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Agronomic Characterization vs. DNA Marker-Based Genetic Similarity of White Lupine (*Lupinus albus* L.) Egyptian Landraces

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ABSTRACT

Knowledge of genetic diversity and relationships among breeding materials are essential to crop improvement. The objective of this study was to determine the association between agronomic characterization and randomly amplified polymorphic DNA-(RAPD) based genetic similarity (GS) estimates for a set of 40 white lupine landraces collected from different Egyptian regions in 1994. The landraces have been conserved properly at the Plant Genetic Resources Research Department, Field Crops Research Institute (FCRI), ARC. Ten agronomic traits were observed throughout the two growing seasons, 2004/2005 and 2005/2006. The dendrogram based on genetic similarity of the agronomic characterization among accessions showed that the 40 landraces formed seven main clusters largely corresponding to the region of collection at level of similarity of 95 % plus five landraces, which were distributed as ungrouped. Meanwhile, RAPD separated the landraces into nine genetically diverted main groups at a level of similarity of 85 % plus four landraces, which were distributed as ungrouped. This investigation supported the use of DNA markers as an accurate approach of measuring genetic similarity. However, the moderate association between both procedures suggested that the agronomic characterization will continue to be useful to inexpensively identify diverse germplasm in breeding programs of lupine.

Keywords: ***Lupine, Agronomic characterization, DNA-RAPD, Cluster grouping, Genetic similarity.***

INTRODUCTION

Leguminous crops, including lupine, are an important source of protein and other nutritious substances. Cultivation of *Lupinus albus* has instituted during Greek and Roman period, and may have been experienced in Egypt as early 2000 B.C. (Gladstones 1970). Lupine possesses high potential human food and animal feed, as well as various cosmetic and medicinal uses (Berville *et al* 2003). Moreover, it substantially improves soil quality through its efficient nitrogen fixation system (El-Sayad *et al* 2002a). Nevertheless, utilization of lupine as an economical crop in Egypt has been constrained and its production failed to face the increasing demand for food and industry (El-Sayad *et al* 2002b). To gain an advantage over the limits that hinder the sustainable use of lupine, the country would exploit its haven genetic diversity.

Utilization of agro-biodiversity seeks to contribute to the improvement of livelihood through conservation of germplasm as well as sustainable use of improved varieties and accessions. Characterization and

evaluation of the available germplasm would facilitate the study of relationship among genotypes and assist in the identification of unique accessions. This will help in the detection of genetic differences among accessions and in the identification of duplicate accessions (Bretting and Wilrlechner 1995 Mosjidis and Klingler 2006). Moreover, the study of genetic diversity is important in a breeding program for the selection of the suitably diverse parents to obtain heterotic hybrids as well as for the conservation and characterization of germplasm (Asins and Carbonell 1986 & 1989 and Van Becelaere *et al* 2005).

Genetic similarity among genotypes can be estimated by different approaches, which include the use of agronomic characterization and DNA fingerprinting (Helms *et al* 1997; Kim and Ward 1997 and Van Becelaere *et al* 2005). However, the level of association between agronomic characterization and DNA marker-based genetic similarity may vary among different crop species. In corn, a close association was found (Messmer *et al* 1993), but in others such as, wheat, barley, oat and cotton, moderate to low associations somewhat have been observed (Graner *et al* 1994; Kim and Ward 1997; O'Donoughue *et al* 1994 and Van Becelaere *et al* 2005). Therefore, it is necessary to determine within each species whether agronomic characterization and DNA marker-based genetic similarity provide similar information about the genetic distance among available germplasm.

Genetic resources in lupines have been well described (Buirchell and Cowling 1998). Moreover, agronomic characterizations of lupine have been assembled and published (Christiansen *et al* 1999; Christiansen *et al* 2000 and Razza *et al* 2000) and have been utilized in several breeding programs (El-Sayad *et al* 2002 a and b). On the other hand, large-scale DNA marker data were not abundantly available. Nevertheless, some research works which discussed DNA marker-based genetic similarity in lupine have recently been released (Talhinhas *et al* 2003; Vaz *et al* 2004 and Yang *et al* 2001).

Collection missions all over Egypt were extensively manipulated during 1990's to amass diverse lupine germplasm from farmers' fields, where the white lupine (*Lupinus albus* L.) is very ancient and adapted to Egyptian conditions. Variable Egyptian landraces were undertaken as a result of these missions (Christiansen *et al* 1999). This study aimed to screen some of these collections to identify their genetic diversity potential, as well as to determine the association between agronomic characterization and DNA marker-based genetic similarity.

MATERIALS AND METHODS

Plant materials

A collection of 40 white lupine (*Lupinus albus* L.) landraces from ten Governorates representing Northern, Middle and Upper Egypt was characterized in Bahteem Agricultural Research Station during 2004/2005 and 2005/2006 growing seasons. Passport data, including collection number and information provided by the Plant Genetic Resources Research Department (PGRRD), Field Crops Research Institute (FCRI), ARC, Giza, Egypt are presented in Table (1).

Field experiment

The design used was randomized complete block with three replications. Each plot consisted of three ridges, three m long and 60 cm apart. Seeds were sown in hills 20 cm apart with two seeds per hill. Agricultural practices were applied as recommended.

Table 1. Collection information of 40 Egyptian landraces collected from ten Governorates in 1994

No.	Collection No.	Origin	No.	Collection No.	Origin
1	1	Belbies/SH[1]	21	22	Fayed/ISM
2	2	Belbies/SH	22	23	Kantra/ISM
3	3	Belbies/SH	23	24	Kantra/ISM
4	4	Belbies/SH	24	25	Ismailia/ISM
5	5	Belbies/SH	25	26	Abo-Soeir/ISM
6	6	Belbies/SH	26	27	Abo-Soeir/ISM
7	7	Belbies/SH	27	28	Algarb/ISM
8	8	Belbies/SH	28	29	Algarb/ISM
9	9	Abo-hamad/SH	29	30	Meet-
10	10	Abo hamad/SH	30	31	Badrashein/GZ[4]
11	11	Abo hamad/SH	31	32	El-Aiat/GZ
12	12	Fakous/SH	32	34	Beni-Salh/FAY[5]
13	13	Fakous/SH	33	35	Beni Suef/BNS[6]
14	14	Fakous/SH	34	36	Beni Suef/BNS
15	15	Fakous/SH	35	39	Beni Suef/BNS
16	16	Fakous/SH	36	40	El-Minia/MIN[7]
17	18	Ismailia/ISM[2]	37	41	Aswan/ASWN[8]
18	19	Ismailia/ISM	38	42	Aswan/ASWN
19	20	Fayed/ISM	39	43	Sohag/SOH[9]
20	21	Fayed/ISM	40	44	Assiut/ASS[10]

1: Sharkia, 2: Ismailia, 3: Dakhlia, 4: Giza, 5: Fayoum, 6: Beni Suef, 7: El-Minia, 8: Aswan, 9: Sohag, 10: Assiut

Plant characteristics

Data were collected on five randomly taken guarded plants from the middle row of each plot. Data were scored according to International Plant Genetics Resources Institute (IPGRI) and International Union for The Protection of New varieties of Plants (UPOV), lupine descriptor, 1997 Table (2).

Table 2. Score levels for agronomic characteristics of studied landraces

No.	Characteristic	Abbreviation	Score
1	Grain bitter principle (Ruiz 1977)	GBP	1 = absent , 9 = present
2	Plant height three weeks after seedling emergence	PH3-w	3 = short, 5 = medium, 7 = tall
3	Plant height at beginning of flowering	PHBF	3 = short, 5 = medium, 7 = tall
4	Plant height at green ripening	PHGR	1 = very short, 3=short, 5 =short, 7 =medium, 9 = very tall
5	Flower color	FC	1=white, 2= bluish white, 3= blue, 4 =pink, 5 =brimstone-colored, 6=chrome yellow
6	Pod length (at green maturity	PL	3 = short, 5 = medium, 7 = long
7	1000-seed weight	SW	1 = very low, 3 =low, 5 = medium, 7 =high, 9 = very high
8	Time of beginning of flowering	TBF	3 =early, 5 = medium
9	Time of green ripening	TGR	3 =early, 5 = medium, 7 = late
10	Time of ripening	RT	3 =early, 5 = medium, 7 = late

UPOV (1997)

DNA fingerprinting

The forty lupine accessions were subjected to the randomly amplified polymorphic DNA (RAPD) system for assembling genetic distance according to Tao *et al* (1993). This work analysis was done in Field Crops Research Institute (FCRI) lab. Five seeds from each lupine accession were germinated in plastic pots for 2 weeks. DNA isolation from plant tissues was done using DNeasy plant Mini Kit (QIAGEN). PCR reaction was conducted using seven arbitrary 10/mer primers. Their names and sequences are shown in Table (3).

Table 3. List of primer names and their nucleotide sequences used in the study

No.	Name	Sequence
1	OP/A12	5′ TCG GCG ATAG3′
2	OP/A19	5′CAA ACG TCGG3`
3	OP/B07	5′GGT GAC GCAG3`
4	OP/C09	5′CTC ACC GTCC 3`
5	OP/C15	5′GAC GGA TCAG3`
6	OP/L13	5′ACC GCC TGCT 3`
7	OP/Z07	5′CCA GGA GGAC3`

The amplification was carried out in a DNA thermocycler (MWG/BIO TECH Primuse) programmed as follows: one cycle at 940C for 2 min, 40 cycles each of= 940C for 45 sec; 360C for 1 min; 720C for 2 min and one cycle of 720C for10 min, then 40C infinite. Gels were photographed and scanned with Bio/Rad video densitometer Model 620, at a wave length of 577. The similarity matrices were done using Gel works 1D advanced software UVP/England Program

Data Analysis

Diversity values based on phenotype classifications were calculated throughout cluster analysis, which is commonly used when gene bank accessions are evaluated. Classification methods, such as hierarchical cluster analysis (Kaufman and Rousseeuw 1990), were used to investigate patterns of phenotypic diversity exist in germplasm collections and to separate homogeneous subgroups from a heterogeneous population to form core subsets (Brown 1989 and Franco *et al* 2006). Group average hierarchical cluster analysis using GenStat C (version 7) program was used to develop dendrogram subgroups.

A binary matrix reflecting the presence (1) or absence (0) of each RAPD band was generated for each landrace. Genetic distances among landraces as well as similarity matrices were done using Gel works 1 D advanced software UVP-England program. The distance matrix generated was used to obtain a dendrogram by the un-weighted paired-grouping method with arithmetic averages (UPGMA) using also GenStat C (version 7) statistical program.

RESULTS AND DISCUSSIONS

Agronomic characterization

The dendrogram based on genetic similarity of the agronomic characterization among accessions showed that the 40 landraces formed seven main clusters largely corresponding to the region of collection (Tables 4, 5 and Fig. 1), indicating that lupine landraces would provide a representative sample on the phenotypic diversity in entire Egypt (Christiansen *et al* 1999; Christiansen *et al* 2000 and Razza *et al* 2000). Cluster I contained eight accessions all of them were collected from Belbeis/SH. Moreover, the two accessions in cluster II were collected also from SH region, but from Abo-Hamad area. These two clusters originating from SH region seem to be closely related. On the contrary, higher grain bitter (GBM) was dispersed in cluster I and cluster II furnished blue flower color (FC) rather than bluish white (Table 4). Most of studied accessions possessed moderate GBP, but Bellies/SH group was comparatively higher (present) in GBP. The 15 accessions in cluster III were a mixture of

Table 4. Averages of some agronomic characteristics from group average cluster analysis on 40 white lupine landraces collected from different regions of Egypt

Clusters	# of AC*	GBP	PH-3W	PHBF	PHGR	FC	PL	SW	TBF	TGR	RT
I	8	8	7	6	7	2	6	6	6	6	6
II	2	5	6	6	7	3	6	6	6	6	5
III	15	4	5	5	6	2	6	6	4	6	6
IV	2	3	6	6	6	2	7	6	4	5	6
V	2	4	4	4	5	2	5	6	5	6	5
VI	4	4	6	5	7	2	5	6	5	4	4
VII	2	4	6	6	7	2	5	7	4	4	4
Ungrouped											
# 11**		5	5	5	5	3	7	6	4	4	4
# 17		1	3	3	3	1	7	6	4	4	3
# 20		3	5	5	5	2	7	5	5	4	5
# 32		4	4	5	7	2	5	7	4	6	6
# 40		2	6	6	8	2	7	8	5	6	6

* Number of Accessions ** Accession Number (Characteristic Abbreviations as in table 2)

Table 5. Summary of the cluster analysis based on agronomic characteristics of 40 white lupine landraces collected from different regions of Egypt

Group No.	No. of Accessions	District/Governorate and accession number
I	8	Belbies/SH (1, 2, 3, 4, 5, 6, 7 and 8).
II	2	Abo-hamad/SH (9 and 10).
III	15	Fakous/SH (12, 13, 14, 15 and 16), Fayed/ISM (19), Kantra/ISM (22)Abo-Soeir/ISM (25), Algharb/ISM (27 and 28), Meet-Ghamr/DEK (29), Badrashein/GZ (30), El-Aiat/GZ (31), Aswan/ASWN (37 and 38).
IV	2	Beni Suef/BNS (33 and 34).
V	2	Ismailia/ISM (18), Beni Suef/BNS (35)
VI	4	Fayed/ISM (21), Kantra/ISM (23), Ismailia/ISM (24), Abo-Soeir/ISM (26).
VII	2	El-Minia/MIN (36), Sohag/SOH (39).
Ungrouped		Abo-hamad/SH (11), Ismailia/ISM (17), Fayed/ ISM (20), Beni-Salh/FAY5 (32), Assiut/ASS (40).

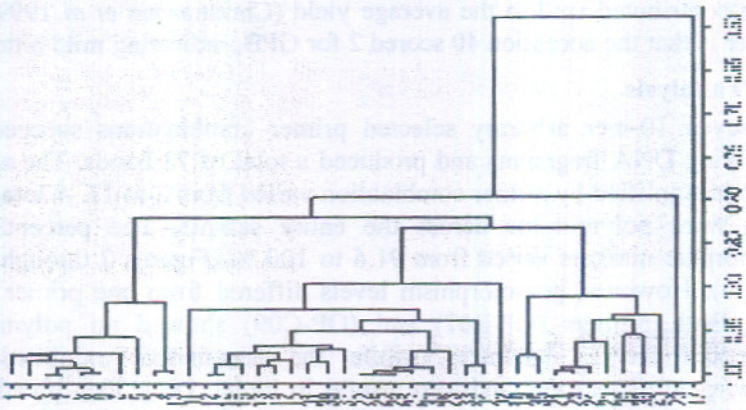


Figure 1: Dendrogram for the genetic distances between the forty lupine landraces based on morphological traits analysis.

landraces collected from Eastern Delta, Middle and Upper Egypt (Table 4). This group (III) possessed an average level for all studied characters, whereas, bluish white flower color (FC) was dispersed and reminder characters contributed moderately score levels Table (4). Cluster IV is considered distinguishable for BNS region containing only two accessions collected from this area. This cluster exhibited somewhat absent GBP (scored 3), long pod length (PL) and relatively early flowering (FT). Cluster V contained distantly far two accessions; numbers 18 and 35 collected from ISM (Eastern Delta) and BNS (Middle Egypt) regions, respectively. These two landraces (18 and 35) had short plant height at all plant growth stages. Moreover, the far distance between the two regions revealed the possibility of moving landraces from one region to another through immigration and/or during the Suez Canal digging. Cluster VI included four accessions was authentic predominantly from ISM region and was reasonably characterized by earliness. Cluster VII plainly roofed two accessions which were collected from Southern Middle (ElMinia) and Northern Upper (Sohag) Egypt, was also characterized with fairly earliness. Moreover, five unique accessions showed no similarity with other cluster groups Table (4). The accession number 11 collected from Abo-Hamad/SH was fairly earlier than other collections from the same area. The accession number 17 from ISM region absolutely exhibited absent GBP and RT and it could be introduced to farmers' fields as sweet lupine from outside Egypt. The accession number 20 was also gathered from ISM, but contributed less GBP and longer PL than ISM group (Cluster VI). The advantage of the ungrouped accessions; number 32 from FAY and number 40 QEN was producing highest SW,

which contributed well to the average yield (Christiansen *et al* 1999). The wonder is that the accession 40 scored 2 for GPB, indicating mild bitter.

RAPD analysis

The seven 10-mer arbitrary selected primer combinations succeeded in amplifying DNA fragments and produced a total of 73 bands. The number of bands amplified by primer combination varied from 2 to 17. A total of 66 bands were polymorphic across the entire sample. The percentage of polymorphic markers varied from 91.6 to 100 % (Figures 2 through 8 and Table 6). However, polymorphism levels differed from one primer to the other. Both primers (OP-B07) and (OP-C09) showed no polymorphic differences among landraces, while the remainders exhibited high polymorphic differences and were useful in lupine DNA identification. In *Lupinus angustifolius*, Yang *et al* (2001) developed DNA fingerprints produced by PCR allowing co-amplification of over 100 DNA fragments. On the other hand, RAPD-PCR analysis requires either cloning or sequencing of DNA so that this can be detected by utilization of the positive and negative specific markers found in the accessions 24 and 35, respectively (Hash and Bramel-Cox 2000).

Cluster analysis (similarity index) based on RAPD-GS analysis using UPGMA computer analysis was described as a dendrogram for the genetic relationships among the 40 lupine landraces Figure (9). The 40 lupine landraces were separated into nine genetically diverted main groups at a level of similarity of 85.0% plus four landraces, which were distributed as ungrouped Table (7). On the basis of this genetic distance, estimates as revealed by DNA markers can be fairly used to assess diversity in germplasm collections (Chaumane *et al* 2004). In general, the nine clusters were also corresponding to regions of collection as in using the agronomic traits Table (7). Increasing the number of cluster groups when using genetic molecular basis than that when using agronomic characterization could largely recommend the use of DNA marker-based genetic similarity to estimate genetic resemblance among germplasm collections rather than using data of agronomic characterization (Abdallah *et al* 2001; Liu *et al* 2000 and Van Becelaere *et al* 2005).

Based on DNA markers, cluster I was also from Belbies/SH containing five accessions rather than eight in the case of using the agronomic data. The three accessions in cluster II were a mixture of Abo-Hamad/SH (accessions; 9 & 10) and Belbies/SH (accession 2), while the two remainder accessions (1 & 3) from Belbies/SH showed no similarity with any cluster group. The third ungrouped accession (11) was also ungrouped in the estimates of genetic distance through the agronomic characterization (Table, 5 & 7). The cluster analysis based on DNA markers

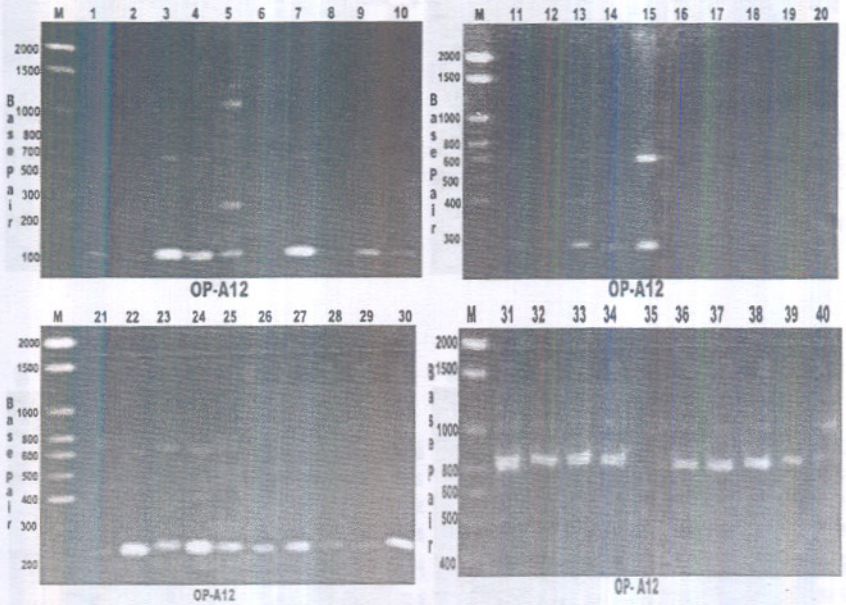


Figure 2: DNA polymorphism of the forty lupine landraces amplified with primer OP-A12

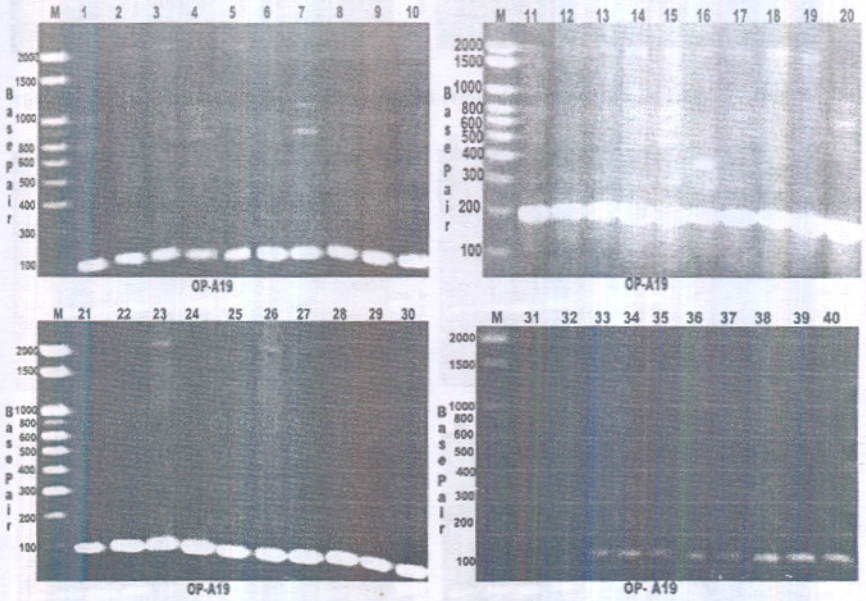
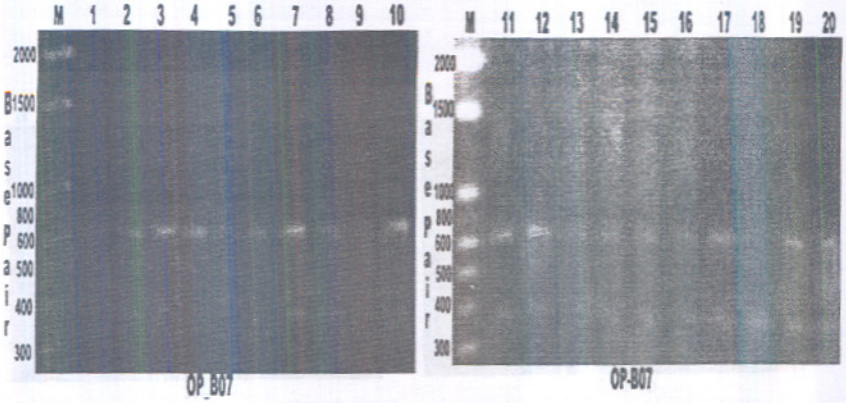


Figure 3: DNA polymorphism of the forty lupine landraces amplified with primer OP-A19.



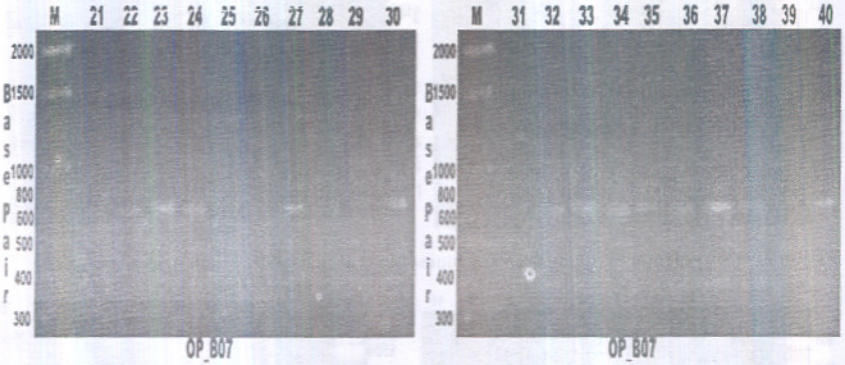
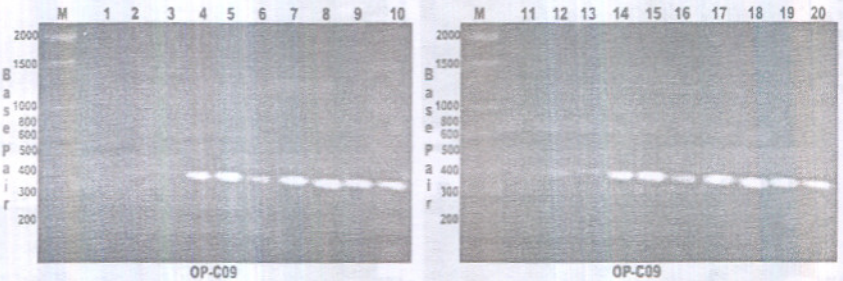


Figure 4: DNA polymorphism of the forty lupine landraces amplified with primer OP-B07



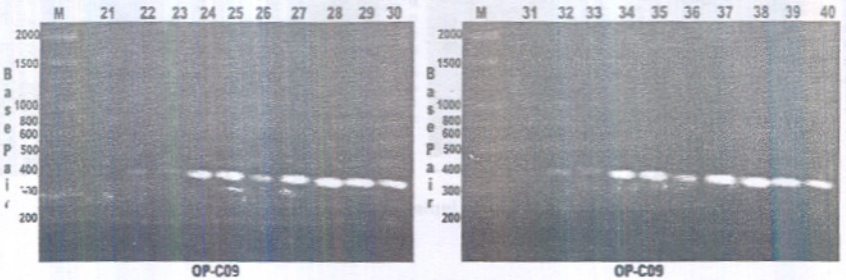


Figure 5: DNA polymorphism of the forty lupine landraces amplified with primer OP-C09.

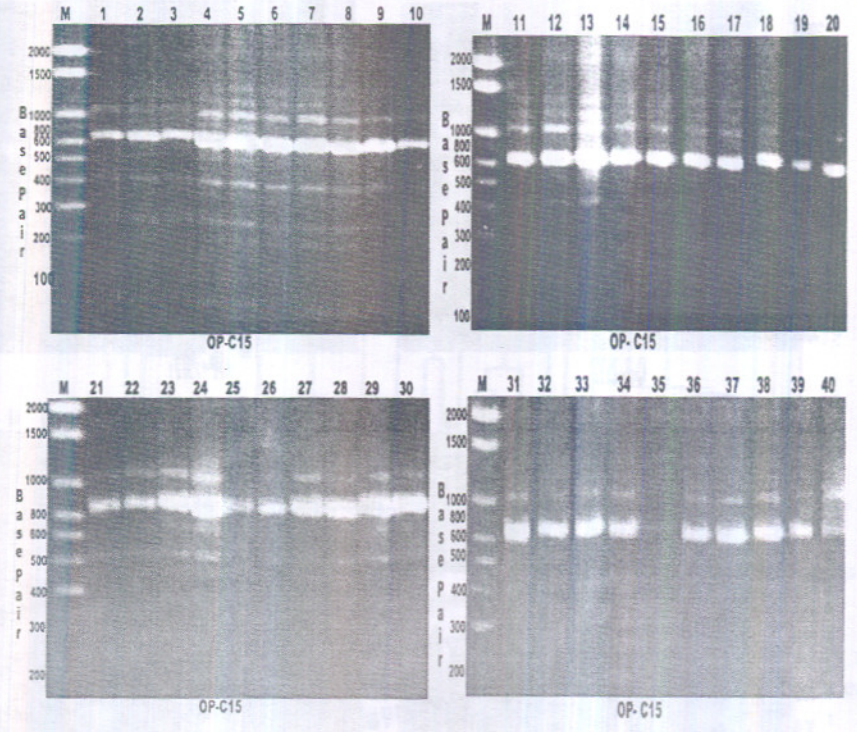


Figure 6: DNA polymorphism of the forty lupine landraces amplified with primer OP-C15.

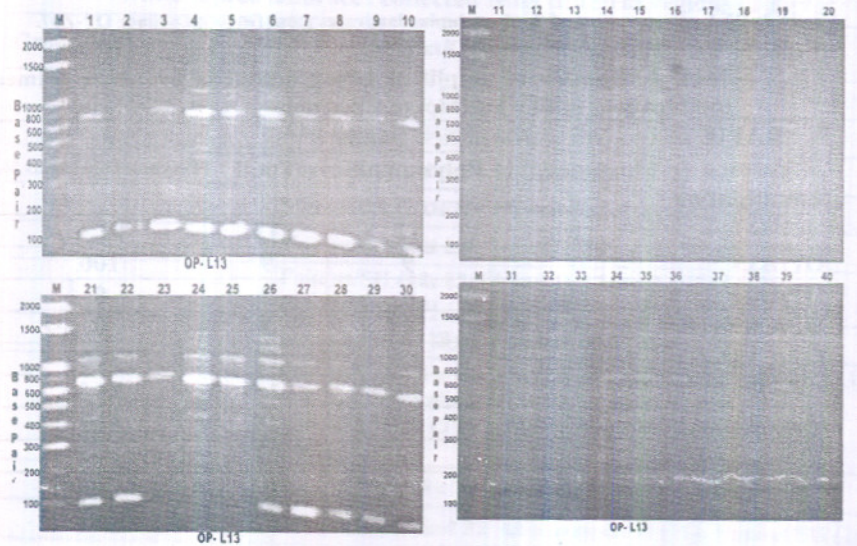


Figure 7: DNA polymorphism of the forty lupine landraces amplified with primer OP-L13.

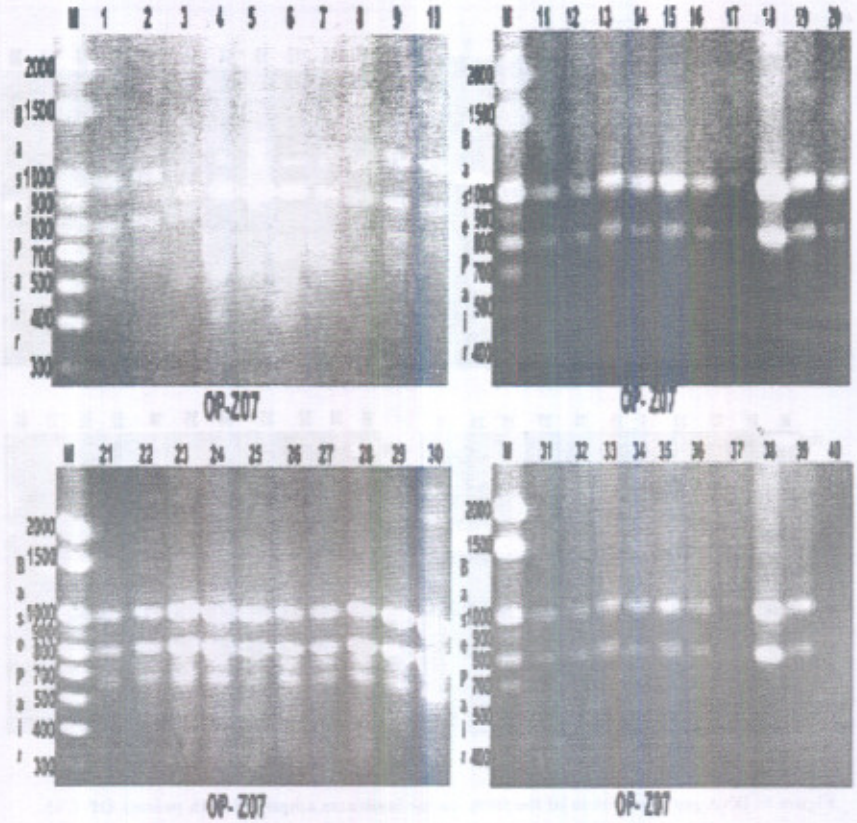


Figure 8: DNA polymorphism of the forty lupine landraces amplified with primer OP-Z07.

Table 6. Number of amplified bands produced by seven primer combinations in 40 white lupine accessions

RAPD primer combination	Amplified bands			Polymorphic bands %
	Monomorphic	Polymorphic	Total	
OP/A12	1	11	12	91.6
OP/A19	0	9	9	100
OP/B07	2	0	2	0
OP/C09	3	0	3	0
OP/C15*	0	17	17	100
OP/L13	1	14	15	93.3
OP/Z07**	0	15	15	100
Total	7	66	73	

* Negative marker in accession number 35. ** Positive marker in accession number 24.

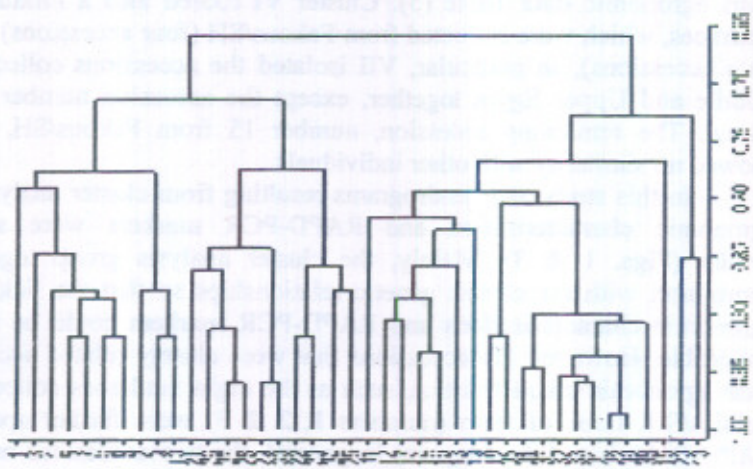


Figure 9: Dendrogram for the genetic distances between the forty lupine landraces based on RAPD analysis

exhibited two unique clusters (III & V) for ISM region rather than only one cluster group in the case of the agronomic data. The two accessions in each of cluster IV and VII were distantly different Table (7).

Table 7. Summary of the group cluster analysis based on RAPD-PCR of 40 white lupine landraces collected from different regions of Egypt

Group No.	No. of Accessions	District/Governorate and accession number
I	5	Belbies/SH (4, 5, 6, 7 and 8).
II	3	Abo-hamad/SH (9 and 10); Belbies/SH (2)
III	4	Fayed (21); Abo-Soeir (26); Algharb (27 and 28)/ISM
IV	2	Meet-Ghamr/DEK (29) and Badrashein/GZ (30).
V	4	Kantra (22 and 23); Ismailia (24); Abo-Soeir (25)/ISM
VI	5	Fakous/SH (12, 13, 14 and 16); Ismailia/ISM (17); Fayed/ISM (19)
VII	2	Ismailia/ISM (18); Beni Suef/BNS (35)
VIII	8	El-Aiat/GZ (31); Beni-Salh/FAY5 (32); Beni Suef/BNS (33 and 34), El-Minia/MIN (36); Sohag/SOH (39); Aswan/ASWN (37 and 38); [Middle and Upper Egypt group]
IX	2	Fayed/ ISM (20); Assiut/ASS (40)
Ungrouped		Belbies/SH (1 and 3); Abo-hamad/SH (11); Fakous/SH (15)

Cluster VII detached the same two accessions as cluster V on the basis agronomic data Table (5). Cluster VI roofed also a mixture of six landraces, which were collected from Fakous/SH (four accessions) and ISM (two accessions). In particular, VII isolated the accessions collected from Middle and Upper Egypt together, except the accession number 40 from Assiut. The remaining accession, number 15 from Fakous/SH, however showed no similarity with other individuals

In this study, the dendrograms resulting from cluster analysis of the agronomic characterization and RAPD-PCR markers were somewhat similar (Figs. 1 & 3). Mainly, the cluster analysis group together the germplasm with the closest genetic relationships so that the link between agronomic characterizations and RAPD-PCR markers could be relatively noticeable. However, the accessions that were closely related according to their agronomic characteristics, such as the eight landraces collected from Belbies/SH, three of them (numbers 1, 2 & 3) were further apart in the RAPD-DNA based dendrogram. In general, the two accessions number 18 and 35 and the two accessions 9 and 10 had an apparent relationship in both dendrograms. Cluster VI roofed also mixture of six landraces, which were collected from Fakous/SH (four accessions) and ISM (two accessions). Specifically, some accessions, such as the accessions collected from Middle and Upper Egypt, as well as the two accessions number 20 and 40 were evidently close in the RAPD-PCR based analysis, but were not clearly associated in the agronomic traits based dendrogram. Moreover, the accession number 11 exhibited similarity in both cases. Landgrebe *et al* (2002) indicated that using micro-array data analysis in the comparison of different individuals' profiles is biologically a crucial task. The accessions number 36 (El-Minia/MIN) and 39 (Sohag/SOH) were closely related in the case of agronomic traits based dendrogram. Nevertheless, they teamed up in Middle and Upper Egypt cluster group in RAPD-PCR based dendrogram. The moderate similarity between both dendrograms suggests that the agronomic traits information could still be useful to identify diverse germplasm (Van Becelaere *et al* 2005).

This moderate association between agronomic characterization and DNA markers was not surprising since the estimation of genetic relationship among different germplasm based on both of them is fundamentally different approaches, where they estimate different types of genetic resemblance (Schut *et al* 1997). The first estimates the proportion of loci identical by descent, whereas the second estimates the proportion of alleles alike in state. However, that is indistinguishable by their effects. Thereby, the agronomic traits based dendrogram ignores alleles that are alike but identical by descent, assuming that genotypes, which were not related by using it do not carry homologous fragments (Van Becelaere *et al* 2005). Therefore this kind of difference would be a key, especially in a case as

lupine in Egypt, which may have a narrow genetic base and thus possesses numerous alleles alike in state but not identical by descent. Helms *et al* (1997) revealed that estimates based on DNA markers are more informative in measuring overall genetic similarities since the proportion of alleles alike in state determines the amount of genetic variance among progeny. In general, most authors have recommended the use of DNA-marker based genetic similarity since it reflects the resemblance among germplasm at the DNA level by direct sampling of the genome (Graner *et al* 1994; Kim and Ward 1997; O'Donoughue *et al* 1994; Talhinhas *et al* 2003; Vaz *et al* 2004 and Yang *et al* 2001). However, Cox *et al* (1985) and Schut *et al* (1997) proposed that the estimation of genetic relationships among germplasm might be improved by combining agronomic characterization and DNA marker into an index to decrease the effect of the independent inaccuracies of both of them. However, one must keep in mind that morphological characterization is very helpful to crop breeders, especially in cases of available primers being inadequate to cover the "plant genome".

This investigation suggests the use of RAPD-GS analysis as a more sensible estimate of genetic resemblance. Nevertheless, the agronomic characterization and RAPD-PCR based dendrograms were somewhat similar, indicating that the agronomic characterization information will continue to be useful to inexpensively identify diverse germplasm in breeding programs of lupine and/or other species. The moderate association resulted between the two approaches may be due to contravention data recording. However, each approach to measuring variation and its structure in germplasm populations might still require more investigations and decisions on a specific, optimal, sampling strategy.

REFERENCES

Abdalla, A.M., O.U.K. Reddy, K.M. El-Zik and A.E. Pepper (2001). Genetic diversity and relationships of diploid and tetraploid cottons revealed using AFLP. Theor. Appl.Genet. 102:222-229.

Asins, M.J. and E.A. Carbonell (1986). A comparative study of variability and phylogeny of *Triticum* species. I. Intraspecific variability. Theor Appl. Genet. 72: 551-558.

Asins, M.J. and E.A. Carbonell (1989). Distribution of genetic variability in a durum wheat world collection. Theor. Appl. Genet. 77: 287-294.

Berville, A., B.S. Kuriovich, J. Heinanen, L.T. Kartuzova, M.L. Bematskava and Z.V. Chmeleva (2003). Diversity of lupine (*Lupinus albus* L.) based on biochemical composition. PGR Newsletter. No. 134:42-57.

Bretting, P.K. and M.P. Wilrlechner (1995). Genetic marker and plant genetic resource management. Plant Breeding Rev. 13:11-8.

Brown, A.H.D. 1989. Core collection: A practical approach of genetic resources management. Genome 31:818-824.

Buirchell B.J. and W.A. Cowling (1998). Genetic resources in lupine, In: Gladstones, J.S., Atkins, C.A. and Hamblin, J. (eds) Lupine as Crop Plants: Biology, Production and Utilization, pp 41-66. CAB International , Wallingford.

Choumane, W.P., T. Van Breugel, O. M. Bazuin, M. Baum,G. W. Ayad, and W. Amaral (2004). Genetic diversity of *Pinus brutia* in Syria as revealed by DNA markers. Forest Genetics 11(2) 87-102.

Christiansen J.L., S. Raza and R. Ortiz (1999). White Lupine (*Lupinus albus* L.) germplasm collection and preliminary in situ diversity assessment in Egypt. Genet. Resour. Crop Evol. 46: 169-174.

Christiansen J.L., S.Raza, B. Jornsgrad, S.A. Mahmoud and R. Ortiz (2000). Potential of landrace germplasm for genetic enhancement of white lupine in Egypt. Genet. Resour. Crop Evol. 47: 425-430.

Cox, T.S., Y.T. Kiang, M.B. Gorman and D.M. Rodgers (1985). Relationship between the coefficient of parentage and genetic similarity indices in the soybean. Crop Sci. 25:529-532.

El-Sayad, Z. S., F. Ashmawy, Sabah M. Attia and R.E. El-Lithy (2002a). The relative importance of some characters to seed yield of white Lupine as affected by planting dates and plant densities. J. Agric. Sci. Mansoura Univ. 27(1):71-83.

El-Sayad, Z. S., N. A. Mohamed, R.E. El-Lithy and M. I. Abd el- Mohsen (2002b). Performance of some promising white lupine genotypes and determination of relative importance of yield factors in newly reclaimed lands. J.Agric. Sci Mansoura Univ. 27(10) 6567-6582.

Franco, J., J. Crossa, M.L. Warburton and S. Taba (2006). Sampling strategies for conserving maize diversity when forming core subsets using genetic markers. Crop Sci. 46:854-864.

Gladstones.J. S. (1970). Lupine as crop plant. A review article. Field Crop Abst. 23(2): 123-147.

Graner, A, W.F. Ludwig and A.E. Melchinger (1994). Relationships among European barley germplasm: II. Comparison of RFLP and pedigree data. Crop Sci. 34:1199-1205.

Hash, C.T. and P.J. Bramel-Cox (2000). Survey of marker applications. Training manual for a seminar held at Ibadan, Nigeria 16- 17 August 1999.

Helms, T., J. Orf, G. Vallad and P. McClean (1997). Genetic variance, coefficient of parentage, and genetic distance of six soybean populations Theor. Appl. Genet 94:20-26.

Kaufman, L. and P.J. Rousseeuw (1990). Finding Groups in Data. John Wiley & Sons, New York.

Kim, H.S. and RW. Ward (1997). Genetic diversity in Eastern U.S. soft winter wheat (*Triticum aestivum* L. em. Thell.) based on RFLPs and coefficient of parentage. Theor. Appl. Genet. 94:472-479.

Landgrebe J., W. Wurst and G.Welzl (2002). Permutation-validated principal components analysis of microarray data. Genome Biology 3(4) 1-11.

Liu, S., RG. Cantrell, J.C. McCarty Jr. and J.McD. Stewart (2000). Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Sci. 40:1459-1469.

Messmer, M.M., A. E. Melchinger, R.G. Herrmann, and J. Boppenmaier (1993). Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33:944-950.

Mosjidis J.A. and K.A. Klingler. (2006). Genetic diversity in core subset of the U.S. red clover germplasm. Crop Sci. 46:758-762.

O'Donoughue, L.S., E. Souza, S.D. Tanksley and M.E. Sorrells(1994). Relationships among North American oat cultivars based on restriction fragment length polymorphisms. Crop Sci. 34:1251-1258.

Raza, S., A. AbdeL-Wahab, B. Jórnsgárd and J.L. Christiansen (2000). Calcium tolerance and ion uptake of Egyptian lupine landraces on calcareous soils. African Crop Sci. Jour.9 (2) 393-400.

Ruiz, L.P. JR. (1977). A rapid screening test for lupine alkaloids, New Zealand J. Agric. Res. 20: 51- 52.

Schut, J.W., X. Qi and P. Stam (1997). Association between relationships measures based on AFLP markers, pedigree data and morphological traits in barley. Theor. Appl. Genet. 95:1161-1168.

Talhinhas P., Neves-Martins J. and J. Leitao (2003). AFLP, ISSR and RAPD markers reveal high level of genetic diversity among *Lupinus* spp.. Plant Breed. 122:507-510.

Tao, Y., J.M. Manners, M.M.Ludlow and R.G. Henzel (1993). DNA polymorphisms in grain sorghum (*Sorghum biocolor* L. Moench). Theor. Appl. Genet., 86: 679-688.

Van Becelaere, G., E.L. Lubbers, A.H. Paterson and P.W. Chee (2005). Pedigree- vs. DNA marker-based genetic similarity estimates in cotton. Crop Sci. 45:2281-2287.

Vaz, A.C., C. Pinheiro, J.M.N. Martins and C.P.P. Ricardo (2004). Cultivar discrimination of Portuguese *Lupinus albus* by seed protein electrophoresis: the importance of considering "glutelins" and glycoproteins. Field Crop Res. 87:23-34.

Yang H., M.W. Sweetingham W.A. Cowling and P.M.C. Smith (2001). DNA fingerprinting based on microsatellite-anchored fragment length polymorphisms, and isolation of sequence-specific PCR markers in Lupine (*Lupinus angustifolius* L.). Mol. Breeding 7: 203-209.

مقارنة التوصيف الزراعي بالواسمات الوراثية الجزيئية للحامض النووى DNA في تقدير التشابه الوراثي للسلالات المحلية المصرية من الترمس الأبيض (*Lupinus albus*)

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تقييم المعلومات المتاحة عن التنوع الوراثى والقرابة الوراثية بين المواد المستخدمة فى التربية عملية أساسية في تحسين الأنواع النباتية. الهدف الرئيسي لهذا البحث هو المقارنة بين استخدام التوصيف للصفات الزراعية للتراكيب الوراثية واستخدام الواسمات الجزيئية في الحامض النووي DNA في تحديد التشابه أو التباعد الوراثي. يتم توصيف عشرة صفات زراعية لعدد 40 سلالة محلية من الترمس الأبيض تم جمعها من محافظات مختلفة من مصر وتمت دراسة هذه الصفات لمدة موسمين زراعيين بمحطة بحوث بهتيم (2004/ 2005 ، 2005 /2006). بينما استخدمت طريقة RAPD للتمييز الوراثي الجزيئى بين هذه السلالات. وقد قسم التحليل العنقودى باستخدام الصفات الزراعية للسلالات المحلية تحت الدراسة الى سبعة مجاميع وراثية مختلفة بدرجة تشابة 95% وقد ارتبطت هذة المجاميع إلى حد ما بأماكن هذه السلالات. بينما أدى استخدام الواسمات الجزيئية للحامض النووي الى تقييم السلالات الى تسعة مجاميع وراثية رئيسية بدرجة تشابة 85 % هذا مع وجود أربع سلالات محلية لم تعطى أى درجة من التشابة الوراثى مع باقي المجاميع الوراثية. ويؤكد هذا البحث أهمية أستخدام الواسمات الوراثية للحامض النووى DNA كوسيلة فعالة من ناحية الدقة لقياس التشابة الوراثى. وبالرغم من ذلك فان هناك درجة من التشابة بين نتائج الطريقتين مما يشجع استخدام المعلومات الناتجة من التوصيف الزراعي كوسيلة مساعدة وغير مكلفة ماديا فى تقييم التباين الوراثى فى محصول الترمس لتدعيم برامج التربية.

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