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Study of vegetative propagation by cuttings of *Thymus satureioides*

Mohammed Karimi, Abdelbasset Berrichi, Azzouz Boukroute

Laboratory of Biology of Plants and Microorganisms, Faculty of Sciences of Oujda, Morocco

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**Corresponding Author. Mohammed KARIMI. E-mail: karimi.ef@gmail.com; Tel: (+212661914619)*

Absract

This work led to the vegetative propagation of *Thymus satureioides*, aims to study the effect of the indole 3-butyric acid (IBA) on rhizogenesis of two cuttings types (basal and summit). This plant species is widely used for its medicinal virtues. An experimental device was installed under glasshouse at the Experimental Station of the Faculty of Sciences of Oujda. The results show that untreated basal cuttings have a low average rate of rooting (11%). Moreover, the hormone treatment shows significant differences in the rooting rate (25%) for high concentrations of auxin (500 ppm). These types of cuttings are considered refractory to vegetative propagation. Besides, the hormone treatment significantly improved the rate of summit cuttings rooting (RR=48% for C_0 and 91% for C_3 (500 ppm)). In addition, the hormone has a significant effect on the qualitative aspects of summit cuttings rooting of *T. satureioides*

Keywords: Thymus satureioides, cuttings, IBA, growth hormone

1. Introduction

Medicinal plants are still an important source of a material for the pharmaceutical industry compressing some 25% of prescribed drugs [2]. In morocco the flora includes over 4500 vascular flowering species of which more than 600 are used in traditional medicine. [5, 11]. Thyme (*Thymus satureioides*) is an aromatic and medicinal plant widely used in traditional medicine and culinary, it is also appreciated for its mellific and pastoral properties. Essential oils of this species are used on an industrial scale in the cosmetics and pharmacy [3, 4]. Also, it is an important source of naturel antibacterial agents as reported by Jasna I. et al., [13], his essential oil is an excellent antiseptic; it proved most effective to have unfertilized microbial cultures at large dilutions in comparison to several medicinal species [26].

In the Eastern region of Morocco, thyme cover very important area in the Ait Serghouchen forest at the eastern High Atlas and prosperous naturally with other species such as holm oak, junipers, rosemary and alfa [1, 14]. The propagation of this species, for reconstitution of degraded ecosystems and recovery of other areas concern so many researchers and developers in order to establish a sustainable management of this natural resource with promising potential.

2. Material and methods

The objective of this test is to study the effect of two cuttings type (basal and summit) and auxin treatment on rooting of *Thymus satureioides*. The auxin is one of the factors for stimulating root product in cuttings [10]. The shoot has been collected from thyme tufts of Ait Serghouchen forest. The stems, which are used for cloning, have to be harvested during the dormant stage from the mother plant [25]. Prepared cuttings, having a length between 5 to 7 cm, were dipped at their bases, for 2 minutes in an indole 3-butyric acid solution (IBA 1%) at various concentrations: C_1 (100 ppm), C_2 (250 ppm), C_3 (500 ppm) with C_0 as untreated control. IBA is known to induce a high number of adventitious roots [10]. It proved as the most efficient treatment and it additionally induced earlier root formation [18]. The cuttings were then planted in a pot (2 x 0.7 m2) filled with a substrate composed of 1/3 peat, 2/3 sand with few quantity of perlite and irrigated in a regular manner. The experimental design adopted set in randomized blocks with 14 cuttings per treatment and 4 repetitions. The experiment was installed under glasshouse at the Experimental Station of Science Faculty

during eight weeks, from the end of the second week of March to the second week of May 2013, the ambient temperatures recorded range from 21°C to 26°C, a suitable condition for rooting [17].

3. Results and Discussion

The results obtained after 8 weeks of experiment shows that the average rooting rate (RR) for the summit cuttings is 73%, it varies from 45% for untreated controls (C_0) to 91% for cuttings treated with high concentration C_3 (500 ppm), when basal cuttings rate varies from 11% to 25% for respectively untreated controls (C_0) and (C_3) concentration with an average rate of 18% (Figure1). Nordine A. [21] report that micro-propagated *Thymus satureioides* plants had a 95% survival rate, and showed vigorous and uniform growth. The exogenous application of IBA generally improves rooting in thymus species [22, 12].

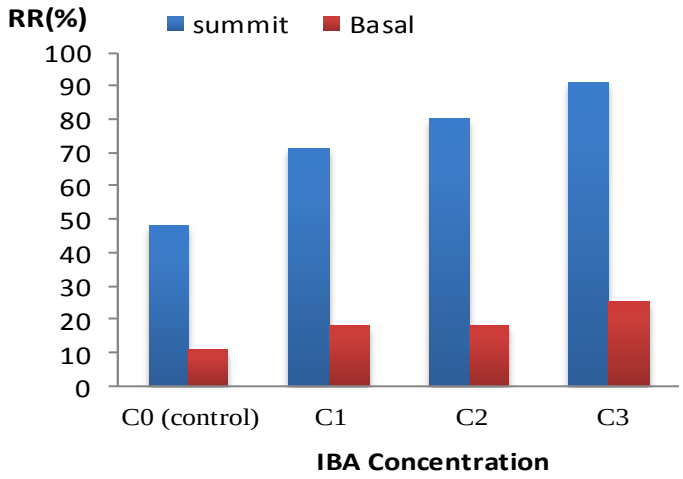

Figure 1: Average rooting rate (RR) for two cuttings types

The analysis of variance with two factors (type of cutting, IBA concentration) shows that the difference is highly significant between the two modality of the first factor (cuttings type) F_{obs} (926.27) >> $F_{calculated}$ (4, 26) at a significance level of 1.1 E -20 (Table 1). This result shows that the rooting ability of *T. satureioides* differ significantly between the two cuttings types. Therefore, the summit cuttings are most adapted to vegetative propagation (average RR = 73%), the basal woody cuttings are considered refractory to this type of multiplication (RR = 18 %). Indeed, gradients of rhizogenesis ability were often observed depending on plant location of the cuttings [17]. Moreover, the study of the second factor shows, that the hormone concentration has a significant effect on the cuttings rooting rate, Fobs (43.82) > F calculated (3,01). Similarly, there is a significant effect on rooting inherent at the interaction between hormone treatment and cuttings types (Table 1). In vitro rooting Nordine A. & al. [20] showed that all tested auxins can induce rooting in *Thymus hyemalis* with a percentage ranging from 81.25% to 100%. So a difference in rooting response is observed between thyme species in several studies. It could be related to multiple factors, such as the genotype, the endogenous cytokinin/auxin ratio, and the influence of shoot multiplication medium and the sensitivity of tissues to absorb or use the exogenous auxin, among others [15, 16].

Table 1: Results of the analysis of variance of two factors

Source of variations	df	MS	F	Probability	critical value for F
Cuttings types	1	472,78	926,27	1,10E-20	4,26
IBA concentration	3	22,36	43,82	6,84E-10	3,01
Interaction	3	6,36	12,47	4,09E-05	3,01
Residual	24	0,51			
Total	31				

Comparison means conducted by student test for hormone concentration of the summit cuttings, shows that at a significance level of 5%, rooting average rates for the three treatments (C_1, C_2, C_3) are individually significantly different from the average untreated cuttings (C_0 control). In addition, and at the same significance level (5%), the difference is significant between the cuttings rooting rate of C_3 and other concentrations (C_1, C_2). However, between these two concentrations (C_1, C_2), the difference observed in the rooting rate is not significant (Table 2).

Table 2: t-Test for means comparison (*CL* = 95%)

IBA Concentration	t (observed value)		t (critical value)	p-value bilateral
	Summit	Basal		
C_0C_1	-5,166	-1,732	3,182	< 0,0001
C_0C_2	-8,332	-1,732	3,182	< 0,0001
C_0C_3	-11,110	-3,464	3,182	< 0,0001
C_1C_2	-2,611	0,000	3,182	< 0,0001
C_1C_3	5,745	-2,449	3,182	< 0,0001
C_2C_3	-4,243	-2,449	3,182	< 0,0001

Thus, the difference between the two concentrations $C_{1\ (100\ ppm)}$ and $C_{2\ (250\ ppm)}$ has no significant influence on cuttings rooting rate; however, at higher concentration of IBA $C_{3(500\ ppm)}$ the rooting rate was significantly improved. Similarly, basal cuttings and at significance level of 5% hormone treatment has a significant effect only for $C_{3(500\ ppm)}$ it means that the induction of rooting for basal cuttings lignified needs high concentrations of auxin. This result shows that mostly, high doses of auxin induced a better rooting. Thus, the scatterplot of the rooting rate depending on the concentration demonstrates clearly this conclusion (Figure 2). Indeed, in practice of the cuttings, meristems initiation is generally favored by a relatively high input of exogenous auxin concentration [17]. Moreover, the difference in rooting ability of treated cuttings can also be influenced by the age difference of mother plants from whom these cuttings were taken. Cuttings from young plants usually have a better ability to rooting than those from older plants [17].

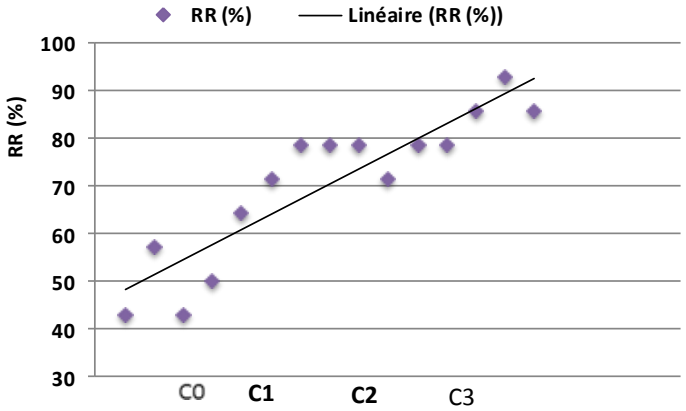

Figure 2: Scatterplot of rooting rate (RR) as a function of concentration

Morphometric study of the aerial part and summit cuttings root, done on a sample of three plants per treatment, shows that plants outcome from cuttings treated with IBA at C_3 concentration are characterized by a more branched root with a larger average length (LR) (Figure 3). In fact, these plants have the highest yields compared to other concentrations, in terms of the number and height of shoots (HS) resulted in a greater aboveground biomass (BR) (Figure 4). IBA also produced stronger shoot growth and the development of an acrobasal type of the rooting system as reported by Mateja et al [18]. IBA has significant effect of the number and height of shoots [8].

The results of the one-way analysis of variance (LR) shows a significant difference between the lengths of the root system (LR) plants observed for four concentrations F_{obs} (5,219)> $C_{\ calculated}$ (2,90) at a significance level of 0,005. Therefore, the hormone has a significant effect on the development and the growth roots rate, expressed by the development of the aerial part observed with a significant difference between the heights shoots (HS) for the four concentrations, F_{obs} (11,80)> $F_{\ calculated}$ (2,90) at a significance level of 0,02 1O-3

(Table 3). Prosad et al [23] report that the application of IBA at 150 ppm resulted an early root initiation (7,8 days), high percentage of rooting (94,3) and more number of roots (13,4) per cuttings of thyme.

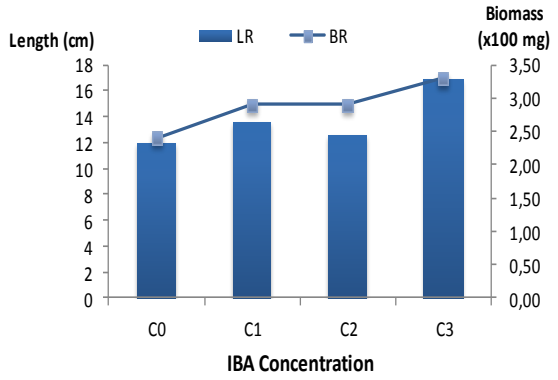

Figure 3: Average length and biomass of the root system of plants

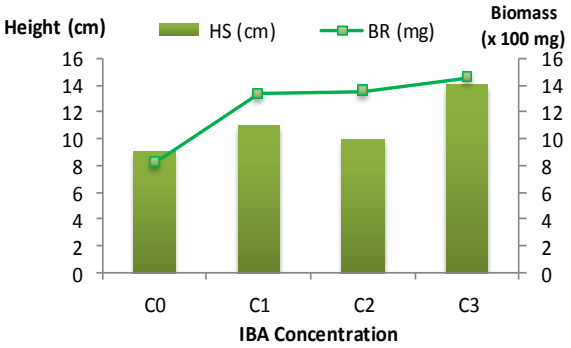

Figure 4: Evaluation of plant biomass after rooting

($C_{3/500ppm}$) ($C_{2/250ppm}$) ($C_{1/100ppm}$) ($C_{1/100ppm}$)

Photos 1: Shoots and Plant roots system of four IBA concentrations

Table 3: Results of variance analysis (root and aerial part growth)

Source of variation	df	CS	CS_R	Fobs	F calculated	Probability
System root length (LR)	3	43,523	8,339	5,219	2,901	0,005
Average height Shoots (HS)	3	44,06	3,73	11,80	2,901	0,00002

Thus, the scatterplot of the shoots height (HS) depending on the length of the root system shows a positive correlation between these two parameters. It means that the development of the root system induced faster growth of the aerial part (Figure 5). The findings from this study agree with Nicola et al. [19] who determined that the use of rooting products had a positive effect on root system development both in terms of root length.

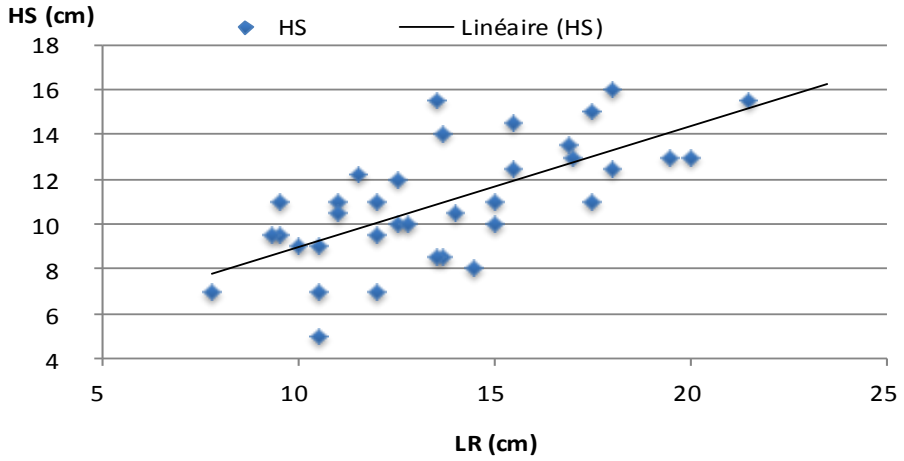

Figure 5: Scatterplot of height shoots (HS) as a function of length roots (LR).

4. Conclusion

The study on the vegetative propagation of *Thymus satureioides* shows that the rooting rate was significantly influenced by the cuttings types (summit, basal) and the hormone treatment (IBA). Basal cuttings have a low average rooting rate (18%), and hormone treatment does not significantly improve their ability to increase until the concentration C_3 (500 ppm). So the inductions of rooting for basal cuttings lignified needs high concentrations of auxin, if not they are considered refractory to this type of multiplication. Moreover, the summit cuttings showed their ability to rooting (RR= 48% for C_0), and was significantly improved by treatment with IBA hormone (RR= 91% for C_3). The hormone has a significant effect on the quantitative and qualitative aspects of rooting cuttings of *T. satureioides*.

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