In vitro Seed Culture of *Rumex dentatus*- An Important Medicinal Herb

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ABSTRACT

A study was conducted to assess the *in vitro* germination and subsequent morphogenetic potential of seeds of *Rumex dentatus* L., a medicinal herb. Murashige and Skoog's (MS) medium was used through out the series of experiments. Seeds inoculated were both with and with out seed coat. Maximum seed germination was observed on basal medium in the seeds which were inoculated with out seed coat. Shoot multiplication response was observed to be maximum on the medium augmented with BAP and NAA. Both elongation of isolated shoots and their subsequent rooting was observed on MS basal medium.

Keywords: Seed culture, *Rumex dentatus*, germination, shoot multiplication, plantlets

Abbreviations: MS- Murashige and Skoog; BAP- 6-Benzyl amino purine; NAA- Napthalene acetic acid; IBA- Indole- 3- butyric acid

INTRODUCTION

Rumex dentatus L. is an annual medicinal herb whose leaves are a rich source of calcium, β-carotene and vitamin C while as roots contain chrysophanic acid and emodin, yield a dye and are used as an astringent in cutaneous disorders (Anonymous, 1988). Leaves are cooked as a vegetable. This plant species is frequently used as a vegetable in most parts of Kashmir but is continuously being eradicated from the fields. No attention is being paid for its cultivation and propagation against the rate at which this plant species is removed from the fields. Hence an attempt was made to study the *in vitro* germination potential and other morphogenetic responses of *R. dentatus* seeds so that possibility of using plant tissue culture technique for micropropagation of plant species is worked out.

MATERIAL AND METHODS

Fresh and healthy seeds of *R. dentatus* were collected from local fields of Chatterhama and its residential areas. The seeds used for culture purposes were soaked overnight in water followed by their washing with 5% labolene containing 2-3 drops of Tween-20. The seeds were rinsed with running tap water for 20-25 minutes so that all traces of detergent are completely removed from the surface of the seeds. Before chemical sterilization of seeds was under taken a rinse with double distilled water was given. Mercuric chloride (0.1-0.2%) and sodium hypochlorite (3-7%) for different time periods (5-10 min) were used for surface sterilization. The best results for sterilization were obtained on 0.1% mercuric chloride (10 min).

The sterilized seeds (with and without seed coat) were then inoculated on MS medium (1962) fortified with 3% sucrose using the procedure as communicated in our earlier publications (Kamili *et al.*, 2005; Qadri *et al.*, 2005).

RESULTS AND DISCUSSION

The main objective of the study was to register the *in vitro* germination response for quick plantlet formation and also to score the data for any other morphogenetic response in seed culture of *Rumex dentatus* for micropropagation of the plant. For this purpose sterilized seeds alongwith seed coats were inoculated on MS basal medium but these did not showed any response. When the medium was augmented with a combination of NAA (5μM) + BAP (5μM), only 25% of seeds germinated after 9 weeks. The germination process was slow and only shoots were produced. After 15 weeks, brown friable callus was produced at the basal end of the hypocotyl on the same medium (Fig.1).

In another attempt seeds without seed coats were used which showed 100% germination response and normal seedling formation on basal medium only after 4 weeks (Fig.2). Normal seedling formation has also been reported on MS basal medium in case of *Prunella vulgaris* and *Cichorium intybus* (Paray, 2002), *Brasicca oleracea* var. acephla (Kamili *et al.*, 2002), *Portulaca oleracea* (Dar, 2002), *Brasicca oleracea* var. gongylodes (Fatima *et al.*, 2003) *Echinacea angustifolia* (Bashir, 2003) and *Vigna radiata* (Fayaz, 2003). More trials were conducted with such seeds when medium was enriched with NAA, BAP and their combination, the results of which are summarized in Table 1. Best result was obtained on MS + NAA (10μM) + BAP (10μM), in terms of multiple shoot induction in 80% cultures only after 4 weeks. Seed germination was incomplete and only shoot formation was noted which was accompanied with compact callus at hypocotylar end (Fig.3). 10% cultures showed only callus formation all over the surface of the seed (Fig4). This is in contradiction to Dar *et al.* (2003) who found stunted growth of seedling of *Portulaca oleracea* on MS medium supplemented with similar hormonal concentrations.

Table 1. Response of shoot tips in different subcultures

Seed	Medium	Germination response	%age response	Multiple shoot induction	%age response	Callus induction	%age response
With seed coat	MS basal	No response	-	-	-	-	-
	MS+NAA(5μM)+ BAP(5μM)	Slow germination response. Only shoot formation (after 9 weeks)	25	-	-	Callus development at the basal end of hypocotyl (after 15 weeks)	25
Without seed coat	MS basal	Seeds showed emergence of plumule and radicle, normal seedling formation with intense root proliferation (after 4 weeks)	100	-	-	-	-
	MS + NAA (10μM)	Emergence of plumule and radicle, normal seedling formation with hairy roots (4 weeks)	80	-	-	-	-
	MS+ NAA (5μM)	Intense root proliferation only along with root hairs (after 5 weeks)	90	-	-	-	-
	MS+NAA (2.5μM)	Emergence of plumule and radicle, normal seedling formation along with root hairs (after 6 weeks)	60	-	-	-	-
	MS+ BAP (5μM)	Stunted growth of seedling (after 4 weeks)	90	Multiple shoot regeneration (4-5) (after 8 weeks)	90	-	-
	MS+BAP (2.5μM)	Stressed growth of seedlings (after 5 weeks)	90	Multiple shoot formation (2-3) (after 10 weeks)	90	-	-
	MS+NAA(10μM)+ BAP(10μM)	Seed germinated to produce only shoot (after one week)	80	Multiple shoot formation (7-8)	80	Compact callus at the basa end of multiple shoots (after 4 weeks).	80
						Moderate callus formation all over the seed surface (after 4 weeks)	10

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Normal seedling formation was also registered with NAA (10µM, 2.5µM) whereas BAP (5µM, 2.5µM) when used alone favoured stunted growth of seedlings but on the other hand induced multiple shoot regeneration from shoot pole after 8-10 weeks.Kamili *et al.* (2005) reported full seedling formation on NAA (5µM) in *Amaranthus hybridus* which is not in accordance with present study where only root formation was observed on NAA (5µM). Present observation is in contradiction to different studies carried out on other medicinal plants like *Cinnamomum zeylancium* (Rai and Chandra, 1987), *Dioscorea floribunda* (Sengugta *et al*., 1984), *Picrorhiza kurroa* (Upadhyay *et al*., 1989) and *Cichorium intybus* (Rehman *et al*., 2002) where shoot multiplication was recorded from explants other than seeds.

Multiple shoots obtained in various trials were isolated and subcultured on MS basal medium which induced both elongation and rooting of these microcuttings and complete plantlets were obtained after 6-8 weeks (Fig.5).Rooting of isolated shoots obtained on MS basal medium is in accordance with the earlier reports of Davies and Dale (1979), Flick *et al.* (1983) and Sen and Sharma (1991) who also observed rooting on hormone free medium in *Solanum lacinatum, Datura* spp and *Withania somnifera* respectively. Tyub *et al.* (2005) used NAA and IBA for rooting of isolated shoots of *Lupinus polyphyllus* which is in contradiction to present study. However, in their study elongation of isolated shoots was achieved on MS basal medium which is in conformity with present study.

For *in vitro* seed germination removal of seed coats and use of MS basal medium has proved to be effective for quick and normal seedling formation, and for multiple shoot induction BAP (5µM, 2.5µM) either alone or in combination with NAA (i.e. BAP 10µM+ NAA 10µM) has been found to be influential. Hence, it is concluded that seeds of *Rumex dentatus* showed the potential for *in vitro* germination and can be used for the seedling production. Moreover, multiple plantlet production can be exploited for mass propagation of the plant. This study can form a basis for the future advanced research programmes in this plant species.

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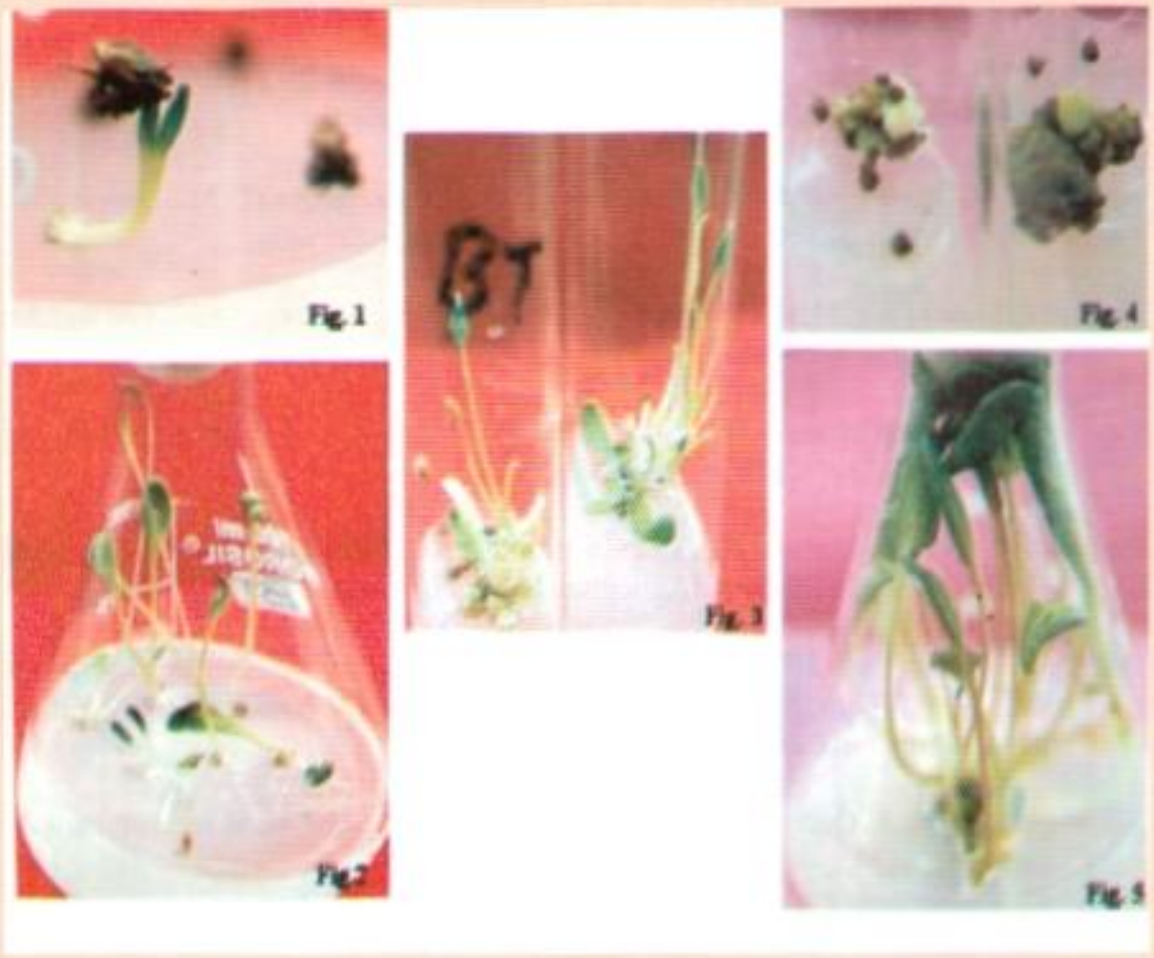

Figs. 1-5 : *Rumex dentatatus*

Fig.1: Seed germination and callus formation from basal end of hypocotyl on MS+NAA (5μM) +BAP (5μM) (after 15 weeks).

Fig.2: Normal seedling formation on MS basal medium (after 4 weeks).

Fig.3: Multiple shoot formation on MS+NAA (10μM) +BAP (10μM) (after 4 weeks).

Fig.4: Callus formation on MS+NAA (10μM) +BAP (10μM) (after 4 weeks).

Fig.5: Elongation and rooting of isolated shoots on MS basal medium (after 8 weeks).

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