International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 12 No. 1, 2026
https://theaspd.com/index.php

Study of the Genetic Diversity of some Ecotypes of a Triplex Halimus from Western Algeria using Isoenzymatic Markers

Reguieg Yassaad Athmen[*1], **Belkhodja Hamza**[2]
[1]Laboratory of amelioration of plants and in vitro culture, Department of Biology, Faculty of Nature and Life Sciences, Abdelhamid Ibn Badis University of Mostaganem, Algeria. E-mail: bioathmane@yahoo.com / ORCID: https://orcid.org/0000-0001-9556-1027
[2]Laboratory of Bioconversion, Microbiology Engineering and Health Safety, Department of Biology, Faculty of Nature and Life Sciences, University of Mustapha Stambouli, Mascara, Algeria.E-mail:hamzabelkhodja@yahoo.fr / ORCID: https://orcid.org/0000-0002-1937-2897

Abstract
In arid and semi-arid regions, Atriplex species constitute excellent forage for livestock, especially during droughts. Due to their hardiness, palatability, and edibility, as well as their richness in proteins. Equipped with a very significant biomass, they constitute an effective and relatively inexpensive tool in the fight against erosion and desertification, especially in steppe areas. The trials aimed at characterizing the genetic diversity of these species are therefore very useful for their classification, conservation, improvement, and selection.
Our work is part of the characterization of the genetic polymorphism of six Atriplex halimus ecotypes from various Algerian thru the separation of isoenzymatic markers on electrophoresis gel represented by peroxidases and glutamate oxaloacetate transaminase. knowing that the three alleles are expressed for the ecotypes of Bechar, Ain Sefra, Mascara, Arzew, and Mostaganem, but for the Chleff ecotype, all the alleles are expressed.
The results of this study showed that glutamate oxaloacetate transaminase is not very polymorphic among the studied ecotypes, with the presence of three isoenzymes visualized in Béchar, Ain Sefra, Mascara, Arzew, and Mostaganem, and an additional isoenzyme characterizing the Chleff ecotype, demonstrating its genetic diversity.
Peroxidases have also demonstrated low interspecific diversity, which characterizes the Chleff ecotype compared to other ecotypes. the zymogram of peroxidases presents two active zones each composed of two bands, which suggests the presence of two loci each containing two alleles.
Generally speaking, the results revealed a non-negligible threshold of genetic polymorphism, both inter- and intra-species, among the studied ecotypes of Atriplex halimus.
Keywords: *Atriplex hailmus, genetic diversity, ecotype, electrophoresis, isoenzymes.*

INTRODUCTION

In Algeria, Atriplex halimus is a cosmopolitan species found from the coast to the extreme south (Al-Turki et al., 2000; Walker et al., 2014). It is planted in inter-dune spaces where it helps stabilize the dunes and on saline soils to improve pastures, used in the phytoremediation of contaminated soils and soil remediation (Chisci et al.2001, Gharibeh et al.2023). These shrubs can play a role in the development of regions where vegetation has been severely degraded. (Mulas ,2004). The organic composition of Atriplex halimus depends on several parameters, such as climate, plant age, and season. (Mkaddem et al., 2024). Many species resemble each other, even from one genus to another, and especially some of them exhibit astonishing polymorphism, which causes the plant's appearance to vary from one individual to another depending on the stage of development and the season. The species of the genus Atriplex exhibit pronounced morphological polymorphism, manifested in the size and shape of the leaves, fruit valves, and seeds, as well as in overall biomass production. (Boules 1999, Piotto et al. 2003).
That is how we became interested in studying the genetic polymorphism of this plant to identify individuals with interesting characteristics for potential selection work. Our work aims to contribute to the evaluation of the genetic variability of Atriplex halimus at the biochemical level, represented here by peroxidases and glutamate oxaloacetate transaminase.

MATERIAL AND METHODS

Plant material

In order to obtain young Atriplex halimus seedlings that will be used in the enzyme extraction step for the study of isoenzymatic polymorphism, Atriplex halimus seeds were collected from the following sites: Bechar (Abadela), Ain Safra (Tiout), Mascara (Tighennif), Mostaganem (Matarba), Arzew (the port of Arzew), Chleff (Soubha) (figure1)..

The map below shows the geographical location of the harvesting sites.

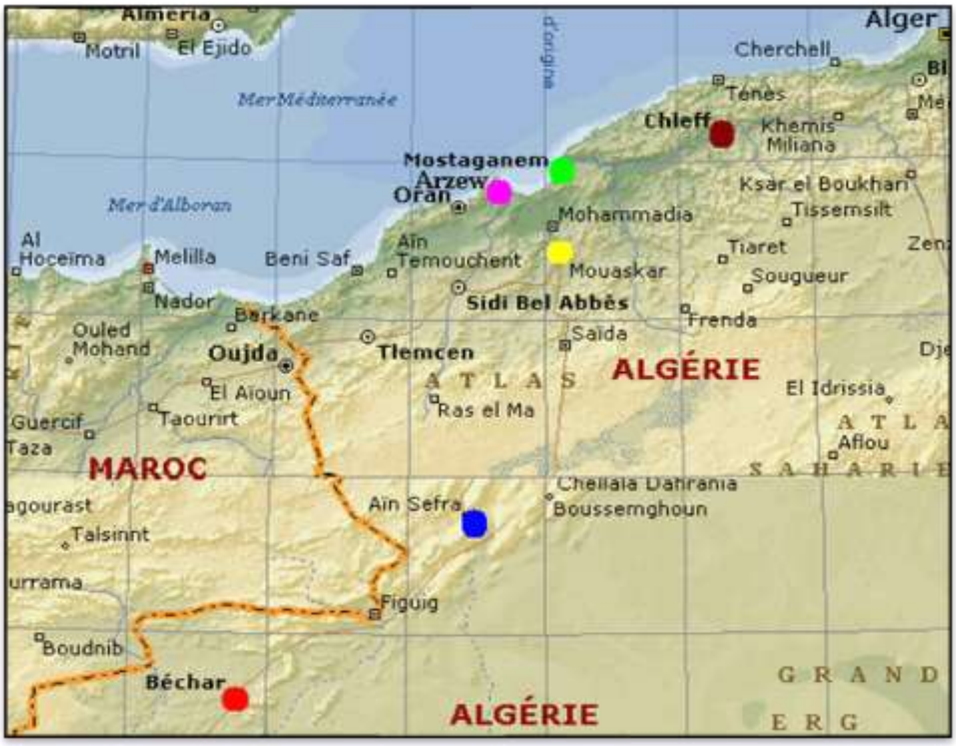

Figure 1. Geographical location of the harvesting sites
Source: Microsoft Encarta 2008; 1993-2007 Microsoft Corporation All rights reserved

The choice of harvesting sites was based on the existence of a more or less marked phenotypic polymorphism, and it was with the aim of verifying whether it finds an explanation by studying it thru biochemical markers.

The panicles containing the seeds were rubbed against a sieve to separate the seeds, which are very small in size. Obtaining a large number of seeds without using this method is difficult. The seeds are stored in separate boxes.

The sowing takes place on filter paper in petri dishes placed in an incubator at 25°C to accelerate germination. The entire 7-day-old seedlings are used for protein extraction for the study of enzymatic systems (Sabu et al., 2000; Tadesse and Bakele,2001).

The extraction of enzymes from Atriplex halimus seedlings

The seedlings are cleared of any remaining seed coats by retrieving the seedlings, which are then counted and weighed. The enzyme extraction is done on a mortar cooled with ice to avoid any possible denaturation.

Ascorbic acid as well as 2-mercaptoethanol are antioxidants that partially prevent the oxidation of phenols into quinones, which can lead to enzyme inactivation. By adding the extraction buffer at a ratio of (1ul / mg) (Sandmeier et al.,1981). The obtained extracts are transferred into Eppendorf microtubes (1.5 ml), then centrifuged for 20 minutes at 14,000 rpm at 40°C: (Thongthieng and Smitamana 2003). After centrifugation, the supernatants (crude extracts) are collected in other microtubes of the same volume, they can be used immediately for electrophoresis or frozen at – 200 C; for later use (Haddioui and Baaziz , 2001; Martinez et al., 2001).

The extraction buffer used is the 0.1 M Tris-HCl buffer, pH 8 (Kaplan et al., 2001), which consists of the following reagents: Tris Hcl 0.1M, B. mercaptoethanol 70 mM, Sodium metabisulfite 26 mM, Ascorbic acid 11mM, PVP (polyvinylpyrrolidone) 4% w/v.

Preparation of polyacrylamide gel

The buffer system used is discontinuous. The use of discontinuous buffers, whose pH and ion nature allows for the prior concentration of the sample to be separated.

Polyacrylamide gels have a thickness of 1 mm and are made using two glass plates measuring 20 x 20 cm. The upper part of the gel, where the enzymatic extracts are deposited, has a large-pore texture (concentration gel, 5% polyacrylamide). This gel allows for the concentration of the extracts before their separation on the narrow-pore gel (separation gel, 8% polyacrylamide). (Wiley Liss 1966). The composition of the two gels is summarized in the following table:

Table 01: Composition of discontinuous gel.

	Gel of concentration 5% (20ml)	Gel of separation 8% (50ml)
Distilled water	11,68 ml	24,2 ml
Polyacrylamide solution	3,32 ml	13,3 ml
Tris-HCl 0,5 M pH 6,8	5 ml	
Tris-HCl 1,5 M pH 8,8		12,5 ml
Ammonium persulfate	100 µl	250µl
TEMED	10 µl	25 µl

The gel buffer

The same gel buffer is used as the tank buffer (Vallejos, 1983). This buffer is composed of: 30g of tris-HCl, pH=8, 14.4g of glycine, 1L of distilled water.

Vertical electrophoresis

This type of setup is mainly used for matrices like polyacrylamide gels. The samples generally move within the matrix. The matrix is in the form of gelled material, between two glass plates. It is prepared shortly before use by forming a "sandwich" of glass plate/gel/glass plate. During gelation, care will have been taken to create wells where samples will be placed. Each end of the gel will be placed in contact with a buffer containing electrolytes which, when subjected to an electrical potential, will allow the propagation of a current thru the gel. This current will drive the molecules that make up the sample. This migration will allow for the separation of various molecular species that will migrate at different speeds. (Kamoun, 1977). We use an "electrophoresis tank" essentially consisting of two containers holding the buffer and an electrode. The anode and cathode are connected to a direct current generator. The ends of the solid support are dipped in the buffer (Guastalla, 2003).

The migration 30 ul of protein extract are introduced into the wells (one ecotype per well) using Hamilton syringe. Bromophenol blue 0.05 mg/ml (Vallejos ,1983) is used to indicate the migration front. The tanks of the device are filled with buffer until both ends of the gel are submerged. The electrodes are connected to the generator that provides a continuous current of 50 mA intensity and 300V voltage. During the manipulations, the device is placed in a refrigerator to keep the gel at a temperature of 4° C, in order to prevent the denaturation of enzymes due to the heat generated by the passage of current thru the gel (Zeidler 2000). The operation continues until the migration front reaches the lower end of the gel.

The revelation

The in situ revelation of isoenzymes is carried out by incubating the gel in a reaction mixture containing the substrates of the studied enzyme.

The gel containing the investigated enzymes is carefully retrieved, rinsed, then placed in a box where the enzymatic reaction and specific staining for each enzymatic system will take place, it is then immersed in a fixation solution composed of 7% acetic acid, then stored in cellophane film and digitized (by Scanner).

Statistical study

All tests were performed in duplicate or triplicate. Results are presented as mean± standard deviation of two or three independent determinations. All statistical analyses were carried out by Graphpad prism 5 using analysis of variance (ANOVA) and differences among the means were determined for significance at p≤0.05 using least significant.

RESULT

Glutamate oxaloacetate transaminase

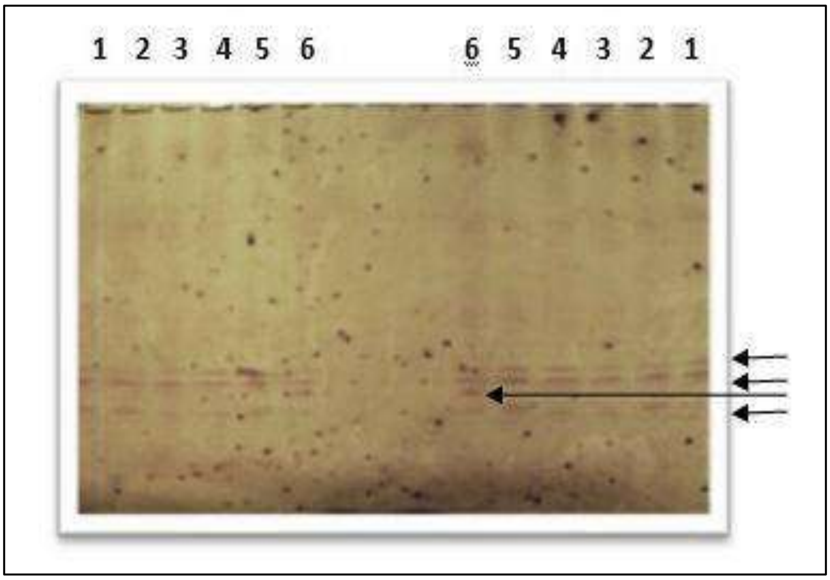

Figure 2. Zymogram of glutamate oxaloacetate transaminase. Bechar(1), Ain Sefra(2), Mascara(3), Arzew(4), Mostaganem(5), Chleff(6)

The observation of the zymogram shows that the resolution was perfect, which made the presence of two zones of enzymatic activity visible. The observed bands were clearly separated and they were colored pink and black with almost identical intensity for the different ecotypes (figure 2).
At the level of the first activity zone, three bands are exceptionally counted in the Chleff ecotype and only two bands for the other ecotypes, whose relative separation profile shows that the two presented bands are identical for the studied ecotypes, whose frontal ratios are 0.73±0.01 and 0.75±0.05 respectively, and which are cathodic.
The additional band of the Chleff ecotype shows the diversity it presents compared to the others with a Frontal Ratio equal to 0.81 ±0.02. The second zone contains a single black, fast, and anodic band common to the studied ecotypes with the same migration or a frontal ratio equal to 0.85 ± 0.06. The results obtained allow us to conclude that for glutamate oxaloacetate transaminase, the two highlighted zones correspond to two loci with two alleles each, determined by Haddioui and Baaziz (2001), and that the structure of this enzyme is monomeric.
Peroxidases

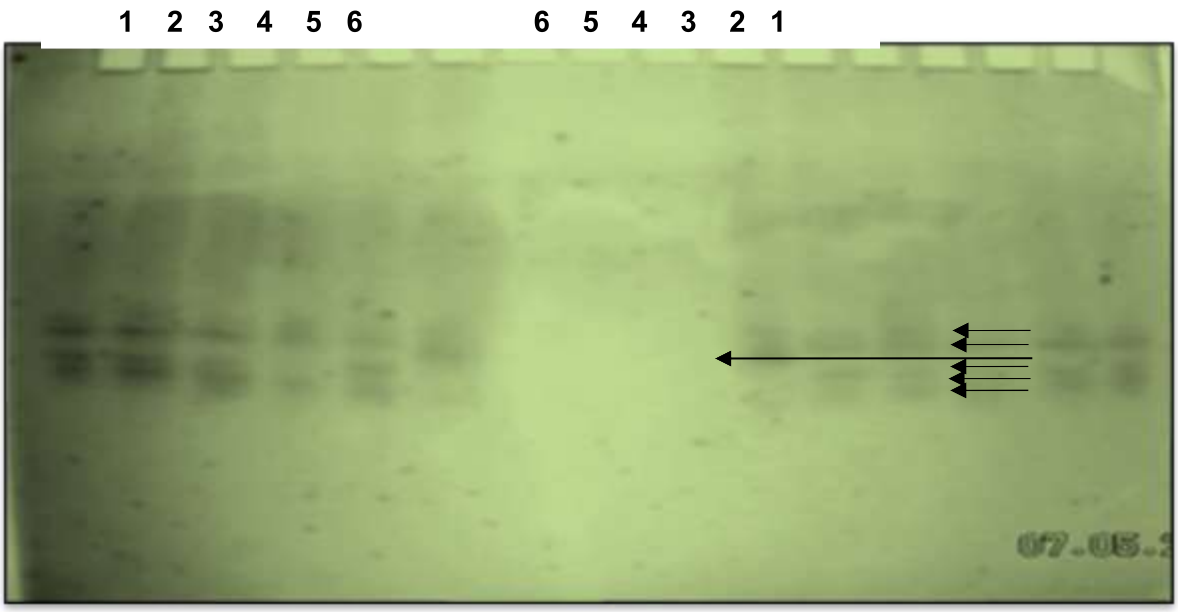

Figure 03. Zymogram of peroxydases. Bechar(1), Ain Sefra(2), Mascara(3), Arzew(4), Mostaganem(5), Chleff(6)

Electrophoretic analysis on discontinuous polyacrylamide gel of peroxidases using their substrate allowed for the separation of the isoenzymes of this system.

The analysis of the zymogram shows that the number of bands revealed varies from four to five per ecotype, these bands are distributed over three zones, each of these three zones is represented by one to three bands, slow bands in the first zone, fast bands in the third zone, and intermediate bands in the second zone (Figure 03).

The first zone contains two common cathodic bands for all populations with a frontal ratio of 0.55 ± 0.04 and 0.56 ± 0.07 respectively. In this zone, there is also a third one with a frontal ratio equal to 0.61 ±0.02, which characterizes the Chleff ecotype, thus showing its genetic polymorphism compared to the other ecotypes. The second intermediate zone contains two bands with frontal ratios of 0.63 ±0.002 and 0.65 ±0.01 respectively, existing in all the studied ecotypes except the Chleff ecotype.

In the third zone, we observe a rapid and identical migration profile of an anodic band where the frontal ratio is 0.67 ± 0.02 across all the studied ecotypes.

DISCUSSION

The enzymes are made visible on the gel by using chromogenic substrates that produce colored products after the enzyme's action. Thus, for example, the release of α or β naphthol allows for its subsequent combination with an Azody (Fast blue RR) to produce a colored precipitate (Baaziz 2006). This is the case with our studied systems.

The Glutamate oxaloacetate transaminase

For the glutamate oxaloacetate transaminase system, the appearance of pink bands and black bands was observed. This color difference highlights the specificity of the enzymes (Vallejos, 1983). Pasteur et al. (1987) reported that this color difference also indicates the presence of different loci.

The presence of three bands common to the different ecotypes demonstrates the homogeneity of the species for this enzymatic system. On the other hand, the presence of an additional band specific to the Chleff ecotype reveals the existence of genetic diversity. Although this variability is low, it is not negligible. According to Micales and Bonde (1995), the origin of isoenzyme diversity may be due to the presence of a single locus coding for multiple alleles. So the presence of three common bands for all the ecotypes of Bechar, Ain sefra, Mascara, Arzew, and Mostaganem translates to the presence of three alleles and four alleles for the Chleff ecotype if the enzyme is monomeric.

Other authors report that glutamate oxaloacetate transaminase can also be dimeric. In this context; the appearance of three bands for the ecotypes: Bechar, Ain sefra, Mascara, Arzew, and Mostaganem reveals the presence of two alleles. Regarding the Chleff ecotype, three alleles responsible for the four bands found were identified.

From a karyological point of view, the ploidy level appears to be highly variable, with diploid, triploid, and tetraploid plants having been identified during the experiments (Barrow, 1987), so the origin of the variation in these isoenzymes may be due to the presence of multiple loci that code for a single allele.

If we consider that the enzyme has a monomeric structure, the presence of three bands for the ecotypes of Bechar, Ain sefra, Mascara, Arzew, and Mostaganem can be explained by the existence of three loci, and the four bands require four loci for the Chleff ecotype.

If the enzyme is dimeric; the three bands indicate two loci in the ecotypes of Bechar, Ain sefra, Mascara, Arzew, and Mostaganem, while the four bands characterizing the Chleff ecotype are explained by the presence of three loci.

Haddioui and Baaziz (2001) consider that in Atridex halimus, glutamate oxaloacetate transaminase is monomeric, and that these enzymes from foliar extracts exhibit four active zones with two bands each, suggesting the presence of four loci with two alleles. From this perspective, the appearance of the bands is explained by the presence of two loci with two alleles each. Only three alleles are expressed for the ecotypes of Bechar, Ain sefra, Mascara, Arzew, and Mostaganem. Whereas for the Chleff ecotype, all the alleles are expressed.

Peroxidases

Peroxidases are Michaelian enzymes with two substrates, catalyzing an irreversible reaction according to a ping-pong mechanism (Ngo, 2010). They have a variety of substrates (aromatic phenols, phenolic acids, indoles, amines, and sulfonates, etc.) (Veitch, 2004, Vlasova, 2018).

The enzymatic activity of peroxidases depends on several factors, the most important of which are pH and temperature. (Diao et al., 2019). Peroxidase exists in several isoforms within the same plant with similar

molecular weights, which are easily revealed by electrophoresis under non-denaturing conditions. These iso-enzymes catalyze the same reaction but differ in some physicochemical properties. (Marzouki, 2012).
The zymogram of peroxidases is simple and easy to read; it probably suggests the presence of five alleles encoded by the same locus for the ecotypes of Bechar, Ain Sefra, Mascara, Arzew, and Mostaganem, and four alleles for the Chleff ecotype, if the enzyme is monomeric and the origin of the variability of isoenzymes is due to the presence of several alleles encoded by a single locus.
In this same context, if the enzyme is dimeric, the appearance of five bands in the ecotypes of Bechar, Ain Sefra, Mascara, Arzew, and Mostaganem requires the presence of three alleles, and the four bands visualized in the Chleff ecotype originate from three alleles.
If we assume that the origin of isoenzyme diversity is due to the presence of several loci that code for a single allele and the enzyme is monomeric, the different levels of band migration can be attributed to several loci, the number of which is five in the ecotypes of Bechar, Ain Sefra, Mascara, Arzew, and Mostaganem, and four in the Chleff ecotype. In this same context, if we consider that the enzyme is dimeric, the five bands visualized by the zymogram in the same five ecotypes indicate the existence of three loci. However, the four visualized bands originate from three loci.
Let's compare our results with those obtained by other authors: Haddioui and Baaziz (2001) and who were able to highlight that in Atriplex halimus, peroxidases are considered monomeric enzymes, these same authors report that the zymogram of peroxidases presents two active zones each composed of two bands, which suggests the presence of two loci each containing two alleles, the existing difference between our results and theirs is mainly due to the structure of the enzyme which can be dimeric or it is a post-translational modification.
The change in edaphic and climatic conditions can force populations of certain species to evolve functional characteristics and express their enzymatic systems (Hegazy ,2001 ; Lobo et al., 2003).
Nowadays, the biotechnological interest in enzymes is growing due to economic and environmental advantages. Among these enzymes of interest are peroxidases, which are heme enzymes. They are capable of catalyzing the oxidation of a wide range of organic or inorganic compounds (Adeniyi et al., 2009; Zia et al., 2011). They are ubiquitous in plants such as chenopods (Quiroga et al., 2000). They participate in the healing of injured tissues and the protection of the plant against pathogenic microorganisms (Pandey et al., 2017). These enzymes have long been studied for their industrial and analytical applications (Agostini et al., 2002; Lu et al., 2016; Sonet et al., 2018).

CONCLUSION

The observation of enzymatic polymorphism is a powerful indicator and tool for studying the genetic diversity of Atriplex halimus, it is also a particularly interesting aspect of phenotypic polymorphism, directly representative of the genetic heritage.
The results obtained with the two enzymatic systems allow us to draw the following conclusions: For glutamate oxaloacetate transaminase, we can assume that the two highlighted zones correspond to two loci with two alleles each, determined by Haddioui and Baaziz (2001); however, the structure of this enzyme is probably monomeric.
For peroxidases, three zones were visualized in the electrophoresis gel, but no hypothesis on the number of loci given the existing difference between our results and those determined in 2001 by Haddioui and Baaziz, which may be the origin of the dimeric structure of the enzyme or it may be a post-translational modification.
However, the estimation of electrophoretic diversity for a population of a given species must be based on the examination of a sufficiently large number of individuals to better analyze the diversity of populations. Finally, extending this study to other enzymatic systems and even to other ecotypes would allow for a better evaluation of genetic variability.
Competing interests: The authors declare no competing interests.

Data availability: The data presented in this study are included within the article. Additional datasets used for visualizations are available from the corresponding author upon reasonable request.
Acknowledgements: We would like to warmly thank the Ministry of Higher Education and Scientific Research for funding this work.

REFERENCES

- Adeniyi SA, Orjiekwe CL, Josiah SJ. 2009. Kinetics of the reaction of compound III of horseradish peroxidase with hydrogen peroxide and NADPH. Int. J. Biol. Chem. Sci., 3(3): 415-421. DOI: http://dx.doi.org/10.4314/ijbcs.v3i3.4535 0
- Agostini E, Hernandez-Ruiz J, Arnao MB, Milrad SR, Tigier HA, Acosta M. 2002. A peroxidase isoenzyme secreted by Turnip (Brassica napus) hairy – root Cultures: inactivation by hydrogen peroxide and application in diagnostic Kits. Biotechnol. Appl. Biochem., 35(1): 1-7. DOI: https://doi.org/10.1042/BA20010049
- Al-Turki T A, Omer S and Ghafoor A (2000) A synopsis of the genus Atriplex L (Chenopodiaceae) in Saudi Arabia. Feddes Repertorium 111 (5-6): 261-293. Illus. Distr. Maps. https://doi.org/10.1016/j.plaphy.2016.02.037.
- Alvarez A, Fuentes J, Deus J, Duque M et Cornide M (2000) - Genetic diversity analysis in rice mutants using isozyme and morphological markers. Cultivos Tropicales vol. 21, no. 4, October-December, 39-44. https://doi.org/20.500.11766/7512
- Chisci GC, Bazzoffi P, Pagliai M, et al (2001) Association of sulla and atriplex shrub for the physical improvement of clay soils and environmental protection in central Italy, Agriculture, Ecosystems and Environment 84, 45-53. . https://doi.org/10.15379/ijmst.v10i3.1606
- Diao M., Dembele RH., Konate K et Dicko MH. (2019). Etude comparative des peroxydases de dix (10) plantes supérieures couramment rencontrées au Burkina Faso. International Journal of Biological and Chemical Sciences 13: 2533-2545.
- Gharibeh OE, Shiyab SM, Saoub H (2023) Atriplex (Atriplex halimus): Distribution, morphology, physiological tolerance to salinity and drought: A review. International Journal of Membrane Science and Technology 10, 860-868. https://doi.org/10.15379/ijmst.v10i3.1606
- Guastalla J (2003) – Electrophorese.2003 Encyclopedia Universalis France S A.
- Haddioui A et Baaziz M (2001) - Genetic diversity of natural populations of Atriplex halimus L in Morocco: An isoenzyme based overview. Euphytica 121: 99-106-2001. 2001 Kluwer Academic Publishers. . https://doi.org/10.1016/j.jaridenv.2013.09.004
- Hegazy AK (2001) Reproductive diversity and survival of the potential annual Diplotaxis harra (Forssk.) Boiss (Brassicaceae) in Egypt. Ecography 24, 403-412. . https://doi.org/10.15379/ijmst.v10i3.1606
- Lobo JA, Quesada M, Stoner KE, et al (2003) Factors affecting phenological patterns of bombacaceous trees in seasonal forests in Costa Rica and Mexico. American Journal of Botany 90, 1054- 1063. https://doi.org/10.3732/ajb.90.7.1054
- Lu Y, Ye W, Yang Q, Yu J, Wang Q, Zhou P, Wang C, Xue D, Zhao S. 2016. Threedimensional hierarchical porous Pt Cu dendrites: A highly efficient peroxidase nanozyme for colorimetric detection of H2O2, Sensors and Actuators B. Chemical, 230: 721-730. DOI: https://doi.org/10.1016/j.snb.2016.02.130.
- Marzouki SM., Almagro L., Sabater-Jara AB., Barceló AR et Pedreño MA. (2010). Kinetic Characterization of a Basic Peroxidase from Garlic (Allium sativum L.) Cloves. Journal of Food Science, 75(9): C740–C746.
- Micales JA, Bonde MR, Peterson GL(1987)- The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon ; 27: 405–49. https://doi.org/10.1007/978-3-662-04480-3
- Mkaddem-Guedri, M., Krir, N., Clausell Terol, C., et al. (2024). Phytochemical Analysis, acetylcholinesterase inhibition, antidiabetic and antioxidant activity of Atriplex halimus L. (Amaranthaceae Juss.). Chemistry & Biodiversity, e202301941.
- Mulas M, Mulas G (2004) - Potentialités d'utilisation stratégique des plantes Atriplex dans la lutte contre la désertification.Short and medium-Termpriority environnemental action programme (SMAP). https://doi.org/10.1016/j.jphotobiol.2018.10.021
- NGO TT. (2010). Peroxidase in Chemical and Biochemical Analysis. Analytical Letters, 43(10-11), 1572–1587. DOI: 10.1080/00032711003653874.
- Nicole Pasteur, Georges Pasteur, François Bonhomme, Josette Catalan (1987)- Manuel technique de génétique par électrophorèse des protéines . Paris : Tec & Doc , DL. https://doi.org/10.1016/0003-2697(76)90527-3
- Pandey S, Fartyal D, Agarwal A, Shukla T, James D, Kaul T, Negi YK, Arora S, Reddy MK. 2017. Abiotic stress tolerance in plants: myriad roles of ascorbate peroxidase. Frontiers in Plant Science, 8: 581-593. DOI: https://doi.org/10.3389/fpls.2017.00-581
- Piotto B, Bartolini G, Bussotti F, García A, Chessa I, Ciccarese C, Tranne G and Todeusz Tylkowski T (2003) - Fact sheets on the propagation of Mediterraneantrees and shrubs from seed. Seed propagation of Mediterranean trees and shrubs. APAT - Agency for the protection of the environment Purification and characterization of a potato tuber acid phosphatase having significant phosphotyrosine phosphatase activity. Plant Physiol. 106, pp 223 – 232. https://doi.org/10.1016/j.plaphy.2016.02.037
- Quiroga M, Guerro C, Botella MA, Barcelo A, Amay I, Medina MI, Alonso FJ, de Frochetti SM, Tigier H, Valpuesta V. 2000. A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiol., 122: 1119-1127. DOI: https://doi.org/10.1104/pp.122.4.1119
- Sabu K, Padmesh P, Seeni S (2000) Intraspecific variation in active principle content and isozymes of Andrographis paniculata Nees (Kalmegh): Journal of Ethnopharmacology 109: 348- 353. https://doi.org/10.1007/978-3-662-04480-3
- Sonet J, Bierla K, Bulteau AL, Lobinski R, Chavatte L. 2018. Comparison of analytical methods using enzymatic activity, immunoaffinity and seleniumspecific mass spectrometric detection for the quantitation of glutathione peroxidase 1. Analytica Chimica Acta, 1011: 11-19. DOI: https://doi.org/10.1016/j.aca.2018.01.068.
- Tadesse W et Bekele E (2001) - Isozymes variability of grasspea (Lathyrus sativus L.) in Ethiopia. Lathyrus Lathyrism Newsletter 2.Technol., 27 : 489-496.
- Thongthieng T et Smitamana P (2003) - Genetic Relationship in Strawberry Cultivars and Their Progenies Analyzed by Isozyme and RAPD. Plant Biotechnology Research Centre,Chiang Mai University,Chiang Mai 50202,Thailand.tolérance à la salinité chez l'orge. Rev. Biol . Biotech. Vol.4, No1, January. . https://doi.org/10.1016/j.jaridenv.2013.09.004.
- Vallejos, C.E. (1983) Enzyme activity staining. Isozymes in Plant Genetics and Breeding. Part A. Elsevier, Amsterdam, 469-515. https://doi.org/10.6018/analesbio.44.03.
- Veitch NC. (2004). Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry 65: 249-259. Vlasova I. (2018). Peroxidase Activity of Human Hemoproteins: Keeping the Fire under Control. Molecules, 23(10), 2561. DOI:10.3390/molecules23102561.
- Walker DJ, Lutts S, Sánchez-García M, et al (2014) Atriplex halimus L.: Its biology and uses. Journal of Arid Environments 100-101, 111-121. https://doi.org/10.1016/j.jaridenv.2013.09.004
- Zeidler M (2000) Electrophoretic analysis of plant isozymes. Biologica 38:7-16. https://doi.org/10.1016/j.plaphy.2016.02.037