

## Full Length Research Paper

# Break dormancy, germination capacity of medics after different techniques of scarification (Physical, Chemical and Mechanical)

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Medics are annual forages very interesting in pastures of Mediterranean and steppes rangelands. Such as other leguminous, they are subject to dormancy which delay and reduce germination. The response of these species (*Medicago polymorpha*, *Medicago intertexta*, *Medicago ciliaris*, *Medicago truncatula*, *Medicago muricoleptis*) to mechanical, physical and chemical scarification, applied for break dormancy, had been studied. Germination is almost full (reaching 100%) is fast (24 h) after scarification with sand-paper, which demonstrates that dormancy is exclusively imposed by teguments of the grain. Soaking in boiled water (20 s by population), passing through sulphuric acid during 5 min and 180 mn, passing in freezer and in refrigerator (respectively 2 h and 5 days), in drying oven at 115°C during 20 mn eliminates partially dormancy. However, passing through ovine digestive tract followed by germination test possesses the same effect as the witness. In exposure in ultra-low temperature at 196°C (liquid nitrogen) during 25 mn improves germination and grains germination speed to all quoted species which is comparable to scarified grains results by sand-paper.

**Keys words:** Scarification, medics, populations, germination, dormancy.

## INTRODUCTION

Fertilisation is a result of seed, in flowers plants, angiosperms, the fertilisation is double. The grain is constituted of several different types of origin tissues: embryo, albumen and teguments.

Embryo and albumen are tissues of fertilisation. At the periphery of the grain, we find teguments, protecting sleeves being more or less resistant. They are maternal origin and drift from ovary tissues (Brunel, 2008). These

organs are intended to give birth to a new generation of plants; during germination process they ensure the survival of species. They are adapted organs to resist in adverse conditions. Such resistance allows them to reach, often for a very long time, in substantially inert state, conditions that will allow them to germinate (Mullon and David, 1982). These transient aptitudes to the germination typically called dormancy.

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**Table 1.** Code and origins of studied populations and so average weight of 1000 grains.

Species	Populations code	Weight of de 1000 grains	Origins
<i>M. truncatula</i>	Tr201 Tr55	3.58 g	Algeria (2004 and 2012)
<i>M. intertexta</i>	I756 I52	15.77 g	Algeria (2004 and 2012)
<i>M. ciliaris</i>	S7 S5	13.21 g	Algeria (2004 and 2012)
<i>M. polymorpha</i>	poly 60 Poly 205	3.24 g	Algeria (2004 and 2012)
<i>M. muricoleptis</i>	Aus106	6.26 g	Turkey (2004 and 2012)

We can consider it as an exceptional resistance of seeds to unfavourable conditions in exterior environment but also, and above all a very high level of dehydration state of the seed. Furthermore, desiccation seems to be more active than grain's drying. It is an active phase in terms of gene and metabolism expression. Which implied that preparation of grains for germination is already starting during desiccation of seeds (Angelovici et al., 2010). According to Pinfield and Dungey (1985) a lot of time and efforts had been made to try to understand and the difference between regulation mechanisms of maintenance dormancy, perception of inductive conditions, the break dormancy and the germination process. Mayer and Poljakoff-Mayber (1982) explained; dormancy may be on account of the embryo's immaturity, imperviousness of seed coat to water or to gas, preventing of embryo development because of mechanical reasons, of particular requirements of temperature or of light, or presence of inhibitory substances of the germination. Others authors attribute dormancy to physiological properties and / or morphological of the seed. According to Baskin and Baskin (2004), it may be classified in five large categories: physiological, physical, morphological, morpho-physiological and combinatory (physical and physiological). Generally, physical dormancy is caused by one or several waterproof layers of palisades cells macrosclerides in tegument (Baskin and Baskin, 2004). However observations by scanning electron microscope revealed a thicker layer of palisades cells in tegument of *Medicago orbiculairis* compared to *Astragalus hamosus* which can explain the higher resistance of its grains facing chemical and physical agents of scarification and of hot water (Patane and Gresta, 2006). In Mediterranean environments, grains of numerous species of leguminous must survive in hot and dry summers to allow seedlings, avoid temperatures potentially lethal and to delay germination until the next spring. Grains of those species developed a resistance mechanism to this stressful condition and complex machinery we can perceive when environmental conditions are favourable to break dormancy (Scippa et al., 2011). Collection, evaluation and valorisation of local populations of *Medicago* in Algeria began from 1971/1972 by Abdelguerfi. As a matter of fact, he identified 17 species belonging to different bioclimatic stages (Abdelguerfi, 1989). Although those species showed an interesting use in agriculture

and a large adaptability with a wide range of environmental conditions (Chebouti and Abdelguerfi, 1999), little information is available in literature on characteristics of the grain's germination and techniques to break its seed coat imposing dormancy (Chabouti and Abdelguerfi, 1999). Several treatments had been proposed to reduce and improve tegument's hardness and rise germination rate in others species of leguminous notably *Acacia* (Danthu and Neffati, 2003). In this context this research aimed to evaluate response of grains' germination of *Medicago* originating of two different harvesting years (2004 and 2012) to different treatments of scarification, in order to promote their use in improvement of natural rangelands of steppes and Mediterranean areas.

## MATERIALS AND METHODS

Vegetable material or seed come from collection of National Higher Agronomic School (NHAS). The code and weight of 1000 grains of used populations are shown in Table 1. This seed is harvested in 2004 and 2012. The storage of the seed of 2004 is made in an ordinary room requiring no conditioning. We wanted to test germination of this seed with one of year 2012. The studied species are classified according to the colour and size into three groups. Species with big grain (*Medicago intertexta*, *Medicago ciliaris*) of black colour, species with small grain (*Medicago polymorpha*, *Medicago truncatula*) of yellow-brown colour and species with middle size *Medicago muricoleptis* of black colour. The shape of all species is feeding nozzle.

## Treatments of populations

- (i) Seed treatment with sulphuric acid during 5 mn for the first test and during 180 mn for the second test. After we rinse under running water.
- (ii) Passing of the seed through the drying oven at temperature of 115°C during 20 mn.
- (iii) Isolation of an animal and introduction in its diet of 500 gr of pods by meal, twice a day during six days (*M. polymorpha*, *M. ciliaris* and *M. truncatula*). Then liberation of ovine and recuperation of faeces' seed by soaking in tap water and sifting.
- (iv) Passing of the seed into boiled water (100°C) on the fire during 20 s.
- (v) Passing of the seed in the freezer during 1 h, but for both steppe species during 5 days in the refrigerator.

It should be noted that seed of each population is put in small perforated closed cages which we have made with grid and sieve. Cages are denominated by codes of corresponding population. So

**Table 2.** Role of the seed coat on germination and survival of the species.

Populations and harvest year	Number of seeds	sprouts	%germination
Aus106/2004	8	0	0
S5 et S7/2004	12	0	0
Poly201/2004	8	7	87,5
Aus106/2012	4	0	0
S5 et S7/2012	8	0	0
Poly201/2012	8	8	100
I11/2012	20	1	5
Moyenne			27,5

during passing in chemical products, cages are gathered and put in a recipient containing the adequate product (acid, liquid nitrogen, boiled water or passing in freezer). After the timing, rinsing is made under tap running water. Drying is made in the open air. As for passing through the oven, seed is put in paper bags. Witnesses undergo no treatment.

#### Seedling after seeds treatment

After all treatments, seed (treated and witness) is sown in Petri boxes full of cotton. As for recuperated seed from faeces of ovine a second seedling is made, it is about of alveolus bearing a mixture of ground and compost to stimulate germination. It should be noted that seed without teguments is also sown to know their effect on the germination. Histological cuts have been performed on teguments by method of double coloration (methyl green and red congo). Observations have been made by optical microscope. The seed irrigation in Petri boxes is done with tap water. The rate calculation of the germination begins at the stopping of appearance of rootlets by counting number of germinated grains x 100 divided by the total number of grain contained in Petri box. Analysis of variance is carried out by statview software.

## RESULTS AND DISCUSSION

As for grains without teguments, all studied species gave a germination rate of 0% except Poly<sub>201</sub> in the two study years which gave 100 and 87.5% of germination respectively for year 2012 and 2014 (Table 2). Also, the recuperated seed of ovine's faeces (Photos 1 and 2) gave 0% germination; we can say that passing of the seed in digestive tracks of ruminants has no scarifier effect. However; animal by its displacements may have a disseminator effect of the seed especially during its transhumance.

Analysis of the variance showed that storage years of seed have no influence on different rates of obtained germinations. Since, according to Tran and Kavavgh (1984) the waterproof coat protects embryo from unfavourable storage and environmental conditions and extend actively longevity of seeds. A combination of two mechanisms: closer shrinking of grains envelope cells and impregnation of cells with hydrophobic substances may exist and operating mechanisms may vary between different species. Observation of histological cuts by

optical microscope, after passing of seeds in boiled water and after coloration in green of Methyl and in red Congo gave Photos 3 and 4. These photos showed three different layers responsible of protection and of dormancy of seeds. The best results are obtained by mechanical scarification (with sandpaper). The percentage of germination reaches until 100% (Table 3).

If we compare this method to other chemical and thermal ones, most of the results are lower than its. Nonetheless, the results average of Figure 1 and Table 4 show that the passing in liquid nitrogen gives higher results close to results of scarification with sandpaper. While witness exceeds average of other methods, In fact, result of passing of the seed in sulphuric acid at 98% during 180 mn is approaching the witness. This result eventually may be improved by decreasing the pause time at 60 mn. According to Patane and Gresta (2006) in *M. orbicularis* even after 2 mn at 100°C, 97.9% of grains remained more difficult to germinate. Exposure at ultra-low-temperature (-196°C) in liquid nitrogen finally improves germination and speed of grains germination of *M. Orbicularis* (Photos 5, 6 and 7). Chemical scarification with sulphuric acid was also efficient in the reduction of hard grains but has the higher concentration (70%) and the longest exposure time (60 mn) (Photos 8 and 9).

With regards to both steppe populations, their passing in refrigerator during five days gives a positive effect only for one but nothing for the other. That means 0% of germination for *M. truncatula* and 68% of germination for *M. polymorpha*. While witness scarified with sandpaper gave 69.84 and 90.57% respectively for *M. truncatula* and *M. polymorpha* (Photos 10 and 11).

Since the variance anyalisis showed no significant difference, thus does not influenceon result of different used tests; we take in consideration values of populations of both years as repetitions which gives  $P < 0.0001$  for all methods. Therefore, difference is very highly significant between species and between populations for the different tests (Table 5).

All seeds from a same lot do not present the same aptitude to the germination. This means that as seeds of a lot are dormant as germination is heterogeneous. Seeds considered as dormant are not necessarily seeds



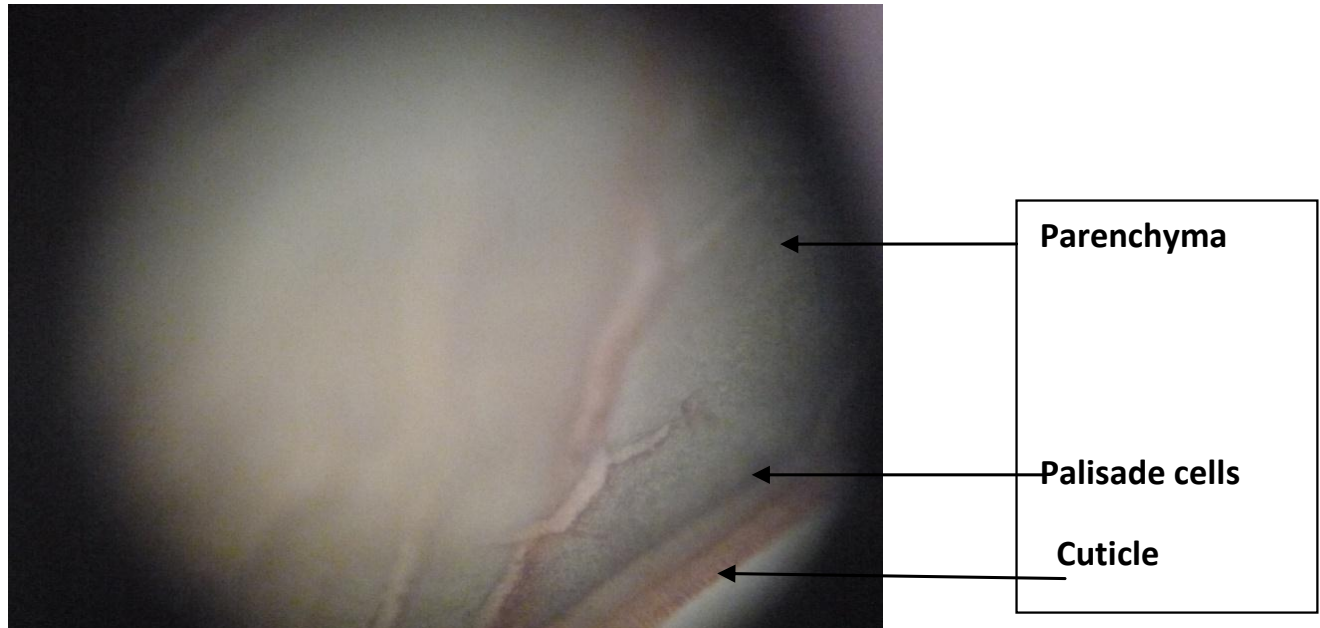
**Photo 1.** Isolation of the animal and introduction pod 500 g per meal.



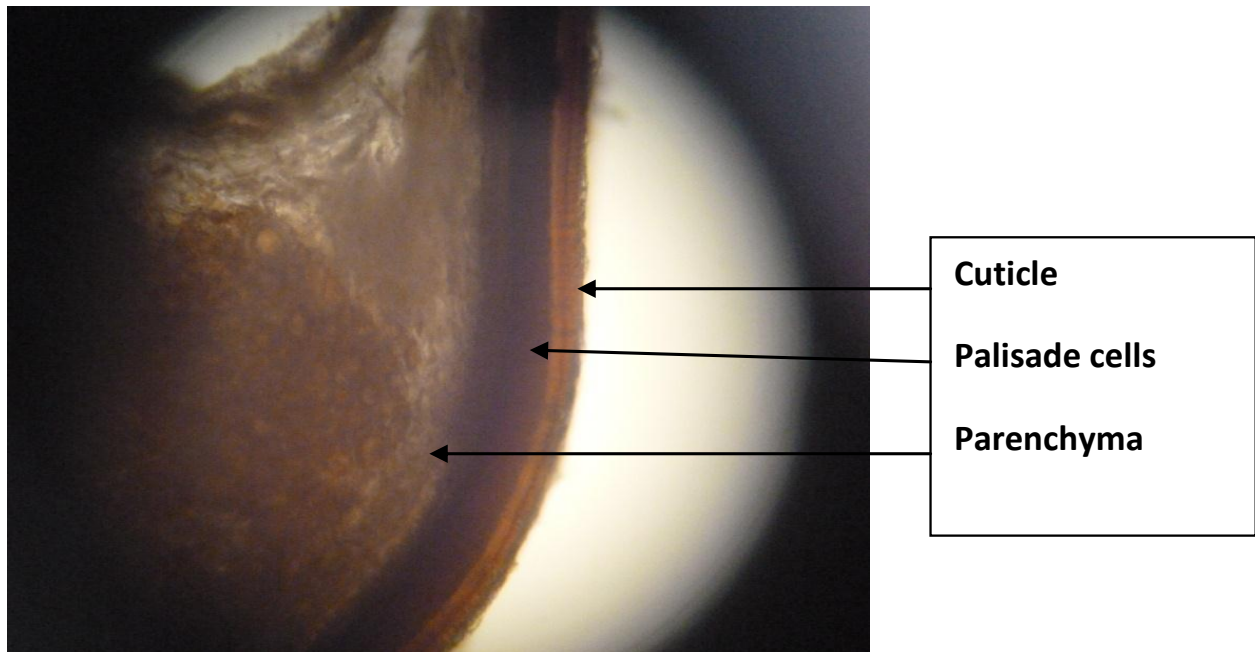
**Photo 2.** Recovery of semen by screening feces.

which do not germinate at all. Dormancies show up most often by a low and heterogeneous germination (Mullon and David, 1982). Causes of variation of the dormancy

are various. First of all there are genetic, because dormancy varies according to species, within the same species depends on variety (Mullon and David, 1982).



**Photo 3.** Histological cut at level of the seed tegument of *M. muricoleptis*.



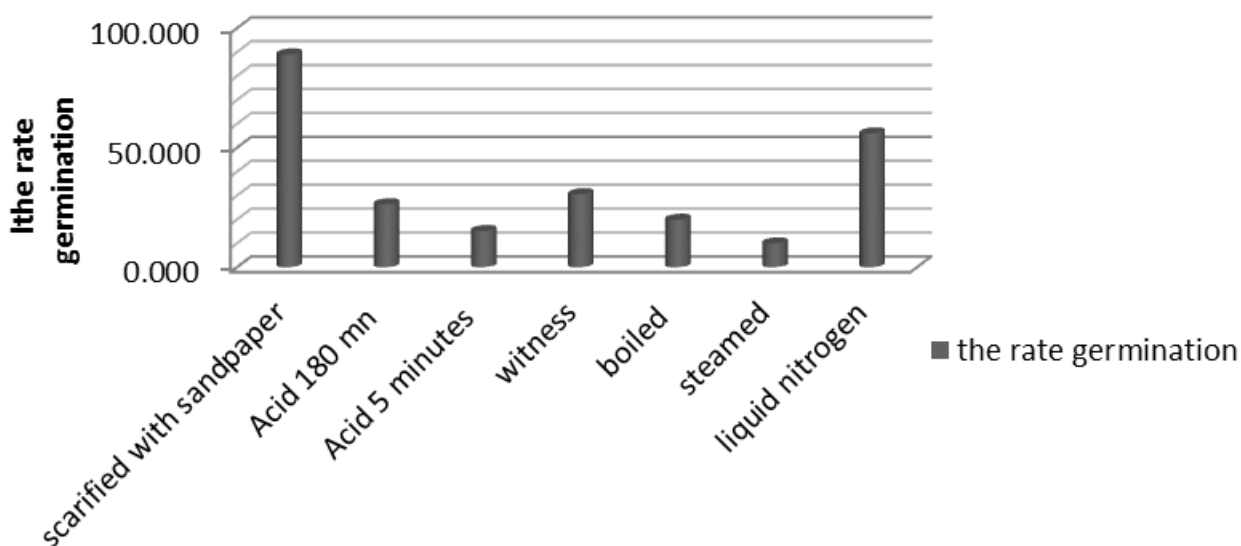
**Photo 4.** Histological cut at level of the seed tegument of *M. intertexta*.

The Medics do not germinate because their embryo has no possibility to be soaked. If we take the mechanical method, we noticed that this dormancy differs between species with big grain and small grain which that means: *M. ciliaris*, *M. intertexta* and *M. polymorpha*, *M. truncatula*. Within the small grains polymorph have a very

high germination rate compared to *M. truncatula* (Table 2). However, within big grains, germination rate is very close between *M. ciliaris* and *M. intertexta*. Therefore, *Medicago* produces a strong proportion of dormant grains, also called "hard" grains which germinate after several years. For that reason it is not necessary to

**Table 3.** Results of two examples of scarification.

Harvest seed	Populations	scarification avec papier verre			scarification avec acide (5-6mn)		
		Number of seeds	Sprouts	% germination	Number of seeds	Sprouts	% germination
2004	Tr55	19	10	52,632	18	6	33,333
2004	Tr2012	14	13	92,857	21	2	9,524
2004	S5	9	9	100,000	42	0	0,000
2004	S7	15	14	93,333	38	0	0,000
2004	Poly205	15	14	93,333	24	6	25,000
2004	Poly60	32	32	100,000	9	0	0,000
2004	I756	24	24	100,000	45	3	6,667
2004	I52	24	24	100,000	28	2	7,143
2004	Aus106	25	25	100,000	55	2	3,636
2012	Tr55	18	18	100,000	8	2	25,000
2012	Tr201	63	45	71,429	3	2	66,667
2012	S5	11	11	100,000	20	0	0,000
2012	S7	16	12	75,000	20	5	25,000
2012	Poly205	26	23	88,462	18	1	5,556
2012	Poly60	17	17	100,000	13	3	23,077
2012	I756	16	16	100,000	23	6	26,087
2012	I52	16	16	100,000	16	1	6,250
2012	Aus106	16	14	87,500	14	1	7,143
Average of five species				91,919			15,005

**Figure 1.** Germination rate of medicos after different treatments of scarification.

reseed rangelands with medicago after implantations. Dormant grains ensure regeneration of rangelands during several years, in spite of seasonal rainfall fluctuations (FAO, 1997). A Medicago's rangelands characteristic consist in their production capacity of abundant supply of green forage richer in proteins during winter and spring with a duration varying according to species and cultivars

of the same species. The question is to know if these species adapt better the African semi-arid surroundings. In our test on the field, we noted that crawling species with small grains such as *M. polymorpha* and *M. truncatula* sown in reduced space between grains, give interwoven plants in spider's web shape which can help contributing to erosive soils retention. We think they

**Table 4.** Germination results in % of Medics' populations after different tests of scarification (chemical, physical and mechanical).

Populations	Year	Witness	Scarified with sandpaper	Boiled	Steamed	5 mn Acid	180 mn Acid	Cold	Liquid nitrogen
Tr55	2004	25	52,632	-	3,571	33,333	11,765	0	89,66
T201	2004	36,111	92,857	4,167	0	9,524	0	25	78,45
S5	2004		100	11,111	8,333	0	16,129	2,941	20,97
S7	2004		90,476	14,286	5,263	2,632	0	9,524	23,9
Poly205	2004		93,333	11,429	1,754	25	8,824	33,333	63,83
PoLY60	2004		100	6,452	8,955	0	44,444	14,286	43,75
Aus106	2004		92,593	3,448	12,5	3,636	0	8,108	62,07
I756	2004		100	50	3,704	6,667	95,24	37,931	71,76
I52	2004		100	22,222	1,282	7,143	30,435	23,810	55,1
Tr55	2012		100	20,3704	19,44	25	27,273	28,571	68,42
T201	2012		71,429	12,5	23,256	66,67	28,125	24,138	85,2
S5	2012		100	10	6,122	0	-	13,636	29,41
S7	2012		75,000	14,286	23,529	25	59,091	9,524	32
Poly205	2012		46,939	6,897	3,226	5,556	80	20	70
PoLY60	2012		100	16,667	14,706	23,077	-	13,636	43,75
Aus106	2012		87,500	14,286	2,703	7,14	1,429	18,919	39,08
I756	2012		100	50	14,815	26,087	10,417	23,077	61,64
I52	2012		100,000	70	26,923	6,250	8,571	9,524	68,22
<b>Average</b>		30,556	89,042	19,889	10,005	15,151	26,359	17,553	56,002

should be sown in arid zones to improve soils structures through the contribution of organique mater vegetable and animal after pastures.

Environmental factors are known to influence the mother plant, provoking thus changes in characteristics of the progeny germination (grains). However, types of changes which may occur in grains, as they are developing under different environmental conditions, should also be taken in consideration. So, research is often necessary to *determine the relative importance* of each one of these two factors in dormancy variation of grains and germination of particular species (Baskin and Baskin, 1998).

### Conclusion

The dormancies are very profitable to wild species, as they contribute ensuring their maintain in ecological conditions and under difficult climatic conditions. But sometimes there are important problems with cultivated species for which obtainment of germination necessitates research of adjusted treatments in each case. The dormancy is heterogeneous in Medicago, varies between species and within populations, the variance is high. Effect of physical treatment or chemical gives a germination rate which varies between 0 and 95.34% for I<sub>756</sub> (acid 180 mn), 50%

for I<sub>756</sub>, 70% for I<sub>52</sub> boiled and 66, 67% for Tr<sub>201</sub> (acid 5 mn) while higher to 50% case of cold in steppes species. The same observation is made within populations. Thus, this is an inter-specific variation and inter-population.

Furthermore, both treatments which offer germinations rate potentially high and fast in all populations which are: Scarification with sandpaper and passing in liquid nitrogen. In spite of this latter gives lower result than the first one, it is more practical and easier to perform than all others means. It is about of a fast passing of a limited number of grains to be sown, but scarification with sand- paper, number of grains is



**Photo 5.** Bottle of liquid nitrogen.



**Photo 6.** Effect of liquid nitrogen on the cages containing the seed.





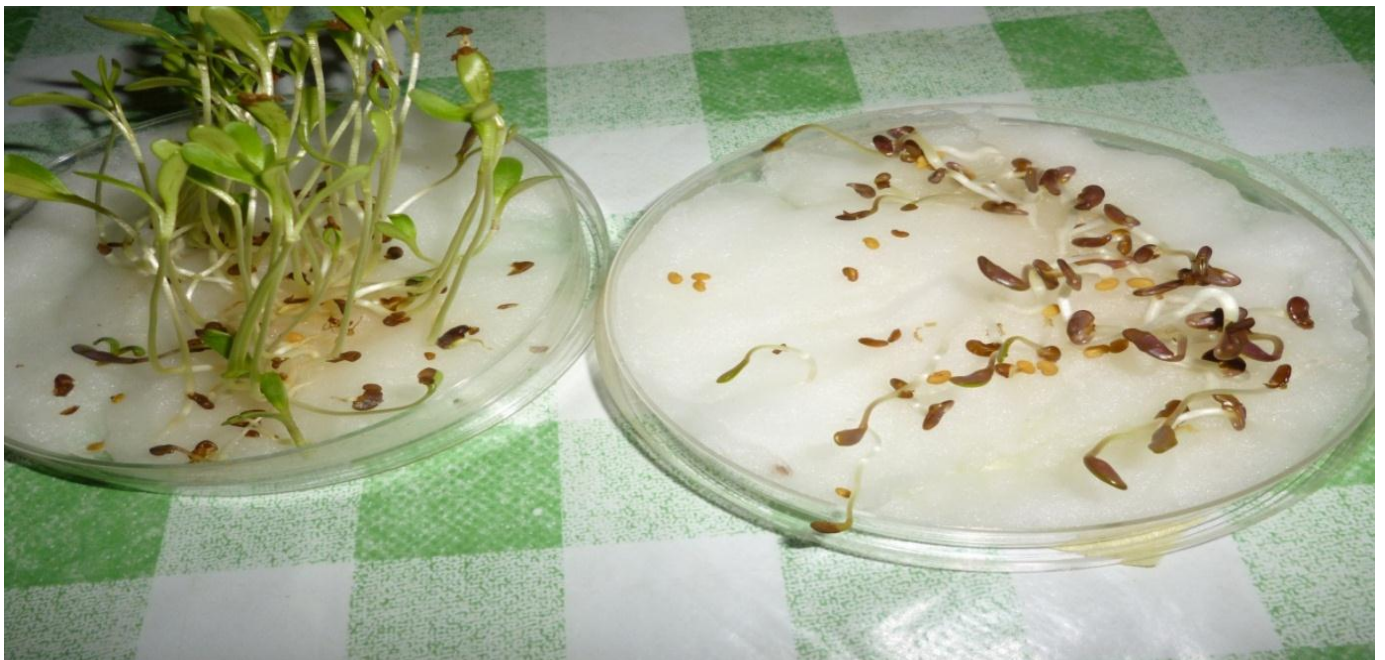
Photo 7. Seven days after sowing.



Photo 8. Seed in acid.



**Photo 9.** Rinçage seed.



**Photo 10.** Germination of *M. polymorpha* right after refrigeration and left after scarification.



Photo 11. *M. truncatula* left after scarification and right after refrigeration has not germinated.

Table 5. Variance analysis of scarified Medics by different methods.

Parameter	Variance	DOF	P	Significance
Scarified seed	277,738	17	<0.0001	***
Boiled seed	350,159	16	<0.0001	***
Steamed seed	73,605	17	<0.0001	***
Passing in acid 5 mn	284,673	17	<0.0001	***
Passing in acid 180 mn	862,836	15	<0.0001	***
Passing in freezer 2 h	106,329	17	<0.0001	***
Passing in liquid nitrogen	440,899	17	<0.0001	***
Witness	61,727	1	<0.0001	***

DOF degree-of-freedom\*\*\* very highly significant.

limited and uses a long time. The germination percentage of these scarified populations by sand-paper in 2013 and sown in 2014 gave the following results: 21.92% of germination in ciliaris, 15% of germination in cultivar Gemalong, 18.5% in *Truncatula*, 26% in *Intertexta* and 7.5% in *Granadensis*. Therefore, we note that *Intertexta* keep their germination power more than others species.

### Conflict of Interests

The authors have not declared any conflict of interests.

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