Isoenzyme diversity for peroxidase among Libyan thyme (*Thymus capitatus* (L.) Hoffm & Link.) Populations

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ABSTRACT: Thyme (*Thymus capitatus* **(**L.) Hoffm & Link) is an endemic wild plant in southern parts of Al-Jabal Al-Akhdar region, Libya. There are five phenotypes (populations or accessions) of thyme that show flower color polymorphism. Difference in flower color among the five phenotypes is due to genetic variation. This study was conducted to determine the pattern of peroxidase isoenzymes in thyme which growing in south parts of Al-Jabal Al-Akhdar . Laves samples of the five *Thymus capitatus* **(**L.) Hoffm & Link genotypes (populations) were evaluated for peroxidase isoenzyme system by horizontal starch gel electrophoresis. All five phenotypes had different number and relative mobility of electrophoretic bands. This variability enabled the five phenotypes of *Thymus capitatus* (L.) Hoffm & Link to be distinguished.

Key words: electrophoresis, genetic diversity, endemic species, peroxidase , *Thymus capitatus* (L.) Hoffm & Link.

INTRODUCTION

Systems of classification and identification based on quantitative and/or qualitative morphological characters were used by researchers to study genetic diversity (Trujillo *et al*., 1995; Ali *et al*, 2019). Although these methods were effective, they presented practical drawbacks due to effect of environmental fluctuations on expression of most morphological traits (Degani *et al*, 1995; Ali and Mustafa, 2019). The use of biochemical markers such as isoenzymes overcome these problems since they are little affected by the environment and can easily be detected in a variety of tissues by relatively simple, rapid and inexpensive procedures (Weeden and Lamb, 1985; Obara-Okeyo *et al*., 1997).

The fact that different molecular forms of enzyme which catalyze the same reaction (isozymes) can occur in the same organism is providing to be not only an especially valuable aid in many biological studies, but it is also providing a new and exciting perspective for the interpretation of a number of central problems to modern biological thoughts such as cellular differentiation, genetics and evolution (El-Wakil *et al*, 2000).

Horizontal starch gel electrophoresis, which uses the migration of proteins in an electric field to detect small differences in their charge and shape, has proved to be useful tool for identification of genetic diversity (Cyrus and Cummins, 1992; Ali, 1999). Isoenzymes analysis by horizontal starch gel electrophoresis can help validate plant pedigree, phylogeny, polyploidy, and mating system and help distinguish among populations (Peirce and Brewbaker, 1973).

Thyme (*Thymus)* is a genus containing about 350 species of aromatic perennial herbs and sub-shrubs to 40 cm tall, belong to the family Lamiaceae. This family is distributed throughout the arid, temperate and cold regions including Europe, North Africa and Asia. It is in leaf all year, flowering from July to September (Gruenwald *et al*., 2004).

Thyme (*Thymus capitatus* (L.) Hoffm & Link) is a perennial woody plant, 15 cm tall, with abundant stoloniferous branches. The inflorescences are composed of whorls of small, zygomorphic flowers. The pollination in *T.capitatus* is entomophilous, as in most *Thymus*

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species. The main pollinators are *Apis mellifera*. *T. capitatus* is gynodioecious, as for most species of *Thymus* (Ali and Mustafa, 2019). *Thymus capitatus* is an endemic wild plant in southern parts of Al-Jabal Al-Akhdar, Libya. Thyme growing in southern parts of Al-Jabal Al-Akhdar showed flower color polymorphism which results five different phenotypes: white- flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered populations (Ali and Mustafa, 2019).

Isozyme analysis which has been successfully applied to identify several plant species, offers a possible alternative method for identification of different thyme populations. The aim of the present study was to identify thyme (*Thymus capitatus*) populations growing in south parts of Al-Jabal Al-Akhdar region, Libya by using isoenozyme analysis (peroxidase).

MATERIAL AND METHODS

The study was carried out to investigate the biochemical genetic markers (peroxidase isozyme) in natural populations of *Thymus capitatus* (L.) Hoffm & Link which growing in southern parts of Al-Jabal Al-Akhdar, Libya. *Thymus capitatus* (L.) Hoffm & Link shows a stable and dramatic flower-color polymorphism (Ali and Mustafa, 2019). There are five patterns: white-flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered plants present in natural populations of *Thymus capitatus* (L.) Hoffm & Link.

Peroxidase isozyme system were resolved by horizontal gel electrophoresis, using 12% hydrolyzed potato starch from Sigma. Leaf samples from fifteen plants for each population were homogenized using 0.1 ml of 0.23M Tris-citrate buffer (pH 8.0) in a prechilled pestle and mortar. The homogenate was absorbed onto a small rectangle **(**4x2mm**)** of filter paper **(** Whatman No. 1**)**. These strips of filter paper were placed on the origin line of agar gel plates, for about 30 min. at 4°C. The filter paper strips were removed, then, an electrical constant current of 15-18 V/ cm was applied for 90 min. at 4°C using 0.23 M Tris-citrate buffer (pH 8.0), as an electrode buffer. After separation of the peroxidase isozymes, the gel plates were incubated at 4°C for 10 min. and were stained with peroxidase staining solution.

Staining solution for peroxidase was consists of 100ml. of 0.01Msodium acetate buffer (pH5.0), containing 0.1g benzidine and 0.5% hydrogen peroxide (H_2O_2), which is added immediately before staining.

RESULTS

Staining for peroxidase (PX) after horizontal starch gel electrophoresis resulted in obvious phenotypic differences among natural populations or accessions of Libyan thyme (*Thymus capitatus* (L.) Hoffm & Link) when leaf tissue was used. The zymogram produced from isozymatic analysis clearly indicated that there was considerable variation among populations (accessions) of *Thymus capitatus* (L.) Hoffm & Link for peroxidase (PX). All the bands were consistent and repeatable in all different accessions (Fig. 1).

Two polymorphic regions of peroxidase (PX) activity were observed on the gel. One moved cathodally and the other moved anodally. The zones of activity (bands) for peroxidase were numbered according to their proximity to the original line (O.L.). The intensity of the bands was illustrated with different strips: black, gray and white, which indicate high, medium and low activities of peroxidase, respectively (Fig. 1). This figure was more illustrated by figure 2.

Polymorphism was observed in peroxidase system for all natural populations of thyme (*Thymus capitatus* (L.) Hoffm & Link) growing in southern parts of Al-Jabal Al-Akhdar region, Libya. The present data identified five isoenzymes of peroxidase expressed as different banding patterns **(**Fig.1, 2**)**. Three bands were anodal (PX_1 to PX_3) and one band was cathodal (PX_4).

Results of the present study showed that populations of Libyan thyme had different relative mobility (Rf) of electrophoretic bands. PX zymogram gave five different banding patterns composed of one to four bands, with the fastest band (PX1) at 0.5 Rf and the slowest band (PX3) at 0.2 Rf. The values of relative mobility for PX2 and PX4 were 0.38 and 0.25, respectively (Table 1).

Except violet-flowered population, all populations of *Thymus capitatus* (L.) Hoffm & Link in the present study had PX3 band (Fig. 2), while only two populations (purple-flowered and mosaic-flowered accessions) carried a fast band (PX1). Moreover, the PX4 band was absent in white-flowered and violet-flowered plants. On other hand, the PX2 band was present in all populations except white-flowered and mosaic-flowered populations (Table 1).

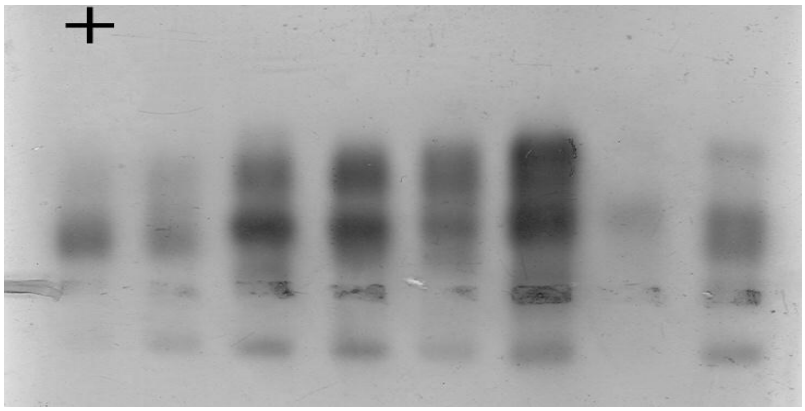

Fig (1): Photograph of banding patterns for peroxidase in *Thymus capitatus* (L.) Hoffm & Link populations.

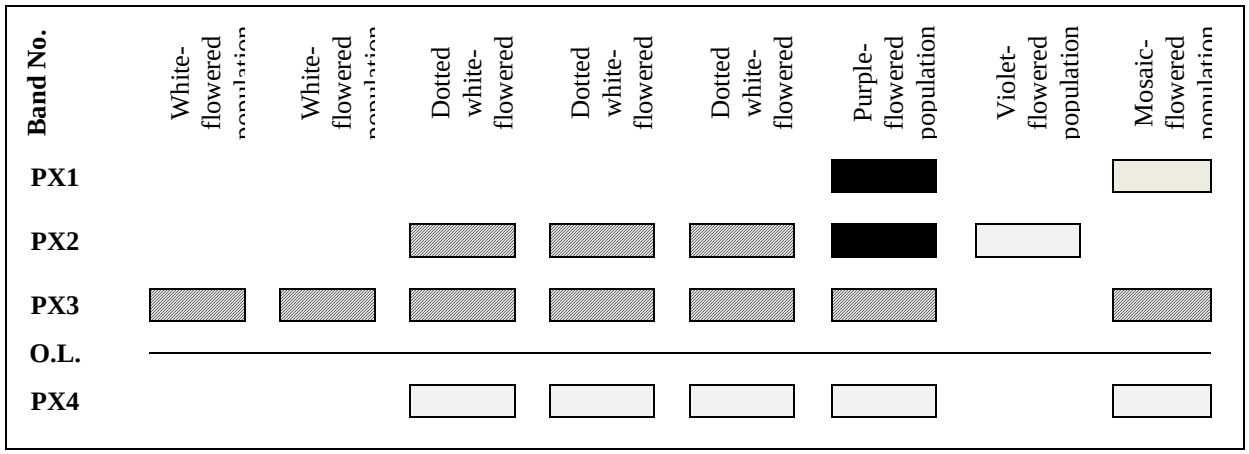

Fig (2): Diagrammatic representation of banding patterns and populations observed in *Thymus capitatus* (L.) Hoffm & Link for peroxidase.

Low activity Medium activity High activity

Table (1): Activity and Relative mobility of banding patterns for peroxidase in *Thymus capitatus* (L.) Hoffm & Link populations.

		Anodal direction			Cathode direction
	Band	Px1	Px2	Px3	Px4
	Relative mobility	0.50	0.38	0.20	0.25
Populations	White-flowered			B	
	Dotted white-flowered		B	B	C
	Purple-flowered	A	A	B	C
	Violet-flowered		C		
	Mosaic-flowered	C		B	C

A= High activity B = Medium activity C = Low activity

DISCUSSION

Plant peroxidases are contained mainly in cell walls and such localization results in their exhibiting activity already at early stages of defense reactions of the plant to pathogen attacks (Rautela and Payne 1970; Van-Geyt,1986; Cook *et al*., 1995; Harrison *et al*., 1995; Curtis *et al*., 1995; Thordal-Christensen *et al*., 1992). The reactions are revealed by accelerated lignification (Vance *et al*., 1980; Gaspar *et al*., 1982; Walter, 1992), and suberisation (Goldberg *et al*., 1981), as well as production of anti-microbial radicals (Peng and Kuc, 1992; Kobayashi *et al*., 1994). Besides, peroxidases can produce oxidized phenolics or other substances toxic for invading pathogens (Gaspar *et al*., 1991). Peroxidases are ubiquitous in plants. They have become standard isoenzyme markers due to their easy detection. In addition, the enzymes are extremely stable (Rasmussen and Kerby,1993; Kolodziejczak and Krzakowa, 2003).

In this work no inheritance study was done for making the different between allozyme and isoenzyme bands. So the genetic differences among different populations were mainly depended on the difference between populations in their banding patterns as a whole.

This study shows that isozymes can be used for identification of Libyan thyme populations at the vegetative stage. This should be useful to germplasm repositories for early detection of mislabeled plants in natural populations of thyme and therefore help maintain the purity of thyme stocks. Moreover, the observed variability can be useful in germplasm evaluation. Also, precise identification of thyme natural populations by isozymes can be useful for patent purposes. These observations agree with those of others (Loapez-Pujol *et al*, 2004; Bartošova *et al*., 2005). Although morphological and physiological markers occasionally may be sufficient for identification, isoenzyme is essential when these markers are not available (Degani and Blumenfeld, 1986).

The present investigation showed that natural populations of Libyan thyme can be distinguished on the basis of peroxidase isozyme system making the identification to be economical and applicable in laboratories. Peroxidase system seemed to be useful in discriminating among thyme populations because of a variety of banding patterns. These findings are in agreement with findings of many previous studies (Byrne and Littleton, 1988; Hannan and Orick, 2000; Stoyanov and Stoyanova, 2007).

The current study indicated that peroxidases of Libyan thyme (*Thymus capitatus* (L.) Hoffm & Link) also exhibit cathodal and anodal migration. These results agree with many researches which reported that most plants contain peroxidases of cathodal and anodal migration. Peroxidases of cathodal and anodal migration were detected in different, taxonomically very distant groups of plants, e.g. in bryophytes (Krzakowa, 1991), grasses

(Felder, 1976; Krzakowa, 1996), peanut (Wan and Vanhusystee, 1993), and in radish (Kim and Park, 1996).

Furthermore, Peroxidase profiles have been used as taxonomic markers and measures of inter-population variability in the *Sphagnum subsecundum* complex, where the lack of morphological discontinuities renders the separation of taxa based only on these features controversial and pointless (Abe and Tsuda, 1987; Abe *et al*., 1993; Krzakowa and Melosik, 2000).

The Libyan thyme populations that examined in this study exhibited substantial isoenzymic variability that proved useful in identification and verification. This variability is a reflection of diverse and complex species background and its out-crossing nature. These results showed that isoenzyme analysis not only provides genetic markers for identification purpose but is also a procedure for analyzing the origin of species (Hauagge *et al*, 1987; Byrne and Littleton, 1988; Volis *et al*., 2002; Kolodziejczak and Krzakowa, 2003) .

These results are expected because these populations are reproduced by seeds. Therefore, the genetic variability among thyme populations is mostly due to genetic segregation produced from sexual reproduction. These findings were confirmed by several studies on different plant species (El-Wakil *et al*., 2000; Loapez-Pujol *et al*., 2004; Talukdar, 2010).

المستخلص: الزعتر (*Thymus capitatus*) نبات بري متوطن في الجزء الشرقي من ليبيا. يوجد خمسة أشكال مظهرية لنبات الزعتر البري تنمو بمنطقة جنوب الجبل الأخضر. الأشكال المظهرية الخمسة لهذا النبات أظهرت تعدد أنماط بالنسبة لصفة لون الأزهار. الاختلاف في لون الأزهار بين الأشكال المظهرية الخمسة يرجع إلى التباين الوراثي. هذه الدراسة أجريت لغرض تحديد مشابهات أنزيم peroxidase في نبات الزعتر المنتشر بمنطقة جنوب الجبل الأخضر. عينات الأوراق للأشكال المظهرية الخمسة جمعت لتحديد نمط مشابهات أنزيم peroxidase باستخدام تقنية الهجرة الكهربائية الأفقي بهلام النشا. الأشكال المظهرية الخمسة لنبات الزعتر أظهرت اختلاف في العدد و الحركة النسبية لحزم التفريد الكهربائي. هذا الاختلاف يجعل من الممكن التمييز بين الأشكال المظهرية الخمسة لنبات الزعتر البري.

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