Effect of growth regulators on *Carpobrotus edulis* rapid micropropagation and molecular analysis

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Abstract: Knowledge concerning *in vitro* regeneration and developmental responses of *Carpobrotus edulis* is limited. Adventitious shoots were regenerated from leaf explants on MS media supplemented with (0.05) µM TDZ. Aseptic seedlings were successfully raised on MS medium containing half strength salts. MS medium supplemented with 4.0 mg/L 2iP proved to be the best for multiple shoot induction. MS supplemented with 0.4 mg/L NAA was found suitable medium for root induction in excised micro-shoots. The plants were successfully acclimatized in the greenhouse at 100% survival rate. *In vitro* derived plants were analyzed using SDS-PAGE of soluble protein extracts and RAPD fingerprints. SDS-PAGE protein and randomly amplified polymorphic DNA (RAPD) analysis confirmed that all the regenerated plants from leaf and shoot tip were genetically identical to their donor plants, suggesting the absence of detectable genetic variation in the regenerated plants.
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1. Introduction

Family *Aizoaceae* is one of the most abundant and diverse plant families in Southern Africa (Van der Watt and Pretorius, 2001). The genus *Carpobrotus* falls within this family and is well identified by its trailing structure and thin, blade-like succulent leaves (Springfield and Weitz, 2006). *Carpobrotus edulis* is a perennial, mat-forming herb. It is a robust, flat-growing, trailing perennial, rooting at nodes and forming dense mats (Malan and Notten, 2006, Thomas, 1990).

For decades, traditional healers all over South Africa have been utilizing various species of *Carpobrotus* genus to treat fungal and bacterial infections (van der Watt and Pretorius, 2001). *C. edulis*, also referred to as sour fig or Hottentot's fig, is widely utilized as a traditional remedy for a wide range of bacterial and fungal infections (Smith *et al*. 1998) including the treatment of eczema, burns, wounds, tuberculosis, vaginal thrush, toothache and earache (Van Wyk *et al*. 1997; Buwa and Afolayan, 2009). Traditionally the propagation of this species is made through cuttings (Johnson and Emino, 1979a and b). Seed propagation involves different problems such as slow initial growth and susceptibility to damping-off (Ault and Blackmon, 1987). For further research into the biochemical compositions and potential medicinal values of this plant, an efficient *in vitro* regeneration system for the production of plants is required because field grown plants may be subject to seasonal and somatic variations and environmental pollutions that may affect the medicinal value of the harvested tissues (Geng *et al*. 2001). In addition, *in vitro* propagation methods offer powerful tools for germplasm conservation and mass-multiplication of many threatened plant species (Murch *et al*. 2000). Establishment of system for plant regeneration from *in vitro* cultured cells and tissues is necessary for micropropagating new cultivars and conserving the valuable old cultivars in danger of extinction. To date, micropropagation techniques are employed to produce a large number of new and true-to-type plants in a relatively short period of time. Recent developments in direct gene transfer are techniques of interest to extend the genetic variability in plant species. To establish a gene transfer system, it is essential to increase the rate of regeneration. *In vitro* shoot tip culture has been proposed as a method that offers several advantages for mutation inductions in vegetatively propagated plants.

Recently, many techniques have been developed to detect and identify genetic variations (Ruibal-Mendieta and Lints, 1998). Molecular techniques such as RAPD, for instance, are a quick and reliable method that could significantly detect small genetic changes in plants, (Williams *et al*. 1990). RAPD analysis using PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals (Rani *et al*. 1995). RAPD markers have been used widely in studying the genetic diversity of somaclonal variations in various plant species (Soniya *et al*. 2001).

Up to our knowledge this is the first introduction of an *in vitro* propagation system for *Carpobrotus edulis*. Efficient direct shoot

regeneration from leaf explants suitable for genetic transformation was also introduced in this report. Detection of the probability of the presesnce of any somaclonal variations in *the in vitro* derived plants was also investigated depending on the morphological characters. Comparing the DNA profiling of normal and variant plants with the aim to monitor the uniformity of plants multiplied *in vitro* was performed as well.

2. Materials and Methods

2.1. Seed germination

Seeds of *Carpobrotus edulis* were purchased from Flora Frey GmbH, Solingen, Germany. The seeds were sterilized by 70% ethanol solution for 30 sec and then were rinsed in 5% (v/v) sodium hypochlorite solution for 5 min and washed three times with sterile tap water under laminar air-flow hood to remove all the traces of sodium hypochlorite. Surface sterilized seeds were cultured into 40 ml capacity jars containing 10 ml MS medium containing half strength salts (Murashige and Skoog, 1962) basic salts and vitamins, supplemented with 2% (w/v) sucrose, 7.0 g/L agar. Medium was adjusted to pH 5.7, prior to autoclaving at 121°C and 1.2–1.3 kg/cm^2 pressure for 20 min. One hundred seeds were cultured with only one seed/jar. The cultures were incubated under growth room conditions (22 ± 2°C, 16 h photoperiod and light intensity of 4000 lux provided by florescent lamps (Phillips TLM 40W/33RS). After eight weeks from seed culture, the rate of germination was determined.

2.2. The effects of BA on shoot induction

Eight weeks old *in vitro* shoot tips (0.5 cm height) were excised and transferred to MS medium containing 3% (w/v) sucrose, 7.0 g/L agar and different concentrations of benzyladenine (BA) (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L). Cultures were established in 40 ml culture vessels. After eight weeks the shoot tip explants from these regenerated shoots were transferred to 40 ml culture vessels containing MS medium to be used as explants for studying the effect of different concentrations of benzyladenine (BA), kinetin (Kin) and 2-isopentenyladenine (2iP) and the following data were recorded on number of shoot regenerated from explants, plant height and plant weight.

2.3. Proliferation of shoots from shoot tip explants

24-weeks-old shoot tips with the height of (0.5 cm) from *in vitro* culture explants were cultured onto MS medium containing 3% (w/v) sucrose, 7.0 g/L agar and different concentrations of BA, Kin and 2iP (0.0, 1.0, 2.0 and 4.0 mg/L), medium was adjusted to pH 5.7, prior to autoclaving at 121°C and 1.2–1.3 kg/cm^2 pressure for 20 min. Every jar contained three explants and each treatment had ten replicates. The cultures were grown for eight weeks before data were recorded based on number of shoots, plant height, plant weight as well as the number of roots.

2.4. Proliferation of shoots from Leaf and root explants

Fully developed leaves and roots excised from 24-weeks–old *in vitro* culture explants were cut into leaf discs and root segments of about 1.0 cm^2 in size. A third group of developed leaves (1.0 g) were cut to very small pieces under sterile conditions. The root segments, leaves and the small pieces of leaf explants were distributed separately on Petri dishes (92x16 mm) containing 30-35 ml of MS media (Murashige and Skoog, 1962) basic salts and vitamins supplemented with 3% (w/v) sucrose, 6.0 g/L agar. The pH of the medium was adjusted to 5.7. The medium was autoclaved for 20 min at 121°C and different concentrations of thidiazuron (TDZ) (0.0, 0.005, 0.01, 0.02, 0.04 and 0.05 µM), were added to the medium after being autoclaved and cooled to 47°C after filter sterilization as required. The explants were placed on Petri dishes with adaxial surface in contact with the medium. Every Petri dish was inoculated with five explant discs, each treatment containing five replicates (Petri dishes). After four weeks the number of shoot out of explant were calculated and the regeneration explants were transferred to the rooting medium.

2.5. Rooting of shoots and acclimazation

For rooting, 24-weeks-old *in vitro* shoots (0.5-1 cm in length) were excised and transferred to MS medium supplemented with different concentrations of IAA, IBA or NAA (0.0, 0.1, 0.2 and 0.4 mg/L) and placed under same growth room conditions for root formation. After four weeks of culture, data were recorded on number of root, root length, plant fresh weight and plant height.The rooted plantlets were transferred to greenhouse for acclimatization in pots with a moist mixture of (1:1) sand and perlite and maintained inside a plant growth chamber and irrigated with a fine mist of water for three weeks.The percentage of survival plant was determined after four weeks.

2.6. Protein extraction

SDS-polyacrylamide gel electrophesis (SDS-PAGE) was used to study the pattern of total soluble proteins of seven *in vitro* derived plants as well as control plants according to the method described by Laemmli (1970). Half gram of fresh leaves was ground to a fine powder and then mixed with 1.5 ml extraction buffer (10 g sucrose, 5 ml 2-mercaptoethanol, 2.0 g SDS and 2.422 g Trizma base, pH 8.5 and made up to 100 ml with distilled water), vortexed and left overnight at 4°C. It was then centrifuged at 5000 rpm for 20 min and the supernatant was transferred to a fresh tube. Aliquots

of the supernatant were analyzed by slab gel electrophoresis (Laemmli 1970) using 12% polyacrylamide gels. A wide range of standard proteins of known molecular weights (20.6, 28.9, 34, 49.7, 80, 124, 209 KDa) were run on a corresponding gel and used for characterization and determination of molecular mass of *Carpobrotus edulis* polypeptides. The protein of each individual plant was extracted separately and applied to the electrophoresis unit in a separate lane, a consistent protein pattern (molecular weight and concentration) was found for all individual plants.

Following electrophoresis the gel was stained with a solution containing 0.002% Commassie Blue-R-250 (National Diagnostics), and then destained with a mixture of glacial acetic acid, methanol and water. Once the position and matches of fingerprint bands had been scored, the data were ready for scanning using a LKB Recording Laser Densitometer equipped with LKB Recording Integrator (El-Manar Co., Cairo, Egypt).

2.7. Genomic DNA extraction

DNA was extracted from fresh leaves of ten *in vitro* derived plants as well as control plants using a standard CTAB extraction procedure (Wolff *et al.* 1994, modified after Saghai-Maroof *et al.* 1984). Cleaning with ammonium acetate was necessary. Samples were diluted with half the volume of 7.5 M, cold ammonium acetate, cooled in a fridge for 15 min, followed by spinning for 15 min at 5000 rpm. The supernatant was taken and two volumes of cold 96% ethanol gently mixed and left for 30 min in a freezer. After spinning for 15 min, the precipitate was taken, and 500 µl of cold 70 % ethanol added for washing. The supernatant was removed and the precipitate left to air-dry at room temperature for 10-20 min, and then dissolved in a suitable volume of TE buffer (1L 12.11 g Tris + 3.7 g EDTA + 800 ml dd. H_2O + HCl until pH 8.0 + dd.H_2O). DNA concentration was determend by NanoDrop 3300 (Thermo Scientific, Wilmington, USA).

2.8. Random Amplified Polymorphic DNA(RAPD)

RAPD analysis was performed in 25 µl volume reactions according to Wolff and Peters Van Rijn (1993). A reaction mixture (17.5 ng genomic DNA, 12.5 REDTaq ReadyMix (Sigma) [20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3.0 mM $MgCl_2$, 0.002% gelatin, 0.4mM mix dNTP (dATP, dCTP, dGTP, dTTP) and 0.06 unit/µl Taq DNA polymerase] and 0.4 p mole was prepared for each primer sufficient for all samples plus one negative control to which water was added instead of DNA. All reagents were centrifuged and kept on ice during the preparation of the master mix. Amplifications were carried out in a Mastercycler gradient programmed according to Wolff (1996) [the initial denaturation for 3 min at 94°C was followed by 45 cycles of denaturation (30 sec at 94°C), annealing (45 sec at 36°C), extension (1.5 min at 72°C)]. PCR products were analyzed by gel electrophoresis (Shelton Scientific, France) on 1.4% agarose gel prepared in 0.5 X TBE buffers, DNA ladder (Fermentas, USA) was used as a standard with molecular sizes.The gel was stained with ethidium bromide for 20 min and examined using UV cabinet unit and photographed with a Polaroid camera connected to a computer system with analytical software (GelDocu Advanced version, Wasserburg, Bodensee). Five oligonucleotide random primers (A1) 5'AGACGTCCAC3', (A2) 5ACGCGCATGT3', (A3) 5'AATGGCGCAG3', (A4) 5'GAATCGGCCA3' and (A5) 5'GGGAGACATC3' were used for RAPD analysis.

2.9. Statistical analysis

Experiments were set up in completely randomized design. Data were statistically analyzed using ANOVA\MANOVA of Statistica 6 software**,** StatSoft company (Statsoft, 2001), the significance of differences among means was carried out using the Least Significant Test (L.S.D) at $p = 0.05$.

3. Results and Discussion

At the end of eight weeks, seeds cultured on half-strength MS medium showed 65% germination. General aseptic techniques concerning *in vitro* culture of the explants were followed in the first experiment when MS supplemented with different concentrations of BA (Table 1). We found that at these levels of the cytokines we were able to have multiplication of explants without changing the plant growth. Data presented in Table (2) and Fig. (1B, C) show the effect of BA, Kin and 2iP concentrations on the *in vitro* shoot multiplication of *Carpobrotus edulis*. The control treatments without cytokinin were able to induce shoot proliferation. All the concentrations of BAP, Kin and 2iP facilitate shoot differentiation but 2iP being the most efficient in terms of number of shoot per explant. Shoots number increased with the increase of 2iP concentration. There was positive effect of 2iP on the capacity to induce plant regeneration (Fig.1A). 2iP at the highest concentration (4.0 mg/L) gave the highest number of shoot per explant 47.17 (Table 2). Number of shoots decreased with the increase of BA and Kin concentration, in contrast, the shoot length and plant weight were decreased with further increase in the concentrations of BA and Kin (Table 2). The positive effect of 2iP on the capacity to induce plant regeneration has been reported previously for plant species (Signe *et al*. 2007; Zhao *et al*.2008; Al-Sulaiman and Barakat, 2010).

The preliminary experiment using leaf, small pieces of leaf and root segments cultured on

hormone-free MS basal medium revealed that all explants did not regenerate shoots effectively and became pale and necrotic after 6-8 weeks period. At the fifth week of culture, the explants turned brown and small granulated callus formed at the margin of the leaves, shoot regeneration was not observed on root culture. Most leaves formed good callus on the MS media enriched with TDZ. In the beginning, callus growth was rather slow but after 4-weeks culture period the speed of callus formation was fast and small green nodules was observed. Eight weeks after initial cultures numerous adventitious shoot formations took place readily from these nodules (Fig. 2 B, C). The number of adventitious shoots varied depending on the concentrations of TDZ (Table 3, Fig. 2 B, C). TDZ at higher concentration (0.05 µM) proved to be the best treatment for direct shoot regeneration from leaf disc (2.8 shoots) and small pieces of leaves (13.4 shoots) (Table 3, Fig. 2).

Root formation was 100% in response to the application of IAA, IBA and /or NAA to the culture media. The MS basic medium without IAA, IBA and /or NAA also revealed root formation (Table 4). Of the three kinds of auxin tested, IBA and NAA at 0.4 mg/L induced the maximum number of roots (30 and 33.8 root/explants) respectively (Table 4 and Fig. 3 A, B). Further increase in the concentration of IAA had no effect on the number of root (Table 4). In contrast, the enhanced level of IBA and NAA had stimulating effect on the total number of root of *Carpobrotus edulis*. Although excessive auxin is commonly characterized by callus formation, no callus formation was detected in rooting stages of *Carpobrotus edulis*. The absence of callus at shoot base is an important observation because it can be excluded that auxin treatments were supplied in improper high supplements (Nerman *et al*., 2009).

The success of any *in vitro* regeneration protocol largely depends on the survival and growth performance of propagated plantlets *ex vitro* (Joshi and Dhar, 2003). In the present study, the acclimatization procedures applied was successful. *In vitro* regenerated plantlets showed 100% survival when transferred to soil (Fig. 3 C, D, E).

Maintaining genetic stability in regenerated plants is essential for species conservation (Quiala *et al.* 2009). In order to investigate whether somaclonal variation was evident in the *in vitro* regenerated plants, both protein and RAPD-DNA marker were used. SDS-PAGE has been used widely in many studies to identify variation in protein pattern of many species (Kitamoto *et al.* 1998; Lortal *et al.* 1997; Kamikouchi *et al.* 2004). The results of protein marker using SDS-PAGE of present work revealed a total of 10 monomorphic bands from seven random *in vitro* derived plants as well as control. These bands were distributed along the gel with molecular weights ranging from 394.52 to 6.67 KDa and the number of bands obtained from each plant were equal (Fig. 4). The result showed no evidence for genetic variations when compared to the protein profile of the *in vivo* control plants (Fig. 4).

DNA sequence of individual is unique, this sequence information can be exploited for any study of genetic diversity and relatedness between organisms. To confirm our data on the DNA level, RAPD-DNA marker was used. RAPD marker has been revealed to be a potential marker for distinguishing genetic variation (Piccioni *et a*l. 1997; Raimondi *et al*. 2001) and is more sensitive to detect the genetic variations than protein (Freire *et al.* 2001). Five random primers (A1, A2, A3, A4 and A5) have been tested with this plant and the best primers (A1, A2 and A4) which gave good banding patterns have been selected (Fig. 5). The banding profile based on RAPD (Fig. 5) showed that both *in vitro* derived plants and the donor plants shared the same banding patterns, implying that they are genetically identical to each other and no evidence for genetic variation on the DNA level. Generally, it is important to make sure that the regenerants were genetically true-to-type of their donor plants with respect to genetic fidelity. Although protein and RAPD are polymorphic markers, the results of this study using both markers showed that no genetic variations at all between the individual of *in vitro* regenerated plants and all of the regenerants showed genetic stability in our regeneration system. Therefore, we can conclude that direct regeneration from leaf and shoot tip explants did not induce any somaclonal variation that has been depicted in other explants-mediated culture (Cassells and Curry, 2001; S˘us˘ek *et al*. 2002).

4. Conclusion

A protocol for *in vitro* propagation of *Carpobrotus edulis* was established. Our data showed that plant regeneration through *in vitro* shoot and leaf explants have been successfully achieved, although the efficiency was affected by different cytokines. The *in vitro* regeneration of adventitious shoots from leaf offers a potential alternative for *Carpobrotus edulis* genetic transformation and clonal propagation. Maintaining genetic stability in regenerated plants is essential for endangered species conservation.

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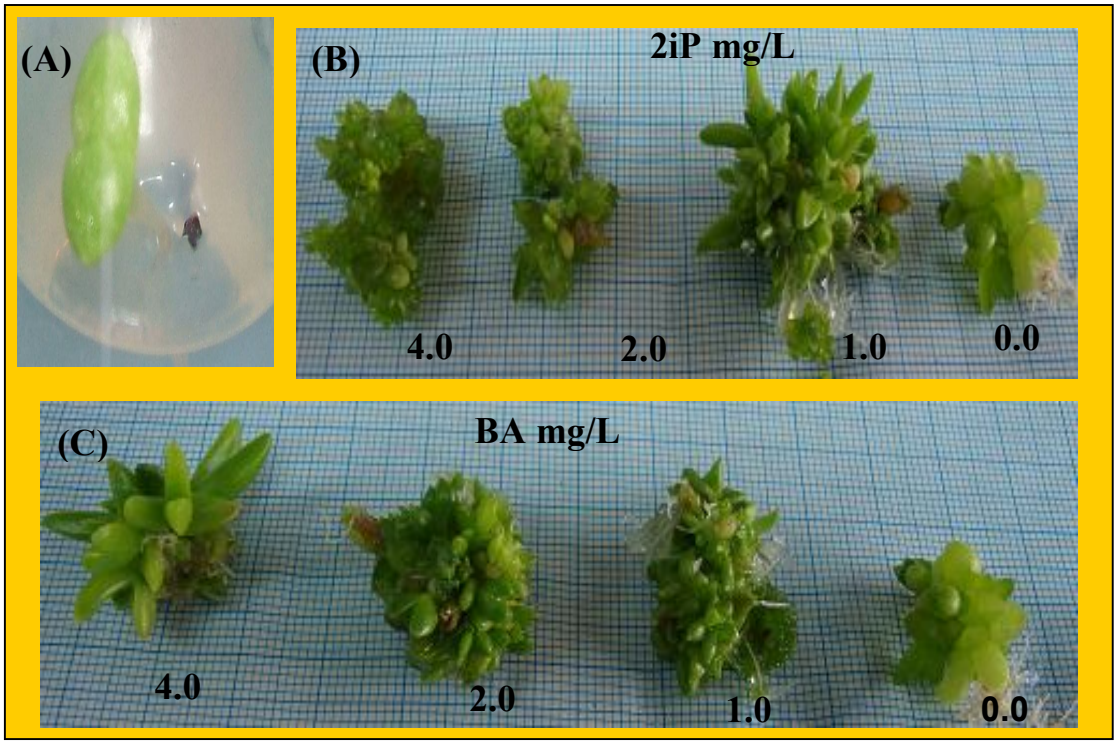

Fig. 1**:** (A) seed germination of *Carpobrotus Edulis,* (B and C) Shoot regeneration on MS medium containing different concentrations of 2iP and BA eight weeks after subculture, respectively

Table 1: The effect of different BA concentrations on multiple shoot induction of *Carpobrotus edulis*

BA concentration (mg/L)	**No. of shoots/ explant**	**Length of the longest shoot (cm)**	**Explant fresh weight (g)**
0.0	1.0^{c}*	0.5^{b}	0.6^{ab}
0.5	3.5^{bc}	0.5^{b}	1.2^{ab}
1.0	14.0^{a}	2.2^{a}	1.8^{a}
2.0	1.5^{c}	0.5^{b}	0.3^{b}
4.0	5.3^{b}	0.4^{b}	0.3^{b}

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

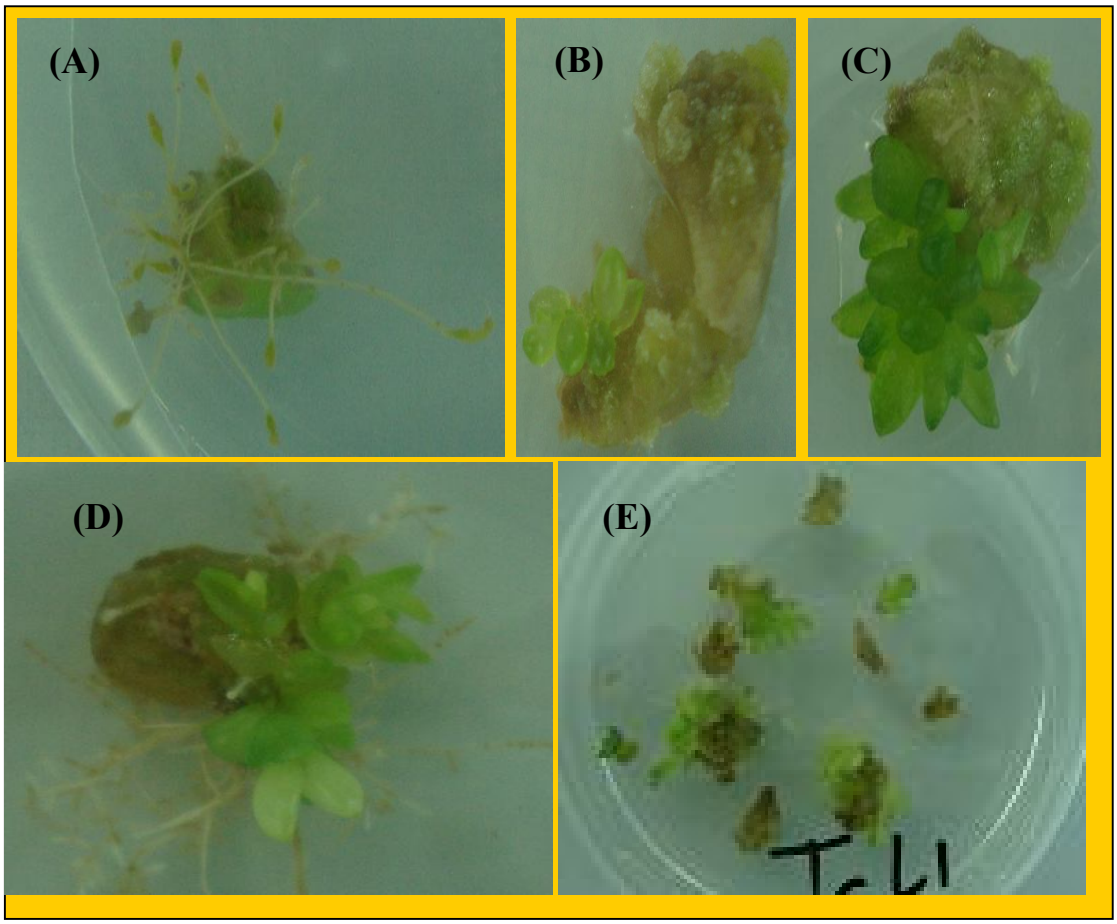

Fig. 2: (A, B, C and D) Adventitious shoot and root regeneration from a leaf explant on MS medium containing (0.02, 0.04 and 0.05 µM TDZ, respectively) and (E) Adventitious shoot regeneration from small pieces of leaves on MS medium containing 0.05 µM TDZ eight weeks after subculture

Table 2: The effects of BA, Kin and 2iP concentrations on multiple shoot induction of *Carpobrotus edulis*

Growth regulator (mg/L)			No. of shoots/ explant	Length of the longest shoot (cm)	Explant fresh weight (g)	No. of root/ explant
BA	2iP	Kin				
0.0	0.0	0.0	4.80^{e*}	0.78^{e}	0.29^{b}	1.0^{b}
1.0	0.0	0.0	38.56^{a}	1.28^{bcd}	1.38^{b}	0.0^{c}
2.0	0.0	0.0	24.00^{bc}	1.36^{bc}	0.89^{bcd}	0.0^{c}
4.0	0.0	0.0	14.33^{de}	1.00^{cde}	0.63^{cd}	0.0^{c}
0.0	1.0	0.0	43.43^{a}	1.64^{ab}	2.21^{a}	5.43^{a}
0.0	2.0	0.0	46.43^{a}	1.21^{bcde}	1.13^{bc}	0.0^{c}
0.0	4.0	0.0	47.17^{a}	1.83^{a}	1.13^{bc}	0.0^{c}
0.0	0.0	1.0	43.00^{a}	1.17^{cde}	1.03^{bc}	2.83^{ab}
0.0	0.0	2.0	28.00^{b}	1.00^{cde}	0.79^{bcd}	0.60^{c}
0.0	0.0	4.0	16.00^{cd}	0.87 ^{de}	0.57^{cd}	1.67^{bc}

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Fig. 3: (A and B) rooting of elongated shoots four weeks after subculture on MS + NAA and IBA concentrations and (C, D, and E) plants in greenhouse after eight weeks of acclimatization

Table 3: Comparison of shoot regeneration efficiency from different explants*

TDZ (µM)	Explants									
	Leaf discs						Small pieces of leaves (1.0 g)			
	No. of shoots/ explant	Length of the longest shoot (cm)	Explant fresh weight (g)	No. of roots/ explant	Length of the longest root (cm)	Callus %	No. of shoots/ explant	Length of the longest shoot (cm)	Explant fresh weight (g)	Callus %
0.0	0.0 ^{b}**	0.0^{f}	0.0^{e}	0.0^{d}	0.0^{c}	0.0^{d}	0.0 ^{b}	0.0^{f}	0.0^{de}	0.0 ^{d}
0.005	1.0 ^{b}	0.5^{de}	0.2^{cde}	2.3^{bc}	1.0^{b}	0.0^{d}	0.0 ^{b}	0.0^{f}	0.0 ^{de}	0.0 ^{d}
0.01	2.5 ^{b}	0.3^{ef}	0.1 ^{cde}	0.6^{cd}	0.0c	0.0^{d}	0.0 ^{b}	0.0^{f}	0.0 ^{de}	0.0 ^{d}
0.02	1.4 ^{b}	0.2 ^{ef}	0.2 ^{cde}	2.6^{b}	1.8^{a}	25.0^{c}	0.0 ^{b}	0.0^{f}	0.0 ^{de}	0.0 ^{d}
0.04	2.7 ^{b}	1.2 ^{bc}	0.6^{a}	4.8^{a}	2.3^{a}	4.2^{d}	3.3 ^{b}	0.8^{cd}	0.3^{bcd}	75.0^{a}
0.05	2.8 ^{b}	1.0^{b}	0.5^{ab}	0.6^{cd}	0.5^{bc}	56.3^{b}	13.4 ^{a}	1.6^{a}	0.4^{abc}	20.0^{c}

*Regenerated shoots were scored after 8 weeks of culture on MS basal medium.
**Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

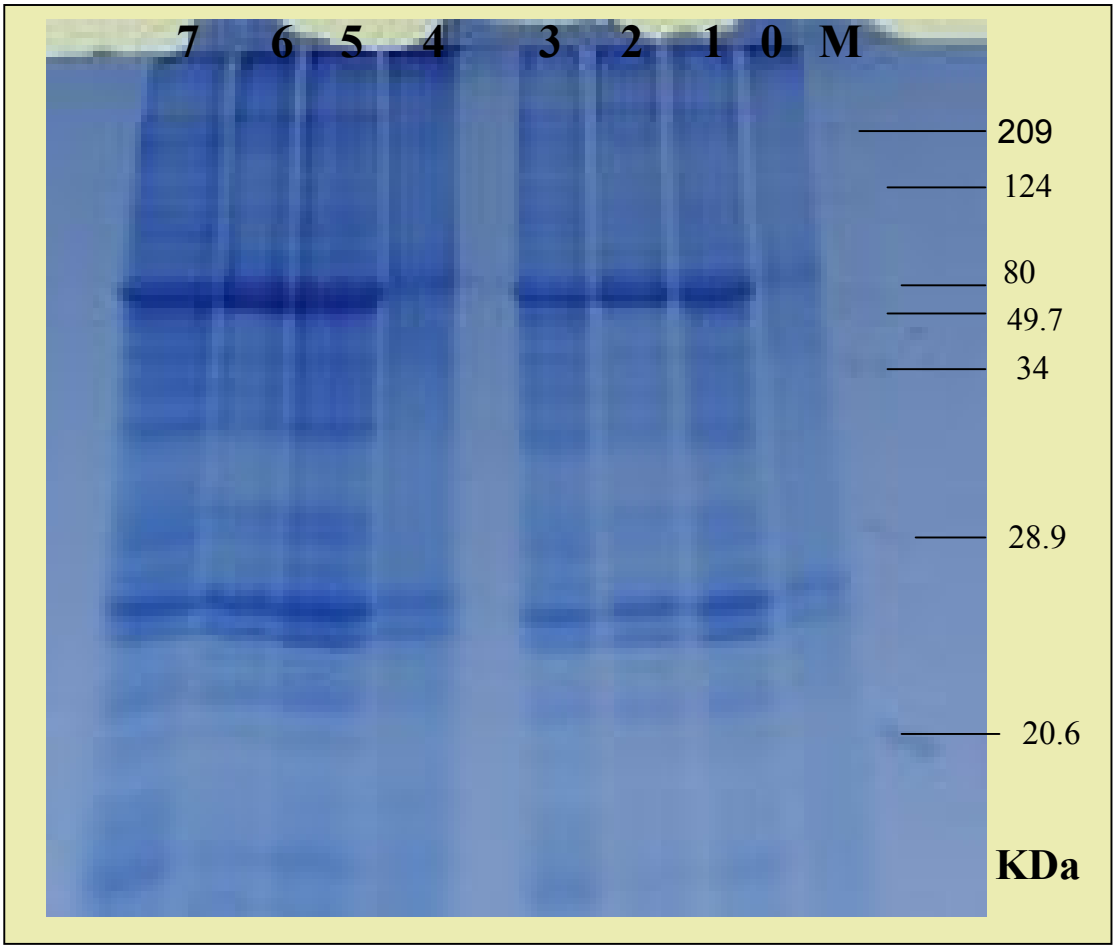

Fig. 4: Electrophoretic patterns of *Carpobrotus Edulis* based on SDS-PAGE of total plant proteins. M-Molecular weight marker, (1– 7) refer to sample plants, and (0) is the control

Table 4**:** The effects of concentrations of IAA, IBA and NAA on root induction of *Carpobrotus edulis*

Growth regulator (mg/L)			No. of roots/ explant	Length of the Longest root (cm)	Plant fresh weight (g)	Plant height (cm)
IAA	IBA	NAA				
0.0	0.0	0.0	1.75^{f}*	0.34^{cd}	0.27^{d}	0.99^{c}
0.1	0.0	0.0	5.00^{f}	0.70^{bcd}	0.62^{cd}	0.98^{c}
0.2	0.0	0.0	14.33^{de}	0.79^{bc}	1.13^{c}	1.42^{bc}
0.4	0.0	0.0	0.13^{f}	0.06^{d}	0.49^{cd}	1.00^{6c}
0.0	0.1	0.0	2.33^{f}	0.76^{bc}	0.53^{cd}	1.00^{6c}
0.0	0.2	0.0	20.00^{cd}	2.00^{a}	1.98^{ab}	1.60^{b}
0.0	0.4	0.0	30.00^{ab}	1.10^{b}	2.21^{a}	1.80^{ab}
0.0	0.0	0.1	25.00^{bc}	1.30^{b}	1.19^{bc}	1.63^{ab}
0.0	0.0	0.2	12.60^{e}	0.60^{bcd}	0.73^{cd}	1.50^{b}
0.0	0.0	0.4	33.80^{a}	1.10^{b}	2.23^{a}	2.10^{a}

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

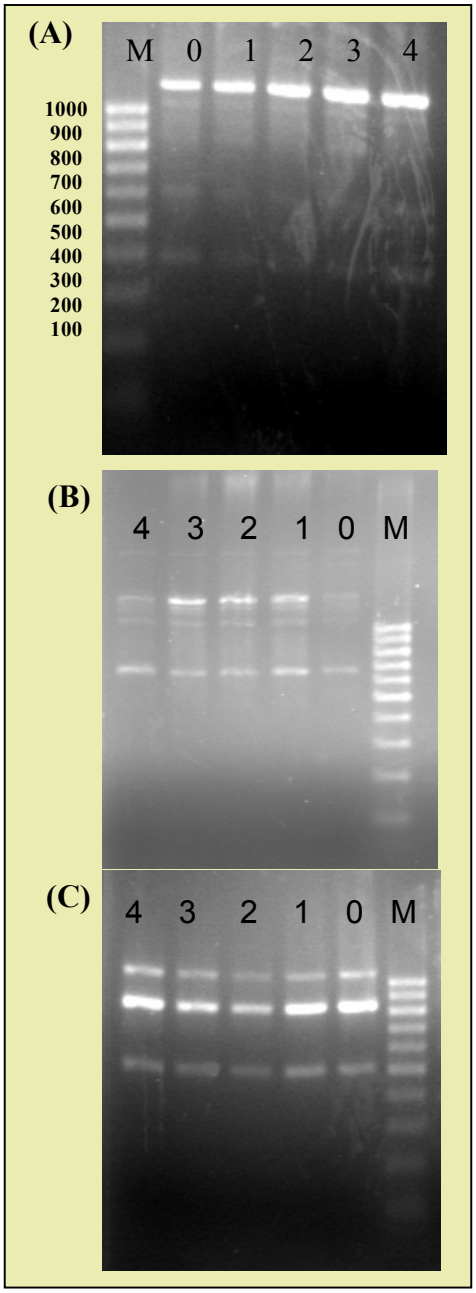

Fig. 5: RAPD pattern of *Carpobrotus edulis* plant A, B and C represents banding pattern with primer, A4, A2 and A1 respectively. (1– 4) refer to sample plants, (0) is the control plant and M refers to the DNA marker

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