RESEARCH ARTICLE

In vivo Rooting, Acclimatization and *Ex situ* Conservation of *Opuntia ficus-indica* (L.) Mill. (Beles) in Tigray Region of Ethiopia, Africa

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Abstract

Opuntia ficus-indica (L.) Mill. commonly known as prickly pear or Beles is a xerophytic, succulent, CAM (Crassulacean Acid Metabolism) plant introduced in Ethiopia (Africa), particularly to Tigray region between 1848 and 1920. It is known as a multipurpose plant since it can be used as human food (fruits and vegetables), medicine and ornamental plants, fodder, natural wind barrier, soil stabilizer, re-vegetation resource in eroded soils. Stem of this plant has been reported to treat diabetes and useful in the cure of hyperlipidemy (excess of lipids in the blood) and obesity. It is well recognized for their wound healing properties and anticancer effects. Conventionally, it is propagated by seeds but physiological limitations of the seeds, such as; low germination rate, genetic segregation, less guarantee of genetic stability, a long juvenile stage, less availability and low viability, and slow seedling growth rate are major constraints in the multiplication of this species in nature. In the present study we developed an efficient procedure for mass multiplication, acclimatization and *ex-situ* conservation of this succulent plant in Adigrat region of Ethiopia (Africa). In the present study out of six used *in vitro* - raised clones, maximum average height of the plantlet (13 cm.) and width (8.5 cm.) were observed in the plantlets (X_6) and (X_5) clones at 30.29 and 29.15°C temperatures, respectively on a modified substrate. Here, the effects of temperature and duration were significant in the increment of clones height and width. Thus, the developed efficient acclimatization techniques of this crop will ensure the supply of the *in vitro*-raised plantlets throughout the year to the consumers in Tigray region of Ethiopia.

Keywords: Biodiversity, CAM plant, Conservation, Micropropagation, *Opuntia ficus-indica*.

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Introduction

Cactus has about 130 genera and 1,500 species within Cactaceae family (Shetty *et al*. 2012). Of these, *Opuntia ficus-indica* (L.) Mill. commonly known as prickly pear or "Beles" is a tree-like cactus native to Mexico and it was introduced in Ethiopia, particularly to Tigray region between 1848 and 1920 (Neumann, 1997, Habtu, 2005). The region is largely covered by cactus (*O. ficus-indica*) fruits, locally known as "Beles" is the only source of food for many farmers during raining season (June-September), just before the harvesting of staple crops (Habtu, 2005). The cactus is a xerophytic, succulent, CAM (Crassulacean Acid Metabolism) plant (Bravo-Hollis, 1997). Over the last few decades, interest in Beles as food and feed has increased due to its drought resistance, high biomass yield, high palatability and tolerance to salinity (Ben *et al*., 1996).

Opuntia ficus-indica can attains the maximum height of about 15 ft tall and a multipurpose plant, can be used as human food (fruits and vegetables), medicine and ornamental plants, fodder, natural wind barrier, soil stabilizer, re-vegetation resource to control water and wind erosion in eroded soils (Nobel, 1994, Rodriguez-Felix, 2002, Russell and Felker. 1987; Pimienta-Barrios and Munoz-Urias, 1995). The sweet fruit of this plant is known as "Tuna" useful as human food and entire plant can be cultured as raw-industrial material to produce several sub-products such as jam, wine, candies and jellies etc. (Hegwood, 1990, Flores-Valdez, 1995, Saenz-Hernandez, 1995). Besides, *Opuntia ficus-indica* has been grown from pre-Columbian times as a host plant for cochineal insects (*Dactylopius coccus*) for the production of valuable, vivid red and purple dyes (Donkin, 1977; Nobel, 1994). It is also source of natural-dye, carminic acid which is used for coloring fabrics, food, and cosmetics (Flores-Flores and Tekelenburg, 1995).

In addition the tree is also known for its medicinal properties as it is a rich source of flavonoids having affirmative health-benefits (Knishinsky, 1971; Iwashina *et al*., 1984; Wang, 1988; Goycoolea and Cárdenas, 2003; Tesoriere *et al*., 2004). It also contains antioxidants, primarily pectin, carotenes, betalains, ascorbic acid, quercetina and quercetin derivatives which is useful in the protection of mammalian cells and organs and slow the aging process, illness and disease (Kuti, 2004). Stem of this plant has been reported to treat diabetes and useful in the cure of hyperlipidemy (excess of lipids in the blood) and obesity (Saenz, 2000). Alcoholic extract of this plant is useful in treating a number of diseases and conditions, including anti-inflammatory effects (Park *et al*., 1998; Sahelian, 2001), hypoglycemic effects (Frati *et al*., 1990), inhibition of stomach ulceration (Galati *et al*., 2003) and neuroprotective effects (Dok-Go *et al*., 2003). In Chinese medicine, fruits of this species are considered weak poisons and are used for treatment of inflammation, pain and as detoxification agents for snake bites (Wang, 1988). A decoction of the bark, which contains about 10% of tannin, is used as an

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astringent lotion in leucorrhoea. The leaves of the plant are heated and applied as a poultice to abscesses to promote suppuration and discharge of pus (Galati *et al*., 2003). *O. ficus-indica* is well recognized for their wound healing properties and anticancer effects (Zou *et al*., 2005). Conventionally, *Opuntia ficus-indica* is propagated by seeds but physiological limitations of the seeds, such as; low germination rate, genetic segregation, less guarantee of genetic stability, a long juvenile stage, less availability and low viability, and slow seedling growth rate are major constraints in the mass production of this species in the nature (Escobar *et al*., 1986; Soltero, 1996). In addition, it is asexually propagated by "areoles" (main meristematic tissue in stems) and cladodes which is time taking process. Furthermore, propagation by cuttings is not always feasible and often leads to a low multiplication rate (Akram *et al*., 2013). Vegetative propagation, which is widely used, can be performed through the rooting of single or multiple cladodes, small portions of mature cladodes comprising two or more areoles (Estrada-Luna *et al*., 2008). All these methodologies require large spaces for propagation and has threat of a low propagation rate. Therefore, the micro propagation is a feasible alternative option for the rapid multiplication and maintenance of germplasm, because it provides high propagation rates, reduced requirements for space, the production of healthy and pathogen-free plants (Johnson and Emino, 1979; Smith *et al*., 1991). The first study on *Opuntia (*prickly pear cactus) micropropagation reported by Sachar and Iyer (1959) and various successful strategies have been described for different species of Opuntia, including *O. dillenii* Haw, *O. amyclaea* Tenore, *O. ficus indica* Linn Mill, *O. streptacantha* Lemaire, *O. robusta* Wendl, *O. cochinera* Griffiths, *O. leucotricha* De Candolle, *O. albicarpa* Scheinvar and *O. ellisiana* Griff. (Mauseth and Halperin, 1975, Mauseth, 1977, 1979, Estrada-Luna, 1988, Mohamed-Yasseen *et al*., 1995, Estrada-Luna and Davies, 2001), however, an efficient *in vitro* protocol is not yet available for commercialization because most cactus plant responses to tissue culture are highly dependent on the genotype. Some important modifications and adjustments might be needed when a new species or cultivar is considered for tissue culture, especially to optimize the overall environmental culture conditions, media, plant regulators (type, concentration, and combination), during the shoot proliferation stage (Aliyu and Mustapha, 2007). Rooting and plantlet acclimatization conditions might also be investigated since they may limit the success of micropropagation (Hartmann *et al*., 1997). Acclimatization is an important step in micropropagation study to produce a well-developed of *ex vitro* plantlets that can successfully survive in the field environment (Pospóšilová *et al*., 1999). It determines the quality of the end product and, in commercial production, the economic viability of the enterprise (Conner and Thomas, 1982). Thus, *Opuntia ficus-indica* is an economically important plant in Ethiopia and undoubtedly, several difficulties are in its cultivation. In this paper authors propose a novel method of mass multiplication and acclimatization of *O. ficus-indica*. The *in vitro*- raised plants are subjected to treatment of various temperature and soil condition which yielded good height, width and percent of survival in Ethiopia.

Materials and Methods

Plant material for tissue culture

One-year-old *in vitro*-raised plantlets of Beles were collected from Tigrey Biotechnology Centre, Mekelle (Ethiopia) in the second week of December 2018 which in turn were procured from Michael Technology Charitable Organization, USA. Few more plantlets were collected from Mekelle University which procured by Dry Grow Foundation Company of Italy in 2017. All the plant materials were maintained in the shade house of Beles Institute, Adigrat University, Adigrat (Ethiopia) prior to the experiment.

Initiation of growth experiment

To know the effects of microclimatic conditions and substrate on acclimatization the *in vitro*-raised plantlets were washed carefully in running tap water to remove the adhering agar and transferred to earthen pots as procedures reported by Shukla and Khare (2012). The experiment was carried out in laboratory of Beles Institute, Adigrat University, Adigrat with six clones of cultures, i.e., X1 (4.26 and 1.56), X2 (8.91 and 4.93), X3 (5.73 and 2.89), X4 (9.57 and 4.52), X5 (10.66 and 5.56) and X6 (11.93 and 4.73) (average length and width in cm). On 15-12-2018, 60 *in vitro*-raised rooted shoots with different length (10 shoots of each clone) were transferred in to earthen pots containing two types of substrate which composed of sand only as substrate I and materials listed in Table 1 as substrate II respectively.

Soil preparation

The soil is composed of different materials which have great importance in the growth of plantlets. The soil was homogenized using spade, then filled to black bottom perforated polyethylene bag with its diameter of 50cm. The single filled bag weighed 7.4 kg. The percentage of the soil composition are shown below in the Table 1.The plants were treated with 16:8hr of light and dark conditions and watered twice a week.

End of experiment

The growth experiment was completed on 15-03-2019 (after three months). Plantlets were initially covered with polythene sheets in order to maintain a high humidity (~ 90%) and kept in the culture room at temperature range of 20-32^{o}C and photoperiod of 16h/day and watered twice weekly. After one week, the polythene covers were removed and plantlets were moved to glass house after transferring to thirteen pots containing sand and soil respectively. The survival rate of plantlets was recorded. Here, some of plantlets get light from Florence lamp and the others get from cactus growth lamp.

Statistical analysis

The collected data's were analyzed statistically to determine the level of significance through Analysis of Variance (ANOVA) among the growth of plantlets through the effects of temperature and soil by using SPSS version 16 software.

Result and Discussion

In the present study out of 6 used *in vitro* -raised clones, maximum average length of the plantlet (13 cm.) and width (8.5 cm.) has been

Table 1: Material type and their proportion used for soil preparation.

No.	*Materials*	*Weight (kg)*	*Percentage (%)*
1	Sand	19.2	25.93
2	Compost	22.2	29.99
3	Ash	7.5	10.12
4	Clay	18.5	24.99
5	Crushed stones	2.22	2.99
6	Banana peel	2.22	2.99
7	Egg shell	2.22	2.99
	Total	74.06	100

observed in X_6 and X_5 clones at 30.29 and 29.15°C temperatures, respectively on substrate II composition (Fig. 1, Table 2). After three months of acclimatization highest survival rate of the plantlets was 95%. Most of the plantlets grew well and showed similar phenotypic homogeneity such as, color, yield etc. as in the case of plants grown in natural environment.

Statistically, the effects of temperature and duration were significant factor in the increment of all clones, expect X_4 with P<0.05 was 0.08 and X_6 with P<0.05 was 0.296 respectively (Table 3). Here, the increased temperature and prepared soil from different materials has significant in clones' height. The optimum temperature for the growth of cactus plant found in between 25-35°C (FAO, 2017). Similarly, temperatures and soil for the width growth of the plantlets had also effect. From the result and observation the temperature fluctuation and plant grew in soiled pot shown good progress than plant grew in sand pot only. The Analysis of variance (ANOVA) results also shown as X_1(P P<0.05=0.00), X_2(P<0.05=0.00), X_4(0.012) and X_5(P<0.05=0.00) (Table 3).

Besides, the increment of width in clones of X_3 (P<0.05=0.06) and X_6 (0.218) had not significant difference (Table 3), this may be due to placement and obtained the same light energy and soil materials.. Thus, this showed better acclimatization percentage than earlier report on prickly pear cactus species (Escobar *et al.,* 1986; Estrada-Luna, 1988; Mohamed-Yasseen *et al*., 1995) and

Fig. 1: Acclimatization of *in vitro*-raised plantlets of *Opuntia ficus-indica* (L.) Mill. in culture room. a) Multiple shoots b) Rooted plantlet c) Plantlets transferred from the medium to the polyethylene bags d) Plantlets after three weeks (e and f) after 40 days.

Table 2: Effect of microclimatic conditions on the growth of *in vitro*-raised plantlets of beles (After 40 days).

	Plantlet growth							
	Substrate I (sand only)				*Substrate II(Soil which prepared from the seven materials as Table-1)*			
Clones	*Average height of shoots (cm)*	*Average width of shoots*	*Temp.*	*%culture response*	*Average height of shoots (cm)*	*Average width of shoots*	*Temp.*	*% culture response*
X_1	3.75 ± 0.43	2.75 ± 0.41	22.62 ± 0.13	35.2 ± 0.12	6.75 ± 0.17	4.75 ± 0.16	24.78 ± 0.08	40.3 ± 0.02
X_2	1.67 ± 0.18	2.13 ± 0.00	21.45 ± 0.15	40.4 ± 0.11	3.67 ± 0.13	5.13 ± 0.12	25.92 ± 0.12	45.2 ± 0.34
X_3	3.66 ± 0.19	1.50 ± 0.12	20.23 ± 0.14	60.3 ± 0.01	4.66 ± 0.17	2.00 ± 0.42	25.14 ± 0.14	65.4 ± 0.21
X_4	6.50 ± 0.39	4.00 ± 0.63	22.34 ± 0.12	65.1± 0.23	8.00 ± 1.54	6.00 ± 1.09	28.55 ± 0.13	70.6 ± 0.43
X_5	9.35 ± 0.48	3.50 ± 0.44	23.20 ± 0.08	70.4 ± 0.13	12.33 ± 1.43	8.50 ± 1.04	29.15 ± 0.11	80.5 ± 0.54
X_6	12.00 ± 1.26	6.43 ± 0.54	26.16 ± 0.05	75.6 ± 0.21	13.00 ± 1.41	6.83 ± 0.70	30.29 ± 0.10	95.4 ± 0.04

NB: X_a - the "X" refers to the name of plantlet; "a"- subscript refers to treatment number.

Table 3: Analysis of Variance (ANOVA) summarized results.

	Effects of temperature and soil	
Clones	*Height (P<0.05)*	*Width (P<0.05)*
X_1	0.000	0.000
X_2	0.000	0.000
X_3	0.000	0.060
X_4	0.080	0.012
X_5	0.011	0.000
X_6	0.296	0.218

most micropropagated cacti (Johnson and Emino, 1979; Ault and Blackmon, 1987). Several research papers on the micropropagation of Cactaceae can be found, but *in vivo* acclimatization of the plantlets is not always reported, and in some cases this is shown to be critical (Smith *et al*., 1991; Rodriguez-Garay and Rubluo, 1992; Rubluo *et al*., 1993). Due to its recalcitrant property, cactus is not a good choice for tissue culture. Only a few scattered studies have been reported on *in vitro* regeneration and acclimatization of this plant. Rooting and *in vivo* establishment have proven difficult for several species of micropropagated cacti (Smith *et al*., 1991; Rodriguez-Garay and Rubluo, 1992; Rubluo *et al*., 1993; Llamoca-Zarate *et al*. 1999). The method of acclimatization presented in this article is different from earlier reported work in terms of plant growth regulators or biostimualtors and aeration were not used. Air humidity was also not maintained at a certain level mechanically or otherwise.

Conclusions

The acclimatization methods presented in this paper are cheap and feasible which can be used by local person of the Tigray region of Ethiopia because it eliminates the necessity of special fertilizers and plant growth regulators. They do not even require reliable irrigation systems, because the constant availability of water is ensured by its presence in the tubs. By this method we can produce a huge amount of acclimatized plantlets of Beles in a limited place and shorter time. In Tigray about 83% of the population lives in rural areas and their main source of livelihood is based on agriculture. Regular income and food security is highly dependent on the annual rainfall resulting in Tigray region with the highest harvest failures and lowest per capita income/expenditure (Giuseppe De Bac, 2009). Present study will be helpful in order to strengthen the link between conservation and utilization. Cactus plant has high potential as commercial crop that it can be processed so easily and it has potential in international market and be source of foreign currency for the country. Our developed efficient acclimatization techniques of this crop will ensure the supply of the *in vitro*-raised plantlets throughout the year to the consumers.

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