Journal of Medicinally Active Plants

Volume 4
Issue 3 *Vol 4 Issues 3-4*

January 2015

Biochemical Markers and Seedling Characteristics Identify Lupine (Lupinus termis L.) Genotypes

Recommended Citation

Eldanasoury, Mohamed; Rehab Tawdy Behairy; Azzam A. Ashrie; and Lyle Craker. 2015. "Biochemical Markers and Seedling Characteristics Identify Lupine (Lupinus termis L.) Genotypes." *Journal of Medicinally Active Plants* 4, (3):30-36.
DOI: https://doi.org/10.7275/R58P5XFT
https://scholarworks.umass.edu/jmap/vol4/iss3/3

Journal of Medicinally Active Plants

Volume 4
Issue 3 *Vol 4 Issues 3-4*

December 2015

Biochemical Markers and Seedling Characteristics Identify Lupine (Lupinus termis L.) Genotypes

Mohamed Eldanasoury
Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, mdanasory@yahoo.com

Rehab Tawdy Behairy
Seed technology Research Department, Field Crop Res., Institute, Agricul. Res. Cen., Giza, Egypt

Azzam A. Ashrie
Food Legumes Research Department, Field Crop Research, Institute, Agricul. Res. Cen., Giza, Egypt

Lyle Craker
Medicinal Plant Program, University of Massachusetts, Amherst, MA, U.S.A

Recommended Citation
Eldanasoury, Mohamed, Rehab Tawdy Behairy, Azzam A. Ashrie, Lyle Craker. 2015. "Biochemical Markers and Seedling Characteristics Identify Lupine (Lupinus termis L.) Genotypes," *Journal of Medicinally Active Plants* 4(Vol 4 Issues 3-4):30-36.
DOI: https://doi.org/10.7275/R58P5XFT
Available at: http://scholarworks.umass.edu/jmap/vol4/iss3/3

Biochemical Markers and Seedling Characteristics Identify Lupine (*Lupinus termis* L.) Genotypes

Mohamed Eldanasoury[1], Rehab Tawdy Behairy[2], Azzam A. Ashrie[3] and Lyle Craker[4]

[1]Biochemistry Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt
[2]Seed technology Research Department, Field Crop Res., Institute, Agricul. Res. Cen., Giza, Egypt
[3]Food Legumes Research Department, Field Crop Research, Institute, Agricul. Res. Cen., Giza, Egypt
[4]Medicinal Plant Program, University of Massachusetts, Amherst, MA, U.S.A.

***Corresponding author:** mdanasory@yahoo.com

Date received: December 23, 2014.

Keywords: Genotype, isozymes, lupin, lupine, *Lupinus*, protein

ABSTRACT

To distinguish among five lupine (*Lupinus termis* L.) genotypes, biochemical markers and seedling characteristics were studied, using electrophoresis of seed and leaf proteins and four isozyme systems[esterase (EST), catalase (CAT), peroxides (Prx), and glutamate oxaloacetate transaminase (GOT)]. A total of 21 and 13 polymorphic bands were detected in the seed and leaves, respectively. Molecular weights ranged from 183.82 to 11.14 kDa for the seeds and 148.52 to 8.17 for the leaves. Among the genotypes, seed storage protein bands ranged from 10 in genotype Giza-1 to 13 in genotype Giza-3, while the total number of leaf protein bands ranged from six in genotype Giza-2 to nine in genotype Giza-1. Specific, characteristic bands could be used to identify and differentiate some genotypes from among others. At the isozyme level, a similar number of bands were produced, but the location and Rf values of the bands differed, enabling identification among the lupine genotypes

INTRODUCTION

White lupine (*Lupinus termis* L.), a crop plant grown as a traditional human food and animal feed since ancient times, is extensively cultivated in Egypt and other Mediterranean countries as a component of sustainable farming systems (Musquizet al., 1993; Hefny, 2013). The plant is a source of protein (33-47%) and contains a high concentration of polyunsaturated fatty acids, but is relatively low in starch, lysine, and sulfur containing amino acids. Oil content ranges from 6-13%, and alkaloid content can exceed two percent.

While a number of researchers have used seed characteristics, seedling morphology, and chemical tests for varietal identification, these characteristics have not been wholly reliable. Indeed, the continuing increase in varieties of several crops has made reliance on plant appearance to distinguish among plant varieties quite difficult. Over the past several years, electrophoresis, a relatively sophisticated and reproducible technique, has gained extensive use for varietal identification in other crop species, replacing morphological characteristics (Cooke, 1987, 1993; Naguib et al., 2011; Vanangamudi et al., 1988; Varier, 1993; Vishwanath et al., 2011).

Differentiating among lupine genotypes using biochemical markers and seedling characteristics will enable the use of plant breeding and seedling selection to improve lupine yields and constituency. The aim of the present investigation was to differentiate between five lupine genotypes, using seedling growth characteristics and protein and isozyme constituents.

MATERIALS AND METHODS

Plant material. Seeds of five lupine *(Lupinus termis* L.) genotypes (Family-9, Mutation–33, Giza-1, Giza-2, and Giza-3) obtained from the Leguminous

Crops Department of Research (LCDR), at the Field Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt were used in this study.

Seedling characteristics. To determine the germination percentage and seedling characteristics of the lupines, 25 randomly selected seeds of each of each genotype were tested as recommended by ISTA (1999). In preparation for the germination tests, all seeds were surface sterilized by immersion in 0.5% sodium hypochlorite (NaOCl) solution for 5 min to prevent fungal infections and then rinsed three times with sterile water to remove any residual NaOCl.

The sterilized seeds were then scattered on the upper surface of two sheets of sterile Whatman No. 1 filter paper that had been premoistened with 10 mL of sterile, distilled water and placed in separate, sterile Petri plates (150 mm in diameter x 15 mm deep). The plates containing the seeds were placed in a controlled environment chamber (Conviron Model EF7) containing a mixture of fluorescent and incandescent light at 20 ± 2ºC for germination under an 18 h light-6 h dark cycle (PAR =135 µmol m^2s-^{1} and a R-FR ratio = 1.92). Seed germination was observed daily with water added to each Petri plate as necessary to maintain moisture levels.

Seedling development was measured at 21 days after transfer into the Petri plates by monitoring seed germination (ISTA, 1999), by measuring seedling stem and root lengths, and determining seedling fresh and dry weights of ten randomly selected seedlings. Seedling vigor index following the procedure (seedling length in cm x germination percentage) outlined by ISTA (1999). Seedling dry weights were determined after drying the plant seedlings to a constant weight in a hot air oven at 85ºC (12 h) (Krishnasamy and Seshu, 1990).

Seed storage and leaf proteins. Protein extracts from seeds and leaves of the various genotypes were characterized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), following the procedure of Laemmli (1970) as modified by Studier (1973). The seed and leaf from each genotype were ground with a mortar and pestle and a 0.02 g sample of each genotype was dried, defatted with hexane, and completely mixed in 0.2 mL of sample buffer before storing overnight at 4ºC.A 400 µL sample of the extract (equivalent to about a 0.2 absorbance value per 10 µL) was thoroughly mixed and heated in a boiling water bath for 5 to 10 minutes, and then centrifuged at an RCF of 7,000 g for five minutes. The supernatant was transferred to clean, cold sample tubes and maintained at 4ºC until analysis.

The pre-prepared separating gel (Table 1) was poured into the space (2 mm) between two glass plates held in casting frames to a height of 12 cm (1.5 cm below the comb bottom) and then overlaid with isopropanol to insure the top of the separating gel was horizontal. After the separating gel had solidified, the isopropanol was removed and the stacking gel was added at the top of the separating gel. The well forming comb was inserted into the stacking gel for a total of 15 min to insure complete polymerization of the stacking gel before removal of the comb.

Table 1. Composition of the separating and stacking gels.

Stock solution	Separating gel (10 %)	Stacking gel (5.6 %)
Monomer solution	10 mL	1.67 mL
Resolving buffer (pH 8.8)	3.75 mL	---------
Stacking buffer (pH 6.8)	---------	2.5 mL
Distilled water	14 mL	5.19 mL
SDS (10 % w/v)	300 µL	100 µL
APS (10 % w/v)	1.5 mL	500 µL
TEMED[1]	300 µL	10 µL
Final volume	30 mL	10 mL

[1]N,N,N,N-tetramethylethylenediamine

After the comb was removed, a 20 to 30 µL sample of each prepared genotype extract was carefully added to a separate comb well to avoid any air bubbles and provide sharp separation of protein bands. The upper and lower buffer tanks were filled with the running buffer and attached each other so that the gel was completely covered with the buffer. Bromophenol blue in lane one was used to mark the protein separation front.

The proteins were separated by attaching the negative electrode to the bottom tank, the positive electrode to the top tank, and then applying 100 volts until the dye entered the resolving gel. The voltage was then increased to 250 volts until the dye front reached the bottom of the resolving gel and electro-

phoresis was stopped by disconnecting the electrodes. To ensure the orientation of the gel was not lost during staining and handling, a small triangle was cut at one corner of the gel.

After completion the of the protein separation, each gel was placed overnight in a separate resalable, clear plastic bag containing staining solution. The stained gels were then transferred into individual reclosable plastic bags containing a destaining solution and gently agitated on a shaker. The destaining solution was changed several times until the gel background was clear except for the protein bands.

The Rf values of the stained bands were calculated and along with the approximate molecular weights were used to determine the position of the protein bands. The gels were subsequently scanned densitometrically using a color flatbed scanner (Epson GT 8000, Epson, Japan) connected to a computer and printer using peak scanner2 software that was downloaded from the WEB. The estimation of molecular weights of different protein bands was automatically calculated by comparison to a protein marker.

Isozymes electrophoresis. Native polyacrl-amide gel electrophoretic techniques were used to identify the isozyme fingerprint of lupine genotypes esterase (EST), peroxidase (Prx), catalase (CAT), and glutamate oxaloacetate transaminase (GOT).Isozyme fractionation was done on a vertical slab (19.8cm x26.8 cm x 0.02cm) using a Labconco gel electrophoresis apparatus, following the procedure outlined by Jonathan and Wendel (1990).

A total of five seedlings from each lupine genotype were extracted with 1 mL extraction buffer (pH 7.5) (1:3 w/v). Each sample was vortexed for 15 sec and centrifuged for 10 min at a g-force (RCF) of 8,600 at 5^{o}C to remove any tissue remains. The supernatant was transferred to a new Eppendorf tube and kept at -18^{o}C until used in the electrophoretic analysis. The standard polyacrylamide8% gel (pH8.6) was made using 25 mL of a30% acrylamide-bisacrylamide solution, 75 mL of gel buffer, 30 mg sodium sulfate, 4 mL of ammonium persulfate, and 100 µL of TEMED. The gel was poured on the plate and 10 well combs were placed immediately. The gel polymerization took place in approximately 30min.

Extract (50 µL) from each sample was mixed with 10 µL of bromophenol blue and added to each comb well. The gel was then completely covered with electrode buffer and electrodes were connected to a power supply and adjusted at 200 volts for 2h.Upon completion of the electrophoretic separation, the appropriate substrate and staining solution was added to each gel and the gels were incubated at 37°C in the dark until the bands appeared.

Table 2. Isozyme staining solutions.

Enzymes	Compounds	Amount
Esterase	Sodium phosphate (100 mM) pH 6	50 mg
	α-Naphthyl acetate	25 mg
	Fast blue RR salt	50 mg
Catalase	α-Na-thiosulfate 60 mM	30 mL
	H_2O_2 3%	30 mL
	β-KI 90 mM	100 mL
	Acetic acid	0.5 mL
Peroxidase	α-sodium acetate (1M, pH4.7)	50 mL
	Acetic acid	20 mL
	3,3,5,5 tetramethyl benzidine (TMBZ)	125 mg
	B-0.30 % H_2O_2	2 mL
Glutamate oxaloacetate transaminase	α-Tris (1M,pH 8.5)	50 mL
	α-Ketoglutaric acid	50 mg
	Aspartic	100 mg
	β-Pyrodxal-5- phosphate	5 mg
	Fast blue BB salt	150 mg

After the enzyme bands appeared, the reaction was stopped by washing each gel two or three times with tap water followed by submerging the gel in a fixative solution consisting of 9 parts ethanol and 11 parts of 20% glacial acetic acid. Each gel was kept in the fixative solution for 24h and upon removal rinsed two times with tap water, photographed, and scanned using a Gel Doc-2001 gel documentation system (Bio-Rad Laboratories, Inc., Hercules, CA) to determine the density of each band.

The densitometry scanning of the bands was focused on the length, width, and intensity of each band to ensure full recognition of the isozymes. Relative amounts were quantified and scored. The Rf values and approximate molecular weights were used to determine the position of the protein bands for identification of cultivars (Vishwanath et al., 2011).

RESULTS

Seedling characteristics. Differences in seed germination, shoot and radicle length, fresh and dry weights, and seedling vigor were observed among the five tested lupine genotypes (Table 3). Seed germination ranged from a low of 89% to a high of 98% in the genotypes Family-9 and Giza-1, respectively. In contrast, while genotype Family-9 had the highest seedling fresh weight among the lupine genotypes, this selection also had the lowest seedling vigor index.

Table 3. Germination and seedling characteristics of lupine genotypes.

Genotypes	Germination (%)	Radicle length (cm)	Shoot length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Seedling vigor index
Family-9	89	11.33	13.00	34.12	3.50	2163
Mutation-33	92	11.43	12.16	27.52	2.82	2170
Giza-1	98	12.00	11.25	26.50	3.00	2278
Giza-2	90	13.40	11.30	23.75	2.77	2223
Giza-3	95	14.25	13.50	28.00	3.08	2663

Seed storage proteins. Electrophoresis with SDS-PAGE revealed a total of 21 polymorphic bands with molecular weights ranging from 183.82 to 11.14 kDa in the seed storage proteins (Table 4). Distinct differences in SDS protein banding patterns were observed among the genotypes with 10 bands in genotype Giza-1 and 13 bands in genotype Giza-3. Some genotypes contained specific bands that could be used to identify and characterize specific genotypes. Family-9 genotype, for example, contained protein bands with molecular weights of 136.92, 50.72, and 27.13kDa. Genotype Giza-3 produced three specific protein bands with molecular weights of 183.82, 41.65, and 26.97 kDa.

The protein band (MW = 179.15) was present in all genotypes. Bands with a MW of about 21.33 kDa were present in all genotypes except for genotype Giza-1. The absence of a band common to all the other genotypes could be considered a negative marker.

Table 4.Molecular weights and the presence or absence of genotypes for seed storage proteins in lupines.

Bands	Mol. Wt. (kDa)	Fam.-9	Mut.-33	G - 1	G- 2	G - 3
1	183.82	-	-	-	-	+
2	179.15	+	+	+	+	+
3	165.34	-	-	-	+	-
4	151.80	+	+	-	+	-
5	140.24	-	-	+	+	+
6	138.35	-	-	+	+	+
7	136.92	+	-	-	-	-
8	85.90	+	+	+	-	+
9	81.34	-	+	+	+	+
10	74.22	+	+	+	+	-
11	53.88	-	-	+	+	+
12	50.72	+	-	-	-	-
13	41.65	-	-	-	-	+
14	31.65	+	+	+	+	-
15	27.13	+	-	-	-	-
16	26.97	-	-	-	-	+
17	25.10	+	-	+	+	+
18	23.80	+	+	+	-	+
19	21.33	+	+	-	+	+
20	17.66	+	+	-	-	+
21	11.14	-	+	-	-	-

[1]Fam.-9 = Family-9; Mut.-33 = Mutation-33.
[2](+) =band present; (-); band absent.

Leaf proteins. A clear variation among the lupine genotypes for production of the leaf proteins was visible. Separation of the leaf proteins by electrophoresis using SDS-PAGE produced 13 bands with molecular weights ranged from 148.52 to 8.17 kDa (Table 5). Within the 13 bands, four were common (MW = 133.17, 131.03, 118.64 and 78.36 kDa) and observed in all the tested genotypes (Table 5).

Specific protein bands were associated with various genotypes. For example, genotype Giza.-1 contained a protein band with a molecular weight of 92.13 kDa, while the genotype Family-9 was characterized by a protein band with a molecular weight of 28.40 kDa. The protein bands with molecular weights of 77.57 and 16.12 kDa were present in all the genotypes except Giza-1 and Giza-3.Genotypes Mutation-33 and Giza-1 had the most bands with nine each. Genotype Giza-2 only had six bands, the least number of bands.

Table 5. Molecular weights and the presence or absence of genotypes for leaf proteins in lupines.

Band number	MW (kDa)	Fam.-9	Mut.-33	Giza-1	Giza-2	Giza-3
1	148.52	-	+	-	-	-
2	141.56	-	-	+	-	+
3	133.17	+	+	+	+	+
4	131.03	+	+	+	+	+
5	118.64	+	+	+	+	+
6	116.52	-	+	+	-	-
7	92.13	-	-	+	-	-
8	78.36	+	+	+	+	+
9	77.57	+	+	-	+	+
10	28.40	+	-	-	-	-
11	16.12	+	+	+	+	-
12	10.82	-	-	-	-	+
13	8.17	+	+	+	-	-

Genotypes: Fam.-9 = Family-9; Mut.-33 = Mutation-33;
+ = band present; - = band absent.

Isozyme electrophoresis. Esterase (EST), catalase (CAT), peroxidase (PRX), and glutamate oxaloacetate transaminase (GOT) extracted from the leaf tissue of the five studied lupines were analyzed and used for identification and characterization of the genotypes through polyacrylamide gel electrophoresis (PAGE) profiles.

Esterase bands differed according to the lupine genotype (Table 6). Characterization the genotypes was possible according to the number of esterase band patterns. The genotypes Family-9 and Giza-3 contained only seven bands each to distinguish between these two genotypes, depending on the R_f values.

Table 6. R_f of esterase isozyme bands in lupine genotypes

Genotypes	EST-1	EST-2	EST-3	EST-4	EST-5	EST-6	EST-7
Family-9	0.061	0.104	0.174	0.240	0.335	0.569	0.885
Mutation-33	0.061	0.104	0.175	0.243	0.335	0.571	---
Giza-1	0.177	0.243	0.338	0.558	0.608	---	---
Giza-2	0.080	0.156	0.291	0.558	0.608	---	---
Giza-3	0.075	0.138	0.216	0.287	0.566	0.617	0.874

The Mutation-33 genotype contained six bands of which the bands withR_f= 0.061, 0.104 and 0.335 were identical to those of EST-1, EST-2, and EST-5 in genotype Family-9. Except for EST-5, The esterase bandsof the Giza genotypes differed from each other in R_f values and from the genotypes Family-9 and Mutation-33 in number of bands, except for Giza-3 that had the same number of bands as Family-9.

Catalase isozyme bands were present in all five genotypes, but the number of bands differed (Table 7). Giza-1 had only three catalase isozyme bands in the five lupine selections that analyzed. The genotypes Family-9 and Mutation-33 had four catalase isozyme bands as compared with the five bands present in the Giza-2 and Gisa-3 banding pattern. The genotypes Family-9, Mutation-33, and Giza-2 had the same R_f values of 0.143 and 0.837.

Table7.R_f of catalase isozyme bands in lupine genotypes.

Genotype	CAT-1	CAT-2	CAT-3	CAT-4	CAT-5
Family-9	0.007	0.143	0.430	0.837	---
Mutation-33	0.110	0.143	0.344	0.837	---
Giza-1	0.113	0.335	0.837	---	---
Giza-2	0.107	0.143	0.300	0.355	0.837
Giza-3	0.075	0.138	0.216	0.287	0.566

For the peroxidase isozyme, the maximum number of genotype bands was three. The genotype Giza-2 only had two bands. Matching bands were identified in PRX-1 for the genotypes Family-9 and Giza-1 with an R_f of 0.091, in PRX-2 for genotypes Mutation-33 and Giza-2 with an R_f of 0.39, and in PRX-3 for genotypes Family-9 and Mutation-33 with an R_f of 0.455 and for genotypes Giza-1 and Giza-3 with an R_f of 0.436.

Table8.R_f of peroxidase isozyme bands in lupine genotypes.

Genotypes	PRX - 1	PRX - 2	PRX - 3
Family-9	0.091	0.417	0.455
Mutation-33	0.098	0.397	0.455
Giza-1	0.091	0.412	0.436
Giza-2	0.077	0.397	---
Giza-3	0.086	0.379	0.436

The glutamate oxaloacetate transaminase isozyme bandsin all five genotypes had very similar R_f values (Table 9). The isozymes for Famly-9, Mutatuion-33, Giza-1, and Giza-3 had the same band R_f value of 0.334. For the isozyme GOT-2, the R_f values for Mutation-33 and Giza-1 were the same and the R_f value of 0.334.Genotypes Mutation-33 and Giza-1hadthe same R_f values for all three isozymes and the genotypesFamily-9Giza-1, Giza-2, and Giza-3 had the R_f value of 0.417.

Table9.R_f of glutamate oxaloacetate transaminase isozyme bands in lupine genotypes.

Genotype	GOT-1	GOT-2	GOT-3
Family-9	0.334	0.387	0.417
Mutation-33	0.334	0.378	0.427
Giza-1	0.334	0.378	0.417
Giza-2	0.350	0.392	0.417
Giza-3	0.334	0.392	0.417

DISCUSSION

Desirable seed germination characteristics are those similar to the qualities of other crops. Rapid germination, vigorous seedling growth, and the ability to withstand environmental and pest stress are important characteristics desirable in all crops seeds. In addition to seed germination and growth, however, the desirable lupine plant must be adaptable to growing and producing a seed crop in marginal soils and climates (Sánchez et al., 2005).

A comparison of lupine seedling germination and development within the five genotypes tested in this study demonstrated differences among the collection of seeds. These differences in seed germination and vigorous growth suggest some significant differences within the genotypes that could be used in plant breeding to improve the cultivation of lupine production under adverse environmental conditions.

Lupine seeds, which are relatively high in proteins, lipids and fiber content, make lupine a historical and current valuable food and feed crop, especially in the Mediterranean area (Gladstone, 1974). Thus, genotype selection and plant breeding could be expected to improve plant development and nutritional value.

Biochemical markers can be considered a good tool for identification and genetic evaluation of the conserved material. These biochemical markers can be achieved and identified by protein banding patterns or isozyme polymorphism. Therefore, biochemical genetic fingerprinting can satisfy both adequacy and accuracy for the identification of the conserved material. Furthermore, electrophoresis polyacrylamide gel continues to play a major role in the experimental analysis of protein.

Polyacrylamide gel electrophoresis (PAGE) is still the most widespread from of the technique, since this procedure offers sufficient resolution for most situations and is coupled with simple use and the ability to process many samples simultaneously for comparative purposes (Hames, 1990). Protein banding patterns can be efficiently used to identify and separate genotypes with desirable traits.

REFERENCES

Cooke, R.J. 1987. The classification of wheat cultivars using a standard reference electrophoresis method. Journal of National Institute of Agricultural Botany 17:273–281.

Cooke, R.J. 1993. Variety identification by electrophoresis. Seed Res. 1:369–371.

Gladstone, J.S. 1974. Lupins of the Mediterranean region and Africa. Tech. Bulletin No. 26. Western Australian Depart. of Agric., Western Australia.

Hames, B. D. 1990. One dimensional polyacrylamide gel electrophoresis in gel electrophoresis of protein .Oxford University Press.

Hefny, M.M. 2013. Use of genetic variability estimates and interrelationships of agronomic and biochemical characters for selection of lupin genotypes under different irrigation regimes. African Crop Science Journal 21(1):97 – 108.

ISTA Rules. 1999. International rules for seed testing. Seed Science & Technol. Proc. Int. Seed Test. Assoc. 31(1):1-152.

Jonathan, F.W. and N.F. Wendel. 1990. Visualization an interpretation of plant isozymes. *In*: D.E. Soltis and P. S. Solits, ed. *Isozymes in Plant Biology*. Chapman and Hall London. pp. 5-45.

Krishnasamy, V. and D.V. Seshu. 1990. Phosphine fumigation influence on rice seed germination and vigor. Crop Sci. 30:28- 85.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227: 680-685.

Musquiz, M., C.L. Ridout, K.R. Price, and G.R. Fenwick. 1993. The saponin content and composition of sweet and bitter lupin seed. Journ. of Sci. of Food and Agric. 63: 47-52.

Naguib, A. Neamat, M. Aziza, Hassanein, and R. T.

Behairy. 2011. Description of Five Rice Genotypes Based on Seed, Seedling Characters, Chemical Tests and Biochemical Markers. Egypt. J. Plant Breed. 15 (1): 13-25.

Sánchez, M.C., P. Altares, M.M. Pedrosa, C. Burbano, C. Cuadrado, C. Goyoapa, M. Muzquiz, C. Jiménez-Martínez, G. Dávila-Ortiz. 2005. Alkaloid variation during germination in different lapin species. Food Chem. 90(3):347-355.

Studier, F.W., 1973. Analysis of bacteriophage T1 early RNAs and proteins of slab gels. J. Mol. Biol., 79: 237-248.

Vanangamudi, K., V. Palaniswamy, and S. Natesan. 1988. Variety identification in rice: Phenol and KOH tests. Seed Sci. and Technol. 16: 465–470.

Varier, A. 1993. Identification of varieties of pearl millet and sunflower using electrophoresis methods. In: Plant Breeder's rights, seed certification and storage. Proceedings of Indo British Workshop, 20-22 February ,1992 , IARS, New Delhi, pp. 131-139**.**

Vishwanath K., K.P.R. Prasanna, H.M. Pallvi, Rajendra Prasad S., Ramegowda, P.J. Devaraju, and T.V. Ananthanarayanan. 2011. Identification of tomato (*Lycopersicon esculentum*) varieties through total soluble seed proteins. Res. J. Agric. Sci. 2:08-12.