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Research Article

Evaluation of yield potential and some agronomic traits variability in Mediterranean alfalfa cultivars in the field under Tunisian conditions

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Abstract:
Sixteen alfalfa populations, originating from the Mediterranean basin, were investigated under field conditions in Tunisia.This research aimed to evaluate the adaptation of cultivars to oases conditions of North Africa. The diversity among ecotypes was studied for potential yield and using some agronomic characters. The fall harvest had given the lower fresh yield in all populations and the greatest was recorded in the harvest of spring season. In addition, fresh yields were significantly different ($p < 0.001$) among populations, except at spring harvest. No significant differentiation was observed between populations in dry matter of both summer and spring harvest. Five agronomic characters showed significant differences between populations ($p<0.001$). Weeds and snail presence were negatively influenced to biomass production. However, covering and dormancy were positively related to biomass production and they can be used as early production indicators. Three groups were identified by cluster analysis independently of geographical origins.

Key words *Medicago sativa*, yield, mediterranean basin, diversity.

Introduction

Lucerne (*Medicago sativa*L.) is the largest cultivated forage crop in the world and especially in Mediterranean countries. It is believed to have originated from Caucasus region (Michaud *et al*., 1988). This plant improves the yield and quality of the following crops by atmospheric nitrogen fixation (Bruulsema and Christie, 1987). Furthermore, it reduces diseases and weeds,increases soil organic matter contents and improves water infiltration (Campbell *et al.,* 1990).

It is an auto-tetraploid ($2n = 4x = 32$) (McCoy and Bingham, 1988), cross-pollinated (allogamous) and seed propagated. In Tunisia, lucerne germplasm exists as a cultivated species in the oases with total area of 12000 ha (ODS, 2005) and it is considered an elite plant material (PERMED, 2004). In Morocco, it is the principal cultivated forage crop with 100000 ha (22% of cultivated area of forage crops) and its cultivation is conditioned by irrigation (Bouizgaren *et al.,* 2010).North African oases are likely to be an environment where pure *M.sativa*ssp. *sativa* germplasm has been maintained for thousands of years; besides, soil and irrigation water salinity levels may have exerted a selective pressure towards lucerne populations. Populations of the North of Africa are mainly landraces produced from farmer's seeds, but those of the South of Europe are mostly varieties bred for improved forage yield and adaptation (Julier *et al.,* 2010). The same authors (Julier *et al.,* 2010) affirm that varieties adapted to Mediterranean climates were also selected in the USA and Australia.

Alfalfa is considered as an elite plant material (PERMED, 2004). In the Lucerne breeding programs, the study of variation of lucerne germplasm collected in different Mediterranean zones is essentially in view of improving collection procedures and optimizing the use of this germplasm. Nevertheless, the structure of lucerne (*Medicago sativa* L.) in the Mediterranean Basin is little described (Julier *et al.* 2010). The objectives of the present study were focus for possible structure of 16 Mediterranean lucerne populations. The study was based on two measured parameters, biomass production and agronomic characters. Relationships between green fodder production and agronomic traits were analyzed, to assess the effect of some agronomic traits on fresh matter accumulation. Furthermore, we offer more consideration to populations which can be more adapted and high forage yielding in North African oases.

Material and Methods

Plant material and experimental design: The study was carried out at the experimental field of Arid Crops in the Arid Areas Institute, Gabès (33.50N, 10.06E, 16 m a.s.l.) located in the south-Mediterranean region of Tunisia (Figure 1). The plant materials used were composed of 16 populations from France, Italy, Tunisia, Algeria, Morocco, Australia and the USA (Table 1). They were preserved in our laboratory since the Permed Project (2004).

Seeds were sown in September and a randomized complete blocks design was used at the rate of four replications per population. Plots were arranged in ten rows (L/W = 2 m/1 m) with inter-row spacing of 20 cm(Figure 1). Nitrogen and phosphorus were applied, respectively, at a rate of 30 kg ha^{-1} and 70 kg ha^{-1} prior to sowing.

Forage production: The forage production was estimated during the three harvests: at the first growing season: summer harvest, fall harvest and spring harvest. Fresh weight (FW) was determined on a plot area of 2 m^2 by weighing samples (Fig 1). Dry weight (DW) was determined by drying out samples at 65ºC, grinding and sieving them to 1 mm particle size.

Agronomic traits: Emergence (EM): Final emergence counts were determined after emergence had stopped (two weeks after seedling) by per cent of young plant from total plot area. The seeds were considered as germinated when the first two leaves arise from the ground.

Snail effect (SE): Snail white (*Xeropicta derbentina*) multiplied in the oasis of Gabes and caused enormous damage inseveral herbaceous crops. This species, active in summer, is adapted to high temperature of the Mediterranean regions. The snail attack was assessed by the damage percent in each plot.

Weeds (WE): Two species of weeds were found (*Cynodon dactylon* L. and *Setaria verticillita* L.). The percentage of weeds was estimated after the spring harvest in each plot by percentage of weeds.

Dormancy (DO): The plant dormancy was estimated in winter season: 0=completely dormant; 1= high dormant; 2= low dormant; and 3 = non dormant).

Others traits: The Lucerne covering (LC) in each plot was estimated by per cent from the total area, by two visual observatories. The length of internodes (LI. cm) and the numbers of sheets (NS) were measured, for each plot, in ten individual plants. The LI parameter reflects the length population growth.

Statistical analysis: Data were analyzed through analysis of variance procedure and means comparison (Student test). The ANOVA was used to test the population effects. Correlations between biomass production and agronomic traits were estimated. Cluster analysis classifications of the 16 lucerne populations on measured traits according to the Euclidean distance using dissimilarities algorithm. These analyses were computed on the data using XLSTAT software (www.xlstat.com).

Results and Discussion

Biomass production: Previous studies have underscored with the same measured parameters a high diversity between lucerne populations (Julier *et al.,* 1995, 2000 and 2010). However, Flajoulot*et al.* (2008) reported that differentiation between populations is difficult because of the large within population variation and of the small mean differences among populations. In our results, the variance analysis showed significant differences between Mediterranean lucerne populations for the fresh yield in the summer and fall harvest (Table 2). The greatest Fresh yield in the summer harvest was observed for Sardi population (1684.35 g/m^2) and the lowest for Africaine ones (70.2 g/m^2). In the fall harvest, the fresh yield was exceeded in Siriver population (1110 g/m^2) but very low in the Africaine ones (15 g/m^2) In the spring harvest this parameter ranged from 76g/m^2 (for the Africaine populations) to 1529 g/m^2 (for the Amerist populations) (Table 3). In the harvesting seasons, Africaine (Morocco), Magali (France) and Cossouls (France) were the lowest yielding varieties. These varieties are not well adapted in Tunisian oases conditions. These results are in agreement with those obtained from experiments conducted under irrigation in Morocco (Bouizgaren *et al.* 2010). In addition, these studies indicated that Cossouls was selected for grazing tolerance, and Africaine was sensible to diseases and insects especially (*Hyperavariabilis*) and has a low perenniality. However, they weren't selected for high yield or for adaptation to North African climates. Then, it is necessary to avoid the use of these populations in North Africa areas, because they can increase the genetics pollution with disadvantageous alleles in native populations.

On the other hand, the greatest forage yields were obtained by Sardi and Siriver (Australia) in the summer and fall seasons and by Amerist (USA) especially at spring harvest. Therefore, these populations are more adapted under the North Africa oases (Tunisia, Algeria and Morocco). Amerist is a synthetic variety being non-dormant and well adapted to the Southwest region of the U.S (AOSCA, 2009), where it is selected for its high yield and its resistance to different diseases such as Phytophthora root rot, anthracnose, bacterial wilt, Fusarium wilt, and Verticillium wilt. The similarity between Tunisian conditions and those of Southwest region of the U.S (references) can explain the Amerist high yields at spring harvest. Sardi10 and Siriver varieties are selected in Australia for the highly winter active and the important biomass production. These findings were completely confirmed by our results. But these varieties are susceptible to most major diseases of lucerne (AOSCA, 2009). Accordingly, these populations may have elasticity for several regions and can be used in improvement program for lucerne growth in Mediterranean countries especially in North Africa.

For all sampling date, no significant differences (P = 0.369 for summer harvest and P = 0.356 for spring harvest) in the amount of dry matter per m^2 were found between populations, except in fall harvest when P is lower than 0.05(Table 2). At this last season, three groups were observed (Table 3). Siriver variety recurred, from Australia, had the highest dry matter (224.77 per m^2). Sardi, Amerist, ABT, Prosemet, Mammunts, EScicilia, Rich2, Demnt, Erfoud and Tamantit populations have medium dry matter production comprised between 63 and 123 g/m^2. Magali, Cossouls, Melissa, Africaine and Gabes populations presented the lower values of dry matter (lower than 45 g/m^2).

The annual variation in forage yields in the Mediterranean regions is very variable due to climate variation along the year and environmental differences among zones. Our results show that the lowest fresh yield in all populations was observed in fall season and the greatest was recorded in spring season. Pagliaricci *et al.* (1987) reported that the high concentration of forage in spring represents over 75% of the annual forage offer. Consequently, the spring harvest can provide enough cattle forage. But at winter, the use of non-dormant lucerne populations or/and additional forage from another species are very necessary.

Agronomic characters: The comparison between the mean agronomic characters obtained for the studied populations showed significant differences between populations (P<0.001) for SE, WE, DO, LC, LI and NS parameters (Table 2). The white snail effect, which is estimated by the percentage of visual symptoms, in above ground biomass is highly significantly different among populations ($P \leq 0.001$).The greatest damage was observed in plot of Gabes landraces from Tunisia (93.75%), where this species was intensively represented. However, the lowest damage was observed in the plots of Amersit (USA) and Tamantit (Algeria) populations, respectively with 16.25 and 12.5% (Table 4). Weeds (WE) was high presented in three plots: for Magali, Cossouls and Gabes populations when their values exceeded 74% (Table 4). In the other plots the percentage of WE was lower than 33%. Eight nondormant populations were significantly distinguished: Sardi, Siriver, ABT, Amerist, Melissa, Demnt, Tamantit and Gabes while Africaine variety is completely dormant. The lucerne covering (LC) was significantly different between populations. This percentage, which indicates seeds viability, varied between 6.25 and 100%. The greatest covering was observed in Amerist populations and the lowest in Africaine ones. Also, seven populations exhibited a greater LC, which is higher than 80% (Sardi, Amerist, ABT, Mammunts, Melissa, Demnt and Tamantit). For Gabes landraces, from Tunisian oases, covering percentage was very low with mean value of 20%. The length of internodes (LI) ranged from 1.22 to 5.25 cm. The greater LI was detected by Amerist population and the lower LI was detected in Africaine stems (Table 4). The numbers of sheets (NS) reflected the digestibility. Indeed, Gabes from Tunisia was the most digestible population (22.17 sheets/plant). But, Africaine was the low digestible than all populations with mean value of 6 sheets/plant. Three other populations have shown an important digestibility when sheets number is superior to 17 sheets/plant (Table 4).

Relationship between agronomic characters and biomass production: The correlation coefficients between agronomic traits and biomass production are shown in Table 5. The summer fresh yield was negatively correlated with snail effect and weeds and positively correlated with dormancy level, Lucerne covering, length of internodes and number of sheets ($r>0.25$, $P<0.01$). A high negative correlations between fall fresh and dry yields and weeds were found ($r=-0.38$ and -0.37, $P<0.01$). However, significant positive correlations between fall biomass production (fresh and dry yields) and LC, LI and NS traits were obtained. The correlations between agronomic traits and spring fresh and dry yields are similar to those observed with summer fresh yield except for NS trait. Benabderrahim *et al.*(2009) reported that fresh matter of lucerne was negatively correlated to seed weight and positively to leaf dimension but dry matter was negatively correlated to stem length.

Analysis of correlations let us identify more determining agronomic characters, which may influence biomass production. These characters can be used as indicators for the harvest yields. More the population is non-dormant more the fresh yield is important. However, more we have weeds lesser the yields. It is likely that weeds induce a high competition for assimilates with lucerne plants, which may modify the partitioning of the biomass and covering in plots. The percentage of snail in plots was negatively correlated with the biomass production in major of seasons. It can be concluded that the weeds likely draw snail in lucerne plots and induce the reduction in its annual production. Similar results were found in sugarcane species by Muhammad *et al.* (2010); they have reported that the presence of weeds will lead to a reduction in the crop growth rate and yield components. On the other hand, Muhammad *et al.* (1999) reported that weeds, in general, harbor insects and diseases organisms. Also, our results showed that the winter non-dormant populations accumulated more fresh matters (in summer and spring) than the winter dormant. Hence, the winter dormancy can be used as an indicator for the high biomass accumulation. From the study of correlations existing between agronomic characters and potential yields, we can deduce the diagram in Figure 2, which summarized some factors determining the lucerne biomass production.

Cluster analysis: In order to elaborate synthetic vision regarding the structure of Mediterranean populations, from different zones, and on the basis of agronomic and production characters, clustering analysis using Dissimilarities method and based on Euclidean Distance was applied (Figure3). The clustering analysis shows three groups. The group A clusters nine Lucerne populations from different geographical origins: North Africa (Rich3, Demnt and Erfoud), South Europe (E Sicilia, Melissa and Mamunts), USA (Amerist and ABT805) and Australia (Sardi Ten). Populations grouped in this first cluster (A) have the higher biomass production at spring harvest, important biomass production at summer harvest, elevated growth and were almost non-dormant. The group B clusters one non-dormant population from Australia (Siriver). It was marked by higher production at summer and fall harvests, higher lucerne covering, and the most growth in length and sheets number. The group C clusters three South European (Magali, Prosemet and Cossouls) and three North African (Africaine, Tamantit and Gabes) populations. Populations in this group were high dormant and they have the lowest values of biomass production and lucerne covering, excepting the summer dry matter. Also, they were characterized by the greater weeds percentage and the highest snail damage. The three groups defined by cluster analysis and their characteristics are summarized in Table 6. These results show that the groups defined in clustering analyses were clustered independently of their geographic origins. In a previous study of Mediterranean populations evaluated by SSR markers, the genetic structure did not exactly fit that expected from the geographical origins (Julier *et al.*, 2010). It is also evident by the cluster analysis that populations gathered in the first group, especially Amerist, have an important yield potential. They can be used in future crop forage research to improvement the lucerne biomass production in North African oases.

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Table 1.Geographical origins of the 16 lucerne populations

Population name	Origin	Type of population
Sardi10	Australia	variety
Siriver	Australia	variety
ABT805	USA	variety
Amerist	USA	variety
Magali	France	variety
Prosemet	Italy	variety
Mamunts	Italy	landrace
Cossouls	France	variety
Melissa	France	variety
E. Sicilia	Italy	landrace
Africaine	Morocco	variety
Rich 2	Morocco	landrace
Demnat203	Morocco	landrace
Erfoud	Morocco	landrace
Tamantit	Algeria	landrace
Gabès	Tunisia	landrace

Table 2. Analysis of variance of fresh yield and dry matter accumulated by square meter at three sampling cuts and some agronomic traits

Source for variation		df	Mean Square	F	Sig.
Fresh yield	Summer	17	907066.899	2.486	0.007**
	Fall	17	273054.565	1.982	0.034*
	Spring	17	859688.307	1.106	0.377**ns**
Dry Matter	Summer	17	34369.560	1.116	0.369**ns**
	Fall	17	10697.954	1.966	0.035*
	Spring	17	32976.128	1.131	0.356**ns**
Agronomic traits	Emg	17	253.225	1.714	0.075**ns**
	SE	17	2534.559	6.249	0.000***
	WE	17	2187.453	8.436	0.000***
	DO	17	2.619	14.313	0.000***
	LC	17	4673.813	21.031	0.000***
	LI	17	7.995	552.618	0.000***
	NS	17	75.100	577.458	0.000***

ns: no significant. *: significant. **: high significant. ***: very high significant

Table 3. Biomass production of Mediterranean lucerne populations in the three season harvests

	Fresh Yield (g/m^2)						Dry matter (g/m^2)					
Population	**Summer**	*SNK*	**Fall**	*SNK*	**Spring**	*SNK*	**Summer**	*SNK*	**Fall**	*SNK*	**Spring**	*SNK*
Sardi	1684.35	**a**	442.50	**ab**	1354.90	**A**	116.76	**a**	84.50	**ab**	252.51	**a**
Siriver	1199.10	**ab**	1110.00	**a**	960.60	**A**	159.80	**a**	224.77	**a**	183.08	**a**
ABT	739.53	**ab**	590.00	**ab**	1307.20	**A**	81.75	**a**	109.47	**ab**	240.73	**a**
Amerist	1231.85	**ab**	640.00	**ab**	1529.28	**A**	254.66	**a**	113.10	**ab**	284.48	**a**
Magali	128.15	**c**	85.00	**c**	236.20	**A**	158.28	**a**	16.38	**c**	44.94	**a**
Prosemet	451.03	**ab**	357.50	**ab**	948.73	**A**	271.01	**a**	63.71	**ab**	179.41	**a**
Mamunts	1117.63	**ab**	660.00	**ab**	1293.40	**A**	165.59	**a**	123.54	**ab**	242.22	**a**
Cossouls	187.98	**c**	240.00	**ab**	269.80	**A**	358.00	**a**	44.46	**c**	50.17	**a**
Melissa	1399.10	**ab**	207.50	**ab**	1523.60	**A**	21.80	**a**	38.36	**c**	319.14	**a**
E. Sicilia	1087.78	**ab**	507.50	**ab**	837.45	**A**	123.11	**a**	100.07	**ab**	155.03	**a**
Africaine	70.20	**c**	15.00	**c**	76.00	**A**	250.56	**a**	2.94	**c**	14.82	**a**
Rich2	982.00	**ab**	620.00	**ab**	1392.35	**A**	162.05	**a**	126.78	**ab**	268.20	**a**
Demnt	1304.73	**ab**	360.00	**ab**	1109.30	**A**	226.86	**a**	69.72	**ab**	231.79	**a**
Erfoud	801.20	**ab**	345.00	**ab**	1220.50	**A**	335.84	**a**	64.68	**ab**	231.53	**a**
Tamantit	440.05	**ab**	345.00	**ab**	833.85	**A**	144.27	**a**	64.90	**ab**	155.90	**a**
Gabes	351.23	**ab**	202.50	**ab**	420.80	**A**	253.23	**a**	36.00	**c**	83.85	**a**
Total	823.5		420.5		957.12		192.72		80.21		183.34	

Means within a row followed by a different letter differ significantly (P<0.05).

Table 4.Means of the agronomic traits measured for the Mediterranean lucerne populations

Populations	Emg (%)		SE (%)		WE (%)		DO		LC (%)		LI (cm)		NS	
	Mean	*SNK*	Mean	*SNK*	Mean	*SNK*	Mean	*SNK*	Mean	*SNK*	Mean	*SNK*	Mean	*SNK*
Sardi	76.25	a	31.25	de	13.38	ab	3.00	a	95.00	ab	4.75	bc	17.17	ab
Siriver	61.25	a	57.50	abcde	19.00	ab	3.00	a	96.25	ab	4.65	cd	16.87	ab
ABT	71.25	a	33.75	de	11.63	ab	2.75	a	92.50	ab	3.32	de	14.25	bc
Amerist	77.50	a	12.50	e	10.75	ab	3.00	a	100.00	a	5.27	a	17.07	ab
Magali	58.75	a	83.75	abc	75.63	a	1.75	bcd	6.25	d	2.02	e	12.57	bc
Prosemet	71.25	a	67.50	abcd	32.50	ab	1.25	d	47.50	c	1.67	e	10.35	d
Mamunts	71.25	a	42.50	bcde	15.38	ab	1.75	bcd	82.50	ab	3.57	de	12.63	bce
Cossouls	55.00	a	85.00	abc	81.25	a	1.50	cd	7.50	d	1.77	e	11.45	d
Melissa	71.25	a	38.75	cde	12.63	ab	3.00	a	92.50	ab	4.87	b	13.31	bc
E Sicilia	72.50	a	75.00	abcd	29.75	ab	2.00	ab	63.75	bc	1.67	e	11.50	d
Africaine	61.25	a	77.50	abcd	24.38	ab	0.00	e	6.25	d	1.22	e	6.00	e
Rich2	57.50	a	88.75	ab	27.50	ab	2.25	ab	51.25	c	3.07	de	15.07	bc
Demnt	72.50	a	52.50	abcde	15.50	ab	3.00	a	96.25	ab	4.48	d	13.17	bc
Erfoud	77.50	a	48.75	abcde	16.25	ab	2.50	ab	73.75	abc	3.87	de	12.25	bce
Tamantit	81.25	a	16.25	e	10.00	ab	2.75	a	95.00	ab	3.57	de	12.70	bce
Gabes	57.50	a	93.75	a	74.38	a	3.00	a	20.00	d	4.57	cd	22.17	a

Means within a row followed by a different letter differ significantly (P<0.05).

Table 5. Correlation coefficients (r, P=0.01 and 0.05) between agronomic characters and biomass production. Correlation coefficients presented in bold are significant

	Summer		Fall		Spring	
	FY	DM	FY	DM	FY	DM
Emg	0.175	-0.157	-0.090	-0.109	0.121	0.119
SE	-0.264*	0.116	-0.122	-0.097	-0.281*	-0.263*
WE	-0.379**	0.243	-0.380**	-0.371**	-0.357**	-0.347**
DO	0.395**	-0.206	0.158	0.155	0.266*	0.271*
LC	0.493**	-0.182	0.317*	0.307*	0.360**	0.358**
LI	0.477**	-0.132	0.281*	0.269*	0.355**	0.364**
NS	0.290*	-0.088	0.281*	0.273*	0.213	0.207

(**): significant at the 0.01 level. (*): significant at the 0.05 level

Table 6. Groups of Mediterranean populations defined by cluster analysis and their characteristics

Group 1 (9)		Group 2 (1)	Group 3 (6)	
Sardi	Australia	Siriver Australia	Magali	South Europe
ABT	USA		Prosomet	South Europe
Amerist	USA		Cossouls	South Europe
Mamunts	South Europe		Africaine	NorthAfrica
Melissa	South Europe		Tamantit	NorthAfrica
E Sicilia	South Europe		Gabes	NorthAfrica
Rich3	NorthAfrica			
Demnt	NorthAfrica			
Erfoud	NorthAfrica			
High biomass production		High biomass production	Lowbiomass production	
Non dormant		Low dormant	High dormant	
Moderate damage by snail		Moderate damage by snail	high damage by the snail	
Undominated by weeds		Undominated by weeds	Dominated by weeds	
Abundant coverage		Abundant coverage	Low coverage	

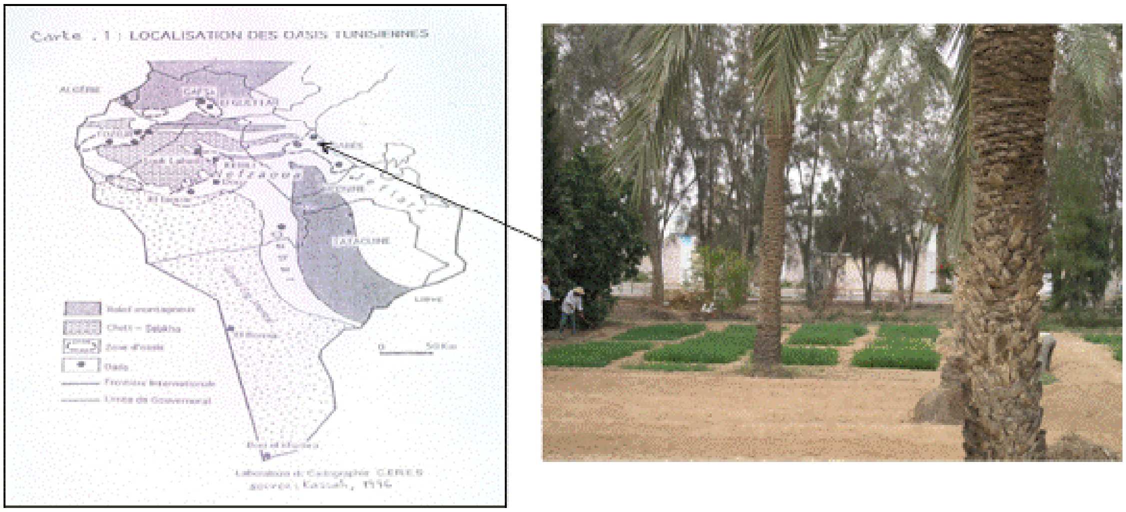

Fig. 1 General view of experimental field in Gabes. South of Tunisia (Only two blocs are presented in this photo)

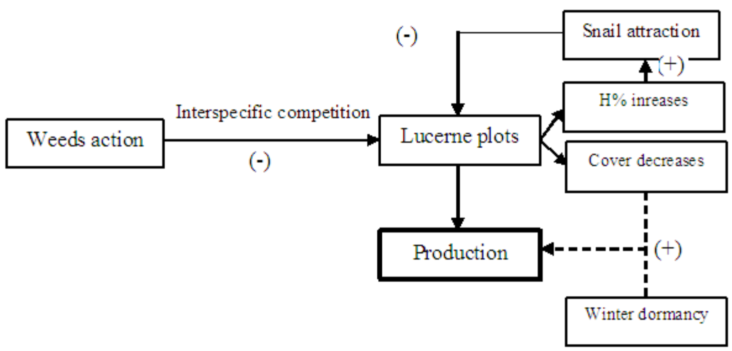

Fig. 2 Diagram summarized the effect of the agronomic traits on Lucerne production. The dashed lines show early indicators of production. H%: relative humidity. (-) and (+): correlations signs

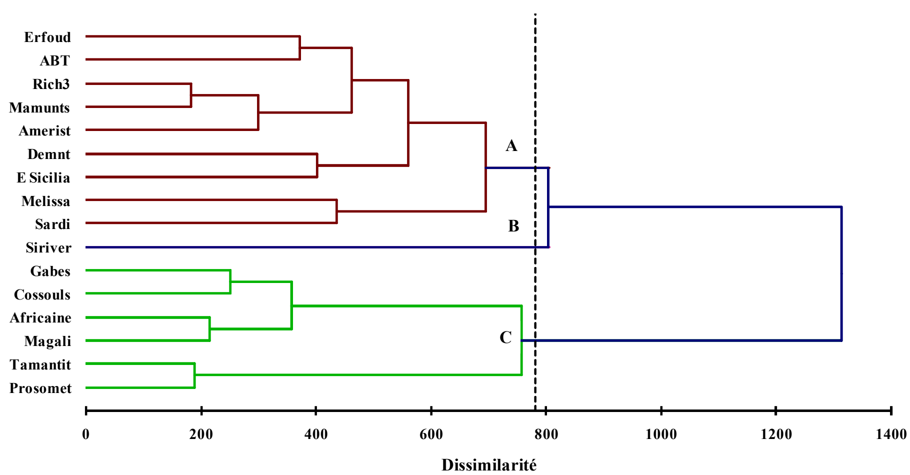

Fig. 3 Dendrogram of 16 Mediterranean Lucerne populations constructed according to Euclidean distance estimated on biomass production and agronomic traits