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Rapid and cost – effective protocol for planting *Arabidopsis thaliana* L. in Iraq

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Abstract

Model organisms are loadstar to the workers in the scientific field, because of its features such as small of chromosomes numbers or short life cycle, in addition it have a complete genomic map. *Arabidopsis thaliana* L. is a member of these groups. This plant is not its exists in Iraqi flora. And because of the difficult and costs of importing seeds from certificated suppliers, this study aimed to put a rapid and cost effective protocol for planting *A. thaliana* in Iraqi laboratories ambient and make it available for plant biology researchers.

The plant seeds of *A. thaliana* (Colombia $_0$) were cultivated in several growth media ; sand – peat moss mix 1:1(V:V), sand – peat moss mix, 1:2 (V:V) and Peat moss only, under growth room conditions; 25±2°C, 8000 Lux and 70% RH). In addition to *Arabidopsis* germination medium as compared medium.

The results indicated that the growth medium of sand -peatmoss at the proportion of 1:2(v:v), was more significantly effective in all growth parameters through vegetative growth, flowering, pods and seeds production followed by sand-peatmoss1:1 (v:v). The second generation of seeds were collected and then tested to ensure its viability.

Key words: Model plants, *Arabidopsis thaliana*, Planting, Seeds production

1- Introduction

- Even though *Arabidopsis thaliana* is described as native plant in Eurasian flora, but it is becoming a remarkable model organism for plant biology**.** *A.thaliana* serves as a focal species for studies of plant physiology, molecular biology and genetics (Dean, 1993; Meyerowitz & Somerville, 1994; Pyke, 1994; Meinke et al., 1998).It has short generation time(6-8 weeks) in the laboratory, thus, Its used as a model species. Also, it gives a large quantity of seeds ,and its reproduction primarily by self-fertilization (Lawrence,1976; Al-Shehbaz & Okane, 2002).*A.thaliana* was the pioneer plant to be fully sequenced (Arabidopsis Genome Initiative, 2000), followed by rice (Yu et al., 2002), poplar (Tuskan *et al.*, 2006). As a member of the mustard (Brassicacea) family *A.thaliana* is closely related to cultivated species, including oilseed rape and cabbages, and offers important advantages for be easily cultivated in restricted space.The plant has a small genome with 100-120 * 10^6 bp compared to other Angiosperm such as tobacco and peas, which have genomes of 1.6 * 10^9 and 4.5 * 10^9 respectively. This facilitates the search for mutations or traits The Arabidopsis genome is about 15 times larger than the E. coli genome and only 8 times larger than the yeast genome, as the reason it has a small genome is because it has fewer duplicate genes (Bennett *et al*.,2003).
- *A.thaliana* is not native to Iraqi flora and there was no evidence of its existence in Iraq. In addition importing the seeds of this plant from certificated supplies is difficult and expensive so this study aimed to put a rapid and cost-effective protocol for planting *Arabidopsis thaliana* in Iraq and make it available for plant biology researchers.

2- Materials and methods

Certificated seeds of *A.thaliana* Col. 0 were brought from Carolina Biological Supply U.S.A and cultured in different types of media cultures as follows:

2-1sand – peat moss mix 1:1(V:V)

2-2-sand – peat moss mix 1:2 (V:V)

2-3Peat moss.

Peat moss either used alone or mixed with sand in proportion mentioned above on volume basis then putted in pots of size10×10 cm and irrigated according to field capacity with Hogland solution. Each pot was provided with five seeds of *A.thaliana* under the growth room condition (25±2°C, 8000 Lux and 70% RH)

2-4*Arabidopsis* germination medium, where brought from Phytotechnology Laboratories Company, U.S.A. the Medium was prepared by adding 12.67g.l^{-1}, pH was adjusted to 5.8 and then 5g.l^{-1} agar was added, heated to 93°C.Then the medium was poured in test tube (10ml/tube) and enwrapped with cotton and aluminum foil. After that the test tubes were sterilized in autoclave for 15 min. (121° C, 0.1 MPa) then cooled to room temperature.

Seeds were sterilized using bleaching fluid (50%) then three rinsed times with distilled water .After that they wear put in alcohol 70% with the addition of 2 drops of tween- 20 for five minutes, then rinsed again with distilled water three times. Each test tube was cultured with 4 seeds and incubated in the growth chamber at 16:8 photoperiod, 25 ±2℃ and 70% RH, for sixty days.

The following parameters wear studied:-

1- Steam diameter (mm) using digital Verniea.
2- Leaf surface area using digital verniea by apply the following equation:

Leaf Area = leaf Length x leaf Width at Center x 0.95 (Robertson & Giunta, 1994)

3- pods number
4- The viability of the seeds produced:

hundred seeds of *A. thaliana* were taken from and planted in four Petri dishes (9 cm in diameter) with the addition of five ml. of distilled water to the filter paper inside each, dish and incubated in the dark at a temperature of (25 ± 2)° C, then the percentage of germination was calculated after 72 hours according to the following equation:

% Germination = Number of Germinated seeds / Total Number of Seeds x 100%.

5-Harvest and seeds storage: mature seeds were carefully collected and packaged in small containers each one with 300 seeds, then stored in the refrigerator for long term storage.

Stastical analysis

All the data were analyzed using one way ANOVA, and means were compared with the least significance difference (LSD) test at P<0.05.

3- Results and discussion

3-1 Growth and development stages of *A.thaliana*

After planting the seeds in the different media, a follow-up was done and the time period for each stage of growth was recorded.Table (1) shows the average times of growth for each stage and for each media of *A. thaliana*. In addition, it shows a comparison among the rates to the rates of the time rates needed for growth in American laboratories according to Boyes *et al*., (2001)

Table (1) Growth and development stages of *A.thaliana*

Stage	Standard Days	Sand - peat moss 1:1	Sand - peat moss 1:2	Peat moss	*A.thaliana* germination media
Seed imbibition	2	2	2	2	2
Radical emergence	4.3	3*	3*	3*	3*
Hypocotyls and cotyledon emergence	5.5	5	4*	5	5
Cotyledons fully opened	6	7**	6	6	6
2 rosette leaves	10.3	11*	10	10	9*
4 rosette leaves	14.4	15	14	16**	12

6 rosette leaves	18.4	19	18	20**	16*
8 rosette leaves	20	21**	20	21**	18*
10 rosette leaves	21.6	24**	23**	25**	27**
12 rosette leaves	23.3	29**	25**	37**	30**
Inflorescence emergence	26	37**	28	40**	31**
First flower buds visible	30	43**	32	43**	35**
First flower open	32	45**	34	47**	37**
Silique ripening	43	50**	40*	54**	47**
First silique shattered	48	53**	43*	53**	50**
Senescence complete ;ready for seed harvest	ND***	75	75	70	60

* Mean decrease from the standard rate** Mean more than the standard rate

*** Mean not determinate

3-2Effect of cultivation medium type on stem diameter of *A.thaliana*

As shown in figure (1), that *A.thaliana* have significantly the best value of stem diameter in cultivation medium of sand- peat moss (1:2) with 1.15mm as compared with 0.8 mm in sand –peat moss (1:1) or 0.7 mm in peat moss alone .While the lesser value of diameter A. artificial germination medium been in (0.48mm).

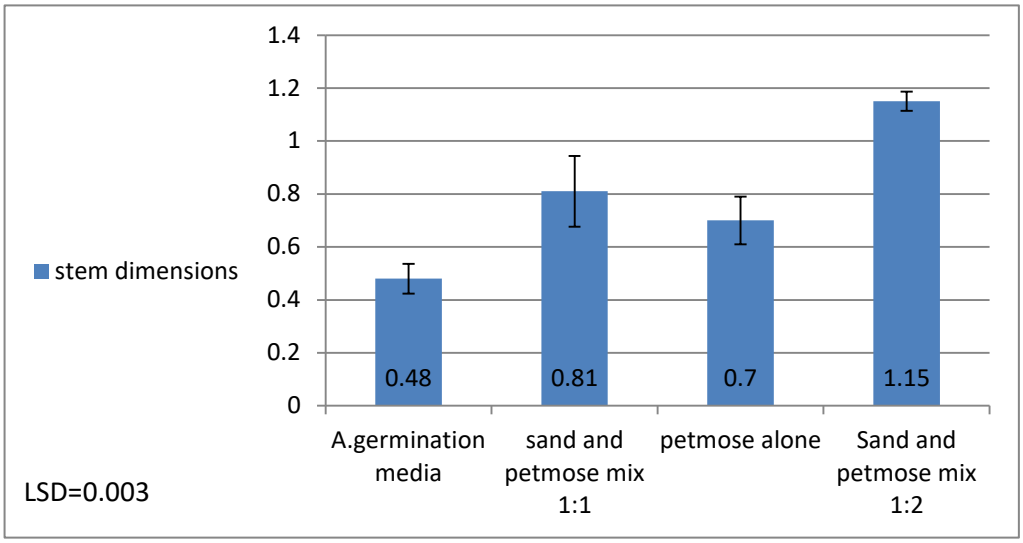
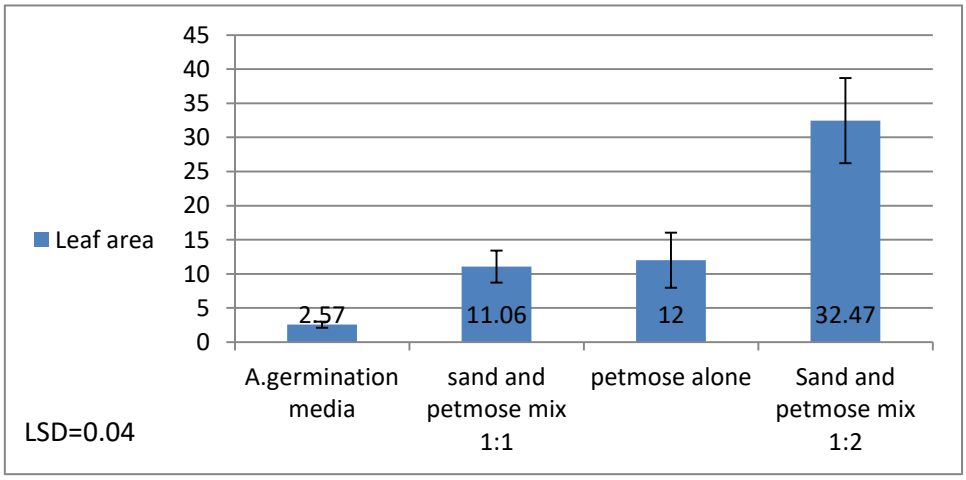


Figure (1) effect of cultivation medium type on stem diameter

3-3Effect of cultivation medium type on leaf surface area of *A. thaliana*

The results shown in figure(2)indicate that *A.thalina* grown in sand-peat moss(1:2) had significantly exceed the other cultivation media in leaf surface area mean .where it was 32.47 cm^2as compared with 12cm^2 ,11.06cm2 and 2.57cm2 in peat moss alone ,and sand-peat moss(1:1) and A. artificial germination medium respectively.



Figure(2) effect of cultivation medium type on leaf surface area

3-4Effect of cultivation medium on pods number (per plant)of *A.thaliana*

Figure (3) expressed that *A.thaliana* grown in sand Peat moss 1:2 significantly produce pods number more than other cultivation medium as follow10.6 pods for sand-peat moss (1:2), then 6 pods in sand peat moss(1:1), 5.2 pods in peat moss alone and 5 pods in artificial germination medi

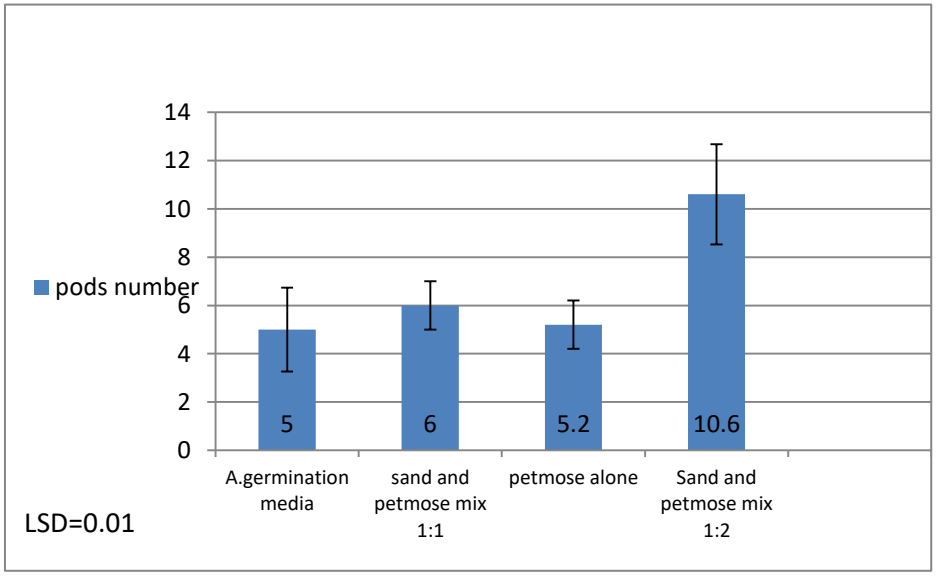


Figure (3) effect of cultivation medium type on pods number

A.thaliana developed well in combination of sand-peat moss (1:2)The most likely cause it was an imitate to the native growing and thrive medium .It was an successful protocol to grow *A.thaliana* and getting viable, true to type and seeds ready to be used by Iraqi botanists.

3-5 The viability of the seeds produced

The percentage of germinated seeds was 96%

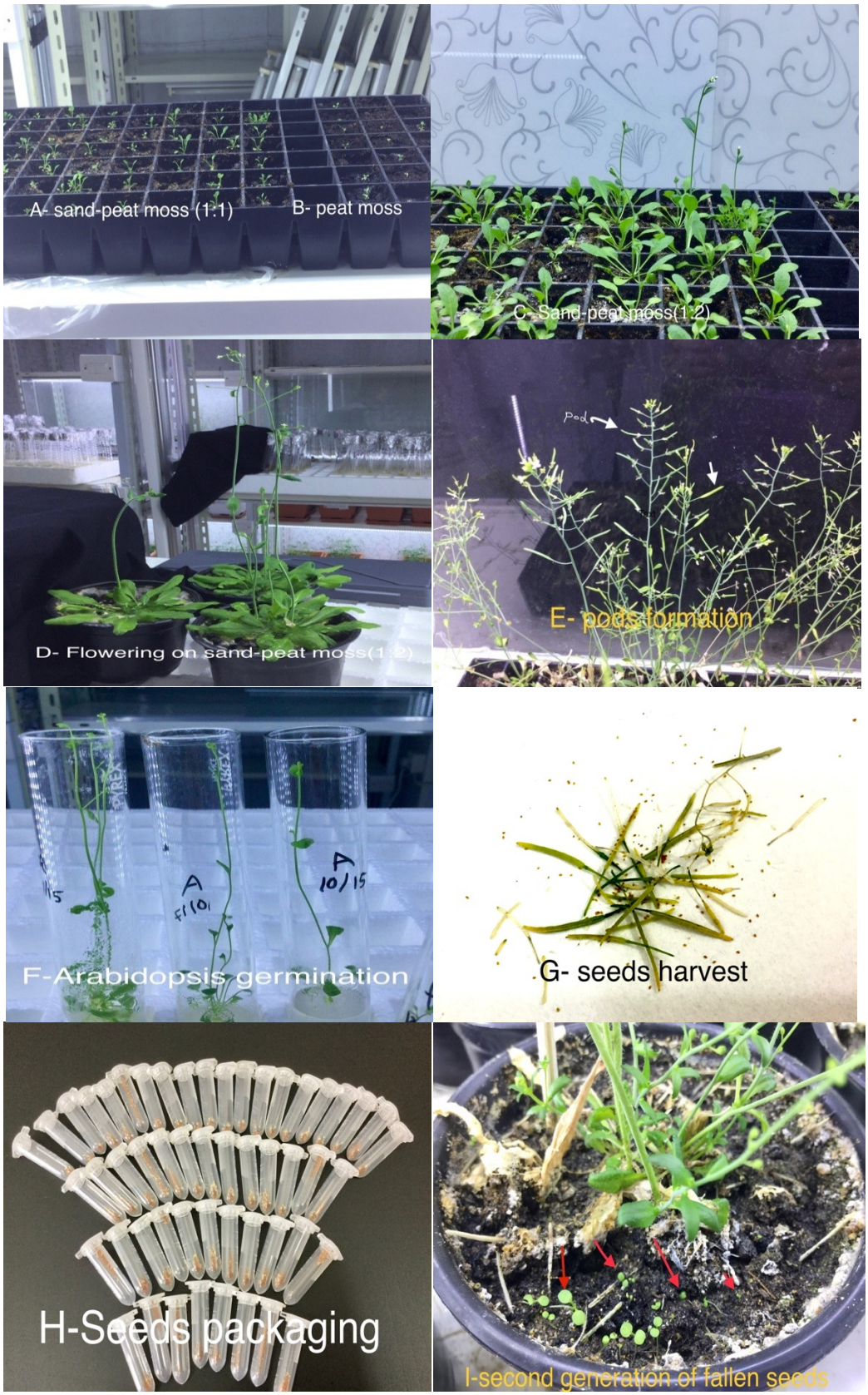
Growth Indicators / media	Leaf area	stem dimensions	pods number

Conclusion

Our results indicated that using a mixture of peatmoss - sand at 1:2 volume basis under laboratory condition of 25±2 ℃, light intensity of 8000 Lux and 16:8 light /dark photoperiod were suitable for the growth, development and mass production of viable and true to type seeds of *A. thaliana* in Iraqi laboratories.

A. germination media	25.7 ±4.4	0.48 ±0.056	5 ±1.73
Sand and petmose mix 1:1	11.06 ±23.4	0.81 ±0.134	6 ±1
petmose alone	12.0 ±40.5	0.7 ±0.09	5.2 ±1
sand and petmose mix 1:2	32.47 ±62.38	1.15 ±0.036	10.6 ±2.08
LSD	0.04	0.003	0.01

Appendix (1) Effect of different media on Leaf area, stem dimensions and pods number



Painting No. (1) includes several pictures:A- plants in sand-peat moss(1:1) B- in peat moss alone C- in sand-peat moss(1:2)D- Flowering on sand-peat moss(1:2)E- pods formation F- plants on A. germination media G- seeds harvest H-seeds packaging I-second generation of fallen seeds.

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طريقة سريعة واقتصادية لاستزراع نبات *Arabidopsis thaliana* L. في العراق

وئام مناضل حسين ولمى حسين عبد القادر وحسين خلف زاير

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الخلاصة:-

ان الكائنات النموذج تعتبر من الجاذبات للعاملين في المجالات العلمية بسبب كونها تتمتع بالعديد من الخصائص مثل العدد القليل للكروموسومات او دورة حياتها القصيرة بالإضافة الى اكمال الخارطة الجينية .وان نبات رشاد الجدران هو احد أفراد تلك المجاميع النموذجية .

ان نبات رشاد الجدران أو أذان الفأر لا يتواجد ضمن الفلورا العراقية وبسبب صعوبة وتكلفة استيراد البذور من المصادر الموثوقة فقد هدفت الدراسة الى وضع بروتوكول سريع وغير مكلف لزراعة النبات في المختبرات العراقية وجعل بذوره متاحة للباحثين في علوم النبات .

تمت زراعة بذور نبات رشاد الجدران صنف كولومبيا في عدة خلطات مثل خلطة الرمل والبتموس 1:1 (حجم :حجم) وخلطة الرمل والبتموس 2:1 (حجم :حجم) والبتموس لوحده تحت ظروف غرفة النمو وهي درجة حرارة 25±2 وأضاءه 8000 لوكس ورطوبة 70% بالإضافة للوسط الخاص بزراعة النبات كوسط مقارنة .

اشرت النتائج ان وسط النمو المتكون من الرمل والبتموس بنسبة 2:1 (حجم: حجم) كان الاكثر كفاءة وبصورة معنوية في جميع مؤشرات النمو اثناء الخضري مثل التزهير وانتاج القرنات والبذور يتبعه وسط الرمل والبتموس بنسبة 1:1 (حجم :حجم) .كما تم جمع البذور المنتجة وتم اختبارها للتأكد من فعاليتها .

الكلمات المفتاحية :نباتات التجارب ،أرابيدوبسس ،استزراع ،أنتاج البذور