IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 3 Ser. IV (May –June 2020), PP 31-35
www.Iosrjournals.Org

Variability in the Activity of the Isozyme Peroxidase in different *Pennisetum glaucum* L. genotypes

Manal A. M. Salih[†1] and Abdalwahab H. Abdallah[2]
1 Department of Botany, faculty of Science, University of Khartoum P.O.Box 321 Sudan
2 Department of agronomy, Faculty of agriculture, University of Khartoum, Sudan

Abstract: *Peroxidase is an ubiquitous enzyme occurring in all higher plants. Multiple forms of peroxidase are well established in various vegetables and fruits. This enzyme is implicated in many physiological functions of plants. In this study the isozyme peroxidase was used to assess the variation of the four pearl millet genotypes collected from Western Sudan at the level of isozymic activity. The results obtained showed a very high value (3.0) maximum velocity for the enzyme extracted from the genotype Bauda leaves, followed by 1.6 for genotype Ugandi, 1.3 for genotype Darmasa and finally 0.5 for genotype Madlkawia.*

Key Words: ***Pennisetum glaucum, genotypes, peroxidase, isozymes***

Date of Submission: 04-06-2020 Date of Acceptance: 20-06-2020

I. Introduction and Literature review

Isozymes and restriction fragment length polymorphism are two classes of molecular markers that have a number of desirable properties when used for breeding purposes. These markers are generally naturally occurring, deleterious, usually have co-dominated expression, can be cytoplasmic or nuclear, and generally are not influenced by environment. Isozymes polymorphisms are readily and early used in plant genetics and have rapidly become popular among plant breeders (Salih, 1997).These isozymic characterizations may be useful for interspecific, introgeneric or intra-familial comparisons, depending on the group (Buth, 1984).Protein polymorphism and consequently isozymic variation is a phase of molecular evolution (Kimura and Ohta, 1971). Salih *et al*., (2000) compared the activity of the isozyme peroxidase in the cultivated species (*P. glaucum*) and its wild relative (*P. violoceum*) using polyocrylamide gel electrophoresis (PAGE).In this study peroxidase enzyme from wild species showed dark brown bands, while peroxidase enzyme from the cultivated species exhibited faint brown bands. The difference in colour intensity between the two species can be explained as, either the concentration of the enzyme was higher in the wild species or both species had the same concentration but the enzyme produced by the wild species had more active sites than that produced by the cultivated species.Chahal*et al*., (1986) also showed considerable variation in the number and intensity of peroxidase isozymes between pearl millet lines susceptible and resistant to ergot disease. This suggests the possibility of using peroxidase isozyme pattern analysis as a technique of rapidly screening millet lines for resistance to ergot at the seedling stage.As in studies of hybridization, isozymes have been valuable tool in identifying the parents of polyploidy plants. Isozymes have supported hypotheses based on other line of evidence and differentiated among alternative hypotheses.Most of the isozymes are genetically determined and they result from the activity of different allelic forms of a gene. Isozymepolymorphism are readily and easily used in plant genetics and have rapidly become popular among plant breeders.So isozymic characterization may be useful for intergeneric or intra-familial comparisons, depending on the group.

II. Materials and Methods

Four millet genotypes, these were Bauda, Darmasa, Ugandi and Madlkawia (table 1) collected from Western Sudan were used in this study.

Table 1: Four genotypes of pearl millet used in the study and their origins

No.	Accession	Origin	Notes
1	Bauda	Darfur/ Western Sudan	Check
2	Darmasa	Darfur/ Western Sudan	Collection
3	Ugandi	Uganda/adopted	Check
4	Madlkawia	Kordofan/ Western Sudan	Collection

Enzyme ExtractionThis was carried out following the method adopted by Sidhu*et al*. (1984). For peroxidase extraction, 15g of leaves from each genotype (Bauda, Darmasa, Ugandi and Madlkawia) at the age of 15 days were collected during the early morning hours, then 100ml of distilled water were added. Crude

extracts of peroxidase enzyme were prepared by crushing the leaves using pestle and mortar; the extracts were filtered and centrifuged immediately at 10,000 rpm for 10 minutes at 4°C, by using sigma Laboratory refrigerated centrifuger. Supernatant of each genotype extracts were collected for the determination of peroxidase activity.

Determination of Kinetic ConstantsThe method used was a modified procedure of Mohamed (1983) and Gadia (1977). One ml of the enzyme was incubated for 10 minutes at room temperature (37°C) with 2ml of different concentrations of catechol (1-10mg/ml distilled water) in 10 tubes + 2ml of hydrogen peroxide (6%) and 2ml of 0.1M acetate buffer (pH 7.0).The absorbance changes due to oxidation of each substrate were followed at 25°C with Genway 6300 spectrophotometer at the wave length of 470nm. Maximum velocity (V_{max}) and Michael's constant (K_m) were determined from the line weaver Burk plots. The experiment was performed in triplicates.

III. Results and Discussion

The results given by the kinetic studies of *pennisetum*peroxidases are reflected in table (2). The table lists the absorbency by different concentrations of the substrate added to one ml of the enzyme extracted from the different genotypes. Four graphs were constructed for the four genotypes, these were: figures 1,2,3 and 4 for Bauda, Darmasa, Ugandi and Madlkawia respectively. In these graphs, 1/concentrations and absorbency (nm.) were constructed in the X and y axis respectively. Maximum velocity (V_{max}) and Michael constant (K_m) were recorded for each graph (table 2) and fig. 5).The highest V_{max} was recorded for the genotype Bauda followed by Ugandi, Darmasa and finally Madlkawia. The Km was more or less of the close value for the genotypes Ugandi and Darmasa (0.14 and 0.18 respectively), while it was of the same value (0.06) for Bauda and Madlkawia.

Table (2): Spectroscopy readings for the four genotypes

Genotype	Substrate concentrations	Absorbency	K_m	V_{max}
Bauda	0.10	51.02	0.06	3.0
	0.1→	34.01		
	0.125	9.009		
	0.1428	8.69		
	0.16→	12.531		
	0.20	714.28		
	0.25	17.85		
	0.30	8.928		
	0.50	3.713		
	1.0	5.405		
Ugandi	0.10	8.403	0.14	1.60
	0.1→	3.203		
	0.125	3.194		
	0.1428	2.985		
	0.16→	4.065		
	0.20	2.890		
	0.25	5.854		
	0.30	3.819		
	0.50	3.625		
	1.0	3.700		
Darmasa	0.10	2.480	0.18	1.30
	0.1→	2.138		
	0.125	2.695		
	0.1428	2.396		
	0.16→	2.523		
	0.20	2.705		
	0.25	2.834		
	0.30	3.287		
	0.50	3.450		
	1.0	2.096		
Madlakawia	0.10	1.395	0.06	0.50
	0.1→	4.409		
	0.125	1.630		
	0.1428	2.035		
	0.16→	2.768		
	0.20	1.474		
	0.25	21.645		
	0.30	6.211		
	0.50	6.157		
	1.0	3.681		

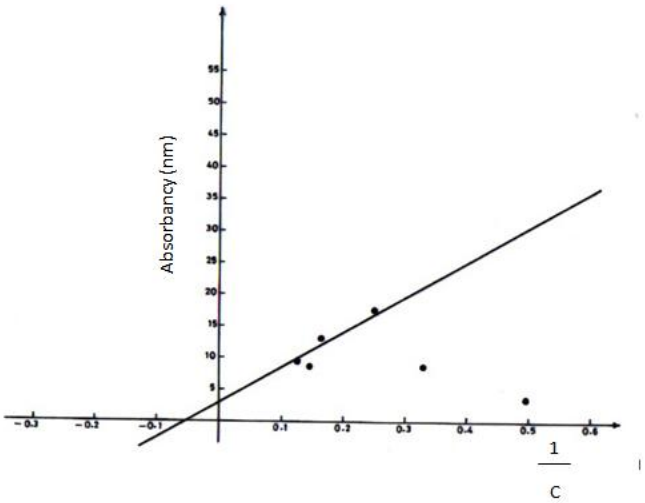

Figure 1. Peroxidase activity plot for the genotype Bauda

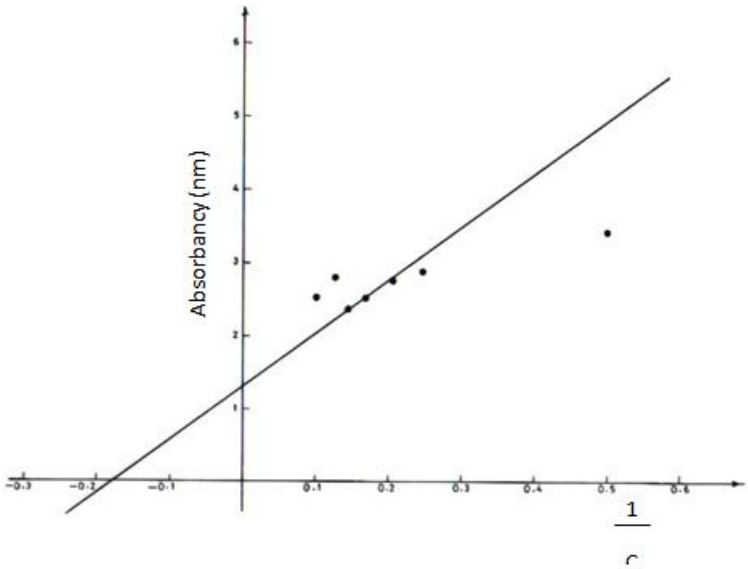

Figure 2. Peroxidase activity plot for the genotype Darmasa

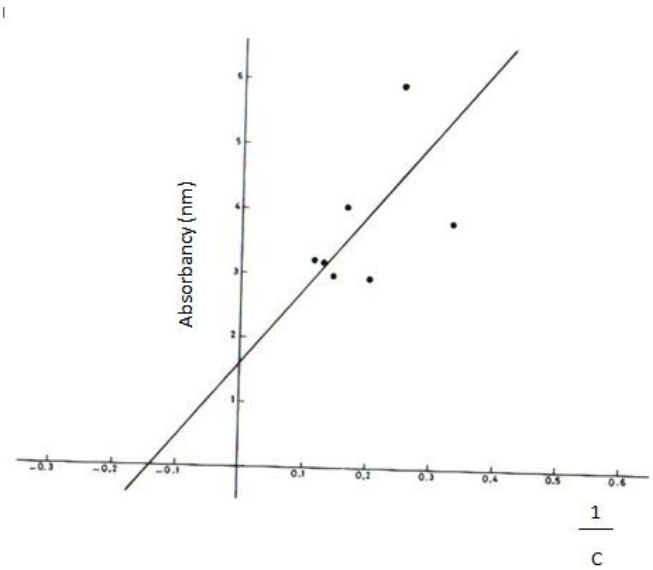

Figure 3. Peroxidase activity plot for the genotype Ugandi

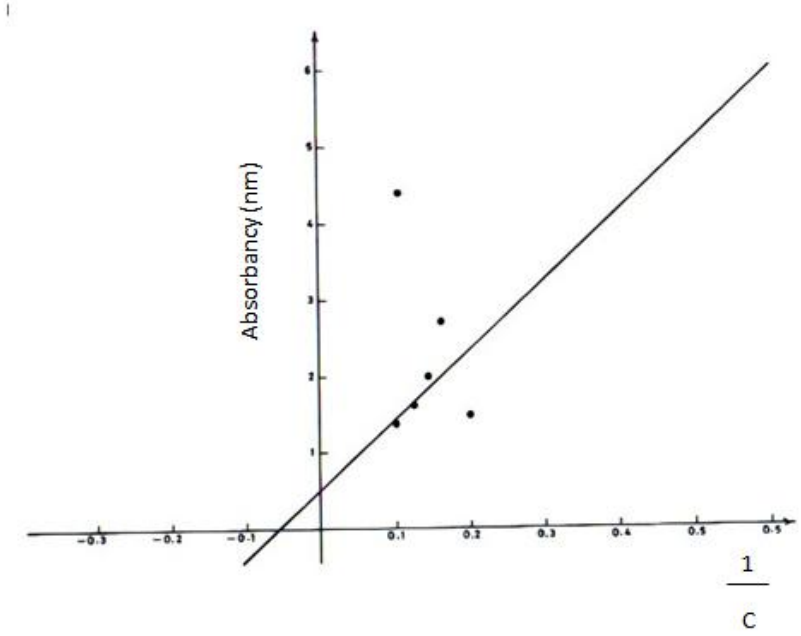

Figure 4. Peroxidase activity plot for the genotype Madlkawia

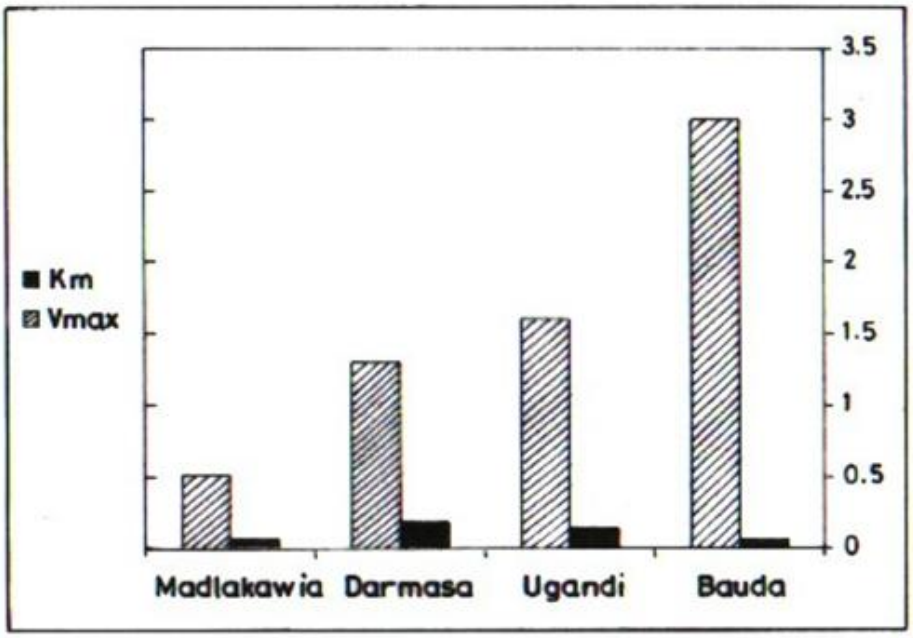

Figure 5. A histogram of spectroscopy results of the four millet genotypes

Every species has revealed considerable genetic variations or polymorphism, either within populations or between populations or both (Muehlbauer*et al*., 1990).Isozymes have been valuable tool in identifying the parents of polyploidy plants. Isozymes have supported hypotheses based on other line of evidence and differentiated among alternative hypotheses.Allozymes have shown that some polyploids have a single origin and that some are autopolyploids. Diversification and speciation in polyploidy have occurred through gene silencing, however, if gene silencing regularly leads to diploidization of entire polyploidy genomes, the value of isozymes for assessing polidy especially in phylogentically ancient groups must be questioned.The conflicts between molecular and morphological evidence have been over-emphasized. The development of molecular systematics has not resulted in wide spread refutation of phylogenetic hypotheses generated by morphologists, although the molecular approach is potentially powerful for generating and testing these hypotheses (Hillis and Mortiz, 1990).

References

[1]. Buth, D. G. (1984).The application of electrophoretic data in systematic studies.Annu. Rev. Ecol. Syst., 15: 501-522.
[2]. Chahal, S. S.; Kumar, R.; Sidhu, J. S. and Minocha, J. L. (1986). Peroxidase isozyme pattern in pearl millet lines resistant and susceptible to ergot. Indian Phytophathol.,39(11): 65-69.
[3]. Gadia, M. (1977).Peroxidase isozymes patterns during germination of four varieties of pea seeds, M. Sc. Thesis, University of III Inios.
[4]. Hillis, M. D. and Mortiz, C. (1990).Molecular Systematics.Sinarruer – Associates, Inc. Publishers.Sunderland, Massachusetts, U. S. A.
[5]. Kimura, M. and Ohta (1971).Protein polymorphism as a phase of molecular evolution. Nature, 229: 467-469.
[6]. Mohamed, E. B. (1983). A Study of Peroxidase of Potato Proctor.Ph.D. Thesis, Department of Food Science, University of Leeds.
[7]. Muehlbaur, F. J.; Simon, C. J.; Spaeth, S.C. and Habdad, N. I. (1990). Genetic improvement of Chick pea: key factors to be considered for a breed through productivity. In the chick pea in the Nineties: proceedings of the second international workshop on chick pea improvement. 4-8 Dec. 1989, Icrisat Centre India.
[8]. Salih, M. A. M. (1997). Cytogenetic Studies in Two *Pennisetum* spp. A Thesis submitted for the degree of Master of Science (Botany) in the Dep. of Botany, Faculty of Science, U. of Khartoum.
[9]. Salih, M. A. M.; El-Tigani, S. and Abdalla, A. H. (2000).Isozyme Patterns of peroxidase and α-amylase in *Pennisetum glaucum* and *P. violoceum*. U. of K. J. of Agric. Sci., 9(2).
[10]. Sidhu, J.; Ravi, S. and Minocha, J. L. (1984). Peroxidase patterns in primary trisomics of pearl millet. Theo. App. Genet., 68: 179-182.

Manal A. M. Salih, et. al. "Variability in the Activity of the Isozyme Peroxidase in different Pennisetum glaucum L. genotypes." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(3), (2020): pp. 31-35.