UDC 575.630
https://doi.org/10.2298/GENSR1902661F
Original scientific paper

ASSESSMENT OF GENETIC DIVERSITY AMONG TWO *in vitro Aole barbadensis* AND *Aloe littoralis* REGENERATED PLANTS USING C-VALUE DNA OF FLOW CYTOMETRY

Farah FARAHANI[1]*, Atena BAYANI[2], Zahra NOORMOHAMMADI[2]

[1] Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
[2] Biology Department, School of Basic sciences, Science and Research Branch, Islamic Azad University (SRBIAU), Tehran, Iran

Farahani F., A. Bayani, Z. Noormohammadi (2019): *Assessment of genetic diversity among two in vitro Aole barbadensis and Aloe littoralis regenerated plants using C-value DNA of flow cytometry*.- Genetika, Vol 51, No.2, 661-674.

Aloe belonging to the family Asphodelaceae family, formerly Liliaceae, is a genus of herbaceous and succulents. Shoot tip of 2-3 cm were collected from offshoot of *Aloe barbadensis* and *Aloe littoralis*, explants were surface sterilized, after were inoculated with MS medium containing various concentrations of BA (0.5, 1, 2 mg/L), IAA (0.5, 1 mg/L). The cultures were incubated at 25 ± 2°C under a 16 h photoperiod. In *A. barbadensis* and *A. littoralis*, the best treatment for highest shoot number and bud proliferation was MS medium containing 2 mg/L BA and 0.5 mg/L IAA. Maximum percentage of proliferated shoot buds (90% and 95%) from a single explant were obtained in MS medium after 4-5 weeks of the second and the first subcultures, respectively. Both of the species were adventitious root induced in MS medium containing 0.5 mg/L BAP and 0.5 mg/L IAA after the second subculture. Genome size of the regenerated plants in *A. barbadensis* and *A. littoralis* species were differed in different treatments (from 30.66 pg to 39.62 pg, 2C value). One sample T-test showed significant difference (P=0.001) between genome sizes of two species studied when *A. littoralis* showed higher C-value in comparison to *A. barbadensis*. Different genome sizes were also indicated among treatments and subcultures. The mixoploids identified in flow cytometry histograms by

Corresponding author: Farah Farahani, Department of Biology, Qom Branch, Islamic Azad University, Qom, Iran, Tel : +989122778171, farahfarahani2000@yahoo.com

the presence of two or three peaks of in *A. barbadensis* and *A. littoralis* histograms in apple regenerated plants in different treatments and subcultures in treatment 2 (BA 1 mg/L, IAA 0.5 mg/L).

Keywords: Aole barbadensis, Aloe littoralis, C-value, Regeneration, Flow cytometry

Abbreviation: BA: 6-Benzylaminopurine, IAA: Indol Acetic Acid

INTRODUCTION

Aloe is an important commercial crop available in a wide range of species and varieties in international markets. *Aloe* belonging to the family Asphodelaceae family, formerly Liliaceae, is a genus of herbaceous and succulents found in tropical and subtropical areas, particularly South Africa and Arabia (NAYANAKANTHA *et al.,* 2010). *Aloe* is comprising more than 350 till 600 species which grow in the warm areas of the world (CANIGUERAL *et al.*, 1993, CAPASSO *et al.*, 1998). The plant prefers sunny weather, requires well-drained soil and can grow in nutritionally poor soil (DWIVEDI *et al.*, 2014). *Aloe vera* (L.) Burm f. Synonyms: *Aloe barbadensis* Miller has become naturalized almost in all parts of India (KLEIN and PENNEYS, 1988).

Aloe littoralis Baker is a unique living member of plants of the genus Aloe in Iran (MAZAFFARIAN *et al.,* 1996). Although *A. vera* as a typical species of this genus is cultivating in Iran, *A. littoralis* wildly grown and distributed in several parts of Iran like southern provinces and Persian Gulf islands (MAZAFFARIAN *et al.*, 1996, SOLTANIPOOR *et al.*, 2006, HAJHASHEMI *et al.,* 2012).

It is a succulent herb that has elongated and pointed leaves. *A. littoralis* and *A. vera* which commonly known in Iran as "Sabre Zard", "Sebr" or "Segel", have been used in Iranian traditional and folk medicine to treat several disorders including some dermatological, gastrointestinal and inflammatory diseases.

The applications of this plant have been recorded in ancient cultures of India, Egypt, Greece, Rome and China (NEJATZADEH-BARANDOZI *et al.*, 2013). *Aloe* has been used for centuries and is currently being actively studied for medicinal purposes (GRINDLAY and REYNOLDS, 1986; DAVIS *et al*., 1994; HEGGERS *et al*., 1993; SHELTON 1991; VISUTHIKOSOL *et al*., 1995).

Almost the two morphologically indistinguishable species are phenotypically identified based on the differences in flower color, aloin content and degree of bitterness.

In nature, *Aloe* is propagated through lateral buds, which is slow, very expensive and low income practice (MEYER and STADEN, 1991, HASHEM ABADI *et al.*, 2010). *Aloe* has been cultured *in vitro* by various researchers (NATALI *et al.*, 1990; ROY and SARKAR, 1991; ABRIE and STADEN, 2001). The technique to tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease-free clones and for progressive valuable germplasm (BHOJWANI & RAZDAN, 1992).

The best explants for micropropagation of *A. vera* are shoot tip and axillary bud (MEYER and STADEN, 1991). Also, the presence of the plant growth regulators is necessary for this purpose (AGGARWAL and BARNA, 2004; DEBIASI *et al*., 2007; LIAO *et al.*, 2004). MEYER and STADEN (1991) reported axillary shoot formation using IBA, whereas ROY and SARKAR (1991) and NATALI *et al.* (1990) obtained shoots on medium containing 2,4-D and Kin.

RICHWINE *et al.* (1995) reported the induction of shoots using zeatin, while DEBIASI *et al.* (2007) and LIAO *et al.* (2004) studied the effects of BA, IAA and NAA on bud initiation.

The present research focuses on the influence of Benzyl Adenine (BA) and Naphthalene Acetic Acid (NAA) on rapid *in vitro* propagation of *Aloe* plants grown in Iran. We also detailed the collection of *Aloe vera* L. and *Aloe littoralis* from the Qeshm Island, micropropagation, its adaptation, conservation and its C-value analysis. Determination of the C-value alterations has been proposed, using flow cytometry analysis, and these variants have been used to evaluate the plant species.

MATERIALS AND METHODS

Aloe tissue culture

Shoot tip of 2.0-3.0 cm were collected from offshoot-derived elite individual of the superior species of *A. barbadensis* and *A. littoralis* on the basis of higher yield of leaf biomass (CAVALLINI *et al.*, 1990). The explants first were washed thoroughly in running tap water for 15 minutes. shoot tip were taken inside the laminar hood for further sterilization, explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of mercuric chloride for 5 minutes, after explants were thoroughly washed for 4-5 times with sterile water to remove any traces of mercuric chloride. MS (MURASHIG & SKOOG, 1962) Media was autoclaved at 121ºC for 20 minutes (HAQUE *et al.*, 2013). Shoot tip explants (2-3 cm) were inoculated with MS medium containing various concentrations of BA (6-Benzylaminopurine) (0.5, 1, 2 mg/L), IAA (Indol Acetic Acid) (0.5, 1 mg/L).

The cultures were incubated in growth chamber maintained at 25 ± 2°C under a 16 h photoperiod with a photosynthetic photon flux density of approximately 50 μmol m^{-2} s^{-1} emitted from cool fluorescent tubes. At every 4 weeks intervals, the cultures were sub-cultured in their respective fresh media. After completion of every regeneration cycle (8 weeks), each individual shoots (≥ 2.0 cm) were separated from proliferated shoot clumps for in vitro rooting and then pre-existing explants were re-inoculated in their respective fresh media for next regeneration cycle.

Morphologic characteristics such as length of leaves, length and number of new shoots and length and number of roots in cm were evaluated in each subculture for both species. The experiment was repeated for 3 times for each treatment used and morphological data were analyzed by analysis of variance test (ANOVA) followed by least significant difference test (LSD).

Flow cytometry

Sample preparations were carried out in a two-step procedure. In the first step, 50 mg of fresh and non-fixed samples (leaves) were used. In the first step we placed plant tissue onto a plastic petri dish and added 0.5 ml of Cystain DNA and chopped the tissue with a sharp razor blade in order to release the nuclei from cells then added 1.5 ml of Cystain DNA and incubated at room temperature for 5 minutes. The sample was filtered through a Partec 50 μm Cell Trics filter and analyzed in Partec flow cytometer (PI), Cod.05-5004 using UV excitation and measured blue emission. For each individual, the 2C DNA content was estimated by comparing the mean peak intensity of its nuclei with the mean peak intensity of the *Pisum sativa* nuclei (4.40 pg, KOCE *et al.*, 2003).

The resulting values were expressed as a peak ratio, which is a ratio of the mean position of the G0/G1 peak in the DNA histogram of the tested plant to the mean position of the G0/G1

peak in the histogram of the reference plant. To estimate genome size of the samples, each analysis was repeated at least three times (BURES *et al.*, 2004).

The 2C DNA nuclear content of the samples was calculated as follows: Sample 2C relative/sample peak mean 2C DNA content × 2C DNA content of the standard.

Each analysis was repeated at least three times. The experiments were set up in a randomized design. Data were analyzed by ANOVA to detect significant differences between means. Means differing significantly were compared using SPSS ver. (2009) at a 5% probability level.

RESULTS

Morphological characters

The effects of different concentrations on the explant for shoot induction was evaluated up to three regeneration cycles in *A. barbadensis* and *A. littoralis* species (Table 1).

Table1. Comparison of different concentrations of cytokinin and Auxin on shoot proliferation, growth and rooting in Aloe barbadensis and Aloe barbadensis during three subcultures.

Subculture	Treatment	number of microshoot/ per explant		Mean of length microshoot		Mean of number Root/per explant		Mean of length root	
		A. barbadensis	*A. littoralis*	*A. barbadensis*	*A. littoralis*	*A. barbadensis*	*A. littoralis*	*A. barbadensis*	*A. littoralis*
1st	1	1.25 a	7 ab	0.44 a	1.6 a	2.2 e	00	2.7 fg	00
	2	0.55 a	2 a	0.33 a	0.6 a	1. 2 cd	00	1.3 cde	00
	3	1.75 ab	9 b	0.90 ab	1.45 a	0.11 ab	00	0.22 ab	00
	4	0.57 a	6.5 ab	0.35 a	0.8 a	1.2 d	00	1.19 bcde	00
2nd	1	0.71 a	4.2 ab	0.48 a	1.3 a	0.8 bcd	0.14 a	1.5 de	0.28 a
	2	0.70 a	5 ab	0.45 a	1.2 a	0.45 abcd	00	0.69 abcd	00
	3	3.00 bc	6.00 ab	0.93 ab	0.90 a	00	00	00	00
	4	2.00 ab	4.4 ab	0.72 ab	1.14 a	0.54 a	00	0.9 abcde	00
3th	1	0.68 a	4.07 ab	1.09 ab	1.5 a	2.3 e	0.6 a	2.9 g	0.76 a
	2	0.94 a	4.4 ab	1.01 ab	2.1 a	1.2 d	00	1.8 ef	00
	3	3.55 c	5.7 ab	1.42 ab	1.6 a	00	00	00	00
	4	1.05 a	1.65 ab	0.90 ab	1.1 a	0.41 abc	0.13 a	0.54 ab	0.11 a

[Treatment 1: BAP (0.5 mg/L) + IAA (0.5 mg/L); Treatment 2: BAP (1 mg/L), IAA (0.5 mg/L); Treatment 3: BAP (2 mg/L), IAA (0.5 mg/L); Treatment 4: BAP (0.5 mg/l), IAA (1 mg/L)].

ANOVA test followed by LSD showed significant difference ($p<0.05$) in morphological characters among the regenerated plants of different subcultures and treatments in both species. In *A. barbadensis* species, The highest percentage microshoot (90%) occurred in the plants of treatment 3 (MS with BA 2 mg/L, IAA 0.5 mg/L) and the highest values of root length (4.5 cm) occurred in the plants of treatment 1 (MS with BA 0.5 mg/L, IAA 0.5 mg/L) (Fig. A-1, A-2). The highest values of the leaf length, microshoot length and root number (6.1 cm, 3.5 cm and 5, respectively) occurred in the plants of treatment 2 (MS basal medium with BA 1 mg/L, IAA 0.5 mg/L) (Fig. B-1, B-2).

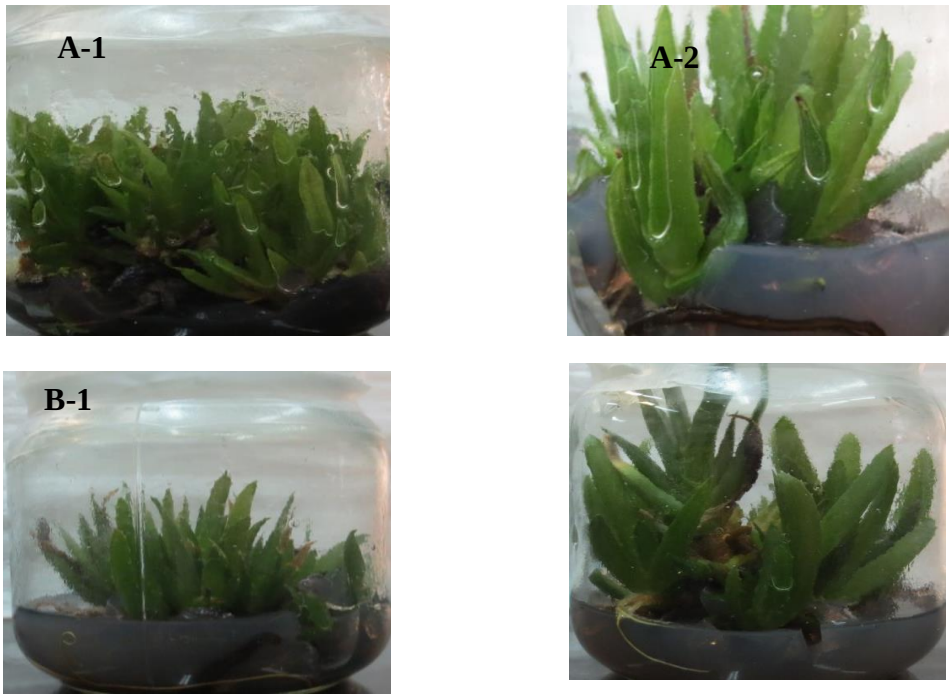

Fig 1. Effect the treatment (MS medium with 2 mg/l BAP, 0.5 mg/l IAA) on the mean number of microshoot (A) and the treatment (MS medium with 1 mg/l BAP, 0.5 mg/l IAA) on the mean length microshoot regenerated plantlets (B), (1) *A. barbadensis* , (2) *A. littoralis*

The highest values of the leaf length occurred in the plants of the second subculture, the highest values of microshoot length, root length and microshoot number occurred in the plants of the third subculture (Fig. 2-A1, B1).

Moreover, a significant positive correlation was observed between the microshoot number and microshoot length ($p<0.05$), while a significant negative correlation occurred between root number and root length ($p<0.05$) (Fig. 2-C1, D1).

Similarly in the species *A. littoralis*, the plants of the subculture third showed the highest values of microshoot length, shoot number and root length and number (Fig. 2-A2, B2). The highest values of the leaf length and percentage microshoot (4.7 cm and 95%, respectively) occurred in the plants of treatment 3 (MS with BA 2 mg/L, IAA 0.5 mg/L) (Fig. 2B1). The highest values of microshoot length, root length and root number (2.5 cm, 2 cm and 3, respectively) occurred in the plants of treatment 1 (MS with BA 0.5 mg/L, IAA 0.5 mg/L) (Fig. 2-C2, D2).

A significant positive correlation was observed between microshoot length and leaf length ($p<0.05$) and between root length and leaf length ($r=0.82$, $p<0.01$) in this cultivar.

In *A. barbadensis* and *A. littoralis*, the best treatment for highest shoot number and bud proliferation was MS medium containing 2 mg/L BAP and 0.5 mg/L IAA. Maximum percentage of proliferated shoot buds (90% and 95%) from a single explant were obtained in MS medium

after 4-5 weeks of the second and the first subcultures, respectively. Both of the species were adventitious root induced in MS medium containing 0.5 mg/L BA and 0.5 mg/L IAA after the second subculture.

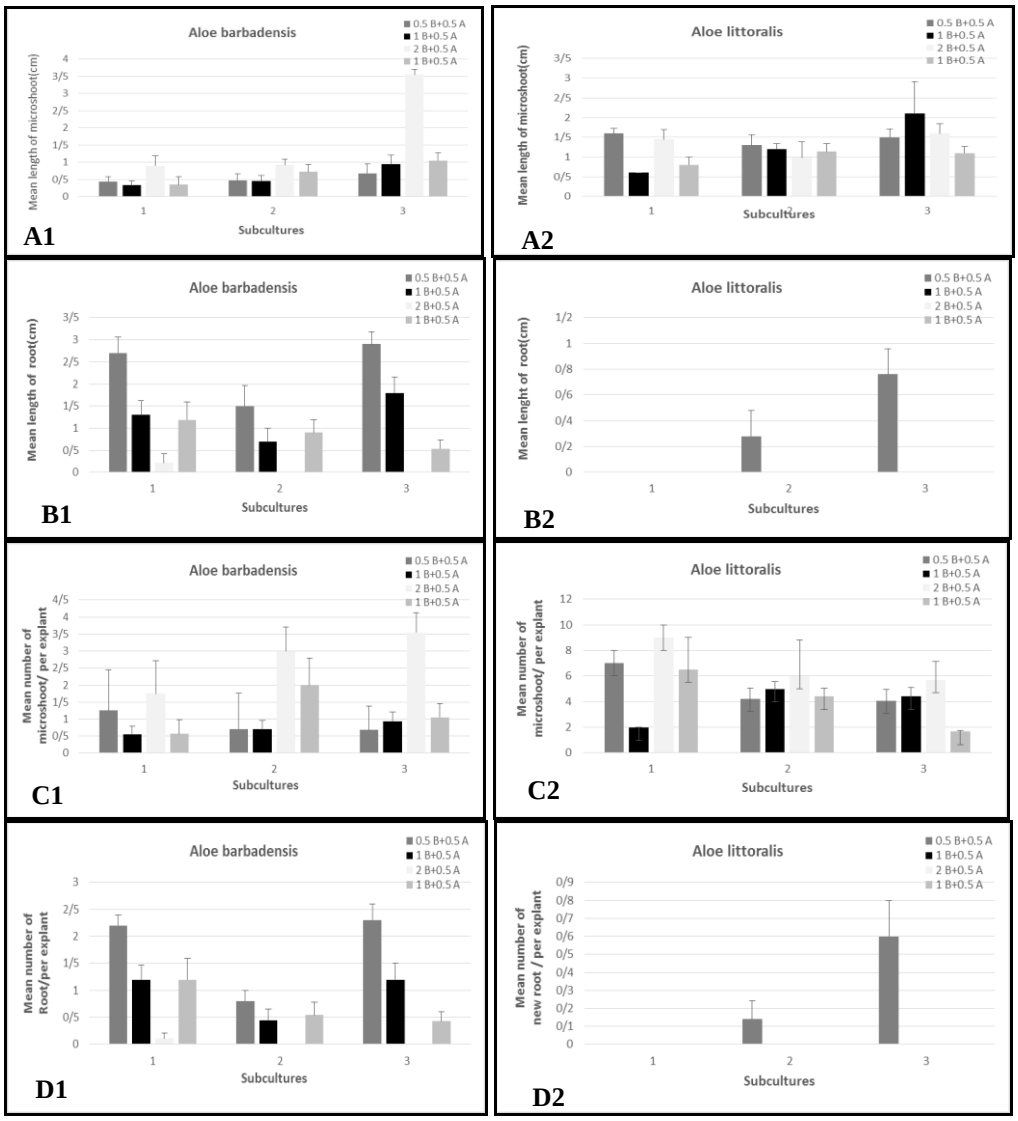

Fig. 2. Comparison of different concentrations of cytokinin and Auxin on A) mean of length microshoot, B) length of root, C) number of microshoot/explant and D) number of root in (1) *A. barbadensis* and (2) *A. littoralis* during three subcultures.

C-value analyses

Genome size of the regenerated plants in *A. barbadensis* and *A. littoralis* species were differed in different treatments (from 30.66 pg to 39.62 pg, 2C value). One sample T-test showed significant difference (P=0.001) between genome sizes of two species studied when *A. littoralis* showed higher C-value in comparison to *A. barbadensis*. Different genome sizes were also indicated among treatments and subcultures.

The mixoploids identified in flow cytometry histograms by the presence of two or three peaks of in *A. barbadensis* and *A. littoralis* histograms in apple regenerated plants in different treatments and subcultures (Fig. 3) in treatment 2 (BA 1 mg/L, IAA 0.5 mg/L).

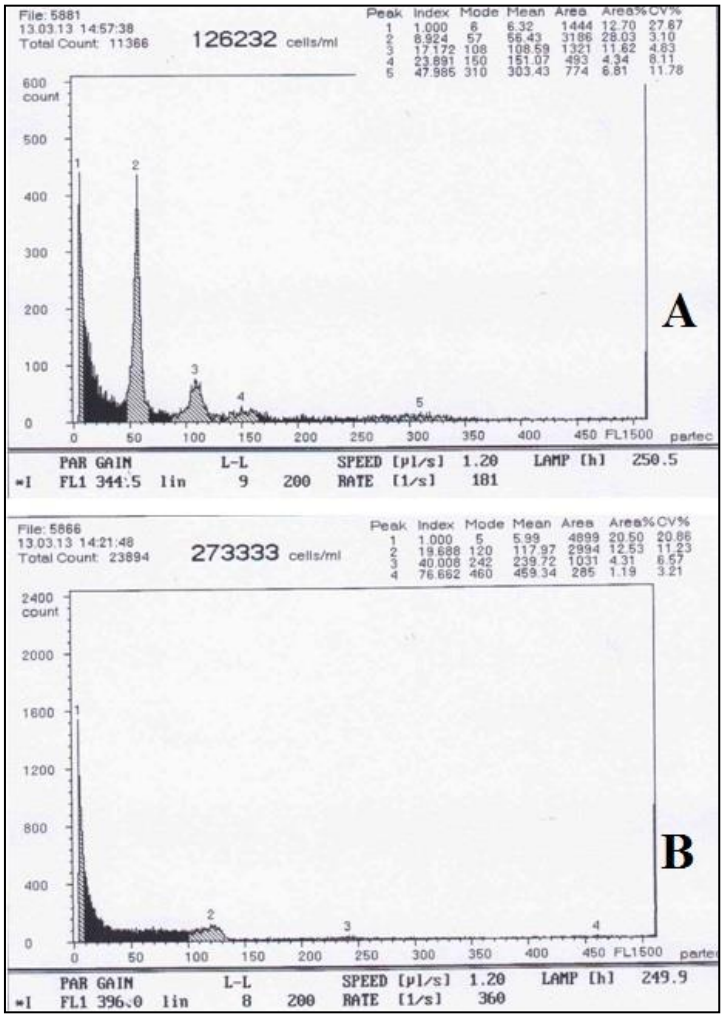

Fig. 3. Flow cytometry histogram of mixoploidy in (A): *A. littoralis* peak 5 = standard reference. (B): *A. barbadensis* peak 4=standard reference

DISCUSSION

Aloe vera syn *barbadensis* Mill. and *Aloe littoralis* are xerophytic medicinal plants of considerable importance. They are widely used in cosmetic and drug industry and these demands are increasing nowadays. Due to widespread male sterility these propagate only through vegetative mode of reproduction. This propagation rate is very slow to meet commercial demand of high quality planting material for its commercial cultivation. The objectives of the present study were to standardize optimum conditions for establishment of axenic culture from elite germplasm, shoot proliferation and rooting of micro shoots.

For shoot proliferation, growth regulators especially cytokinins (LANE 1979, STOLZ 1979, BHOJWANI 1980a, GARLAND & STOLZ 1981) are one of the most important factors affecting the response. A range of cytokinins (Kinetin, 6-Benzylaminopurine (BA), 2-Isopentenyl Adenine (2-ip) and Zeatin) has been used in micropropagation work (BHOJWANI and RAZDAN 1992).

A number of plants has been were successfully multiplied on medium containing BA. In white clover (BHOJWANI 1981) and hybrid willow (BHOJWANI 1980), chickpea (BARNA & WAKHLU 1994). NAIR *et al.* (1979), and *Iresine lendenii* (SEBASTIN & BARNA 2003) BA is the most effective cytokinin for the shoot tip, meristem and bud culture.

Type of explants used, culture condition and composition of culture media play very significant role for in vitro shoot proliferation. The data indicated a great interaction between the two growth regulators for shoot formation (%) and number of shoots and shoot length of *Aloe vera* (ZAKIA *et al.*, 2013).

In present study, Signs of shoot proliferation were showed after 10 days of culturing. Multiplication of shoot was best on MS medium with BA (2 mg/L). In *A. barbadensis* and *A. littoralis* shoot proliferation were 9 and 15, respectively. BA variations affecting shoot proliferation were also reported by HAQUE *et al.* (2013), BHANDARI *et al*. (2010), GANTAIT *et al.* (2010) and Singh *et al.* (2009). ABRIE and STADEN (2001) and CHAUDHURI and MUKUNDAN (2001) had also reported the use of BA in shoot proliferation of *A. polyphylla* and *A. vera*, respectively. It was also reported that highest shoot proliferation in *A. vera* was found in MS medium containing BA and IBA (AGGARWAL & BARNA, 2004; MUKESH KUMAR *et al.*, 2011 and MEYER & STADEN, 1991, ABDI *et al.*, 2013). This is in contrast to earlier reports in *A. vera* by NATALI *et al.* (1990), where better proliferation occurred on medium containing Kinetin instead of BA. BAKSHA *et al.* (2005) also reported that the enhancement of shoots was observed by using BA and NAA.

There was a declining trend of shooting in *Aloe vera* with increasing the concentration of BAP (2 mg/L). Further, there was a declining trend of elongation shoot was observed in *Aloe* with increasing the concentration of BAP (2 mg/L). This decline is also supported by HASHEMABADI and KAVIANI (2008). This decline in the shoot length of *Aloe vera* might be due to the inhibitory effect of BAP, which provoke a little suppression of plant growth and activity of some proteolytic enzymes (PETKOVA *et al.*, 2003). Rooting response of microshoots is reported to be controlled by growth regulators in the medium (BHOJWANI & RAZDAN 1992), basal salt composition (GARLAND & STOLTZ 1981, ZIMMERMAN and BROOME.1981, SKIRVIN & CHU 1979), genotype (RINES & MCCOY 1981) as well as cultural conditions (MURASHIGE 1977).

For most of the species auxin is required to induce rooting. In the present study, rooting was observed in IAA (0.5 mg/L) medium. The highest roots (number >3 and length > 4.5 cm) were obtained in medium with IAA (0.5 mg/L) in 8 weeks of time. Highest root response in *A. vera* result, rooting was not suitable in MS medium with IAA hormone. Cytokinin carry over

effects, perhaps from residual BA in the plant tissue, reduced the numbers of plants that had rooted (ADELBERG *et al.*, 2012).

But rooting was observed better in free hormone medium. These kinds of observations were also earlier by SANCHEZ *et.al* (1988), MEYER & STADEN (1991), and RICHWINE *et al.* (1995) in *Aloe vera.*

The results obtained in our study revealed the no differences in morphological parameters among somaclones and the parental clones (Table 1).

Plants regenerated by tissue culture techniques exhibited various morphological variations due to mutations believed as somaclonal variations (LARKIN & SCOWCROFT 1981). The high variability observed in regenerated plantlets might be triggered by the cytokinin during micropropagation (YIPENG *et al.*, 2005). The frequency of somaclonal variation would depend upon culture protocol applied during *in vitro* process particularly hormone composition and number of subcultures (DUCOS *et al.*, 2003).

The regenerated plantlets reveal no morphological or growth abnormalities. For commercial utility it is therefore imperative to establish genetic uniformity of micropropagated plants to confirm the true-to-type plantlets. KHATUN *et al.,* (2018) reported this implies that micropropagation through shoot tip explants is a safe method for producing true-to-type plants and could be used for commercial plantation of *A. vera*.

NOORMOHAMMADI *et al.* (2018) reported, somaclonal variations on genomic level while no significant differences were observed in the amount of gel among regenerated *Aloe barbadensis* plantlets.

C- value DNA of flow cytometry

In terms of estimating genome size of regenerated plants in different treatment and subcultures, C-value of samples was determined by using flow cytometry. Although we found no changing in the number of chromosome (2n=2X=14, data not shown), the difference in the genome size among some of the regenerated plants indicates that change in genetic structure of plants during tissue culture is also accompanied with quantitative change in DNA. This also happens among plants of each subculture as also was observed in molecular characteristics of these plants. Up to now, there is no reports about genome size changes and mixoploid events among regenerated plants in two *A. barbadensis* and *A. littoralis* species studied. BRANDHAM and DOHERTY (1998) reported genome sizes of 20 Aloe species (except *A. barbadensis* and *A. littoralis*). They suggested "evolutionary increase in DNA amount involves the amplification of many small DNA segments at sites distributed evenly throughout the chromosome complement in numbers proportional to chromosome length, thus maintaining karyotypic uniformity in the Aloe genus".

The reports on genome size change during somaclonal variation vary in different plant species. For example, nuclear DNA content in *Malus* species and cultivated apples (*Malus domestica* Borkh.) by flow cytometry were reported by TATUM *et al.* (2005) with a range of 1.45 pg for *Malus fusca* (diploid) to 2.57 pg for *Malus ioensis* (triploid).

Similarly, Jin *et al.* (2008) investigated cytogenetic and genome size variation in 67 regenerated cotton plants and observed that 2,4-D and kinetin hormone combination could induce relatively high somaclonal variation. They revealed that the number of chromosomes and ploidy levels were nearly stable in all regenerated plants. This result also might mean that the cell lines with variation of chromosome numbers were difficult to regenerate plants (BARANYI *et*

al., 1996). LEAL *et al.* (2006) also investigated genome size of somatic embryo-derived plants obtained from anther culture in *Vitis vinifera* cultivars and found that only one among the 41 analyzed plants (2.4%) presented somaclonal variation (tetraploidy); the other plants were diploid and no significant difference occurred in the nuclear DNA content of these plants.

There were no alterations in chromosome phenotypes, somatic haploid and diploid chromosome count (n = 7; 2n = 14), or meiotic behavior. The analyses revealed there were no somaclonal variations among these regenerants. These results confirm the very reliable method for large scale production that agreement with other researches studies on aloe vera (HAQUE & GHOSH, 2013)

CONCLUSION

In both of species (*A. barbadensis* and *A. littoralis*), a significant positive correlation was observed between the microshoot number and microshoot length, ($r=0.82$, $p<0.01$). Different genome sizes were also indicated among treatments and subcultures.

Received, July 21th, 2018
Accepted May 18th, 2019

REFERENCES

ABADI, G., M. HEDAYAT, M. MODARESSI (2013): Micropropagation of *Aloe vera* – Impacts of Plant Growth Regulators, Media and Type of Explants. J. Biol. Environ. Sci., *7*(19), 19-24.

ABRIE, A., J.V. STADEN (2001): Micropropagation of endangered *Aloe polyphylla*. Plant Growth Regulation, *33*(1): 19-23.

ADELBERD, J., J. NAYLOR- ADELBERG (2012): Effects of Cytokinin on ultiplication and Rooting of *Aloe barbadensis* during Micropropagation on Agar and liquid Medium. Journal of Medicinally Active Plants, *1*(1):1-5.

AGGARWAL, D., K.S. BARNA (2004): Tissue culture propagation of elite plant of *Aloe vera* Linn. Journal of Plant Biochemistry & Biotechnology, *13*: 77-79.

BRANDHAM, P.E., M.J. DOHERTY (1998): Genome Size Variation in the Aloaceae, an Angiosperm Family Displaying Karyotypic Orthoselection. Annals of Botany, *82* (Supplement A): 67-73.

JIN, S., R. MUSHKE, H. ZHU, L. TU, Z. LINL, Y. ZHANG, X. ZHANG (2008): Detection of somaclonal variation of cotton (*Gossypium hirsutum*) using cytogenetics, flow cytometry and molecular markers. Plant Cell Rep., *27* (8): 1303-1316.

LEAL, F., J. LOUREIRO, E. RODRIGUEZR, M.S. PAIS, C. SANTO, O. PINTO- CARNIDE (2006): Nuclear DNA content of *Vitis vinifera* cultivars and ploidy level analyses of somatic embryo-derived plants obtained from anther culture. Plant Cell Rep., *25* (9): 978-985.

BARNA, K.S., A.K. WAKHLU (1994): Whole plant regeration of *Cicer arietium* from callus culture via organogenesis. Plant Cell Rep., *13*: 510-513.

BARANYI, M., J. GREILHUBER (1996): Flow cytometric and Feulgen densitometric analysis of genome size variation in Pisum. Theor. Appl. Genet., *92*: 297-307.

BHAKSHA, R., J. MISKAT ARA AKHTER, K. RAHIMA, J. LITON MUNSHI (2005): Micropropagation of *Aloe barbadensis* Mill. through *in vitro* culture of shoot tip explants. Plant Tissue Cult. & Biotech., *15*(2): 121-126.

BHANDARI, A.K., J.S. NEGI, V.K. BISHT, M.K. BHARTI (2010): *In vitro* propagation of *Aloe vera*- A Plant with Medicinal Properties. Nature and Science, *4*(8): 15-24.

BHOJWANI, S.S., M.K. RAZDAN (1992): Plant tissue culture: Theory and practice, Elsevier, Amsterdam, London, New York, Tokyo.

BHOJWANI, S.S. (1981): A tissue culture method for propagation and low temperature storage of *Trifolium repens* genotypes. Physiol. Plant, *52*:187-190.

BHOJWANI, S.S. (1980): Micropropagation method for hybrid willow (*Salix matsudana x alba* NZ-10002). N.Z.J. Bot., *18*:209-21.

BURES, P., Y.F. WANG, L. HOROVA, J. SUDA (2004): Genome size variation in central European species of Cirsium (Compositae) and their natural hybrids. Ann. Bot., *94*: 353-363.

CANIGUERAL, S., R. VILA (1993): Aloe. British J. Phytother., *3*:67–75.

CAPASSO, F., F. BORRELLI, R. CAPASSO, G. DI CARLO, A.A. IZZO, L. PINTO (1998): Aloe and Its Therapeutic Use. Phytother Res., *12*:S124–S127.

CAVALLINI, A. (1990): In vitro culture of *Aloe barbadensis* Mill.:Micropropagation from vegetative meristems. Plant Cell, Tissue and Organ Culture, *20* :71-74.

CHAUDHURI, S., U. MUKUNDAN (2001): *Aloe Vera* L. Micropropagation and Characterization of its gel. Phytomorphology, *51*(2): 155-157.

DAVIS R.H., DONATO, J.J., G.M. HARTMAN, R.C. HASS (1994): Anti-Inflammatory and Wound Healing Activity of a Growth Substance in Aloe vera. Am. Poddiatr. Med. Assoc., *84*,77-81.

DEBIASI, C., C.G. SILVA, R. PESCADOR (2007): Micropropagation of *Aloe vera* L. Rev Bras PIant Med Botucatu, *9*: 36-43

DUCOS, J.P., R. ALENTON, J.F. REANO, C. KANCHANOMAI, A. DESHAYES, V. PETIARD (2003): Agronomic performance of *Coffea canephora* P. trees derived from large-scale somatic embryo production in liquid medium. Euphytica, 131(2): 215–223.

DWIVEDI, N.K., A. INDIRADEVI, K.I. ASHAL, R. ASOKAN NAIR, A. SUMA (2014): A protocol for micropropagation of *Aloe vera* L. (Indian Aloe) – a miracle plant. Research in Biotechnology, *5*(1): 01-05.

GANTAIT, S., N. MANDAL, S. BHATTACHARYA, P.K. DAS (2010): A Novel Strategy for *in vitro* conservation of *Aloe vera* L. through Long Term Shoot Culture. Biotechnology, *9*: 326-331.

GRINDLAY, A., B. REYNOLDS (1986): Aloe Promotes only Healthy Cell: If a Cell is Cancerous, Growth is not Stimulate. J. Ethnopharmacology, 117-151.

HAJHASHEMI, V., A. GHANNADI, A.H. HEIDARI (2012): Anti-inflammatory and wound healing activities of *Aloe littoralis* in rats. Res. Pharm. Sci., *7*(2): 73–78.

HAQUE, S.K.M., 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. Botanical Studies, *54*(46): 1-10.

HASHEM ABADI, D., B. KAVIANI (2010): *In vitro* proliferation of an important medicinal plant Aloe- A method for rapid production. Australian Journal Crop Science, *4*(4): 216-222.

HAQUE S.M., 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. Botanica Studies, *54*(46): 2-10.

HEGGERS, J.P., R.P. PELLEY, M.C. ROBSON (1993): Beneficial effects of aloe wound healing. Phytotherapy Research, *7*: 48-52.

KHATUN M.M., T. TANNY, S. YESMIN, M.D. SALIMULLAH, I. ALAM (2018): Evaluation of genetic fidelity of in vitro-propagated *Aloe vera* plants using DNA-based markers. ScienceAsia, *44*: 87-91.

KLEIN, A.D., N.S. PENNETS (1988): *Aloe vera.* J. Am. Acad. Dermatol., *18*:714-720.

KOCE, J.D., B. VILHAR, B. BOHANEC, M. DERMASTIA (2003): Genome size of Adriatic seagrasses. Aquat. Bot., *77*: 17-25.

KUMAR, M., SINGH S., S. SATYAPAL (2011): *In vitro* Morphogenesis of a medicinal plant –*Aloe vera* L. Asian Journal of Plant Science and Research, *1*(1): 31-40.

LANE, W.D. (1979): In vitro propagation of Spirea bumalda and Prunus cistena from shoot apics. Can. J. Plant Sci., *59*: 1025-1029.

LARKIN P.J., W.R. SCOWCROFT (1981): Somaclonal variation: A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., *60*: 197–214.

LIAO, Z., M. CHEN, F. TAN, X. SUN, K. TANG (2004): Micropropagation of endangered chinese aloe. Plant cell, Tiss. Org. Cult., *76*: 83-86.

MAZAFFARIAN, V. A. (1996): Dictionary of Iranian Plant Names. Tehran: Farhang-e Moaser Publications; PP. 32.

MEYER, H.J., J.V. STADEN (1991): Rapid *in vitro* micropropagation of *Aloe barbadensis* Mill. Plant Cell, Tissue and Organ Culture, *26*:167-171.

MURASHIGE, T. (1977): Clonal crops through tissue culture. In: W.Barz *et al.* (Editors), Plant Tissue Culture and its in Biotechnological Application. Springer-Verlag, Berlin, 392-403.

MURASHIGE, T., F. SKOOG (1962): A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol. Plant, *115*: 493-497.

NAIR, P.K.R. (1979): Intensic'e multiple cropping with coconuts in India: Principles, programmes and prospects. Verlag Paul Parey, Berlin (West).

NATALI, L., I.C. SANCHEZ, L. NATALI, I.C. SANCHEZ, A. CAVALLINI (1990): In vitro culture of Aloe polyphylla. Plant Growth Regulation, *33*(1): 19-23.

NAYANAKANTHA, N.M.C., B.R. SINGH, A. KUMAR (2010): Improved culture medium for micropropagation of *Aloe vera* L. Tropical Agricultural Research and Extension, *13*(4): 87-93.

NEJATZADEH-BARANDOZI F., L. AKBARI (2013): Karyotypic Variation of the *Aloe vera* L. and *Aloe littoralis* Baker in Iran. Iranian Journal of Biotechnology, *11*(4): 233-37.

NOORMOHAMMADI, Z., B. GHASEMPOUR, F. FARAHANI (2018): Somaclonal variation of tissue culture regenerated plants of *Aloe barbadensis* Mill. Nova Biologica Rep. 5: 72-81.

PETKOVA, S., N. POPOVA, Y. ANGELOVA, B. STEFANOV, L. ILIEV, M. POPOV (2003): Inhibitory effect of some plant growth regulators and chlorsulfuron on growth, protein composition and proteolytic activity of Maize seedlings. Biotechnol and Biotechnol. Eq., *17*(2): 77-83

RICHWINE, A.M., J.L. TIPTON, A. THOMPSON (1995): Establishment of *Aloe*, *Gasteria*, and *Haworthia* shoot cultures from inflorescence explants. Hort Science, *30*(7): 1443-1444.

RINES, H.W., T.J. MCCOY (1981): Tissue culture initiation and plant regeneration in hexaploid species of oats. Crop Science, *21*:837-842.

ROY, S.C., A. SARKAR (1991): In vitro regeneration and micro propagation of *Aloe vera*. Scientia Horticulturae, *47*(1-2): 107-114.

SEBASTINA, J., K.S. BARNA (2003): Plant regeneration through callus culture of *Iresine lindenii*. In Vitro Cellular and Developmental Biology, Communicated.

SHELTON, M.S. (1991): Aloe vera, Its Chemical and Therapeutic Properties. International Journel of Dermatology, *30*: 679-683.

SINGH, M., M.S. RATHORE, D. PANVAR, J.S. RATHORE, R. DAGLA, N.S. SHEKHAWAT (2009): Micropropagation of selected genotypes of *Aloe vera* L. - An ancient plant for Modern Industry. Journal of Sustainable Forestry, *28*: 935-950.

SKIRVIN, R.M., M.C. CHU (1979): In vitro propagation of "Forever Yours" rose. Hort. Sci., *14*: 608-610.

SOLTANIPOOR, M.A. (2006): Introduction to the Flora, Life Form and Chorology of Hormoz Island Plants. S. Iran. Rostaniha, *7*:19–32.

STOLTZ, L.P. (1979): In vitro propagation of *Acalypha wilkesiana.* HortScience, *14*:702-703.

TATUM, T.C., S. STEPANOVIC, D.P. BIRADAR, L. RAYBURN, S.S. KORBAN (2005): Variation in nuclear DNA content in *Malus* species and cultivated apples. Genome, *48*(5): 924-93.

VISUTHIKOSOL, V., B. CHOWCHUEN , Y. SUKWANARAT, S. SRIURAIRATANA, V. BOONPUCKNAVIG (1995): Effect of aloe vera gel to healing of burn wound a clinical and histologic study. Journal of the Medical Association of Thailand, *78*(8): 403–409.

YIPENG, Z., W. BRAIN, W. GROUT, C. PETER (2005): Variations in morphology and disease susceptibility of micropropagated rhubarb (*Rheum rhaponticum*) PC49, compared to conventional plants. Plant cell, Tissue and Organ Culture, *82*: 357-361.

ZAKIA, S., N.A. ZAHID, M. YASEEN, N.A. ABBASI, A.A. HAFIZ, M. NASIR (2013): Standardization of micropropgation techniques for Aloe vera: A pharmaceutically important plant. Pak. J. Pharm. Sci., *26*(6): 1083-1087.

ZIMMERMAN, R.H., O.C. BROOME (1981): Phloroglucinol and in vitro rooting of apple cultivar cuttings. J. Am. Soc. Hortic. Sci., *106*: 648-652.

ISPITIVANJE GENETIČKOG DIVERZITETA DVE REGENERISANE *in vitro Aole barbadensis* I *Aloe littoralis* BILJKE PRIMENOM C-VREDNOST DNK CITOMETRIJOM

Farah FARAHANI[1]*, Atena BAYANI [2], Zahra NOORMOHAMMADI [2],

[1] Departmant za mikrobiologiju Qom Branch, Islamic Azad Univerzitet, Qom, Iran
[2] Biološki Departmant, Škola osnovnih nauka, Nauka i istraživanje, Islamic Azad Univerzitet (SRBIAU), Tehran, Iran

Izvod

Aloe koje pripadaju porodici Asphodelaceae, ranije Liliaceae, je rod zeljastih i sukulenata. Vrh izdanka od 2-3 cm sakupljen je kod *Aloe barbadensis* i *Aloe littoralis*, eksplantati su površinski sterilizovani, nakon što su inokulirani sa MS medijumom koji sadrži različite koncentracije BA (0,5, 1,2 mg / L), IAA (0,5, 1 mg / L). Kulture su inkubirane na 25 ± 2° C tokom 16 h fotoperioda. Kod *A. barbadensis* i *A. littoralis*, najbolji tretman za najveći broj izdanaka i proliferaciju pupoljaka bio je MS medijum koji sadrži 2 mg / L BA i 0,5 mg / L IAA. Maksimalni procenat razmnoženih pupoljaka (90% i 95%) iz jednog eksplantata dobijen je u MS medijumu nakon 4-5 nedelja druge i prve subkulture, respektivno. Obe vrste su imale adventivne korene indukovane u MS medijumu koji je sadržavao 0,5 mg / L BAP i 0,5 mg / L IAA posle druge subkulture. Veličina genoma regenerisanih biljaka u vrstama *A. barbadensis* i *A. littoralis* razlikovala se u različitim tretmanima (od 30,66 pg do 39,62 pg, 2C vrednost). Jedan uzorak t-testa pokazao je značajnu razliku (P = 0,001) između veličina genoma dve ispitivane vrste kada je *A. littoralis* pokazao veću vrednost C u poređenju sa *A. barbadensis*. Različite veličine genoma takođe su naznačene među tretmanima i subkulturama. Mikoploidi identifikovani su u histogramima protočne citometrije prisustvom dva ili tri pika kod *A. barbadensis* i *A. littoralis* u regenerisanim biljkama u različitim tretmanima i subkulturama u tretmanu 2 (BA 1 mg / L, IAA 0,5 mg / L).

Primljeno 21.VII.2018.
Odobreno 18. V. 2019.