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Conservation and multiplication of *Mecardonia procumbens* (MILL.) small through clonal propogation

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Abstract
The IUCN listed plant species *Mecardonia procumbens* was explored for its micropropogation potential in our investigation. The nodal explants of the healthy elite plant were chosen for direct organogenesis of the plant species in the MS basal medium with combination of cytokinin and auxin for shooting and rooting respectively. The concentration of these combinations ranged between 2+1 to 10+5 micromolar of BAP and KIN and NAA and IAA. Of the tested concentration prior results were recorded in 10+5 combination of cytokinin with a maximum of 45.6±0.54 shoots that possessed a length of 6.74±0.28 cm. for rooting the best results were obtained at 6+3 micromolar. The highest root numbers were 11.2±0.83 with 4.18±0.19 cm root length. The well-developed plantlets were taken to field for further process of acclimatization. The study supports in conserving and exploring the potentiality of the plant.

Keywords: micropropogathion, nodal explants, hormones, combination, plantlets

Introduction

Humans had faith in nature from the very beginning of his existence for basic prerequisites, fabrication, foodstuff, shelter, clothing, therapeutics, transportation and lot more. Plants formed the stepping stone for the evolution of modern medicine from traditional systems. Though we live in the materialistic world where the start and end point seems merged, civilization and modernization has occupied every nook and corner still herbal medicine is relied upon by world pharma's for their expansion and survival. Nature still conquers the materialistic world. Nearly 40% or more of the pharmaceuticals formerly used in western countries are calculated or at least partially derived from biological sources (Rout *et al*., 2000). Nature holds number of uncovered truths and therapeutics one such is the plant called *Mecadonia procumbens* (Mill.) Small belonging to the family Scrophulariaceae. The systematic position of the plant is as follows

Kingdom: Plantae
Subkingdom: Viridiplantae
Superdivision: Embryophyta
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Superorder: Asteranae
Order: Lamiales
Family: Plantaginaceae (Scrophulariaceae)
Genus: *Mecardonia* Ruiz & Pav.
Species: *Mecardonia procumbens* (Mill.) Small

The plant is a least concerned plant species as per the IUCN red list from 2009 till date. The plant has not been much explored other than a few morphological characteristics. The plant traditionally used to heal wounds and abscesses. As the plant is placed in the conservation category still we need to unwrap the plant to analyze its potentiality in various fields. A suitable method is *in vitro* cultivation where a small tissue is sufficient to regenerate the whole plant provided the favorable condition in aseptic compartment. As mentioned earlier there are no studies in the tissue culture or other fields on this plant thus this serves as the first report on the plant *Mecardonia procumbens* (Mill.) Small. The aim of the investigation focuses on the multiple plantlet production of the plant from nodal explants and utilizes the *in vitro* grown plant to uncover the other fields and find their beneficiaries to serve humankind.

Materials and Methods

The nodal explant from the field was surface sterilized with tween, running tap water and distilled water for about 30 minutes. The sterilized explants were inoculated in the autoclaved MS medium augmented with MS

salts, B5 vitamins and shooting hormone. The inoculated tubes were incubated at 25 ± 2° C under 45 $m^{-2}s^{-1}$ photon density for a photoperiod of 16/8. The plantlets were transferred to rooting medium and finally hardened and acclimatized.

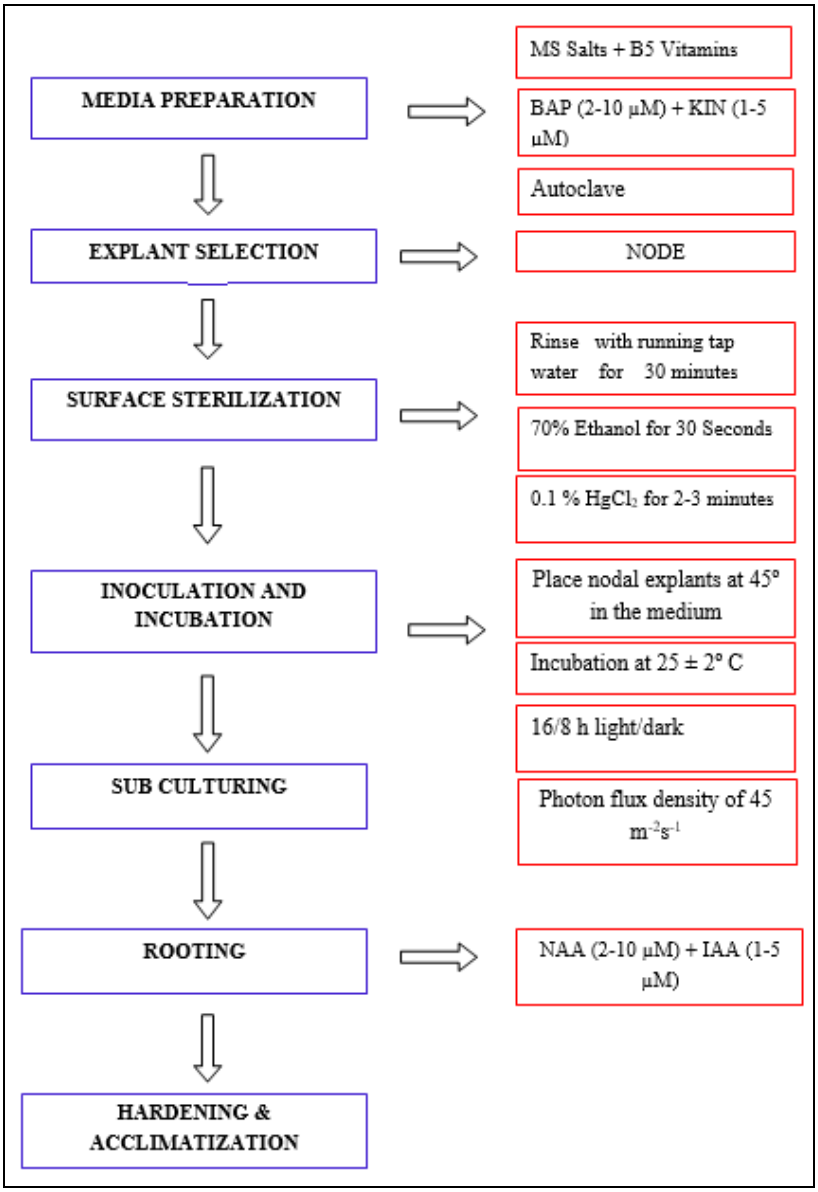

Fig 1

Result and Discussion

The nodal explants of *Mecardonia procumbens* initiated after 2 days from the date of inoculation and it showed a high significance in the regeneration frequency. The initiated shoots developed into plantlets that resembled the *in vivo* plant with no somoclonal variations. The MS medium fortified with combinations of cytokinin and auxin for shootlet and rootlet production in the concentration range of 2+1 to 10+5 micromolar gave finer results beyond our expectations. This is the first report on the tissue culture of this plant. The plant has yet not been studied for its other properties too.

The range of plant induction frequency from shooting and rooting hormones ranged from 97-100 and 96-100% respectively. Most of the studies on other plants of the family reported regeneration below this range or in alignment. Those include *Bacopa monnieri* (Nagarajan *et al.,* 2015), *Rungia pectinata* (Mahipal *et al.,* 2016). The highest combination of cytokinin produced the maximum shoots of 45.6±0.54 with shoot length of 6.74±0.28 cm followed by 8+4 micromolar BAP+KIN which incorporated 42 numbers of shoots with 6.36 cm lengthy shoots. The shoot numbers and the length of the shoot uplifted with the increase in concentration and attained its optimum numbers at the highest concentration. The shoot numbers and length was minimal in the lowest combinations of concentration. This show the plants requires additional hormones or we can say the maximum combination of concentration of cytokinin for high multiple shoot induction is 10+5 of BAP+KIN (Table 1). Mafatlal *et al.,* (2012) reported maximum Shoot multiplication obtained in BAP when both BAP and KIN were used for micropropagation of *Hibiscus radiates.*

Table 1: Hormonal efficiency of plantlet production in *Mecardonia procumbens* (Mill.) Small

Bap+Kin	NAA+IAA	Percentage of Response (%)	Number of Shoots	Shoot Length (cm)	Number of Roots	Root Length (cm)
2+1	-	95	26.4±0.54	4.26±0.48	-	-
4+2	-	97	29.8±0.83	5.08±0.60	-	-
6+3	-	98	35.6±0.89	5.54±0.32	-	-
8+4	-	99	42.6±0.54	6.36±0.20	-	-
10+5	-	100	45.6±0.54	6.74±0.28	-	-
-	2+1	96	-	-	7.2±0.44	2.28±0.20
-	4+2	98	-	-	8.6±0.54	2.64±0.36
-	6+3	100	-	-	11.2±0.83	4.18±0.19
-	8+4	99	-	-	9.8±0.83	3.54±0.36
-	10+5	100	-	-	10±0.70	3.08±0.35

Mean ± Standard deviation of five replicates of three experiments

Fig 2: Effect of Cystokinin on Multiple Shoot Induction from the Nodal Explant of *Mecardonia procumbens* (Mill.) Small a. Shoot Initiation from the Nodal Explant; b. & c. Shoot Multiplication; d. Shoot Proliferation; e. Rooting

The root induction and multiplication was fortified in the MS medium with combinations of NAA and IAA. The rooting medium was inoculated with healthy shoots of above 3 cm in length. The roots initiated and developed within 15 days of inoculation. The rooting frequency of the plantlet was above 95%. The combinational concentrations 6+3 and 10+5 had full-fledged regeneration. The lowest number of roots (7.2±0.44) was recorded in the first concentration of hormones. It also had the lowest length of 2.28 cm with standard deviation 0.20. The optimal concentration for rooting of *M. procumbens* was found to be 6+3 of NAA + IAA after three consecutive experiments with five replicates. It comprises the maximum root numbers of 11.2 and length 4.18 cm followed

by the final concentration with 10 roots. The length of the roots varied and it secured third position from the optimal concentration. The trend of the rootlets hiked and dropped after the optimum concentration.

The highest shoot regeneration frequency (100%) of our study showed much similarity to the study on *Physalis minima* (Jahirhussain *et al.*, 2016). Ma *et al.*, 2010 reported 90.3 shoots from 5.0 µM BAP and 2.5 µM NAA in *Primulina tabacum. Scoparia dulcis* showed better result in the rootlets production with the combination of IAA and IBA from the leaf and nodal explants (Majumder *et al.*, 2011). The shoot tip explants of *P. angulata from the in vitro* seedling was the most responsive in BA+auxin medium (Mastuti and Munawarti, 2017). Sivanesan *et al.*, 2012 in his microprogation work on *Scrophularia takesimensis* achieved highest average number of shoots (7.2) from shoot tip containing 2.0 mg L^{-1} BA and 71.8% percentage shoot induction frequency. They proclaimed BA found to be most effective than 2-iP and TDZ for multiple shoot induction from shoot tip explants of *S. takesimensis.* Among the combinations tested, the greatest average number of shoots (13.0) was obtained on the MS medium supplemented with 2.0 mg L^{-1} BA and 1.0 mg L^{-1} IAA while the lowest mean number of shoots (7.6) was obtained on the MS medium supplemented with 1.0 mg L^{-1} BA and 0.5 mg L^{-1} IAA . The regenerated shoots were transferred to modified MS medium containing 1.0 mg L^{-1} IBA for shoot elongation and rooting. After 4 weeks of culture 100% root induction was achieved. The result of our investigation was better than the other studies in combination of auxin and cytokinin for plantlet regeneration and the combinations can be used for commercialization, conservation and phytochemical studies.

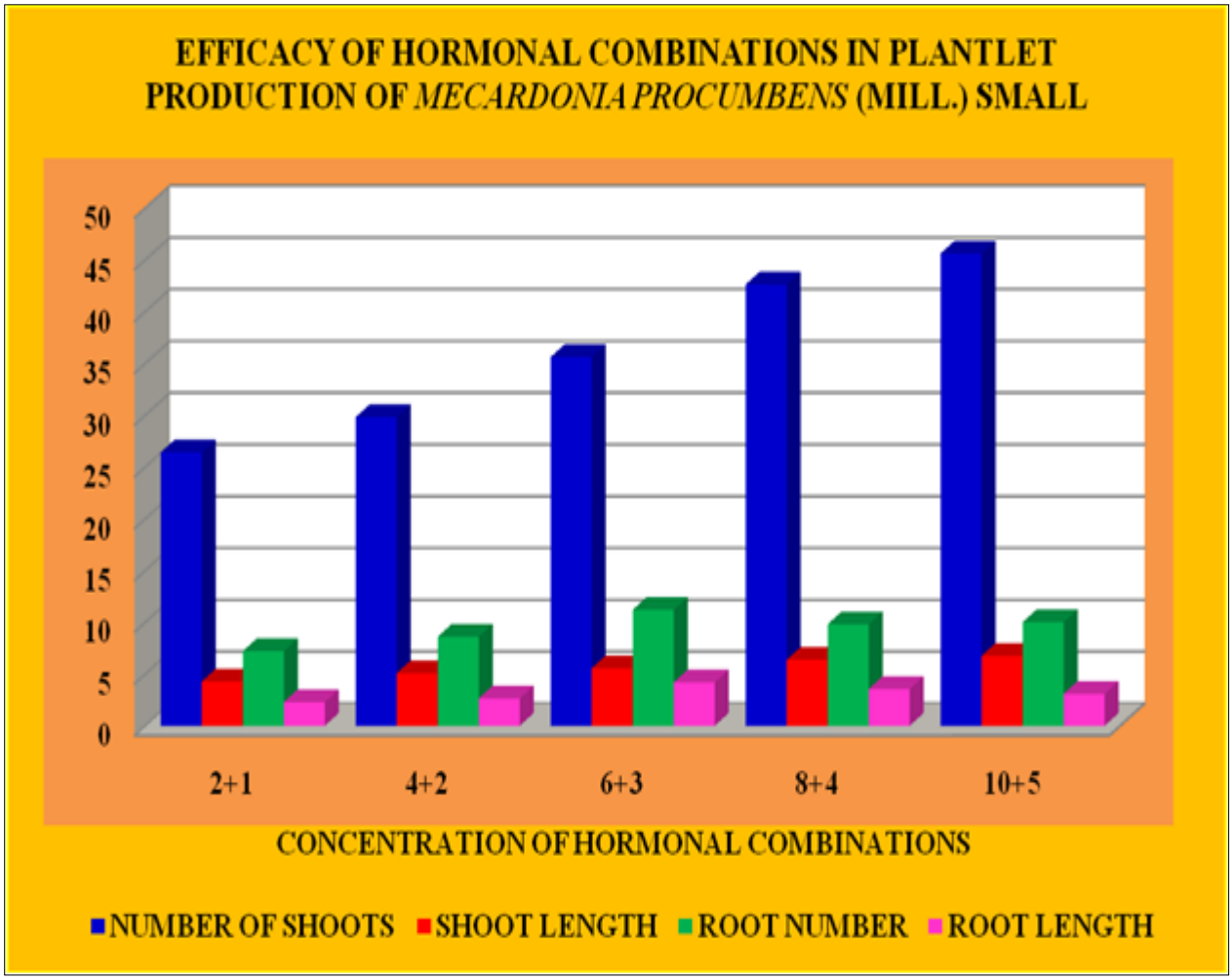

Fig 3

Conclusion

The vast biodiversity of India has a number of unexplored areas where the flora is diversified with its unique medication. Some plants are ignored for being a weed but the constituents in them are much useful for drug production. The plant *Mecardonia procumbens* is also the plant species of the same category. The standard protocol developed has been used to help in finding its phytochemical and pharmacological properties fulfilling the conservation strategy.

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