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Invitro propagation of selected medicinal plants species

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ABSTRACT

The present studies carried out on *Thymus vulgaris*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Ocimum basilicum* and *Ocimum americanum* which showed the effect of different growth hormones (T1: GA3 1ml, T2: BAP 1mg, T3: Agar 5gm/l, T4: Kinetin 1mg/l + 0.3mg/l. GA3 (semi solid media) and T5:MS control) on plant height, number of nodes, number of shoots, number of roots and number of leaves. The shoot tips and seeds were used as explants, which were cultured in all the five different Media. GA_3-1ml showed best response in the multiplication of shoots and plant height, Agar 5gm/l showed best response in root production, semi solid media is best for No. of nodes and No. of leaves as compared to MS control. Afterward adapted and transplanted *invitro* derivative plants were found 100 %strong in *in vivo* environments.

Contribution/ Originality
In the present study, optimized protocol for micro propagation of selected plants provide new means of conserving and rapidly propagating valuable, rare, and endangered medicinal plants and can satisfy the demand of expanding markets for plant-based medicines and the need to protect medicinal biodiversity under disease free state.

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1. INTRODUCTION

For the pharmacological trade and traditional medication medicinal plants are an important foundation of complexes and in emerging countries about 80 percent of the population still use out-dated medicines derived from plants (Cunningham, 1993; De Silva, 1997). Tissue culturing is defined as the competency of plant cells besides tissues emerging up to roots into entire fresh and new plant termed as tissue culturing (Fowler *et al*., 1993). Several plants under certain conditions do not produce flower and seeds or have lengthy time period of growth and in multiplications. Micro propagation is superlative for to supply regular pharmaceutical plants with in fewer space plus time interval (Prakash and Staden, 2007). For giant scale of plant duplication the tissue culture machinery is being broadly recycled and used. In quite little period of time and with beneath explicit surroundings plus nevertheless of a season, solitary explant can be bourgeoned into numerous thousand plants and undertake on a year plump source (Idu *et al*., 2005). In this research work by using nodal explant and seed we recognised a reliable plantlet regeneration practice for extensive production of five medicinal plants species and also the five different medium effect used during experiment for stretched or long term germplasm stowage in *in air-*conditioning behalf of preservation or conservation. In our present work the proprieties which were obtainable should be developed a prised amount of imminent energies at inherited/organic enrichment of these imperative certain medicinal plants.

2. METHODOLOGY

2.1. Materials

In vitro propagation of five medicinal plants species (*Thymus vulgaris*/ Thyme, *Lavandula Angustifolia/*Lavender, *Rosmarinus officinalis/* Rosemary, *Ocimum americanum/* Lime basil) and *Ocimum basilicum*/ Italian basil) were carried out in Plant Genetic Resource Institute (PGRI) in*in vitro* lab at National agricultural research centre (NARC) Islamabad. Mature seeds of *Ocimum basilicum* and *Ocimum americanum* were used as a source of ex-plant where from healthy ex-plants (5-6cm) of *Rosmarinus officinalis*, *Thymus vulgaris* and *Lavandula angustifolia* were used as explants for *invitro* propagation.

2.2. Study design (CRD)

Experiment was achieved by using Complete Randomize Design (CRD). Individually each experiment was repeated three times.

2.3. Ex-plant preparation and pre-soaking of seeds

Leaves present on the herbaceous part of stem were removed. The excised stem was further cut into different segments of 1 cm each. By adding a pinch of detergent and two drops of tween-20 solution (1 drop for 20 ml), shacked for 1-2 minutes, the explant segments and seeds were surface sterilized. Within different beakers the same explants and seeds were then put under running tap water separately for 30-45 minutes. The seeds were washed to remove the gelatinous materials. The nodal segments were then surface-sterilized with 70% ethanol for 3 minutes and in 28% Clorox for 15 minutes and lastly washed 4 or 5 times with autoclaved disinfected water in laminar flow chamber while seeds were pre-soaked in distilled water and 1% GA3 for 72 hour and then disinfected with 70% ethanol for 1minute then in 5% sodium hypochlorite for 10 minutes and then wash systematically with distilled water.

2.4. Culturing

The sprout or shoots tips/tops and seeds were cultured separately on MS medium comprising 30gram sucrose and different concentrations of growth regulators. Five MS media were prepared, T1: MS media with GA3 at the rate of 1ml/l., T2: MS media containing BAP at the rate of 1mg/l., T3: Only MS media., T4: MS media with agar at the rate of 5gm/l and T5: MS media with Kinetin at the rate of 1mg/l + 0.3mg/l GA3.They served as explant sources for consequent experiments. The pH of all the preferred five medium were familiar or adjusted to 5.8 with sodium

hydrochlorides and hydrochloric acids (NAOH and HCL) formerly crystalizing through 7 per gram agar. All the experimentations used analytical grade chemicals. The ex-plants firstly were inserted perpendicularly on culture medium in test tubes then ploughed tightly withnon-absorbentyarn cotton. The light duration were kept as 25±1 ºC in 16 hours light/2,000 lux with cool white florescent for all the cultures (Ahmad *et al.*, 2003).

2.5 *in vivo* propagation

After *in-vitro* propagation the cultured plants were shifted to *in-vivo*condition (Figure 1)

Figure 1: Medicinal plants in organic media

3. RESULTS

On the basis of organs development after four weeks of culturing of seeds and nodal explants phenotypic parameters were noted (Height of plants, Shoots numbers, Leaves numbers, Roots numbers and Nodes numbers).

3.1. Plant height

Our fallouts displayed that determined height among all the medicinal plants were shown by *Thymus vulgaris*(2.16cm) at GA3-1ml as related to MS control and others growth regulators (Table 1; Figure 2; and 7).

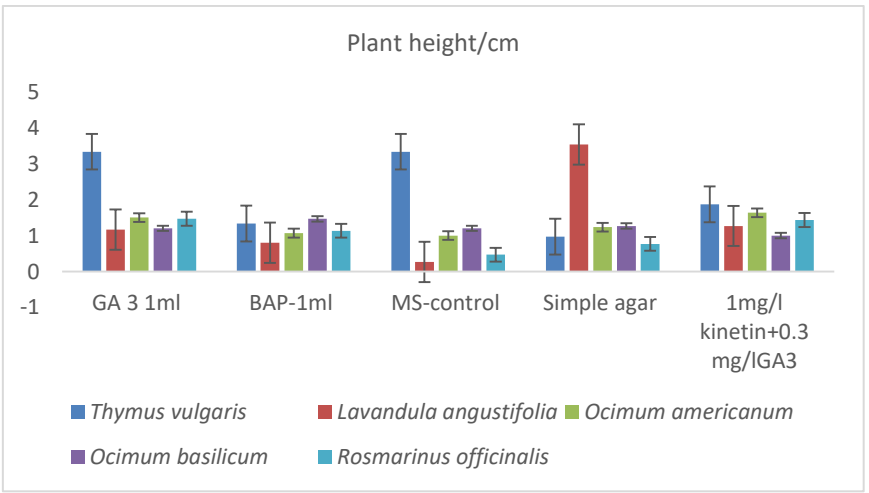

Figure 2: Effects of different hormonal concentrations on medicinal plants height

3.2. No of nodes

Between all the test plants number of nodes were found to be supreme in 1mg per litre kinetin+0.3 mg/l GA3 (semi solid media) in *Thymus vulgaris* (19.06) as compared to MS control and other concentrations (Table 1; Figure 3; and 7).

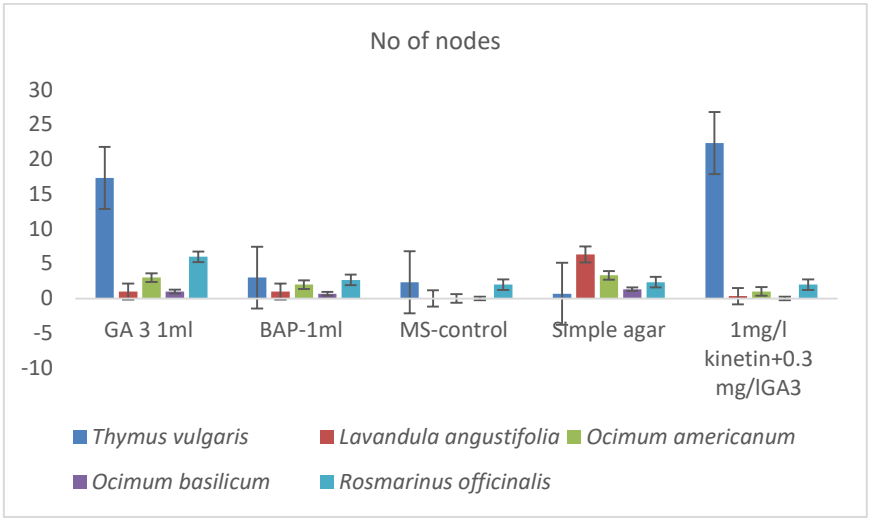

Figure 3: Effects of different hormonal concentration on number of nodes

3.3. No of roots

Ocimum Americanum showed best no of roots in simple agar media (6.7) as compared to MS control and other growth regulators (Table 1; Figure 4; and 7).

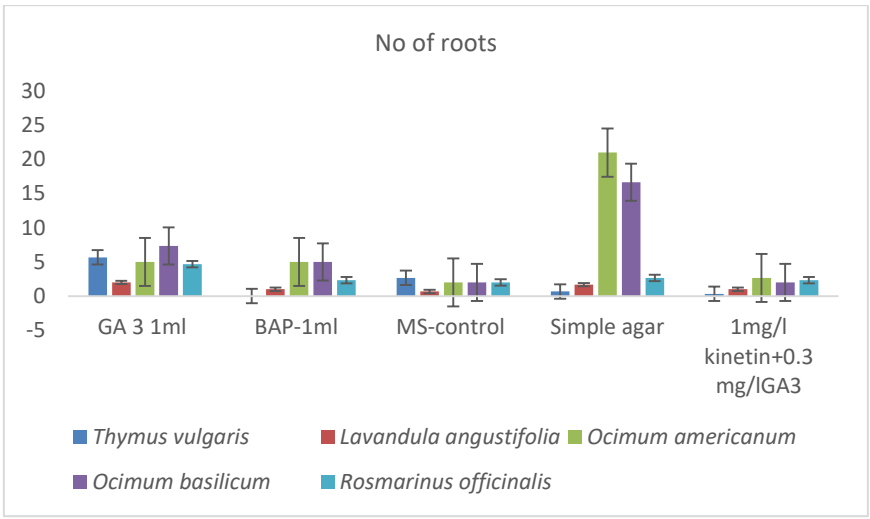

Figure 4: Effects of different hormonal concentrations on number of roots

3.4. No of shoots

Shoots were found best in *Thymusvulgaris* (5.73) in GA3-1ml media as compared to MS control and other concentrations (Table 1; Figure 5; and 7).

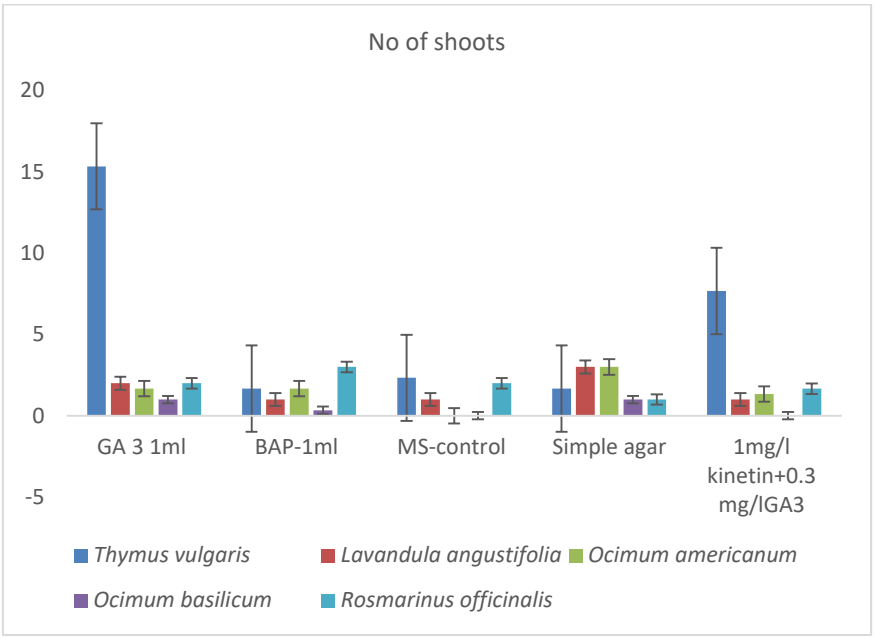

Figure 5: Effects of different hormonal concentration on number of shoots

3.5. No of leaves

Semi solid media (1mg/l kinetin+0.3 mg/lGA3) showed best number of leaves in *Thymus vulgaris* (19.06) as compared to MS control and other concentrations (Table 1; Figure 6; and 7).

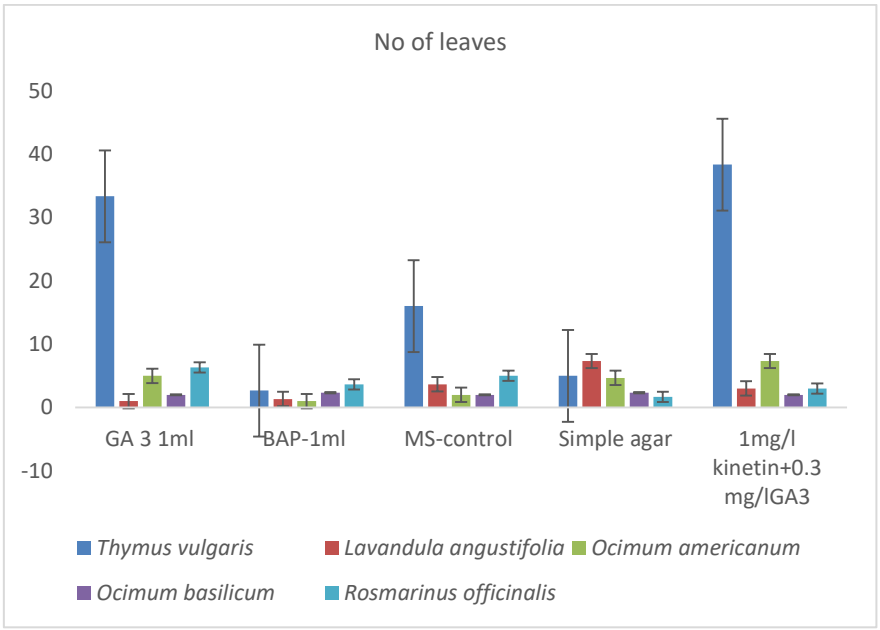

Figure 6: Effects of different hormonal concentration on number of leaves

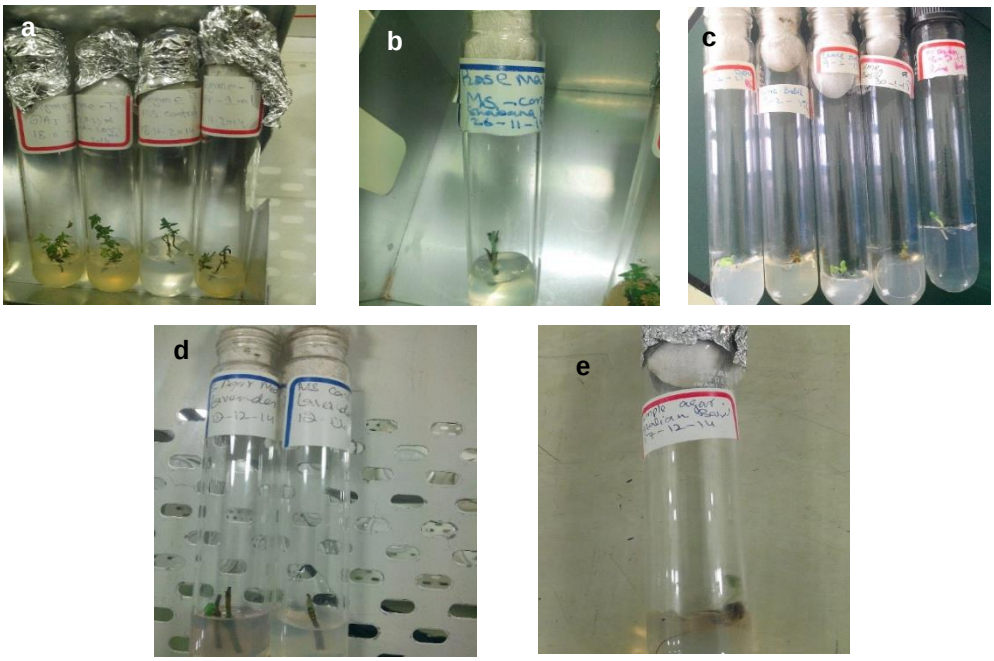

Figure 7: Influence of diverse hormonal applications on

(a). Thymus vulgaris (b). Rosmarinusofficinalis
(c). Ocimum americanum (d). Lavandula angustifolia.
(e). Ocimum basilicum

4. DISCUSSION

Presented work showed that among all the five MS medium includes MS media, MS media with GA3 at the rate of 1ml per litre, MS media containing BAP at the amount of 1mg/litre, MS media with agar 5gm per litre, MS media with Kinetin at the proportion of 1mg per litre plus 0.3mg per litre GA3 were affective for the germination of five medicinal plants. Present work was with in agreement with Aicha *et al*. (2013) while their work showed that great figures of shoots were gotten in GA3 in which media were added with 0.5/1.0/2.5 and 5.0 µM GA3. GA3-1ml media was responsible for maximum plant height while according to Shabnum and Wagay (2011) shoots could be definitely rooted on the MS medium in *Thymus* species with concentration of media is (0.3mg/l IBA + 3mg/l BAP). (Chishti *et al*., 2006) concluded that *lavendula abgustifolia* were showed best shoot production in (BAP 2.0mg/l). According to other works the NAA-2mg/l-1 and KIN-2 mg/l-1 were best for callus growth in *Thymus vulgaris* by former work of (Valizadeh and Kazemitabar (2011). When the GA3 concentrations is increased the inter nodaldistance is also increased which is reported by Ndagijimana *et al*. (2014). Conferring to Al-Bakhit *et al*. (2011) were described that once lavender shoots were refined/cultured at MS medium which is accompanied within 0.4 mg per litre of NAA or at Indole IBA(Butyric Acid) weredeep-rootedhealthy. According to Janarthanam and Sumathi (2012) *Ocimum citriodorum*brought roots/6.0 ± 1.0 onceshifting to partial MS medium perfected with 0.5 mg per litre IBA (Indole-3-butyric acid), however our effectsexposed that enhanced number of roots in *Ocimum basilicum* and *Ocimum americanum*were formed in simple agar media. Our work as compared to other researchers have shown variation in results. This may be due to the collection of ex-plant in various seasons and also the medium used during experiment of *in vitro* (Machado *et al*., 2011)

and also the different ratio of auxin and cytokinin present in media responsible for rooting and the taking of explant (de Klerk *et al*., 1999; de Klerk, 2002).

5. CONCLUSION

In the current study we established the original efficient and consistent micro proliferation etiquette and *in vitro* propagation for *Thymus vulgaris, Lavandula angustifolia, Rosmarinus officinalis, Ocimum basilicum* and *Ocimum americanum* from nodal explants. For huge balance propagation these plants may be recycled and would convert a valued part of approaches for ex situ preservation and conservation of mentioned significant scented and homoeopathic or medicinal herb. It was concluded that GA3 1ml, Semi solid media and Simple agar Media have great effect on organogenesis as compared to other selected media. Plant height and number of shoots were best in GA3 media while number of leaves and number nodes were best in semi solid media (Kinetin 1mg/l+0.3mg/l GA3). Simple agar media was best for number of roots while MS control were best for conservation of plants by keeping their growth in control condition and also should be available for future research studies.

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Contributors/Acknowledgement: Conceived and designed the experiments: Shabana Irum performed the experiments. Shazia Erum and Faisal Nouroz supervised the researcher. Aish Muhammad and Saima Kanwal contributed in downloaded the related literature. Farhat Ali Khan analysed the data (ANOVA and LSD) and Shabana Irum wrote the paper including graphs.

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Appendices

Table 1: All para-wise comparison test for selected medicinal plants

Genotypes	Height/plant	Leaves/plant	Shoots/plant	Roots/plant	Nodes/plant
Rosmarinus officinalis	1.0800b	3.9333b	21.9333b	2.9000bcd	3.0000b
Thymus vulgaris	2.1667a	19.067a	5.7333a	1.8667cd	9.1333a
Ocimum americanum	1.2267b	4.2000b	1.5333bc	6.6667a	1.8667bc
Ocimum basilicum	1.1000b	2.1333b	0.4667bc	5.4000ab	0.6000bc
Lavandula angustifolia	1.4067b	3.2667b	1.6000b	3.4000bc	1.7333bc
LSD	0.4850	4.3839	1.5851	2.6348	2.8538