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Micropropagation of *Rumex vesicarius* L. Through Shoot Tip Culture

Abo El-soud I.H., AL-azab, A.A., Koriesh, E.M., and Atta-Alla, H. K *
Horticulture Dept., Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt

ABSTRACT

Shoot tips of *Rumex vesicarius* L. were excised from *in vitro* seedlings and transferred for shoot multiplication into MS medium supplemented with various concentrations of 6-benzyladenin (BA) or Kinetin (Kin). After 4 weeks, shoot multiplication was best achieved from explants cultured on MS medium containing 8.88 µM BA, with an average of 11.0 shoots per explant. While, the shoot tips cultured on the medium containing 26.63 µM Kin produced 4.6 shoots per explant. For rooting, the proliferated shoots were cultured on rooting medium containing IBA or NAA at different rates. Root induction was successfully occurred on MS medium supplemented with 2.46µM IBA or 7.38µM NAA .The survival rate of transplants during acclimatization reached 87% and the plants grew normally under the greenhouse conditions.

Keywords: *Rumex vesicarius,* Micropropagation, 6-Benzyladenin (BA), Kinetin (Kin), Naphthalene acetic acid (NAA), Indole butyric acid (IBA).

INTRODUCTION

Rumex vesicarius L. is a wild plant grows in Egypt as an annual herb belongs to family Polygoneceae and has different vernacular English names such as "Sorrel", "Ruby Dock", "Blader Dock" and Arabic names as "Hummayd" and "Hanbeit". The plant has the capability of growing during many seasons along the year. (Goodman and Hobbs, *1988;* Boulos, 1999, Al-Rumaih *et al.,* 2003). *Rumex* plant is widely used as food (appetizer and green vegetable). The leaves can be used as a good source of minerals, protein, ascorbic acid and oxalic. The protein value was ranged from 17.1 to 20.1 % based on fresh weight (Al-Fawaz, 2006). Seeds and leaves of *R.vesicarius* contain amino acids like sisten; proline; phenylalanine and other components like histadine and alkaloids. The root of Rumex is used as an astringent and in cutaneous disorders (Chopra *et al*., 1986). In this concern, Shah and Khalil, (1988) suggested the possibility of using this plant as a developing food crop for the production of protein in human food cycle and animal feed . Also, *Rumex* can be used as an antidote to scorpion stings and as a medicinal herb to cure many illnesses such as tooth pain, laxative and warm releaser (Täeckholm, 1974).

Nowadays, the wild flowers and native plants begin to be used as ornamental and cut flowers (Moody and Gollnow, 2008). Thus, *Rumex* plant is strongly recommend being an important plant in the ornamental and cut flowers industry because it produces a large number of inflorescences which have a very attractive pink to purple color and the plants bloom from late January till end of March (Plate 1-1).

Seeds of *Rumex* display variations in their ability to germinate, which are related to the time of the year and depend on the presence of light. The fruiting valves of *R. vesicarius* produce some chemicals that inhibit seed germination. The germination is best during the autumn and winter months but poor during the rest of the year (Schatral and Osborne, 2006).

To conserve and produce *Rumex* plants in a large scale, tissue culture technique is recommended. The primary advantage of micropropagation is the rapid production of high quality plants. No reports on micropropagation of *Rumex* have been published so far. Hence this study aimed to find out an efficient protocol for micropropagation of. *R. vesicarius*.

MATERIALS AND METHODS

Seeds of Rumex plants were extracted from the dried fruits which collected from the wild plants grown in the protected areas in South Sinai, and then used for *in vitro* germination. The seeds were rinsed in running tap water for one hour, surface sterilized by immersion in 70% (v/v) ethanol for one minute, followed by soaking in 20% (v/v) Clorox (commercial bleach containing 5.25% Sodium hypochlorite, NaOCl) supplemented with three drops of Tween-20 as wetting agent for 15 min. and subsequently rinsed three times with sterile distilled water. Sterilized seeds were cultured in culture tubes containing 10 ml of half strength MS medium (Murashige and Skoog, 1962) supplemented with 7.0gl^{-1} agar and 30 gl^{-1} sucrose.

Effect of BA and Kin on the shoot multiplication

Shoot tips (1.0 cm) were excised from *in vitro* seedlings and transferred to jars containing 40 ml MS medium supplemented with sucrose at 30 gl^{-1}, solidified with 7.0 gl^{-1} agar, and BA or Kin at 0.0, 1.11, 2.22, 4.44, 8.88, 13.32, 17.75, 22.19 and 26.63 µM.

Two shoot tips were cultured in a jar and 24 replicates were used for each treatment. After 4 weeks, number of shoots, shoot length in cm, number of leaves /explants, fresh weight of shoot and fresh weight of callus (in the base of shoot tips) in g were recorded.

* *Corresponding Author. Islamhassan2010@hotmail.com*

Effect of IBA and NAA on the *in vitro* rooting.

Micropropagated shoots of 2.0 cm length were individually separated from cultures and were ready to enter the rooting stage. IBA or NAA were added to the MS medium at rates of 0.1, 0.49, 1.23, 2.46, 4.90, 7.38 and 9.84 µM beside the control (without any auxin). One proliferated shoot was placed into a jar containing 40 ml medium supplement with 30 gl^{-1} sucrose and solidified with 7.0 gl^{-1} agar, Each treatment had 16 replicates. After 4 weeks from the culture, rooting percentage, number of roots, root length (cm), shoot length (cm) and callus percentage were recorded.

The culture vessels were maintained in a growth room at 25±1 °C with 16 hours light/8 hours dark cycle at a fluorescent light intensity of approximately 25µmol $m^{-2}s^{-1}$.

For acclimatization, the rooted shoots obtained from the rooting stage were carefully removed from the culture jars, washed with water to remove agar around the roots and then washed using (Rhizolex 0.1%) to reduce the fungal contamination. The plantlets were cultured into small plastic pots (6.0 cm) containing soil mixture of sand: peat moss: vermiculite (1: 1: 1 by volume). The pots were transferred to the greenhouse, The survival percentage of plants was recorded after 4 weeks from the transplantation.

Multiplication and rooting experiments were repeated twice. Data were computed and subjected to statistical analysis using SPSS statistical program (10). The differences between the means of treatments were tested using Duncan Multiple Range Tests at 0.05% according to Snedecor and Cochran (1980).

RESULTS AND DISSCUSION

Effect of BA and Kin on the shoot multiplication.

Generally, the number of shoots in media with BA was greater than those observed in the media supplemented with Kin. Incorporation of BA or Kin in the media was essential for enhancing shoot multiplication (Table 1).

The greatest number of multiple shoots (11.0 shoots / explant) was obtained from shoot tips cultured on medium containing 8.88 µM BA (Plate 1-2). On the other hand, Kin at all concentrations exhibited similar rates of shoot multiplication per explant except with Kin at 26.63 µM which produced 4.6 shoots/explant. It was obviously that the medium without growth regulators significantly gave the tallest shoots as 4.8 cm. Addition either of BA or Kin to the culture media decreased the length of Rumex shoots comparing to the control. However, the treatment with Kin produced longest shoots in contrast with BA at the same concentration. Same results were obtained by Baskaran and Jayabalan, (2005) and Faisal *et al.,* (2005) who stated that higher concentrations of cytokinine reduced shoot number as well as shoot length of *Eclipta alba* and *Ruta graveolens* respectively. Also, Santana-lopez *et al*., (2004) concluded that BA stimulated multiple shoot formation while Kin is more efficient in the elongation process on *Helianthemum inaguae*.

The greatest number of leaves (36.3) and the heaviest fresh weight of shoots (1.75 g) were obtained with MS medium supplemented with 8.88 µM BA. Increasing the leaf number as well as the culture fresh weight may be attributed to increasing the number of proliferated shoots which has been occurred as a result of addition of BA (Table 1).

The presence of either BA or Kin on culture medium induced callus on the base of shoots with no morphogenetic structures in a high proportion. Calli were observed within 2 weeks from inoculated explants on MS medium supplemented with varying concentrations of BA or Kin at different forms, (solid or friable callus) as shown in (Plate 1-2). It was considerable to mention that the shoot multiplication was directed to organogenesis from the shoot tip. Thus, a method of shoot propagation involving no callus formation phase is preferred for production of true type plants.

Table (1): Effect of BA and Kin on the shoot multiplication of *Rumex vesicarius*.

Means with the same letters in the same column are not significantly different according to Duncan's multiple range test at 5%.

Treatments (µM)		No. of Shoots /explant	Shoot length (cm)	No. of leaves /explant	F.W. of Shoots (g)	F.W. of callus (g)
BA	**0.0**	1.8^{f}	4.8^{a}	11.8^{f}	$1.19^{a\sim d}$	0.10^{e}
	0.55	$4.5^{c\sim e}$	$2.6^{c\sim e}$	$17.7^{d\sim f}$	1.65^{ab}	$0.66^{a\sim e}$
	1.11	7.1^{b}	$2.9^{b\sim d}$	26.1^{bc}	$1.64^{a\sim c}$	$0.64^{a\sim e}$
	2.22	8.3^{b}	2.2^{de}	25.4^{bc}	$1.60^{a\sim d}$	1.24^{a}
	4.44	7.9^{b}	2.0^{e}	25.2^{bc}	$1.27^{a\sim d}$	$0.97^{a\sim c}$
	8.88	11.0^{a}	2.3^{de}	36.3^{a}	1.75^{a}	$0.89^{a\sim d}$
	13.32	6.4^{bc}	2.1^{e}	$21.8^{b\sim e}$	$1.19^{a\sim d}$	1.17^{ab}
	17.75	7.5^{b}	1.9^{e}	26.5^{b}	$1.56^{a\sim d}$	$0.76^{a\sim e}$
	22.19	6.3^{bc}	2.0^{e}	$21.6^{b\sim e}$	$1.08^{b\sim d}$	$0.64^{a\sim e}$
	26.63	6.5^{bc}	1.9^{e}	$23.4^{b\sim d}$	$1.63^{a\sim d}$	$0.54^{a\sim e}$
Kin	**0.55**	2.1^{f}	3.6^{b}	12.6^{f}	1.07^{cd}	0.14^{e}
	1.11	1.8^{f}	3.0^{bc}	10.9^{f}	$1.21^{a\sim d}$	0.11^{e}
	2.22	$2.7^{d\sim f}$	3.1^{bc}	15.3^{ef}	$1.19^{a\sim d}$	0.11^{e}
	4.44	2.4^{ef}	3.4^{b}	14.0^{f}	$1.37^{a\sim d}$	0.18^{de}
	8.88	2.4^{ef}	$2.6^{c\sim e}$	13.9^{f}	$1.21^{a\sim d}$	$0.34^{c\sim e}$
	13.32	2.2^{f}	$2.6^{c\sim e}$	12.4^{f}	$1.26^{a\sim d}$	$0.35^{c\sim e}$
	17.75	$3.0^{d\sim f}$	$2.5^{c\sim e}$	15.5^{ef}	$1.22^{a\sim d}$	$0.37^{c\sim e}$
	22.19	$3.6^{d\sim f}$	$2.5^{c\sim e}$	$18.6^{c\sim f}$	1.06^{d}	$0.46^{b\sim e}$
	26.63	4.6^{cd}	2.2^{de}	$23.5^{b\sim d}$	1.71^{a}	$0.56^{a\sim e}$

Effects of IBA and NAA on the *in vitro* rooting

For root induction, multiplied shoots were individually separated and transferred to rooting media containing different concentrations of IBA or NAA. The induction of roots was observed after one or two weeks of inoculation in all studied media. During the third and fourth weeks , the best root growth was obtained (Table 2).

Plate (1) Micropropagtion of *Rumex vesicarius* L.

(1-1), *R. vesicarius* plants at flowering time **(1-2),** *in vitro* multiplication of *R. vesicarius* with the friable callus (arrow) on the base of shoot **(1-3),** *in vitro* rooting of *R. Vesicarius* – **(1-4)**, rooted plantlet d plantlets **.(1-5),**solid callus (arrow) on the base of shoot) **(1-6),** plants grown in the greenhouse

In vitro rooting of *R .vesicarius* plantlets was occurred with or without growth regulators with percentage ranging from 62.5 to 100%. However, significant differences were reported between auxin treatments concerning the number and the length of developed roots.

A greatest number of roots (22.8 roots/plantlet) were obtained when MS medium was supplemented with NAA at 1.23 µM (Plate 1-3;1-4) without significant differences with 2.46 and 4.90 µM, of IBA which produced 19.7 and 19.4 roots/explants, respectively. On the other hand, MS medium without IBA or NAA significantly produced the tallest roots (5.4 cm). The root length decreased with increasing the IBA or NAA concentrations. Such results were noticed by Jahan *et al*. (1998) who reported that about 80% of shoots of *Datura metel* from every subculture were rooted on hormone-free medium.

In addition, higher concentrations of NAA (7.38 µM and 9.84 µM) increased callus formation, which could have a negative effect in the acclimatization process due to the formation of non-functional roots (Plate1-5). Similar results were obtained on *Helianthemum inaguae* by Santana-lopez, *et al*. (2004).

Both IBA and NAA have some different effects on the process of rhyzogenesis and caulogenesis. Thus, roots regenerated from shoots cultured on MS basal medium supplemented with IBA are taller as compared with roots formed from shoots cultured on MS basal medium supplemented with NAA, generally MS basal medium supplemented with either NAA or IBA showed no significant differences for shoot lengths of *Rumex* plantlets (Table 2).

Table (2): Effect of IBA and NAA on the *in vitro* rooting of *Rumex vesicarius*

Treatment µM		Rooting (%)	No. of root/ plantlet	Root length (cm)	Callus .(%)
IBA	0.00	62.5	7.8^{e}	5.4^{a}	0.00
	0.49	68.75	9.1^{de}	3.7^{b}	6.25
	1.23	81.25	12.7^{cde}	3.9^{b}	6.25
	2.46	100	19.7^{ab}	4.0^{b}	12.5
	4.90	100	19.4^{ab}	1.7^{cd}	25.00
	7.38	100	15.6^{bc}	2.1^{c}	50.0
	9.84	100	14.9^{bcd}	1.9^{c}	50.0
NAA	0.49	68.75	12.1^{bc}	2.0^{c}	0.00
	1.23	87.5	22.8^{a}	1.7^{cd}	0.00
	2.46	87.5	15.0^{bcd}	1.2^{c-e}	50.0
	4.90	93.75	12.3^{cde}	1.2^{c-e}	75.0
	7.38	100	9.3^{de}	0.9^{de}	100
	9.84	100	7.0^{e}	0.8^{e}	100

Means with the same letters in the same column are not significantly different according to Duncan's multiple range test at 5%.

The rooted shoots were carefully removed from the jars and placed into plastic pots (6 cm) for hardening off.

Successful acclimatization took 4 weeks. 87% of plantlets were survived and grew normally (plate1-6). Subsequently, the acclimated plantlets were transferred to the field.

In summary, the results of this study showed the efficient of *in vitro* regeneration as a fast and reliable method to conserve Rumex plants.

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الاكثار الدقيق للنبات الحميض بإستخدام مزرعة القمة النامية

إسلام حسن أبو السعود – أمين علي احمد العزب – عيد محمد قريش – حمدي كمال عطا الله
قسم البساتين – كلية الزراعة – جامعة قناة السويس – 41522-الاسماعيلية – مصر

الملخص العربى

تم فصل القمم النامية من نباتات الحميض من بادرات نامية في المعمل وزراعتها على بيئة موراشيج وسكوج (MS) مضافاً اليها تراكيز مختلفة من البنزيل أدنين (BA) او الكينيتين (Kin) و ذلك لأجل دراسة تأثير كل منهم على إحداث التضاعف . وبعد مرور اربعة اسابيع أشارت النتائج إلى أن أضافة البنزيل ادنين بتركيز 8,88 ميكرومول /لتر أعطت أفضل النتائج حيث كانت عدد الفروع الناتجة 11 فرع / قمه نامية مستخدمة . اما بالنسبة لاضافة الكينيتين فقد تم الحصول على أفضل النتائج عند تركيز 26,63 ميكرومول/لتر حيث كانت عدد الافرع الناتجة 4,6 /قمة نامية . تم زراعة الأفرع على بيئة التجذير وكانت أفضل النتائج بعد مرور اربعة اسابيع تلك المزروعة على بيئة اندول حامض البيوتريك IBA بتركيز 2,46 ميكرومول /لتر وعلى نفثالات حامض الخليك NAA بتركيز 7,38 ميكرومول /لتر . تم نقل النباتات المكونة للجذور الى ظروف الاقلمة وكانت نسبة البقاء 87% وقد نجحت النباتات في النمو بشكل طبيعي في ظروف الصوبة .