EFFECTS OF GREEN COCONUT MILK AND ACTIVATED CARBON ON THE *IN-VITRO* MICROPROPAGATION OF TWO IMPROVED *VANILLA* VARIETIES FROM MADAGASCAR « Manitra Ampotony » AND « Tsy Taitra »

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ABSTRACT

On in-vitro culture, green coconut milk is frequently added as a complement or as a substitute for certain phytohormones in the culture medium of orchids as well as activated carbon through its role as an antioxidant. This study concerns the influences of green coconut milk and activated carbon on the in-vitro micropropagation of two improved varieties of Vanilla from Madagascar « Manitra Ampotony and Tsy Taitra ». Different treatments consisting of MS/2 base medium supplemented with 150 ml/l of green coconut milk combined or not with 1 g/l, 2 g/l, and 3 g/l of activated carbon have been studied. The best growth and maximum root production were abtained by combining 150 ml/l of green coconut milk and a dose of 1 g/l of activated carbon. The exclusive presence of 150 ml/l of green coconut milk in the culture medium in both varieties gave maximum root elongation. In the Manitra Ampotony variety, the culture medium with the simultaneous presence of 150 ml/l of green coconut milk and 1 g/l of activated carbon has been shwn to be adequate for phyllogenesis. In the Tsy Taitra variety, the maximum leaf production was obtained with the treatment using only 2 g/l of activated carbon. The optimal in-vitro proliferation of the shoots of these two varieties was recorded in the medium widium without green coconut milk or activated carbon.

Keywords : Activated carbon, Green coconut milk, Microprpagation, Manitra Ampotony, Tsy Taitra, *Vanilla*.

1. INTRODUCTION

In the orchidaceae family, the genus *Vanilla* is the only one used in human food with great economic importance (Joseph, 2012). Madagascar is one of the largest *Vanilla* producers covering about 80% of world production (Raharimanganindriana, 2007). In particular, colossal hydridization work was carried out in Madagascar, at the Ambohitsara station near Antalaha, where more than 30000 hybrids were created and then evaluated in the years 1965-1970 (Dequaire, 1976). Two improved and interesting varieties of *Vanilla* have been selected « Manitra Ampotony » cause of to its higher vanillin content (approximately 6-7%) and « Tsy Taitra » cause of to its more resistant ability to *Fusarium* wilt. They remain among the varieties highly cultivated in the Big Island. Reproduction by the seeds contained in the *Vanilla* is long and uncertain. Vegetative propagation by cuttings is slow and limited due to the limited number of cuttings which could not meet the significant needs demanded by the extension of cultivation and the increased demand for increased production. With the aim of rapid mass production of healthy, good quality plants of these improved *Vanilla* varieties Manitra Ampotony and Tsy Taitra, *in-vitro* micropropagation biotechnology was conducted during this study. Research on matural extracts added as supplements to the culture medium or as substitutes for phytohormones has been carried out in the micropropagation laboratory of the faculty of Sciences (Dinaharilala, 2012 ; Rahelivololona, 2005). Green coconut milk has been frequently added to orchid propagation medium. In addition, activated carbon has been used for its role as an antioxidant (Mazinga, 2014). The effects of green coconut milk and/or activated carbon, used alone or in combination, during the *in-vitro* development stages of two varieties, have been studied in order to establish favorable culture media for each variety.

2. MATERIALS AND METHODS

This study focuses on the two improved varieties « Manitra Ampotony (V1) and Tsy Taitra (V2) ». The explants used are vitroplants, regenerated *in-vitro* by the micropropagation technique at the plant Regeneration and Micropropagation *in-vitro* Laboratory at the University of Antananarivo. They were taken in a very homogeneous way, having a length of 1,5 cm, provided with two pre-leaves. The basal medium of Murashige and Skoog (1962), modified and diluted by half (MS/2) was used. It is supplemented with vitamins and amino-acids (thiamine-HCL, pyridoxine-HCL, glycine, nicotinic acid and myo-inositol), 3 mg/l of benzyladenine (BA) and 0,1 mg/l of indole butyric acid (IBA), 30 g/l (p/v) of sucrose, and solidified with 8 g/l of agar-agar. The Ph of the medium was adjusted from 5.5 to 5,6. The medium is sterilized by autoclaving in the vapor phase at a temperature of 120°C. And under a pressure of 1 barfor 20 min. Three (3) vitroplants were subcultured into each culture dish containing 10 ml of medium. The tests were placed at an illumination of 3000 lux, a temeprature of 24°C, a photoperiod of 16 hours of light and 8 hours of darkness and a relative humidity of 80%. Eight (8) treatments varying according to the presence and concentration of green coconut milk (GCM) and activated carbon (AC) were tested. For each treatment, 4 repetitions were carried out, giving a total of 96 vitroplants per variety. Table 1 presents the different treatments tested. The evaluation parameters relate to the growth in height of the vitroplants, the average number of leaves, shoots and roots as well es the root growth after 10 weeks of culture. The results were statistically analyzed with the XLSTAT software.

Table-1 : Different treatments tested on the micropropagation of the two *Vanilla* varieties « Manitra Ampotony and Tsy Taitra »

Types of backgrounds	Treatments	
	AC (g/l)	GCM (ml/l)
M0	0	0
M1	1	0
M2	2	0
M3	3	0
M4	0	150
M5	1	150
M6	2	150
M7	3	150

3. RESULTS

3.1 Effects of green coconut milk (GCM) and activated carbon (AC) on the height growth of vitroplants

In the two varieties, the height of the tissue culture plants in the two varieties is classified into four significantly different groups according to the treatments. The presence of 150 ml/l of GCM combined with 1 g/l of AC (M5) in the culture medium was very effective on the height growth of the vitroplants. The average height was the highest there, reaching 4,2 cm in the Manitra Ampotony variety and 4,5 cm in the Tsy Taitra variety.

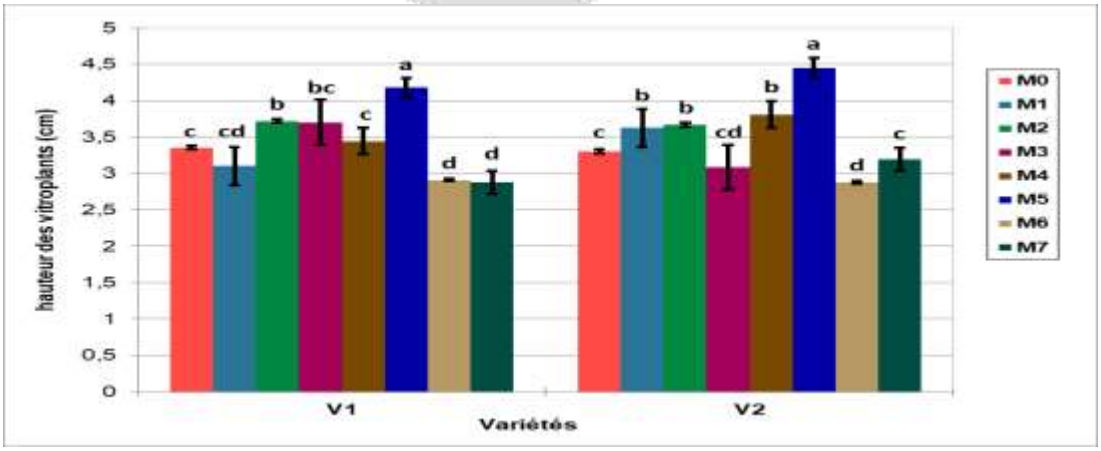

Figure-1 : Average height of tissue culture plants of the Manitra Ampotony and Tsy Taitra varieties after 10 weeks of cultivation according to the treatments

The values followed by the same letter do not show a significant difference according to the Newman and Keuls test at the 5% level.

3.2 Effects of green coconut milk and activated carbon on leaf production

In the Manitra Ampotony variety, all the treatments favored leaf production compared to the control medium. Simultaneous use of 1 g/l of AC and GCM (M5) was appropriate with maximum leaf production (3,1 per vitroplant). As for the Tsy Taitra variety, similar reactions were recorded with respect to the different treatments. However, only the culture medium containing exclusively 2 g/l of AC (M2) gave the maximum mean number of leaves (2,6 per explant).

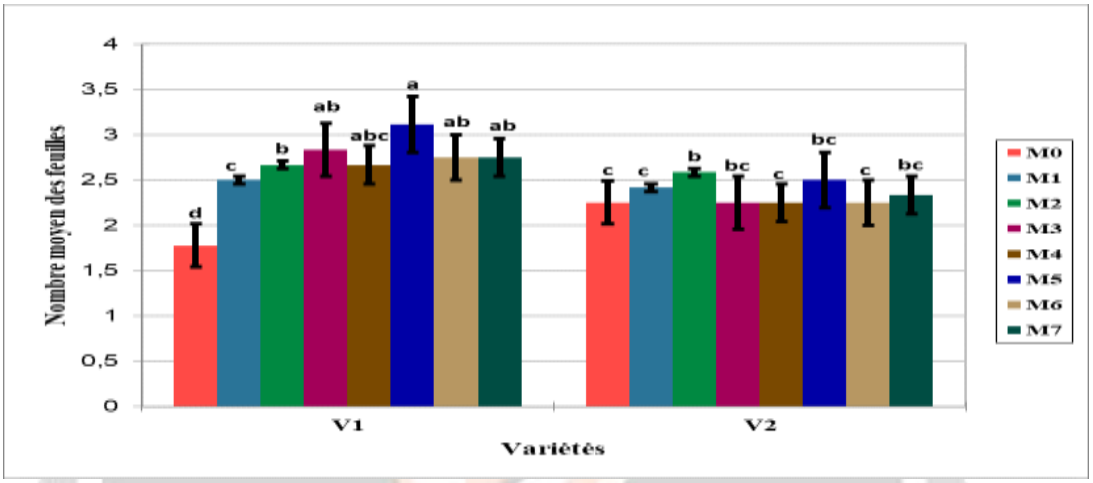

Figure-2 : Average number of leaves produced in the Manitra Ampotony and Tsy Taitra varieties after 10 weeks of cultivation according to the treatments

The values followed by the same letter do not show a significant difference according to the Newman and Keuls test at the 5% level.

3.3 Effects of green coconut milk and activated carbon on shoot proliferation

In both varieties, the addition of AC and GCM negatively influences the production of shoots on cultured tissue culture plants. The maximum production of shoots was recorded in the medium without green coconut milk or activated carbon (M0), respectivevely of 3 shoots for the variety Manitra Ampotony and 3,3 shoots per vitroplants for Tsy Taitra.

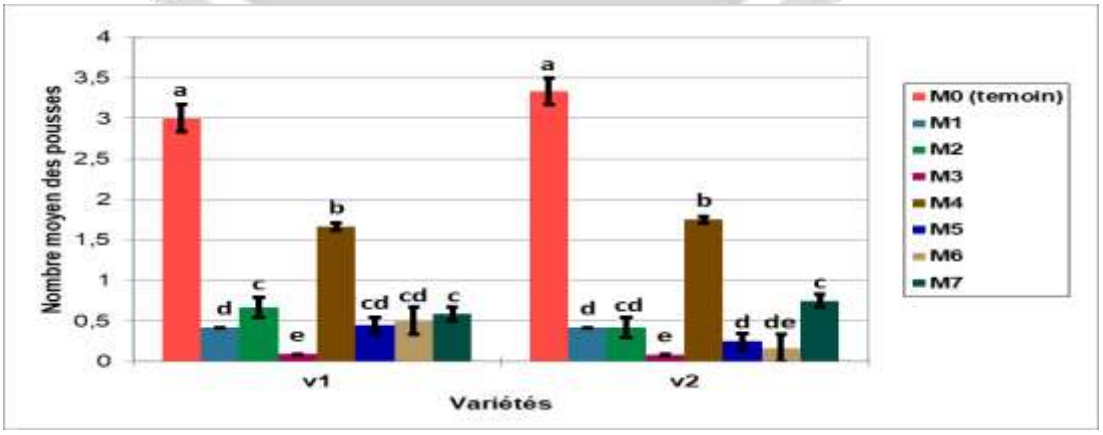

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Figure-3 : Average number of shoots formed in the Manitra Ampotony and Tsy Taitra varieties after 10 weeks of cultivation according to the treatments

The values followed by the same letter do not show a significant difference according to the Newman and Keuls test at the 5% level

3.4 Influence of coconut milk and activated carbon on the number of newly formed roots

The numbers of newly formed roots are significantly different between the treatments in the two varieties. The exclusive presnce of GCM or AC as well as their combination in the culture medium favored rhizogenesis in the two Vanilla varieties. Simultaneous use of GCM with a dose of 1 g/l of AC (M5) remains use the best treatment for rhizogenesis with a maximum average production of root per vitroplant respectively of 3,3 in Manitra Ampotony and 3,1 in Tsy Taitra.

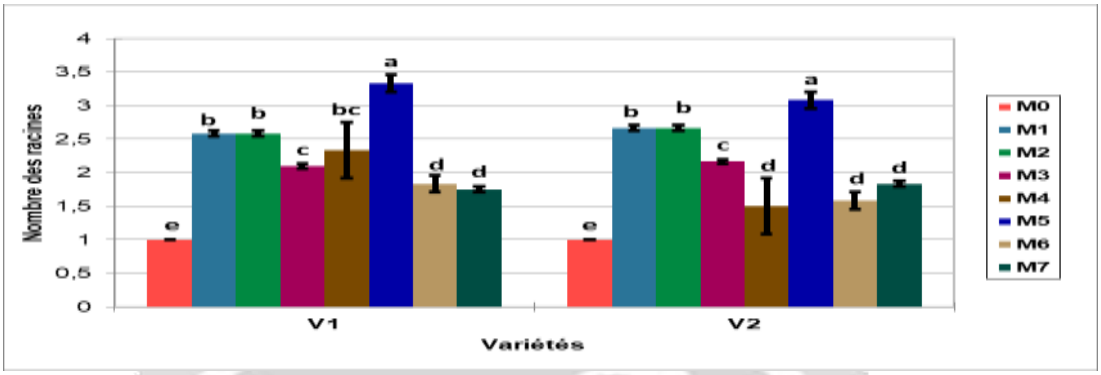

Figure-4 : Average number of newly formed roots in the Manitra Ampotony and Tsy Taitra varieties after 10 weeks of cultivation according to the treatments

The values followed by the same letter do not show a significant difference according to the Newman and Keuls test at the 5% level

3.5 Effects of green coconut milk and activated carbon on root growth

The exclusive presence of GCM or AC in the culture medium favored root elongation. The combination of CA with LCV decreased root growth as the CA dose increased. On the other hand, an inhibitory effect was obseved with the combination of 3 g/l of AC and GCM (M7) in the two varieties. The culture medium supplemented exclusively with 150 ml/l of GCM (M4) gave the best root elongation respectively of 3 cm for Manitra Ampotony and 3,1 cm for Tsy Taitra per vitroplant.

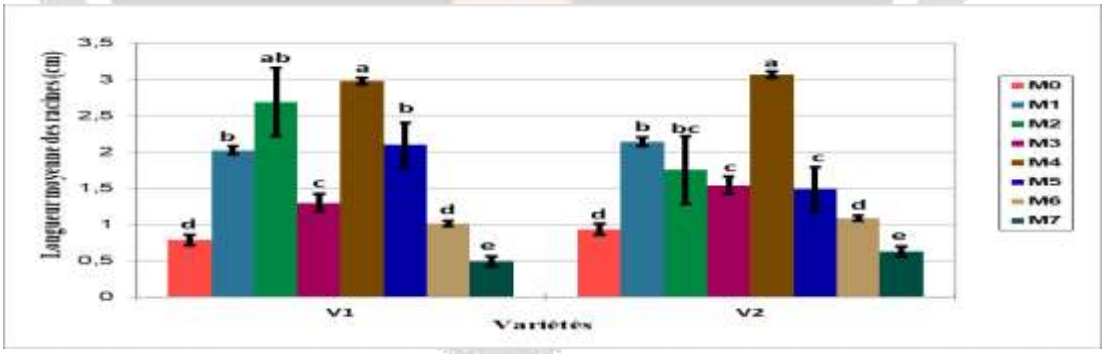

Figure-5 : Average root length of the Manitra Ampotony and Tsy Taitra varieties after 10 weeks of cultivation according to the treatments

The values followed by the same letter do not show a significant difference according to the Newman and Keuls test at the 5% level

4. DISCUSSION

- Regarding the effect of green coconut milk on the growth of vitroplants, the two improved varieties of *Vanilla* studied reacted differently in the presence of green coconut milk (GCM) at a dose of 15 %. GCM showed no effect in the Manitra Ampotony variety, while a stimulatory action was observed in the Tsy Taitra variety. Similar results were obseved by Chenieux and *al*., (1977), according to them, the use of green coconut milk with concentrations varying from 15 to 20% and in the presence of α-naphtyl acétic acid, on the tissue of *Quercus sp*, showed no reaction. But, the tissues of *Robinia pseudoacacia* and *Eucalyptus gomphocephala* in the absence of auxin reacted positively. These results could be due to

the existance of a substance in green coconut milk, including 1-3 diphenylurea, which exerts a stimulating action on the growth of vitroplants tissues (Souidi, 2005). On the other hand, in both varieties, the exclusive presence of activated carbon favored the growth of vitroplants but this depends on its dose. The favorable concentrations are respectively 1 g/l as 2 g/l in the Manitra Ampotony variety, as well as 2 g/l and 3 g/l in the Tsy Taitra variety. According to Kaul, (1990), activated carbon has positive or negative effects depending on its concentration in the culture medium. On the one hand, these results match those of Margara (1989) and Mazinga (2014), according to them, the culture medium containing a concentration of 1 g/l of activated carbon (AC) favored the increase in the size of vitroplants in banana plants. On the other hand, they disagree with those of de Naydenov and *al*., (2006), because the presence of activated carbon in the media showed an inhibiting effect on the growth of jack pine, particularly beyond the equal concentration or greater than 1 g/l. The addition of activated carbon is explained by its capacity to absorb the inhibiting substances diffused in the medium and create an environment favorable to the development of vitroplants (Geerts, 2000).

- Green coconut milk and activated carbon in the culture medium had no effect on the proliferation of shoots per vitroplant in the two varieties. According to Walker and *al*., (1979), the reaction of a cultured explant depends on the auxin/cytokinin ratio in the culture medium. If this ratio is less than one, we obtain the neoformation of buds. On the other hand, if it is greater than unity, we expect the neoformation of roots ; and if this ratio is close to unity, there is callus formation. The results concerning the two varieties could be due to an increase in the BA concentration in the medium. They agree with those of Razafiarison (2010), reporting that the cytokinin including BA only influences bud development and shoot growth in *Solanum tuberosum* species.
- In addition, it appears from this study that activated carbon stimulates rooting in number and growth on both varieties. Our results agree with those of Damiano (1978), announcing that the addition of activated carbon (AC) in the medium had a significant effect on the induction of rhizogenesis in *in-vitro* culture of strawberry. In addition, the exclusive presence of 1 to 2 g/l of AC in the culture medium favored rhizogenesis with optimal root production in both varieties. Similar results were obtained by Bettaieb and *al.,* (2007) on in-vitro rooting tests in the presence of activated carbon at a concentration of between 1 g/l to 2 g/l, those which resulted in a general rhizogenesis of the explants placed in culture. It seems that the realization of an opaque and dark medium thanks to addition of activated carbon would have the effect of imitating the conditions present in the medium where the roots normally evolve (Anonyme, 1999 ; Panis and *al.*, 2002).

5. CONCLUSION

- The addition of green coconut milk and activated carbon in the culture medium improved the growth in height and the in-vitro rooting of the two varieties of *Vanilla* plant studied « Manitra Ampotony and Tsy Taitra ». The eight treatments studied have shown that the appropriate medium for growth of vitroplants of these two varieties is that simultaneously added to 150 ml/l of green coconut milk and 1 g/l of activated carbon. The average heights of the vitroplants were respectively 4,2 cm in the Manitra Ampotony variety and 4,5 cm in the Tsy Taitra variety.
- Regarding phyllogenesis, in the Manitra Ampotony variety the favorable medium for maximum leaf production, on average 3,1 leaves, was that containing 150 ml/l of green coconut milk and 1 g/l of activated carbon, whereas in the Tsy Taitra variety, the culture medium provied with 2 g/l of activated carbon was appropriate with an average production of 2,6 leaves per vitroplant.
- The two varieties have similar physiological reactions regarding *in-vitro* multiplication in the presence of activated carbon and green coconut milk. The addition of two substances to the culture medium inhibits the proliferation of shoots *in-vitro*.
- Regarding rhizogenesis, root production is stimulated by the simultaneous presence of 150 ml/l of green coconut milk and 1 g/l of activated carbon because the average numbers of roots produced there were respectively 3,3 roots in Manitra Ampotony and 3,1 at Tsy Taitra. On the other hand, in both varieties, the optimal growth in root length was obtained by the exclusive presence of 150 ml/l of green coconut milk in the culture medium.

In short, the two varieties have similar physiological reactions in the medium considered favorable, except for phyllogenesis. The addition of green coconut milk and activated carbon is necessary in *in-vitro* growth and rooting media.

6. REFERENCES

- **ANONYME, H., 1999**. Micropropagation for the greenhouse business. Technical reference book. CIDES (Center for Information and l Development Experimenta in Greenhouse).

- **BETTAIEB, T., DENDEN, M., HAJLAOUI, I., MHAMDI, M., METHOUTHI, M., 2007**. *In-vitro* Multiplication and bulbing of G*ladiolus* (*Gladiolus grandiflorus* Hort.). Tropicultura, 25 (4) : 228-231.
- **CHENIEUX, J.C., RIDEAU, M. and GERARD, M., 1997.** Initiation and growth of tissue cultures and cell suspensions of *Choisya ternata* (Rutacées), Bulletin of Society Botanic in France, 124 :5-6, 265-280.
- **DAMIANO, C., 1978**. Il carbone attivo nella coltura *in vitro* de la *fragola. -Frutticoltura*, 40(5) : 49-50. Beltsville, Maryland, USDASEA, Agricultural Research Results, ARR-NE-11, 11-22.
- **DEQUAIRE, J., 1976**. The improvement of Vanilla in Madagascar. Jourmnal of tropical agriculture and applied botanic. 23 (7) : 133-58.
- **DINAHARILALA, M.T.G., 2012**. Substitution of synthetic phytohormones by organic additives on the micropropagation of *Aeranthes antennophora* and *Bulbophyllum peyrotii*, two species of endemic orchids, threatened on the Ambatovy mine site. DEA Mémoir; Faculty of Sciences-Univ. Antananarivo, 47 p.
- **GEERTS, P., SASSI, K., MERGEAI, G. and BAUDOIN, J. P., (2000)**. Development of an *in-vitro* pod culture technique for young pods of *Phaseolus vulgaris* L. *In-vitro* Cell. Dev. Biol. - Plant. **36** : 481-487.
- **JOSEPH, B., 2012**. Presentation during the visit to the greenhouses, 3 March 2012.
- **KAUL, K., 1990**. Factors influencing *in-vitro* micropropagation of *Pinus strobus* L. Biol. Plant 32: 266–272.
- **MARGARA, F., 1982**. The in-vitro multiplication of Orchidaceae. In : the basics of vegetative propagation. Ed-INRA. 142 – 150.
- **MAZINGA, H., 2014**. Effet of activated carbon in the culture medium on the induction of rhizagenesis in the FHIA-01, banana hybrid in *in-vitro* culture ; journal of applied bioscience 76 : 6352-6360, vol. ISSN 1997- 5902.
- **MURASHIGE, T. and SKOOG, F., 1962**. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant, 15 : 473 – 497.
- **NAYDENOV, K., Tremblay, F., Yves, B. Y., Goudiaby, V., 2006**. Influence of activated carbon on the primary growth of jack pine seedlings. Canada. J. For. Res. 36 (3) : 761–767.
- **PANIS, B., STROSSE, H., VAN-DEN, H. S., SWENNEN, R., 2002**. Sucrose preculture to simplify cryopreservation of banana meristem culture. CryoLettres 23 : 375-384.
- **RAHARIMANGANINDRIANA, V. R., 2007**. Malgache Vanilla sector and the growth of export prices ; Master-memoir ; Faculty of Law, Economic, Management and Sociology, Univ. Antananarivo, 46 p.
- **RAHELIVOLOLONA, R., 2005**. Development of vitropropagation techniques for Malahasy orchids : *Eulophiella roempleriana* Rolfe and *Grammangis Lind*. (Rchb). Doctorat ; Faculty of Sciences. Option plant-Physiology, Univ. Antananarivo. 128 p.
- **RAZAFIARISON, N. L., 2010**, Vitropropagation test of *Solanum tuberosum* (solanacées) varieties meva, jengy manga et spunta. Master ; Faculty of Sciences-Univ. Antananarivo, 62 p.
- **SOUIDI, H., 2005**. Contribution to the cloning of racalcitrant varieties in multiplication of *Phoenix* (*Phoenix dactylifera* L.) by somatic embryogenesis. Master II; University of SFAX; 54 p.
- **WALKER, K. A., WENDEL, M. L. and JAWARSKI, E. G., 1979**. Organogenesis in callus tissue of *Medicago sativa*, the temporal repartition of induction process from differentiation process. Plant Science Letters, 16: Pp 23-30.