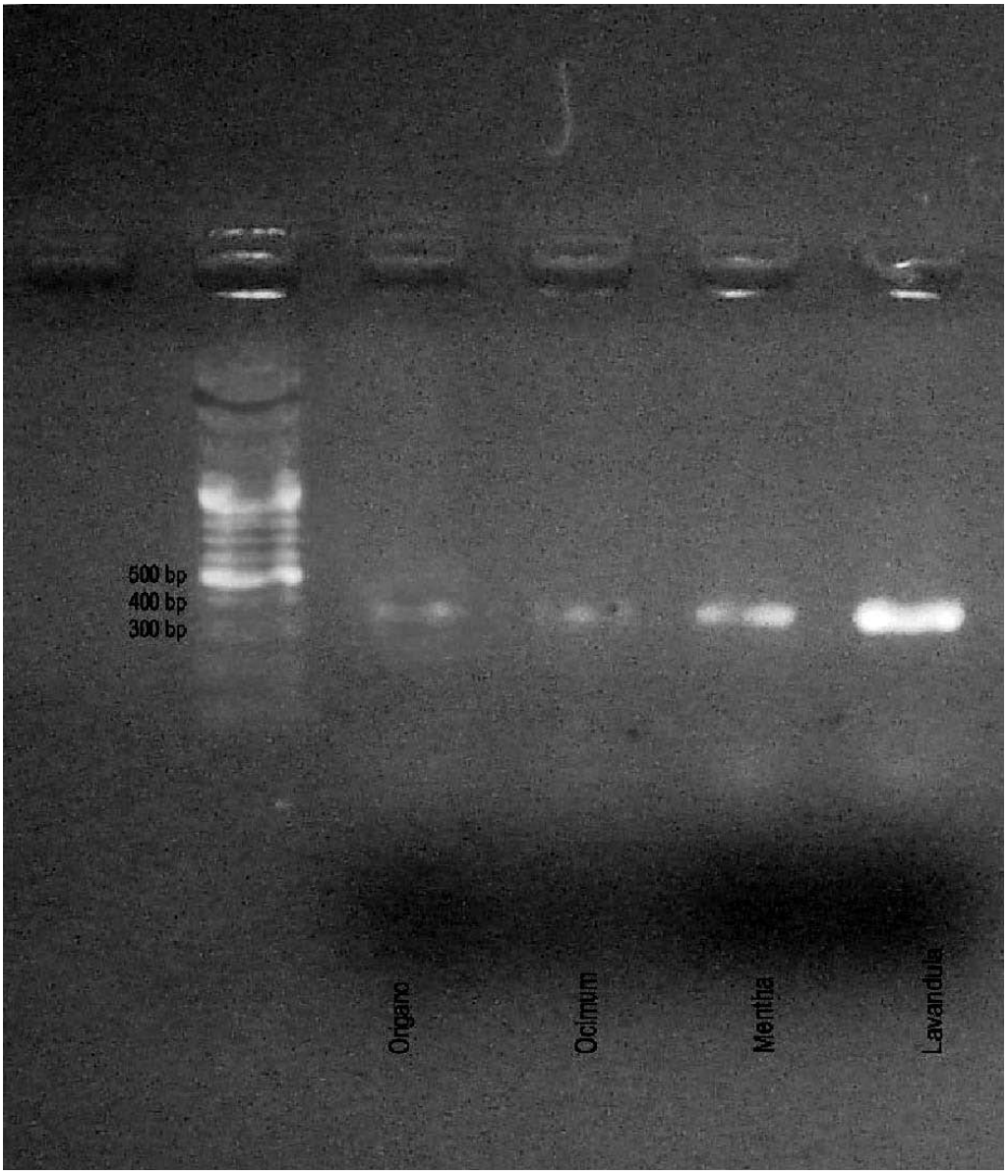

Fig. 2: Gel electrophoresis of PCR reaction.

Discussion:

The highest concentration of phenolic compounds was in *Lavendula angustifalia* , (13.4 mg /gm fw) followed by *Mentha spicata* (11.67 mg/gm fw) and *Ocimum basilicum* (11.36 mg/gm fw) while the lowest concentration of phenolic compounds was in *Thymus vulgaris*, (5.4 mg/ gm fw/plant). these results are in harmony with those obtained by (Wong *et al*., 2006 ; Fing – Lin *et al*., 2010).

The results showed bands between 300 and 400 bp (figure 2) which is in consistent with the PAL gene primer in all four tested species, meaning that the PAL gene existed in all the four lamiaceae members. Using the differences between band intensity as a measure for gene expression, a comparison of the species to each other indicated that the expression of the PAL gene was high in *Lavendula angustifalia.* followed by Mentha, while PAL gene expression was low in Ocimum and Thymus (Fig. 2).

A comparison of the PAL gene expression and the amount of total phenolics produced from the leaves of each species were correlated (Table 1). meaning that the species producing the highest phenolic compounds, Lavendula, had the highest PAL gene expression as evidenced by band intensity, while Ocimum and Thymus, which had a very low PAL gene expression, had the lowest phenolic compound production.

(De – Eknamkun *et al*., 1987). Working on a type of antioxidant (rosmarinic acid) production in *Anchusa officinalis* and *Coleus blumei*, were able to demonstrate PAL activity and the rate of RA synthesis changed in a coordinated manner with the increases in PAL activity producing increased RA levels in tissue.

The enzyme phenylalanine ammonia-lyase (PAL) catalyzes the initial step of the phenylpropanoid pathway leading to the formation of 4-coumaroyl-CoA(De – Ek namkun and Ellis, 1987; Woodrow and Berry, 1988). Also the increase in PAL activity in plant tissues coincides with an increase in antioxidants and total phenolic content, indicating a role for PAL in the regulation of phenolics and antioxidants biosynthesis. (figure 3).

Differential expression of various gene families has been demonstrated, but their role in differentiation processes remains unclear (Pelkonen *et al*., 2003).

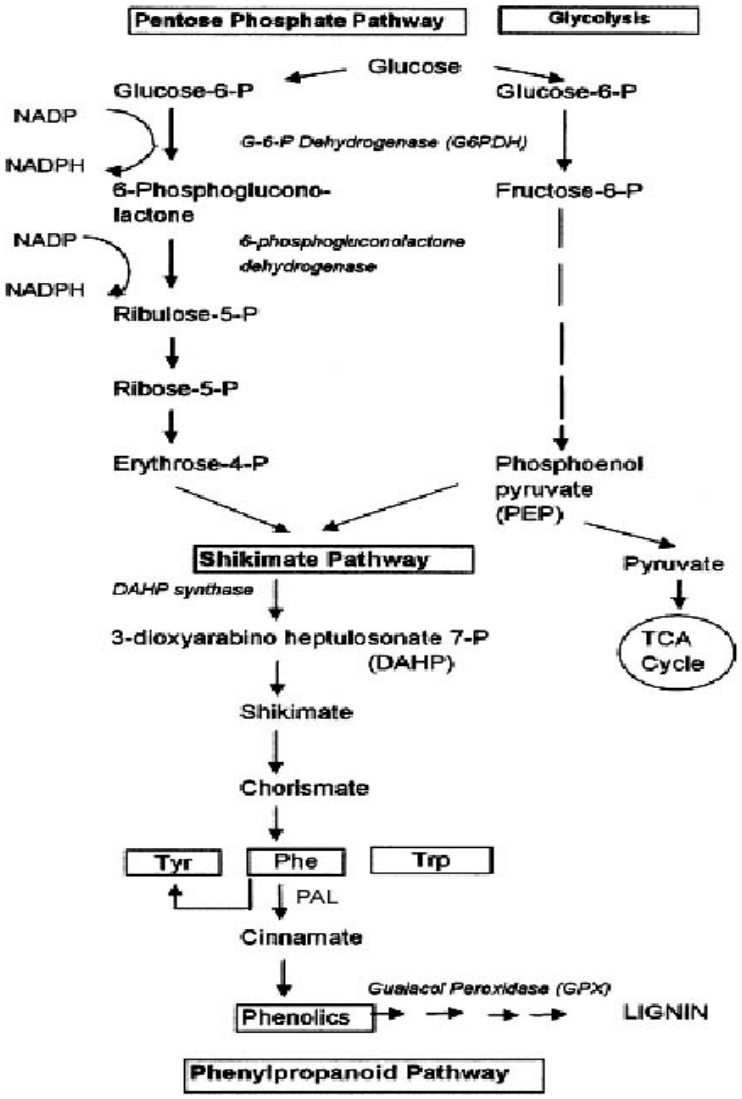

Fig. 3: The biosynthetic pathway for the total phenolic compounds and the role of the PAL gene . (Reena Randhir *et al*., 2004).

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