Effect of mechanical scarification, chilling, and gibberellic acid on germination of *Leucaena leucocephala* seeds

تأثير التخديش الميكانيكي، البرودة ، حامض الجبريلين في انبات بذور نبات اللوسينا *Leucaena leucocephala*.

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Abstract

***Leucaena leucocephala* is a tree species used for several agricultural purposes in Mediterranean region. The seeds of these species exhibit dormancy causing delayed germination. A series of experiments evaluated the effects of various treatments on the germination of *leucaena leucocephela* L. seeds. Germination of fresh seeds was 46% but it was improved (to about 70%) by mechanical scarification using sand paper. A germination of about 60% was obtained when dry seeds were pre-chilled at 4℃ for 2,3 and 4 weeks prior to germination that various storage periods of pre-chilling had no influence upon germination enhancement of seeds. Exposure of seeds to -18℃ inhibited germination in comparison to the control. There was no significant increase in germination percentage after cold treatment. Maximum improvement being achieved when seeds were kept dry under alternating cold/ warm temperatures for 8days at two day intervals prior to germination, since germination percentage increased to 73%. A significant promotion was recorded when seeds treated with 100 mg/l GA3 prior to germination and that pre-chilled treatment improved germination percentage compared with those un-chilled the maximum (70%) at 100 mg/l of GA3.The effect of nutrient media on seed germination was studied on MS and B5 media after being stored for one week in refrigerator (at 4°C) or in the freezer (-18°C). The germination percentage of *Leucaena* seeds cultured on MS medium showed a significant increase over the control for those stored one week at 4°C, when the effect of B5 medium on seed germination was found to be similar to the control (50%) for seeds stored one week at 4°C. These results demonstrate that alternating temperatures (cold/warm) provide faster and highest germination percentage and could be secured at low cost which would be adequate to large scale treatment of *Leucaena leucocephala* seeds.**

المستخلص

تستخدم شجرة الليوسينا للاغراض الزراعية في عدة مناطق من حوض البحر الابيض المتوسط. تدخل بذور هذا النوع في طور السكون مما يسبب انخفاض قابليتها على الانبات تحت الظروف الطبيعية. اجريت عدة تجارب لمعرفة تاثير بعض المعاملات على انبات بذور الليوسينا. بلغت نسبة انبات البذور غير المعاملة 48%، وازدادت هذه النسبة عند تخديش البذور. في حالة خزن البذور الجافة بدرجة حرارة 4م لمدة يومين، ثلاثة او اربعة ايام بلغت نسبة الانبات 60% الا ان خزن البذور في درجة حرارة (-18م) انخفضت نسبة الانبات مقارنة مع معاملة المقارنة. وقد تبين عدم وجود علاقة معنوية بين فترة الخزن على درجات الحرارة المنخفضة وبين نسبة الانبات .

Key words: ***Leucaena leucocephala*,seed germination, Pre-chilling, mechanical scarification, GA3, MS**

تم الحصول على اعلى نسبة انبات عندما خزنت البذور الجافة على درجات حرارة باردة ودافئة على التوالي ولمدة 8 أيام (2/2 يوم) قبل اجراء عملية الانبات. كما كانت هنالك زيادة معنوية في نسبة الانبات عند خزن البذور على درجة حرارة 4م قبل زراعتها على الوسط الغذائي او اذا تم تنقيعها في محلول الجبرلين بتركيز 100%. اظهرت النتائج بان استعمال درجات الحرارة الباردة والدافئة على التوالي اعطت نسبة انبات اعلى واسرع من المعاملات الاخرى، وهذه الطريقة من الناحية العملية تعتبر سهلة وغير مكلفة ولاتحتاج الى جهد عند اكثار النبات على مساحات واسعة .

Introduction

*Leucaena leucocephela*is a plant known as the miracle tree because of its worldwide success as a long-lived and highly nutritious forage tree, and for its wide assortment of uses compered to all tropical legumes [5].In addition it can provide shade and erosion control, capability of surviving in dry regions with poor quality soils because of its nitrogen fixating capability [1,2], it has the ability of being cultivated around roadsides and industrial areas for decorative intentions [3]. The wood, leaves and twigs have a medicinal value [4].The success of plantation program largely depends on prompt, germination of seeds, growth parameters and even on the containers in which seeds are sown [5].Seed germination is a critical reproductive stage. Seeds of *L. leucocephala* were rubbed with sand paper to soften the seed coat [6]. It was reported increased percentage of germination in *leucaena* with grind stone scarification and nicking[7].Villiers [8] suggested that soaking of *L. leucocephela* seeds in 70 ℃ water for 20 min is effective treatment to break seed dormancy and enhanced seed germination of this species. Soil erosion and loss of soil fertiustity and finally the lands are getting barren, researchers have shown that declines in soil fertility due to land degradation can be checked and soil sustainability can be maintained by planting nitrogen fixing tree species like *Leucaena leucocephala*[9].

Present investigation was made to find the suitable method to germinate *l.leucocephala* seeds in low costs. Thus using legumes as alternative fodder resources for livestock (where cost of concentrated feed is a limiting factor in Iraq) is prerequisite for improvement and development of livestock. Besides using this tree as immediate plantations is essential for stability of soils and protects the country from unexpected cyclone and other natural calamities.

Materials and Methods

Plant material

Seeds of *L. leucocephala* were obtained from mature trees grown in the gardens of Al-Nahrain University. All germination tests were conducted at 25°C in controlled environmental chamber using 9 cm diameter glass petri dishes lined with 1 sheet of filter paper, four replicates of 25 seeds each were used and 10 ml of distilled water was added to each dish. Germination counts were made daily. Seeds were considered to be germinated when the radical was emerged from the seed coat [3]. The data obtained was subjected to the statistical analysis.

In an attempt to study the effect of seed size upon germination, seeds were separated into three classes, large (9*5mm), medium (7*4mm) and small (6*3.5mm). The average weight of one seed of each class was 62.7, 43.7 and 26.6 mg for the large, medium and small respectively. Germination tests of the three classes separately indicated that size, in general, has no effect on germination.

Attempts were made to increase the germination of *leucaena* seeds (using seeds of nearly similar size) a number of chemical and physical treatments were tried including:-

1. Effect of mechanical scarification using sand paper or cutting the seeds with hand pruner at the end opposite the micropyle.
2. Pre-chilling treatments: seeds were put in refrigerator at 4°C or in a deep freezer at -18°C for 2,3 and 4 weeks, or subjected to alternating temperature (4°C / 25°C) for 8 days at 2 / 2 day intervals.
3. Pre-chilling the seeds for one week at 4°C and soaked for 24hrs. with gibberellic acid (GA3) using concentrations of 50, 100 and 150 mg/l prior germination.
4. Pre-chilling the seeds for one week at 4°C or -18°C prior to germination on MS or B5nutrient medium.

Results and Discussion

The response of *Leucaena leucocephala* seeds to mechanical scarification is shown in Table (1). The table indicated that there is a significant increase ($p \leq 0.05$) in germination percent of seeds scarified with sand paper (69%) but not with that of hand pruners (48%) in comparison to the control. These results show that germination was improved when the seed coats were damaged. The restricting effect of the seed coats on gas exchange is one of the factors imposing dormancy on the seed which can be released by cut or scratches the coat. In this respect, it was showed that oxygen is necessary for the enzyme oxidation of endogenous germination – inhibiting substances in the embryo tissues [8,10].On the other hand, the effect of seed coats on water absorption during germination is also important in terms of seed dormancy. Other investigators support the present evidence that seed coats do provide a substantial although not total barrier to water uptake necessary for germination [11, 12, 13].

Table (1): Effect of mechanical scarification on germination percentage of *Leucaena leucocephala* seeds.

	Treatment		
Days	**Control**	**Scratched with sand paper**	**Nicked with hand pruner**
2	**8**	**24**	**13**
3	**14**	**33**	**26**
4	**18**	**42**	**40**
5	**26**	**50**	**46**
6	**38**	**66**	**46**
7	**47**	**69**	**48**
LSD 0.05	**8.25 ***	**9.69 ***	**8.04 ***

Germination of *Leucaena* seeds as affected by pre-chilling temperature is presented in fig.1. The figure illustrates that seeds kept dry in refrigerator at 4°C for 2,3 or 4 weeks, their germination was 60,55 and 60% respectively. However, seeds stored in freezer at -18°C for the same periods and under similar conditions germinated very poorly (30, 20 and 20% respectively) in comparison to the refrigerator treatment and to the control as well. These results suggested that very low temperature -18°C probably delay the physiological changes which occur within the embryo during cold storage at higher temperature 4°C and delay production of germination stimulators [14]. Furthermore, the present data figure (2) indicated that various storage periods of pre-chilling had no influence upon germination

enhancement of *Leucaena* seeds. Possible explanation for this behavior is that seeds might require only certain period of cold temperature before germination achievement [15].

Further experiment was conducted where the seeds kept dry under alternating temperature (4 / 28 °C) at 2/2 days interval for 8 days. The data in figure (1) indicated that number of germinated seeds increased to 73% in comparison to 48% of control. These results probably related to the differential sensitivity of the various enzymes and their precursors within the embryo to alternating temperatures[10], and probably be that the pre-chilling plays a vital role in the number of days that will result in the highest percentage germination [16].

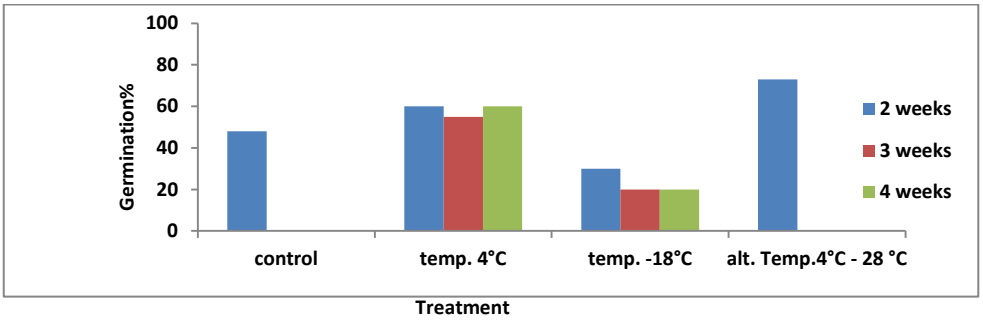

Fig (1): Germination percentage of *Leucaena leucocephala* seeds as affected by cold storage treatments and alternating cold / warm temperatures prior to germination under control condition.

Thereby supporting the results from the first test reported earlier in the present paper, *Leucaena* has an impermeable seed coat, which could breaks down after exposure to cold or fluctuating cold / warm storage temperature over a period of time to allow germination as shown in fig. 2. It was reported that, breaking dormancy of pistachio is probably related to the chilling treatment, and that chilling is concerned with food mobilization and their transfer to the embryo [15]. Food transfer to the embryo resulted in the development of sufficient osmotic and imbibitions forces to enable the embryo to overcome the mechanical resistance of seed coats.

Fig (2): Germination of *Leucaena* seeds as affected by alternating temperature (kept dry in refrigerator at 4 / 28 °C at 2/2 days interval for 8 days).

The effect of exogenous application of gibberellic acid (GA3) on seed germination enhancement is shown in Table (2). Which shows that seed germination was higher when the test was conducted under laboratory conditions than at constant temperature? The table also showed that pre-chilled treatment rose germination percentage of the seeds over those of un-chilled. It was reported earlier that gibberellins are apparently absent from un-chilled dormant seeds but became detectable after 6 weeks of chilling at time seeds were able to germinate. The effectiveness of GA3 in breaking dormancy and accelerating germination

indicates that a chemical inhibitor may have been present and that GA3 was effective, regardless of seed moisture content [17]. Pre-chilled seeds treated with 50 mg/l of GA3 had moderate promoting effect (7% germination over control) as the test conducted under laboratory condition. Soaking pre-chilled seeds with 150 mg/l GA3 induced higher value of germination (12% over control) and maximum reached (70%) at 100 mg/l GA3.

It is assumed, therefore, that 100 mg/l GA3may be adequate to initiate the reaction necessary for germination, whereas the other concentrations were either inadequate (50 mg/l) to promote germination or inhibited germination due to high concentration (150 mg/l) leading to an alteration in the balance of promoters and / or inhibitors [18,19].

Table(2): Effect of gibberllic acid (GA3) treatment on germination percentage of *Leucaena leucocephala* seeds after being pre-chilled for one week at 4°C

GA3 con. (mg/l)	Without chilling		Pre-chilled	
	Constant temp. 25 °C	Lab. Temp. 14-26 °C	Constant temp. 25 °C	Lab. Temp. 14-26 °C
50	31	47	40	55
100	43	52	60	70
150	20	40	30	60
LSD 0.05	6.24 *	5.49 *	7.50 *	5.27 *

Results of other experiments where seeds were cultured on MS or B5 media after being stored for one week in refrigerator at 4°C or in the freezer -18°C are shown in fig. 3. There were highly significant differences ($P \leq 0.01$) between seeds stored in refrigerator and those stored in freezer irrespective of the type of media (MS or B5) used in relation to germination. The results indicated that seeds pre-chilled at 4°C exhibited higher germination percentage than seeds freeze at -18°C where the extent of their enhancement in both media was 30%. These results probably related to the exposure of seeds to low temperature than seeds usually experience in nature. Furthermore, the germination percentage of *Leucaena* seeds cultured on MS media showed a significant increase over the control (fig. 3). This increase in germination could be related to the availability of nutrients in the surrounding tissue of the embryo [20]. The effect of B5 media on seed germination was found to be similar to the control with a slight difference (10%) which might be attributed to the absence of some essential germination stimulating substances or hormones in B5 as compared with MS medium [8].

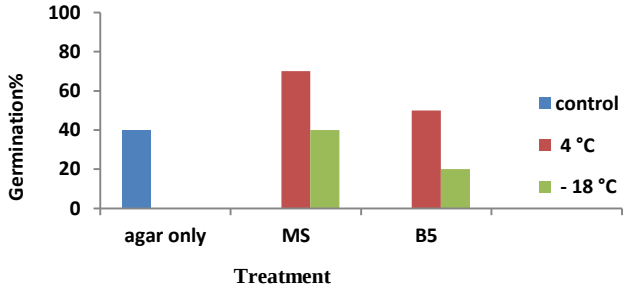

Fig.(3): Germination percentage of *Leucaena leucocephala* seeds subjected to cold treatments (4 and -18°C) for one week prior to germination under control conditions on agar treated with two types of nutrient solutions (MS and B5). No seeds germinated after one week.

On the basis of above results, it is concluded that dry storage at alternating cold / warm temperature is most economic and easy technique, which provides faster and higher germination as compared to other treatments mentioned above.

References

1. Tadros, M. J., Samarah, N. H., Alqudah, A. M. (2011). Effect of different pre-swing seed treatments on the germination of *Leucaena leucocephala* (lam.) and *Acacia farnesiana* L. New Forests. 42: 397 – 407.
2. Paul, D., Paul, N. K., and Basu, P. K. (2008). Seed germination response of *Rauvolfia serpentine* Benth. to certain physical and chemical treatments. Bio-Sci. 16:129-131.
3. Salomão, A. N. and Mundim, R. C. (2000). Germination of Papaya Seed in Response to Desiccation, Exposure to Subzero Temperatures, and Gibberellic Acid. Hort. Science. 35(5):904-906.
4. Iqbal, C. M., Huda, S.M., Sujauddin, M., and Hossain, M. K. (2007). Effect of sludge on germination and initial growth performance of *Leucaena leucocephala* seedlings in the nursery. J. of Forestry Res.18 (3):226-230.
5. Ferdousee,N.,Jabbar, F., Hossain, M. K., and Hoque, A. T. (2010). Comparative growth performance of *Leucaena leucocephala* seedlings raised in nursery bed, polybag and root trainers. Proc. of Intern. Conf. on Envir. Aspects of Bangladesh (ICEAB10). Japan.
6. Iqbal, M.Z., Shazia, Y. (2004). Differential tolerance of *Albizialebbeck* and *leucaena leucocephala* at toxic levels of lead and cadmium. Polish J. of Envir. Studies. 13(4):439-442.
7. Pal, A., and Negi, V.S. S. (2012). Efficient *in vitro* regeneration of *Leucaena leucocephala*using immature zygotic embryos as explants. Agroforst Syst. 34:131-140.
8. Villiers, T. A. (1972). Cytological studies in dormancy. I. Embryo maturation during dormancy in Fraxinus excelsior. In: Seed Biology, T. T. Kozlowski (ed). 11, A. P.
9. Nkomo, M., and Kambizi, L. (2009). Effects of pre-chilling and temperature on seed germination of *Corchoruso litorius* L. (Tiliaceae) (Jew's Mallow), a wild leafy vegetable. Afri. J. of Biotech. (6): 1078-1081.
10. Ghadiri, H., and TorshizB. N. (2000). Effects of Scarification and Temperature on Germination of Licorice (*Glycyrrhiza glabra* L.) Seeds. J. Agr. Sci. Tech. 2:257-262.
11. Tadros, M. J., and Al-Mefleh, N. K. (2011). Preliminary Evaluation of Different Water Qualities on *Leucaena leucocephala* Seed Germination and Seedling Growth. World Academy of Sci., Engi.and Tech. 76.
12. Rehman, S. A., and Iqbal, M. Z. (2010). Growth of *Albizialebbeck* (L.) benth, and *Leucaena leucocephala* (LAM.) de-wit, in different soil composition of Karachi university campus soil.J. Of Basic and App. Sci. 6(1):5-12.
13. Mahmud, S., Hoque, A.T., and Mohiuddin, M. (2005). Germination Behavior and Initial Growth Performance of Seven *Leucaena leucocephala* Varieties in

Chittagong University Sites. J. of App. Sci. Res.1 (2):200-204.

14. Bewley, J. D. (1997). Seed Germination and Dormancy. The Plant Cell. 9:1055-1 066.
15. Andriotis,V. M. Smith, S. B. and Ross, J. D. (2005). Phytic acid mobilization is an early response to chilling of the embryonic axes from dormant oilseed of hazel (*Corylusavellana*). J. of Exper. Botany. 56(412):537–545.
16. Padma, V., Satyanarayana, G., and Reddy, B. M. (1994). Effect of scarification treatments on the germination of *Leucaena leucocephala*, *Albizzia lebbeck* and *Samanea samon*. Seed Res. 22:54-57.
17. Shafiq, M., Iqbal, M. Z., Athar, M. (2008). Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala* . J. Appl. Sci. Environ. Manage. 12(2):61 – 66.
18. Bouteau, H. M., and Bailly, C. (2008). Oxidative signaling in seed germination and dormancy. Plant Signaling & Behavior.3 (3):175-182.
19. Gianinetti, A., and Vernieri, P. (2007). On the role of abscisic acid in seed dormancy of red rice. J. of Exper. Botany Advance Access. P:1-14
20. Lepage - Degivry, M. T., Elude en culture in vitro de la dormance embryonnaire *Chez – Taxusbaccata* L. Biol. Plant. 15: 264-269 (1973).
21. Ferdousee N., Jabbar, F., Hossain, M. K., and Hoque, R. (2011). Comparative Growth Performance of *Leucaena leucocephala* and *Gliricidia sepium* Seedlings Raised in Nursery Bed, Polybag and Root Trainers. Inter. J. of Envir. 1(1):14–20.