Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 13 (2): 66-72, 2014
ISSN 1682-3974 / DOI: 10.3923/ajps.2014.66.72

In vitro Micropropagation of Ere Meraat (*Aloe percrassa* Tod.) Using Explants from Offsets

[1]Haftom Baraki Abraha, [1]Desta Berhe Sbhatu and [2]Manikandan Muthuswamy
[1]Mekelle Institute of Technology, Mekelle University, Mekelle, P.O. Box 1632, Ethiopia
[2]Haramaya University, Haramaya, Dire Dawa, P.O. Box 138, Ethiopia

Abstract: The study aimed at developing a suitable and reproducible protocol of *in vitro* micropropagation of *Aloe percrassa* Tod., locally known as ere meraat using explants from offsets. Mother plants were collected from some rural localities in southeastern Adigrat, northern Ethiopia. Murashige and Skoog (MS) media supplemented with different concentrations and combinations of Plant Growth Regulators (PGRs) were used. The best shooting responses with regard to number of days to shooting (14.10 days), mean shoot number (8.60) and mean shoot length (5.20 cm) were recorded on MS media enriched with 1.0 mg L^{-1} benzylaminopurine (BAP) and 0.5 mg L^{-1} α-naphthaleneacetic acid (NAA) after a month of culture. Similarly, rooting response in terms of number of days to root (14.40 days), mean root number (8.40) and mean root length (6.60 cm) were achieved in half strength MS media enriched with no hormones after four weeks of culture. Regenerated plantlets were transferred into different substrates for hardening and those planted to coco peat alone showed 100% of survival.

Key words: Acclimatization, callusing, micropropagation, offsets, rooting, shooting

INTRODUCTION

Aloes also known as lilies of the desert, plants of immortality and medicine plants are native to Africa (Hansen *et al*., 2007). They grow in many parts of Africa, South and Central America, the Rio Grande Valley of South Texas, Florida, Southern California, Mexico, the Pacific Rim countries, India, the Caribbean and Australia (IASC, 2002). In Africa, they grow over much of the Sub-Saharan region with about 155 species in Southern Africa, 145 species in Madagascar, 85 species in Tropical East Africa, and about 76 species in the Horn (Klopper and Smith, 2010). They grow in diverse habitats such as forests, wooded-grasslands, woodlands, rocky expanses, mountains, cliffs, beaches and waterfalls (Dessalegn, 2006; Demissew and Nordal, 2010). According to the most recent and widely accepted taxonomic information, the genus *Aloe* belongs to Aloaceae (Alessandro and Stefano, 2005).

Aloes include about 400 species throughout the planet and so far 46 species are known in Ethiopia and Eritrea (Demissew and Nordal, 2010); Of which 31 species are endemic. *Aloe percrassa* Tod. also known as *Aloe abyssinica* var. *percrassa baker* is one of the species native to Ethiopia. In some rural locations around Adigrat, a northern Ethiopian town from where mother plants for the study were collected. *Aloe percrassa* is known as *ere meraat*; *ere* means aloe, *meraat* means bride. The name is believed to have been derived from the fact that gel of the plant was widely used as traditional shampoo for brides. *Aloe percrassa* belongs to a group of aloes that includes *Aloe debrana*, *Aloe rivae* and *Aloe trigonantha*. It is stemless plant except some old ones developing thick prostrate stems. Its inflorescences have one level of branching with up to more than 50 racemes. It grows in sparsely vegetated rocky slopes and outcrops between 2100 and 2700 m in Tigrai, Gondar, Ethiopia and Eritrea. *Aloe percrassa* flowers in September and October and sometimes in March and April (Demissew and Nordal, 2010). The botanical descriptions of the species are given in Aloes and Lilies of Ethiopia and Eritrea (Demissew and Nordal, 2010).

It is succulent herb, suckering from base to form small groups, commonly stemless but sometimes developing erect or decumbent stem up to 80 cm long, 10-15 cm thick. It's leaves are crowded, 40-55×13-15 cm or larger, glaucous-green or grey-green, often suffused red, old leaves brown when drying. It's marginal spine are 6-16 per 10 cm 2-3-5 mm long, with pale pink to brown tips. It's inflorescence is 60-80 cm high with 5-12 racemes. It's racemes are cylindrical to conical, 6.5-25 cm long, with 2-5 flowers per cm. It's bracts are ovate acuminate,

Coressponding Author: Desta Berhe Sbhatu, Mekelle Institute of Technology, Mekelle University, Mekelle, P.O. Box 1632, Ethiopia

8-10-16-20×2.5-3-6 mm, Pedicels 11-17-20 mm long, It's perianth are cylindrical, 17-23 mm long, 4-6 mm wide pressed; outer lobes free for 5-7 mm.

Aloes are all-purpose plants and are recorded in the annals of various civilizations (IASC, 2002). They are known for their use in medicine, commerce and horticulture (Carter, 2001) and are sources of various products including medicinal, pharmaceuticals, food and cosmetics (Bhandari *et al*., 2010; Adelberg and Naylor-Adelberg, 2012). Hence, they have become subjects of extensive research in the chemical, pharmaceutical, economic and taxonomic fields (Carter, 2001). Studies revealed that gels of aloes contain amino acids and proteins, enzymes, minerals, vitamins, polysaccharides, and biological stimulators (Surjushe *et al*., 2008). Unfortunately, little is known about the chemistry of Ethiopian aloes (Demissew and Nordal, 2010). Now a days, the aloe-based medicinal and cosmetic industries have established high ethical standards for businesses and their products with the help of the International *Aloe* Science Council (IASC, 2002).

Whereas the needs of the aloe-based industries are growing beyond what can be supplied. Cultivation of aloes via the conventional methods and mechanisms is slow as seeds do not often form and when they do, they take years to germinate (Adelberg and Naylor-Adelberg, 2012). Sexual reproduction of some species of *Aloe* is also ineffective due to male sterility (Bhandari *et al*., 2010; Choudhary *et al*., 2011). Likewise, Demissew and Nordal (2010) reported some anatomical and developmental barriers that make bird and self-pollination of aloes ineffective. And yet vegetative propagation of aloes with offsets is ineffective for commercial cultivation as a single plant can only produce 2-3 offsets per year (Aggarwal and Barna, 2004). Moreover, the collection of offsets (as propagules) may result in some damages exposing them to diseases and desiccation (Saggoo and Kaur, 2010).

It is apparent that all the rationales for micropropagation of any plant species are applicable to *Aloe percrassa*. This being true, there are other more compelling rationales for the present and future studies. First, whereas much of the research on tissue culture and micropropagation of *Aloe* is restricted to few species, many others including *Aloe percrassa* are marginalized. Second, as it is indicated in the preceding paragraphs both sexual and vegetative propagation of *Aloe percrassa* are ineffective for commercial cultivation. Third, there are some efforts in Ethiopia aiming at cultivating *Aloe debrana* at commercial scale for its gel to treat and soften fibers of sisal by which coffee bag is made. *Aloe percrassa*, a morphologically similar species, is an excellent candidate for its relatively massive gel. Fourth, as the gel of *Aloe percrassa* was locally used as traditional shampoo, its commercial potential (for the cosmetic industry) can be claimed to be fairly high. Therefore, the development of micropropagation protocol of this endemic species of high commercial potential is quite timely, if not late. Hence, this article reports the results of a study aimed at developing suitable and reproducible protocol of *in vitro* micropropagation of *Aloe percrassa* using explants from offsets.

MATERIALS AND METHODS

The study was carried out in Mekelle Plant Tissue Culture Laboratory, northeastern Mekelle City (altitude, 1979 m; 13°30' 0"N; 39°28' 11"E), Ethiopia, between September 2013 and February 2014.

Preparation and sterilization of explants: Mother plants of *Aloe percrassa* with no symptoms of diseases were collected from some rural localities in southeastern Adigrat. Offsets were then dug out from the bases of the mother plants ascetically and gently by making sure that mechanical damages and contaminations are minimal. The offsets were trimmed to yield 2-3 cm long explants. The explants were sterilized first by; washing with running tap water for 30 min; dipping and stirring some of them in soup solution and others in Tween 20 for 10 min and rinsing with running tap water four times and with distilled water twice to remove traces of the detergent. Then, the explants were sterilized under laminar air flow by; dipping in 70% ethanol for 30 sec; dipping and stirring in 20% sodium hypochlorite for 5-10 min, rinsing with distilled water three times; surface sterilizing with freshly prepared $HgCl_2$ (0.1% w/v aqueous solution) for 5 min and thoroughly washing with sterile water to remove traces of $HgCl_2$. More or less similar sterilization procedures are reported in various sources (Aggarwal and Barna, 2004; Loyala and Vasques, 2008; Oliveira *et al*., 2009; Jayakrishna *et al*., 2011).

Experimental design: The study looked into the shooting, rooting and acclimatization responses of *Aloe percrassa* Tod. It began with culturing sterile explants in MS (Murashige and Skoog, 1962) media supplemented with 0.2 mg L^{-1} BAP (benzylaminopurine) and 0.2 mg L^{-1} NAA (α-naphthaleneacetic acid) to produce microshoots. Shooting responses of microshoots were studied in MS media supplemented with BAP and NAA at the concentrations of 0 and 0, 0.5 and 0.5, 1.0 and 0.5, 1.5 and 0.5 and 2.0 and 0.5 mg L^{-1}, respectively. All treatments were replicated 10 times. Rooting responses of shoots

were studied in half strength MS media supplemented with 0.0, 0.5, 1.0 and 1.5 mg L^{-1} of NAA. All the treatments were replicated five times. Finally, acclimatization capacity of plantlets was studied in three potted media or substrate, namely coco peat, composted and manured soil media. Whereas, the composted soil medium is made up of garden soil, sand and compost at ratio of 1:1:1, the manured soil medium is made up of garden soil, sand and manure (cow dung) at a ratio of 1:1:1.

Treatment procedures: Sterile initiation media inoculated with sterile explants were kept in growth room for four weeks to yield microshoots. Then, the microshoots were aseptically harvested and transferred into fresh shooting media at a rate of one to two microshoots per bottle and were kept in the growth room for four weeks to grow to shoots-operationally defined as shoots≥1.5 cm. Shoots measuring 2-4 cm were transferred into rooting media at a rate of 1-2 shoots per bottle and were kept in growth room for four weeks to root. The explants, microshoots and shoots were incubated in the growth room in completely randomized designed at a temperature of 25±0.5°C under fluorescent tube light with 16 h photoperiod and 2000-2500 lux light intensity. Rooted shoots, i.e., plantlets, were planted in potted media and were kept in completely randomized design in greenhouse with beginning and ending relative humidity of 90-60% and temperature of 30-31°C for two weeks for primary acclimatization. All the plantlets that survived the primary acclimatization were divided into two groups and were tested for secondary acclimatization. Whereas plantlets of Group 1 were put in direct sunlight, those of Group 2 were put in nursery shades for three weeks provided with adequate amount of water.

Data sources and analyses: Inoculated cultures that established clean microshoots were selected and sorted for further work. The microshoots were let to grow to shoots and the shoots in turn, were let to root. Rooted shoots (i.e., plantlets) were finally sent for acclimatization. Hence, sources of quantitative data included: number of days to shooting and rooting; shoot and root numbers; shoot and root lengths and survival rate of plantlets to acclimatization. Data analyses and comparisons were made using appropriate descriptive and inferential statistical methods.

RESULTS AND DISCUSSIONS

Shooting response of microshoots: As put in the preceding paragraphs, the study began with production of microshoots. Sterile explants inoculated into MS media supplemented with 0.2 mg L^{-1} BAP and 0.2 mg L^{-1} NAA yielded interesting results in four weeks. The explants yielded 1-4 new microshoots. The microshoots were then, inoculated into multiplication MS media supplemented with no PGR (control) and five concentrations of BAP and constant concentration of NAA (experimental). The responses of *Aloe percrassa* microshoots to the multiplication media revealed that days to shooting, number of shoots per micro shoot and shoot length (cm) showed statistically significant ($p \leq 0.05$) and non-significant ($p \geq 0.05$) variations among the treatments (Table 1).

Microshoots of *Aloe percrassa* cultured on MS media supplemented with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA, 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA, 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA proliferated shoots at an average of 14.10, 14.90 and 15.60 days, respectively. All are statistically significantly fewer than the number of days required for the shooting of microshoots cultured on MS medium supplemented with 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (2010 days) and the control (26.20 days) (Table 1). In this regard, whereas Jayakrishna *et al.* (2011) have reported that shooting of explants of *Aloe barbadensis* occurred in two weeks, Saggoo and Kaur (2010) have reported *in vitro* shooting of *Aloe vera* explants by 20th day of culture.

Microshoots cultured on the MS media enriched with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA yielded statistically significantly greater number of shoots (mean = 8.60; $p \leq 0.05$) compared to the other treatments (Table 1). On top of that the shoot were morphologically good-looking, well differentiated and relatively deep green than the shoots in the other treatments. The mean numbers of shoots recorded in those microshoots cultured in growth media supplemented with 1.5 and 2.0 mg L^{-1} of BAP were also high. Other workers have reported similar findings. *Aloe vera* explants cultured in MS media supplemented with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} of NAA yielded more shoots than those cultured in MS

Table 1: Effects of BAP and NAA on shooting of *Aloe percrassa* Tod

PGRs (mg L^{-1})		Mean responses		
BAP	NAA	Days to shoot	Shoot No.	Shoot length (cm)
0.0	0.0	26.20^{a}	2.30^{d}	2.20^{c}
0.5	0.5	20.10^{b}	5.70^{c}	2.70^{c}
1.0	0.5	14.10^{c}	8.60^{a}	5.20^{a}
1.5	0.5	14.90^{c}	7.40^{b}	3.90^{b}
2.0	0.5	15.60^{c}	7.10^{b}	3.40^{b}
Mean		18.80	6.22	3.48
CV		10.66	14.60	20.61
LSD		1.76	0.82	0.65

Mean values in the same column carrying different letters are statistically significantly different at $p \leq 0.05$, CV: Coefficient of variation, LSD: Least significant difference

media supplemented with lower or higher concentrations of BAP (Choudhary *et al*., 2011). Similarly, Aggarwal and Barna (2004) reported maximum shoot proliferation with BAP at 1.0 mg L^{-1} combined with some auxin. Furthermore, *Aloe vera* shoot tip explants cultured in shooting media supplemented with BAP at 2.0 mg L^{-1} and NAA at 0.5 mg L^{-1} yielded 10.10±0.50 shoots (Baksha *et al*., 2005). Many more workers have reported similar findings to that of ours, where no or lower concentration of BAP is less effective in promoting shooting (Choudhary and Mukundan, 2001; Hashem and Kaviani, 2008; Jayakrishna *et al*., 2011; Khalafalla *et al*., 2007; Rout *et al*., 2008). An average of 14 shoots per *Aloe vera* explant was obtained within 8 weeks of inoculation (Dwivedi *et al*., 2014).

Shoots grown in MS media containing 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA yielded significantly higher mean shoot length (mean = 5.20 cm; $p \leq 0.05$) compared to the rest of the treatments (Table 1). The smallest mean shoot length (mean = 2.20 cm; $p \leq 0.05$) was observed in treatments that are not supplemented with PGRs. In one study, highest mean lengths of *A. vera* shoots (4.0±0.16) were observed in shoots cultured in MS medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA as well as lower mean length was obtained in both 0.5 mg L^{-1} BAP plus 0.5 mg L^{-1} NAA and 4.0 mg L^{-1} BAP plus 0.5 mg L^{-1} NAA (Baksha *et al*., 2005). In another study, the maximum and minimum mean shoot length of *A. vera* observed were 4.20±0.03 cm and 0.89±0.009 cm (Das *et al*., 2010). Therefore, it can be claimed that the maximum mean shoot length observed in this study (at 1.0 mg L^{-1} BAP plus 0.5 mg L^{-1} NAA) is profound. Some authors argue that increasing the concentration BAP supplements may lead to negative effects on the morphology of the *in vitro* shoots (Bhatia *et al*., 2005). Baksha *et al*. (2005) argued similarly that increasing the concentration BAP with or without NAA had no effect on the number of shoots. In the works of Haque and Ghosh (2013) shoot production is decreased when BAP concentration is increased. In MS medium containing 10.0 combined with 0.1 mg L^{-1} and 5.0 combined with 0.2 mg L^{-1} BAP and IBA, respectively, *A. vera* explants turned brownish and died after 1-2 weeks (Daneshvar *et al*., 2013).

Rooting response of shoots: The responses of *in vitro* developed *Aloe percrassa* shoots to five different concentration of NAA were tested. Analysis of variance revealed that days to root, number of roots per explant and root length (cm) showed statistically significant ($p \leq 0.05$) and non-significant ($p \geq 0.05$) variations among the treatments (Table 2).

Table 2: Effects of NAA on the rooting of *Aloe percrassa* Tod

	Mean responses		
NAA (mg L^{-1})	Days to root	Root No.	Root length (cm)
0.0	14.4^{c}	8.4^{a}	6.6^{a}
0.5	15.2^{ab}	7.2^{a}	6.0^{a}
1.0	16.6^{ab}	4.2^{b}	3.6^{b}
1.5	17.2^{a}	3.4^{b}	2.1^{c}
Mean	17.80	5.90	4.57
CV	9.70	19.75	23.21
LSD	1.47	1.56	1.46

Mean values in the same column carrying different letters are statistically significantly different at $p \leq 0.05$, CV: Coefficient of variation, LSD: Least significant difference

Rooting of shoots of *A. percrassa* was observed in all the treatments by the second and third weeks of culturing. Shoots cultured in half strength MS medium supplemented with no PGRs rooted quickly (mean number of days to rooting was 14.4) significantly lower than the mean number of days required for the rooting of shoots cultured in MS medium supplemented with 1.0 and 1.5 mg L^{-1} of NAA ($p \leq 0.05$) (Table 2). This observation is in line with other study on *Aloe vera* (Narayana, 2008; Hashem and Kaviani, 2010; Bhandari *et al*., 2010). Rooting response of *Aloe vera* shoots within a week of inoculation and 100% of rooting within 15 days were reported (Aggarwal and Barna, 2004). High rooting rate (97%) of shoots inoculated in NAA supplemented medium is documented (Kalimuthu *et al*., 2010).

The highest means of roots per shoot were observed in shoots cultured with no supplemental PGRs (i.e. 8.40 roots per shoot) and with 0.5 mg L^{-1} of NAA (i.e. 7.20 roots per shoot). Both mean values are statistically significantly greater than the other two treatments (Table 2). Several studies indicated that best rooting of *in vitro* shoots of *Aloe vera* occur under PGRs-free media (Aggarwal and Barna, 2004; Bhandari *et al*., 2010) while others showed that PGRs are necessary (Abrie and Van Staden, 2001; Velcheva *et al*., 2005). Both assertions agree with the findings of the present study. While *Aloe vera* shoots inoculated in hormone free media showed 100% rooting, no rooting was observed in shoots inoculated in MS enriched with IAA (Krishnapuram and Pandey, 2009). *Aloe vera* microshoots cultured in hormone-free media yielded an average of 7.84 roots per explants (Hashem and Kaviani, 2010). The fact that shoots cultured in media supplemented with aloe gel yielded as many as 10.90±0.17 roots per explants (Das *et al*., 2010), it can be hypothesized that many species of *Aloe* have sufficient endogenous auxin to enhance rooting of *in vitro* shoots. Moreover, several workers on *A. vera*

have reported that *in vitro* shoots cultured in half strength MS media supplemented with 0.5 mg L^{-1} of NAA yielded best rooting responses (Narayana, 2008; Bhandari *et al*., 2010; Lee *et al*., 2011). It is useful to note some exceptions (Abdi *et al*., 2013) reported that good rooting responses were observed with 2 mg L^{-1} of NAA. Similarly, an average of 5.5 roots per shoot is observed in *Aloe vera* explants inoculated 1.0 mg $^{-1}$ NAA (Kalimuthu *et al*., 2010). Induction of only one root per shoot of *Aloe vera* culture is documented on MS media supplemented with 2-10 mg L^{-1} NAA (Saggoo and Kaur, 2010).

Likewise, the highest means of root length were observed in shoots cultured with no supplemental PGRs (i.e. 6.6 cm) and with 0.5 mg L^{-1} of NAA (i.e. 6.0 cm). Both mean values are statistically significantly greater than the other two treatments ($p \leq 0.05$, Table 2). Hashem and Kaviani (2010) observed mean root length of 8.75 cm among shoots cultured in PGRs-free media. Observation of the morphologies of the roots in the present study showed that the roots of shoots cultured in PGRs-free media and those that were cultured in media with 0.5 mg L^{-1} NAA were thicker with no branching while the roots of shoots cultured in rooting media supplemented with high concentrations were thinner. The observations that roots of shoots cultured in rooting media enriched with higher concentrations of NAA are shorter, thinner and abnormal/poor quality are noted in the studies of various authors (Kollarova *et al*., 2004; Baksha *et al*., 2005; Lee *et al*., 2011).

Acclimatization of plantlets: The survival capacity of *in vitro* micropropagated plantlets of *Aloe percrassa* transplanted into acclimatization media for primary and secondary hardening were affected by the nature of the media. Total amount of plantlet 15 and 11 to coco peat; 17 and 9 into both composted and manured soil media for primary and secondary acclimatization, respectively were transplanted. Plantlets transplanted for acclimatization into coco peat resulted in 100% survival in both primary and secondary hardening. The primary and secondary acclimatization rates of plantlets transplanted to composted (soil, sand, compost in 1:1:1) and manured (soil, sand, manure in 1:1:1) soil media were 94.10 and 88.90%, respectively. The amount of light in secondary acclimatization (direct sunlight or shade) did not affect the rate of survival of plantlets (Table 3). Morphological observation showed no detectable variations among the regenerated plantlets.

Various studies have reported high rate of survival of *A. vera* (between 70 and 100%) under different light conditions as well as planting substrates (Aggarwal and Barna, 2004; Baksha *et al*., 2005; Hashem and Kaviani, 2008; Narayana, 2008; Oliveira *et al*., 2009; Hashem and Kaviani, 2010; Bhandari *et al*., 2010; Jayakrishna *et al*., 2011). In the study of Molsaghi *et al.* (2014) hundred percent of survival was observed on plantlets transferred to garden soil, sand and compost in the proportion of 1:1:1.

Table 3: Responses of *Aloe percrassa* Tod. plantlets to primary and secondary acclimatization

	Survival rate in acclimatization (%)		
	Primary	Secondary	
Acclimatization substrate	Greenhouse	Light	Shade
Coco peat	100.00	100.00	100.00
Composted soil	94.10	88.90	88.90
Manured soil	94.10	88.90	88.90

CONCLUSION

Aloes have long history in the fields of ethnobotany, traditional medicine, economics and cosmetics around the world. *Aloe percrassa* is one of aloe species native to Ethiopia and widely used in traditional medicine to heal wounds and as shampoos. The local demand for *Aloe* at the industry level is increasing. There are some efforts in Ethiopia aiming at cultivating *Aloe debrana* at commercial scale for its gel to treat and soften fibers of sisal by which coffee bag is made. *Aloe percrassa*, a morphologically similar species, is an excellent candidate for its relatively massive gel. Also, as the gel of *Aloe percrassa* was locally used as traditional shampoo, its commercial potential (for the cosmetic industry) can be claimed to be fairly high. Developing an efficient and reproducible protocol is a pre-requisite for successful use of plant tissue culture techniques.

The present study has developed a reproducible *in vitro* micropropagation protocol of *Aloe percrassa* September 5, 2014Tod. Detailed methods of collecting and sterilizing, initiating and shooting, rooting and acclimatization protocols of the plant are established. The results of the study suggest that *Aloe percrassa* can be successfully micropropagated and acclimatized to field conditions with no or minimum supplements of synthetic inputs. Therefore, future works need to focus on developing low-cost *in vitro* micropropagation where its gel is used as source of endogenous PGRs in proliferation, shooting and rooting. Moreover, chemical characterization of its extracts would help us understand its medicinal and cosmetic importance to drive its propagation and conservation.

ACKNOWLEDGMENTS

This study is financially supported by the Ethiopian Ministry of Education. Laboratory and greenhouse studies were conducted at Mekelle Plant Tissue Culture

Laboratory, Mekelle, Ethiopia. Haramaya University, Ethiopia made learning, research and logistical provisions available.

REFERENCES

Abdi, G., M. Hedayat and M. Modarresi, 2013. *In vitro* micropropagation of *Aloe vera*: Impacts of plant growth regulators, media and type of explants. J. Biol. Environ. Sci., 7: 19-24.

Abrie, A.L. and J. Van Staden, 2001. Micropropagation ofthe endangered *Aloe polyphylla*. Plant Growth Regul., 33: 19-23.

Adelberg, J. and J. Naylor-Adelberg, 2012. Effects of cytokinin on multiplication and rooting of *Aloe barbadensis* during micropropagation on agar and liquid medium. J. Med. Active Plants, Vol. 1.

Aggarwal, D. and K.S. Barna, 2004. Tissue culture propagation of Elite plant of *Aloe vera* Linn. J. Plant Biochem. Biotechnol., 13: 77-79.

Alessandro, B. and S. Stefano, 2005. The Great *Aloe* Book.In: History, Botany, Composition and Pharmacological Aspects of this Legendary Plant, Azzali, A. (Ed.). Zuccari Pty Ltd., Malaga WA., Australia.

Baksha, R., M.A. Jahan, R. Khatun and J.L. Munshi, 2005. Micropropagation of *Aloe barbadensis* Mill. through *in vitro* culture of shoot tip explants. Plant Tissue Cult. Biotechnol., 15: 121-126.

Bhandari, A.K., J.S. Negi, V.K. Bisht and M.K. Bharti, 2010. *In vitro* propagation of *Aloe vera*: A plant with medicinal properties. Nat. Sci., 8: 174-176.

Bhatia, P., N. Ashwath and D.J. Midmore, 2005. Effects of genotype, explant orientation and wounding on shoot regeneration in tomato. *In vitro* Cell. Dev. Biol. Plant, 41: 457-464.

Carter, S., 2001. Aloaceae. In: Flora Zambesiaca, Pope, G.V., S.J. Owens and M.A. Diniz (Eds.). Vol. 12, Royal Botanic Gardens, Richmond, UK., ISBN-13: 9781842460375, pp: 48-98.

Choudhary, A.K., A.K. Ray, S. Jha and I.N. Mishra, 2011. Callus formation, shoot initiation and *in vitro* culture of *Aloe vera*. Biotechnol. Bioinf. Bioeng., 1: 551-553.

Choudhary, S. and U. Mukundan, 2001. *Aloe vera* L. Micropropagation and characterization of its gel. Phytomorphology, 51: 155-157.

Daneshvar, M.H., N. Moallemi and N.A. Zadeh, 2013. The effects of different media on shoot proliferation from the shoot tip of *Aloe vera* L. J. Nat. Pharm. Prod., 8: 93-97.

Das, A., P. Mukherjee and T.B. Jha, 2010. High frequency micropropagation of *Aloe vera* L. Burm. F. As a low cost option towards commercialization. Plant Tissue Cult. Biotechnol., 20: 29-35.

Demissew, S. and I. Nordal, 2010. Aloes and Other Lilies of Ethiopia and Eritrea. 2nd Edn., Shama Books, Canada, ISBN-13: 9789994400423, Pages; 351.

Dessalegn, F., 2006. Taxonomic and demographic studies on three species complexes within the genus *Aloe* L. (Aloaceae) in Ethiopia. Ph.D. Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Dwivedi, N.K., A. Indiradevi, K.I. Asha, R. Asokan Nair and A. Suma, 2014. A protocol for micropropagation of *Aloe vera* L. (Indian *Aloe*)-a miracle plant. Res. Biotechnol., 5: 1-05.

Hansen, D.M., J.M. Olesen, T. Mione, S.D. Johnson and C.B. Muller, 2007. Coloured nectar: Distribution, ecology and evolution of an enigmatic floral trait. Biol. Rev., 82: 83-111.

Haque, M. and B. Ghosh, 2013. High frequency microcloning of *Aloe vera* and their true-to-type conformity by molecular cytogenetic assessment of two years old field growing regenerated plants. Bot. Stud., Vol. 54.

Hashem, A.D. and B. Kaviani, 2010. *In vitro* proliferation of an important medicinal plant *Aloe*-a method for rapid production. Aust. J. Crop Sci., 4: 216-222.

Hashem, D. and B. Kaviani, 2008. Rapid micro-propagation of *Aloe vera* L. via shoot multiplication. Afr. J. Biotechnol., 7: 1899-1902.

IASC, 2002. *Aloe vera*: A long, illustrious history dating from biblical times. International *Aloe* Science Council (IASC).http://docs8.chomikuj.pl/305768720, PL,0,0,aloebook.pdf

Jayakrishna, C., C. Karthik, S. Barathi, D. Kamalanathan and I.P. Arulselvi, 2011. *In vitro* propagation of *Aloe barbadensis* miller, a miracle herb. Res. Plant Biol., 1: 22-26.

Kalimuthu, K., S. Vijayakumar, R. Senthilkumar and M. Sureshkumar, 2010. Micropropagation of *Aloe vera* Linn.: A medicinal plant. Int. J. Biotechnol. Biochem., 6: 401-406.

Khalafalla, M.M., E. Abdellatef, M. Mohamed Ahmed and M. Osman, 2007. Micropropagation of cactus (*Opuntia ficus-indica*) as strategic tool to combat desertification in arid and semiarid regions. Int. J. Sustainable Crop Prod., 2: 1-8.

Klopper, R.R. and G.F. Smith, 2010. *Aloe* L. http://www.plantzafrica.com/plantab/aloe.htm.

Kollarova, K., D. Liskova, D. Kakoniova and A. Lux, 2004. Effect of auxins on *Karwinskia humboldtiana* root cultures. Plant Cell Tissue Organ Cult., 79: 213-221.

Krishnapuram, S. and N. Pandey, 2009. Method for *In vitro* Mass Culture of *Aloe Vera*. Reliance life Sciences Pvt. Ltd., Mumbai, India.

Lee, Y.S., T.J. Yang, S.U. Park, J.H. Baek, S.Q. Wu and K.B. Lim, 2011. Induction and proliferation of adventitious roots from *Aloe vera* leaf tissues for *in vitro* production of *Aloe*-emodin. Plant Omics J., 4: 190-194.

Loyala, V.M. and F. Vasques, 2008. Methods in Molecular Biology: Plant Cell Culture Protocols. Humana Press Inc., Totowa.

Molsaghi, M., A. Moieni and D. Kahrizi, 2014. Efficient protocol for rapid *Aloe vera* micropropagation. Pharm. Biol., 52: 735-739.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Narayana, K.S., 2008. Micropropagation of *Aloe vera*. Project Report, School of Bioengineering, SRM University.

Oliveira, E.T., O.J. Crocomo, T.B. Farinha and L.A. Gallo, 2009. Large-scale micropropagation of *Aloe vera*. Hortscience, 44: 1675-1678.

Rout, G.R., S. Senapati and H. Aparajeta, 2008. Micropropagation of *Acacia chundr*. Hortiscience, 35: 22-26.

Saggoo, M.S. and R. Kaur, 2010. Studies in North Indian *Aloe vera*: Callus induction and regeneration of plantlets. Arch. Applied Sci. Res., 2: 241-245.

Surjushe, A., R. Vasani and D.G. Saple, 2008. *Aloe vera*: A short review. Indian J. Dermatol., 53: 163-166.

Velcheva, M., Z. Faltin, A. Vardi, Y. Eshdat and A. Perl, 2005. Regeneration of *Aloe arborescens* via somatic organogenesis from young inflorescences. Plant Cell Tissue Organ Cult., 83: 293-301.