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INVITRO CLONAL PROPAGATION STUDIES IN *RUMEX VESICARIUS* L

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ABSTRACT

Rumex vesicarius L .commonly known as" Bladder dock or Chukkakura" is a renowned medicinal plant, which belongs to the family *polygonaceae*.It posses anti inflammatory, antimicrobial, analgesic, antiviral, antioxidant, cordial and stomachache properties. An attempt has been made to study clonal propagation of this important medicinal plant from nodal segments as explants. These explants were inoculated on MS medium supplemented with different concentrations of Benzyladenine, Kinetin and Indole-3-butyricacid for invitro regeneration. Maximum numbers of shoots were observed on the medium containing 2.0µM/lt BA and 0.75µM/lt Kn after 2 weeks of culture initiation. Efficient rooting was noted on full strength MS media when supplemented with IBA1.0µM/lt. Invitro regenerated plantlets were transferred onto vermiculite pots, which were further acclimatized in the field.

KEYWORDS: ***Rumex vesicarius*** **L, Murashige and Skoog medium, Clonal propagation, Benzyl adenine, Furfuryl Kinetin and Indole-3-butyric acid.**

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INTRODUCTION

Rumex vesicarius L is an annual plant belongs to family *Polygonaceae*,commonly known as "Bladder dock" or"Chukkakura". It is widely cultivated as a green leafy vegetable in many parts of India. This is an annual, glabrous herb, 15-30 cm in height, branched from the root, with long elliptic, ovate or oblong leaves and monoecious flowers. This can be a wild edible plant used as a sorrel and collected in spring time, eaten fresh or cooked. It shows many important medicinal properties such as treatment of tumors, hepatic diseases, bad digestion, constipation, calcules, heart troubles, pains, diseases of the spleen, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea[1,2,3,4]. *R. vesicarius* L exhibits biological activities like antimicrobial, anti-inflammatory, antidiarrhoeal, antiviral, antioxidant, laxative, stomachache, tonic, analgesic, appetizer, astringent, purgative, antispasmodic and aphrodisiac properties[5, 6, 7]. Finally, this plant can also be used to reduce biliary disorders and control cholesterol levels. The medicinal importance of this plant is a reflection to its chemical composition, since this plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones particularly in roots (emodin and chrysophanol), quinones, carotenoids, vitamins (especially vitamin C), proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterepenoids and organic acids[10,11,12,13].This plant is also a good source of minerals, such as; K, Na, Ca, Mg, Fe, Mn, Cu .The leaves are used as asperient, diuretic, cooling and used as an antidote for snake venom, seeds are also used as cooling agent for curing dysentery and considered as an antidote for scorpionvenom.Thebioactive phytochemicals found in *Rumex vesicarius* L such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenodis, phenolic compounds and flavonoids will lead to the protection against noncommunicable diseases in human beings such as cancer, cardiovascular diseases and cataract [8,9]. Propagation of these medicinal plants by conventional techniques like rooting of cuttings and grafting is inadequate to meet overgrowing demand. Hence, there is an urgent need to develop a protocol for large scale multiplication of medicinally important plant species. To the meet the growing herbal needs of pharma industries and for protecting the genetic erosion, there is a need for mass propagation of this plantlet. Till today there is no report on micro propagation of *Rumex vesicarius,* therefore we made an attempt to develop an invitro protocol from nodal segments and seeds for extensive development and long term germplasm storage.

MATERIALS AND METHODS

Plant material was collected from the Botanical garden at KLUniversity campus, Vaddeswaram, Andhra Pradesh, India. For the initial experiments, young, immature, one week old, healthy nodal segments (0.8cm-1.0cm) with dormant axillary buds, and seeds were excised from plants grown in the botanical garden. We found that the nodal explants were appropriate as they were responding well under invitro conditions showing effective response when compared to seed explants. After selection of nodal segment as ideal explants are washed in tap water for 3-4 times, further rinsed with tween-20 for 5- 10 minutes. Explants were sterilized with 0.1 %(w/v) $HgCl_2$ for 2-3 minutes and then explants were washed in autoclaved distilled water. The explants were flame sterilized with whatman filter and sliced at the terminal ends to a required size and supplemented on the surface of the nutrient culture medium.

CULTURE MEDIUM AND CONDITIONS

The culture medium used for the explant selection is MS (Murashige&Skoog) medium supplemented with 0.8% (w/v) agar, 3% (w/v) sucrose with various concentrations of BA & Kinetin were used to determine optimum growth regulator levels. The concentrations tested for BA were 0.25, 0.50, 0.75, 1.00,

1.25,1.50,1.75,2.0,2.25 and 2.50µM/lt. while those for Kinetin was 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 µM/lt. Each treatment was carried out for three times in 30 replicates. Each data (nodule, shoot bud initiation) were recorded after 2 weeks of culture. The invitro shoot buds when reached to a size of 3-4 cm after an incubation of two weeks in the culture room maintaining controlled conditions were transferred to shoot elongation medium containing BA 2.0 µM/lt and Kn0.75 µM/lt. After attaining 4-5 fully expanded leaves the plantlets were transferred to root induction medium containing full strength MS salts with various concentrations of IBA (0.5,1.0,1.50,2.0 µM /lt). While effective rhizogenesis was noted at 1.0 µM/lt concentrations of auxin in the nutrient media. The rooting results were recorded after 2 weeks of inoculation. The pH of the medium was adjusted to 5.6 with 1 N NaOH or 1NHCl before molten media were dispensed into test tubes (Borosil, India) and the media were autoclaved at 121 ˚C at 15 p.s.i pressure for 15 min. The cultures were maintained at 25±2 ˚C under a 16-h photoperiod of 50μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes.

STATISTICAL ANALYSIS

The experiments were repeated thrice for each and every respective concentration followed by a completely randomized design. Ten explants per replicate were used for each treatment. Data (number of shoots, shoot length and percentage of shooting response) were analyzed by one-way ANOVA technique. The Mean values recorded from the experimental data were compared using Turkeys' HSD test at P=0.05 with SPSS ver.13.0.The results are expressed as Mean±SE of three experiments.

RESULTS & DISCUSSION

The nodal segments were cultured on MS medium without growth regulator gave no regeneration response, therefore two types of cytokines (BA & Kn) were used for shoot regeneration. The explants were inoculated on to MS medium supplemented with various concentrations of BA (0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.0, 2.25 and 2.50µM/lt) and Kn (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 µM/lt) in both the cases shoot buds were observed after 4-6 days of inoculation. No intermediate callus formation was observed. Number of shoots, shoot length and percentage of shooting response were recorded after 4 weeks of culture initiation using different growth parameters. The explants inoculated on to MS medium with 0.25 µM /lt showed 30.0% of shooting response. At 0.50 µM /lt BA 36.6% were observed. When concentration of BA was increased to 0.75 µM /lt 50.0% of shoots were observed. 60.0% of shooting response were observed, when explants inoculated on MS medium with BA1.0 µM/lt. At 1.25 µM /lt 70.0% of shooting response will be observed.76.6% of shooting response will be observed at BA 1.50 µM /lt. 80.0% of shooting response will be observed at BA 1.75µM/lt. Effective shoot growth was observed at BA 2.0 µM /lt with average number of shoots5.44±0.427, average shoot length 7.67 ±0.492 and percentage of shooting response will be 90.0%. At BA 2.25 µM/lt 66.6% of shooting response will be observed. 53.3% of shooting response will be observed at BA 2.50µM/lt. Gradual reduction in number of shoots, shoot length, and percentage of shooting response was observed when treated with lower and higher concentrations of BA 2.0 µM /lt .

The effect of different concentrations of Kn tried on full strength MS medium showed the initiation of shoots. At 0.25 µM /lt Kn 60% of shoots were formed from single nodal explant.When concentration of Kn was increased to 0.50 µM /lt 76.6% were observed. A maximum of 96.6% shoot response in terms of number of shoots, shoot length were obtained in full strength MS media supplemented with 0.75µM/lt Kn. At 1.0µM /lt Kn 83.3% of shoots were formed from a single nodal explants.56.6% of shooting response were observed when explants inoculated on Ms media containing 1.25 µM /lt Kn. At 1.50 µM /lt Kn 40.0% of shoot regeneration observed from nodal explants. Of the 2 cytokinins tested, Kn-treated explants achieved higher regeneration than those treated with BA.Among different selected concentrations of Kn 0.75µM/lt treatment yielded higher regeneration (96.6%) with average number of shoots 6.620±0.430.After 4 weeks, longest shoot (12.70±0.796) were

developed in this medium. At Kn concentrations higher than 0.75 µM /lt, the numbers of shoots as well as the percentage of response were lower. The shoots were incubated more than three weeks of duration under strictly controlled conditions for shoot elongation medium containing 0.75 µM /lt Kn (Table 1, Graph 1& Figures A,B&C).

ROOTING OF SHOOTS

Elongated shoots (3-5cm) were excised and placed on full strength MS medium supplemented with various concentrations of IBA for rhizogenesis. Where MS medium with IBA1.0 µM /lt showed 100% of root development in the shooted explants, within 6-8 days. Whereas MSmedium with IBA0.50 µM /lt showed root initiation after 15 days of time but the percentage of root generation was lower 63.3%.Whereas the shoots shifted onto MS medium with IBA 1.50 µM /lt showed only 80.0% of the results in two weeks of time. Whereas MS medium with IBA 2.0 µM /lt showed very poor root development accounting for 56.6%. Efficient rooting occurred on MS medium supplemented with IBA1.0 µM /lt.While increasing the concentration of IBA above 1.0 µM /lt the percentage of rooting response will be lower. Shoots formed roots 100% on media containing IBA1.0 µM /lt, In this medium the number of roots was highest 8.07±0.444 and their length measured11.30±0.547, it is evident from (Table2, Graph 2& Fig D &E).Hence MS medium with BA2.0 µM /lt,Kn 0.75 µM /lt and IBA1.0 µM /lt was found to be ideal concentrations for complete organogenesis and healthy plantlet regeneration in *Rumex vesicarius L*.

ACCLIMATIZATION AND FIELD ESTABLISHMENT

Well developed rooted plantlets were gently removed from the test tubes and thoroughly washed with sterile water to remove adhered agar and traces of medium to avoid contamination. plantlets were transferred to plastic pots containing autoclaved and annealed soil with nutrient rich vermiculite (1:1) (Fig F). In the first week of transplantation the plantlets were encapsulated with polyethylene sheet rinsed with 70% ethanol to provide high humidity, allow sufficient light and to curb the affect of contaminants. The polyethylene sheet was removed periodically and progressively whenever leaves appeared to be wet. The polyethylene sheet was withdrawn completely after 2–3 weeks of hardening. After 3 weeks the plants were transferred to larger pots filled with soil and organic manure for further growth. Finally the acclimatized plants were shifted to field conditions, 81.13% of them having survived. The growth characteristics of plants raised in vitro did not show any significant morphological variations from those of the natural habitat.

Table 1

Influence of different concentrations of Benzyladenine and Kinetin on invitro shoot induction in Rumex vesicarius L (Each treatment consists of 10 explants and data were recorded after"3" weeks of culture).

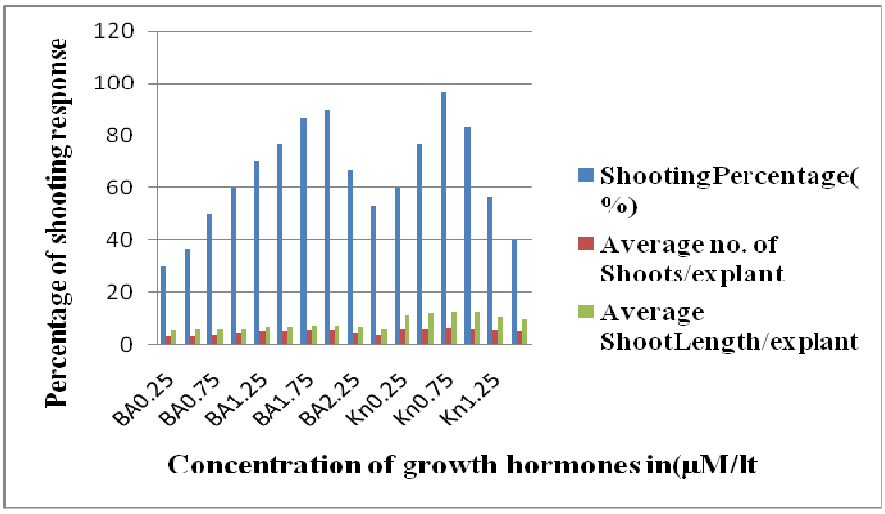
S.No	Growth regulator concentration in (µM/lt)	Shooting Percentage(%)	Average no. of Shoots/explant	Average Shoot Length/explant
1	BA0.25	30.0	3.22±0.277	5.44±0.466
2	BA0.50	36.6	3.45±0.349	5.82±0.533
3	BA0.75	50.0	3.93±0.376	6.13±0.577
4	BA1.0	60.0	4.38±0.411	6.44±0.600
5	BA1.25	70.0	4.86±0.436	7.04±0.621
6	BA1.50	76.6	5.08±0.483	7.23±0.604
7	BA1.75	86.6	5.23±0.452	7.42±0.513
8	**BA2.0**	**90.0**	**5.44±0.427**	**7.67±0.492**
9	BA2.25	66.6	4.53±0.438	6.95±0.663
10	BA2.50	53.3	3.87±0.395	6.06±0.589
11	Kn0.25	60.0	5.83±0.575	11.39±1.140
12	Kn0.50	76.6	6.04±0.535	12.08±1.068
13	**Kn0.75**	**96.6**	**6.62±0.430**	**12.70±0.796**
14	Kn1.0	83.3	6.28±0.518	12.36±1.002
15	Kn1.25	56.6	5.47±0.535	10.94±1.095
16	Kn1.50	40.0	5.17±0.479	9.83±0.864

Table 2
Influence of different concentrations of IBA on root induction from invitro regenerated shoots.

S.NO	Growth regulators Concentration in(µM/lt)	Rooting(%) percentage	Average no.of roots/explant	Average root length/explant
1	IBA0.50	63.3	6.95±0.651	10.21±1.045
2	**IBA1.0**	**100.0**	**8.07**±0.444	**11.30**±0.547
3	IBA1.50	80.0	7.83±0.683	10.84±0.940
4	IBA2.0	56.6	6.47±0.629	9.65±0.933

(10 cultures were maintained in each treatment and data (SE) were recorded up to four weeks of culture).

Graph 1
Average number of shoots, length and percentage of expression in invitro Rumex vesicarius L



Graph 2
Average number of roots, length and percentage of expression in invitro Rumex vesicarius L

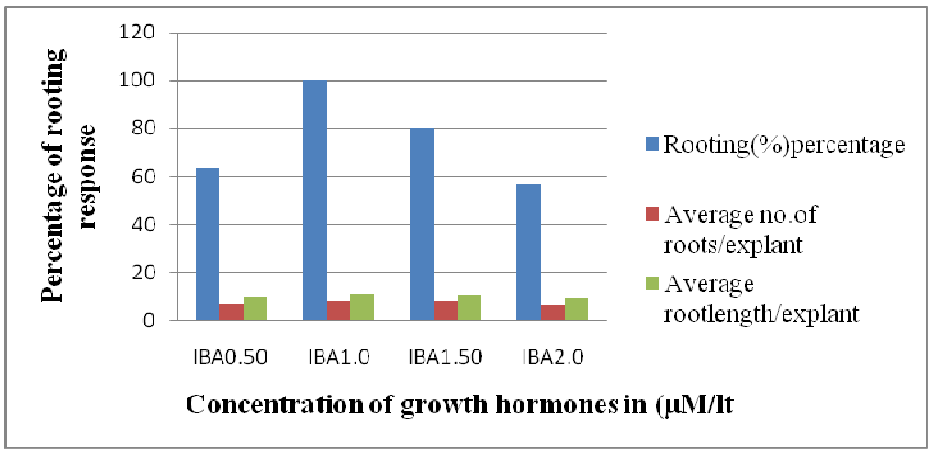
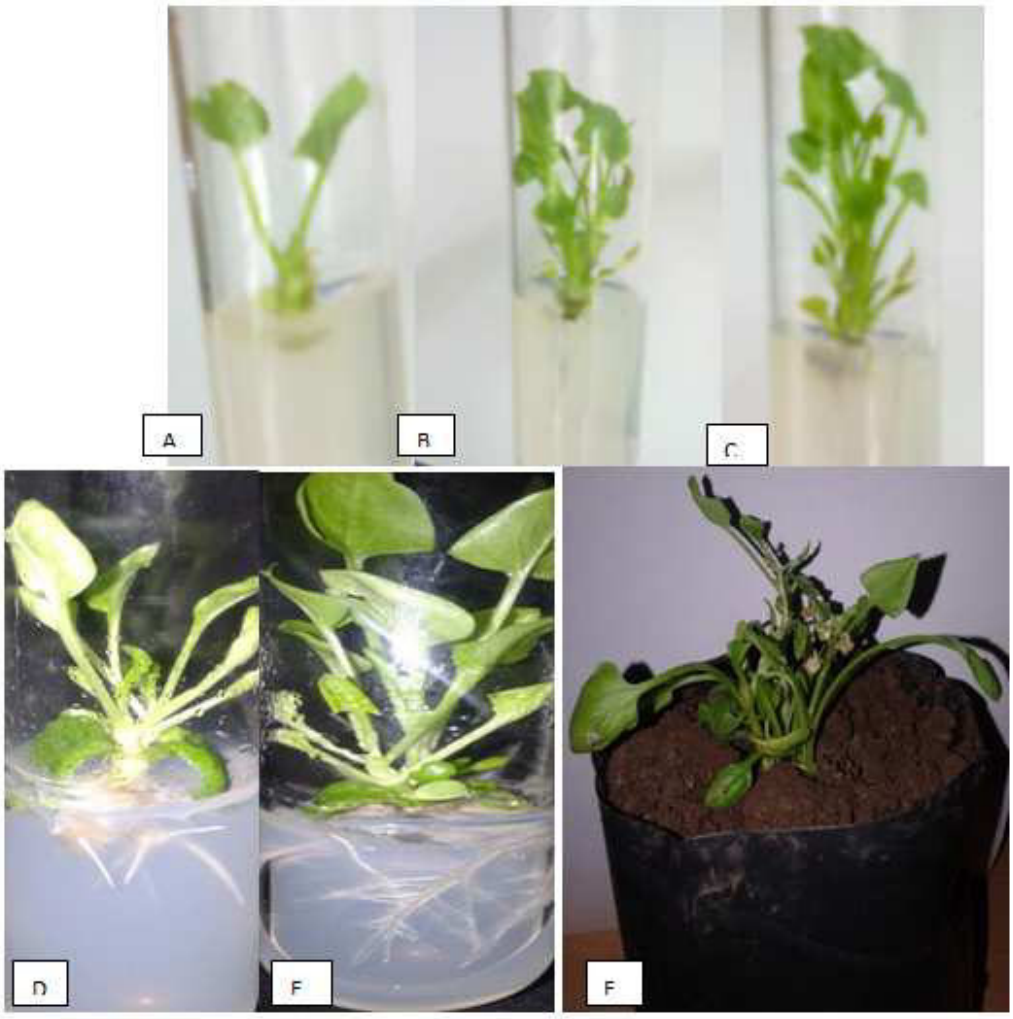


FIGURE 1



Invitro clonal propagation of *Rumex vesicarius L* through direct regeneration.(A)-Shoot bud induction from nodal explants in *Rumex vescarius* when supplemented on MS+BA2.0µM/lt.(B& C) - Well developed shoots from the nodal explants on MS +Kn0.75µM/lt.(D)- Root bud initiation from nodal explants when supplemented on MS+IBA1.0µM/lt.(E)- Invitro plantlet with well developed shoot and root bud induction.(F)- Successful pot culture establishment of invitro grown *Rumex vescarius*.

CONCLUSION

The micro propagation protocol reported here will be useful for mass multiplication and production of genetically uniform plants from limited donor plants within short time. Invitro culture of *Rumex vesicarius L*. provides a new system that could be explored in several ways, especially in the production of flavonoids, tannins, terpenoids, and other compounds of medicinal value for cultured cells/tissues/organs of plantlets could be explored.

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