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Effect of phytohormone on shoot generation potentiality in rucola

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ABSTRACT

The present investigation was carried out to study the in vitro regeneration in Rucola. Different concentrations and combinations of BA and IBA were used for regeneration of root and shoot in rucola. Cotyledonary nodes were used as explants. The highest number of shoots (4.60) and leaves (6.4) were recorded in treatment 4.0 mg/l BA with minimum (9.00) days. The maximum days (14.00) was noticed in a simple MS medium to regenerate lower no. of shoots. The treatment BA 4.0 mg/l produced the highest number of the main root (4.60) while it was lowest in the control treatment. The highest number of shoot (3.40) was noticed from the BA 4.0 mg/l + IBA 3.0 mg/l. The treatment BA 4.0 mg/l + IBA 4.0 mg/l gave the highest number of leaves (11.00) and maximum number of roots (3.60). The regenerated plantlet was acclimatized in natural pot and soil conditions. Finally, a convenient protocol technique has been established for in vitro regeneration of rucola, which can be used for large scale plantlet production.

Key Words: Phytohormone, Cotyledonary Nodes, Regeneration, Rucola, IBA and BA

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I. Introduction

Eruca sativa commonly known as rocket, rucola, arugula, rucoli, etc. is an edible annual plant under the family Brassicaceae is used as a leafy vegetable for its fresh, peppery flavor and is geographically confined to the Mediterranean region (Chopra et al., 1996 ; Yaniv et al., 1998). The Rocket was used medicinally at once, although it is now used commonly as a salad herb (Lamy et al., 2008). All parts of the plant except roots are edible (leaves, flowers, siliquas, young and mature seeds). The plant is reported to have antiscorbutic, diuretic, stimulant, stomachache, and rubefacient activities (Songsak and Lockwood, 2002). Alqasoumi et al. (2009) recently found anti-ulcer activity and anti-inflammatory effects of *Eruca* extracts (Yehuda et al., 2009). Rucola controls human diseases and disorders like

diabetes, cancer, cardiovascular diseases that have already been proven from scientific research. The conventional techniques used the seed as planting material, which shows the low viability and less regeneration capacity. The traditional method is also time consuming and slow growing process. Therefore, Rucola is also susceptible to many diseases and insect attacks. For solving this problem and large-scale production, tissue culture technology can be the best way. It will give disease free healthy seedling for higher yield. Although Rucola has so many uses, in Bangladesh no commercial production is reported yet. So, for starting large-scale commercial production, tissue culture method (micropropagation) should be used. It is possible now to obtain a large number of plants from one explant in vitro. In Bangladesh, till now no evidence has been reported on the in vitro rapid multiplication capacity of Rucola which is very much essential to be known for its commercial production.

II. Materials and Methods

The present experiment was carried out in the Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of February 2016 to June 2017. The planting materials of *Eruca sativa* were collected from Horticulture Department and Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. The cotyledonary nodes were the explants. Explants were cultured on MS medium supplemented with different concentrations of BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and IBA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and their combination. The temperature of the growth room was maintained within $21\pm 1^\circ\text{C}$ by an air conditioner and a 16 hour photoperiod was maintained along with light intensity of 3000-5000 lux for proper growth and development of culture.

After planting, the plantlets were thoroughly watered and were kept at $23\pm 2^\circ\text{C}$ with light intensity varied from 3000–5000 lux. The photoperiod was generally 14 hours light and 10 hours dark and 70% RH for 7 days with consecutive irrigation. Then the plants were shifted to shade house with less humidity and indirect sunlight. The top of the pots was covered with a transparent plastic sheet and grew at room temperature and 70% RH for 14 days with periodic irrigation (2 days interval). After 3 weeks, the plants were transferred to the soil following deposing and potting into different pots having bigger size. The plants were watered periodically and upper layer of the soil was mulched occasionally whenever necessary.

The experiment was conducted in the laboratory and arranged in Completely Randomized Design (CRD) with five replications. The significant difference between the pair of means was evaluated at 1% level of significance by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

III. Results and Discussion

Days to shoot induction

The maximum (14.00 days) to shoot induction were recorded in control followed by 1.0 mg/l (11.60 days) and 2.0 mg/l BA (10.80 days). On the other hand, minimum (6.40 days) was required in 3.0 mg/l BA followed by 5.0 mg/l (7.20 days) and 4.0 mg/l (9.00) days (Figure 01).

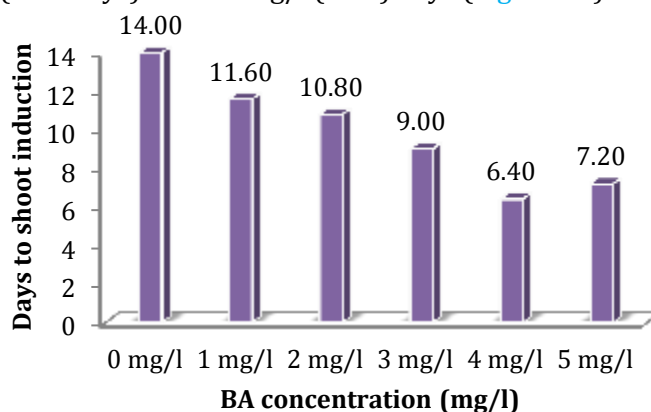


Figure 01. Effect of BA on days to shoot induction in Rucola

Number of shoot

The highest number of shoot (2.60, 3.60 and 4.60) at 14, 21 and 28 days after induction (DAI) respectively were noticed from the 4.0 mg/l BA which was statistically similar with 3 mg/l BA (2.40, 3.00 and 4.00) and 5.0 mg/l BA (2.20, 3.20 and 4.00) at 14, 21 and 28 DAI (Table 01). Whereas, the lowest number of shoot (1.00, 1.60 and 1.60) at 14, 21 and 28 DAI, respectively were noticed in control without hormone (Table 01). Cuce et al. (2017) found that the highest shoot multiplication successes were obtained in the lowest BA treatments with 37.88 mm shoot length. The findings of this study are partially similar to their results.

Number of leaves per shoot

There was a significant influence of different concentrations of BA on the number of leaves per shoot. BA 4.0 mg/l gave the maximum number of leaves (2.60, 4.20 and 6.40) and the second highest leaves number (1.80, 3.20 and 5.20) was found in BA 5.0 mg/l at 14, 21 and 28 DAI, respectively (Table 01) whereas, the control treatment showed the lowest number of leaves (1.00, 1.20 and 1.60) at 14, 21 and 28 DAI (Table 01) respectively.

Table 01. Effect of different concentration of BA on number of shoot and number of leaves at different days after induction (DAI) of shoot

BA (mg/l)	Number of shoot			Number of leaves/shoot		
	14 DAI	21 DAI	28 DAI	14 DAI	21 DAI	28 DAI
0	1.00f	1.60e	1.60d	1.00c	1.20c	1.60e
1.0	1.60e	2.00d	2.60c	1.20c	1.80c	2.20de
2.0	2.00d	2.60c	3.20bc	1.20c	1.80c	2.80d
3.0	2.40b	3.00b	4.00ab	2.20ab	3.40ab	4.20c
4.0	2.60a	3.60a	4.60a	2.60a	4.20a	6.40a
5.0	2.20c	3.20b	4.00ab	1.80b	3.20b	5.20b
CV (%)	30.60	24.54	17.75	25.69	26.27	20.16
LSD (0.05)	0.134	0.357	0.772	0.558	0.892	0.982

In a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT

Days to root induction

Significant variations were observed among different concentrations of IBA on days to root induction. The maximum days (21.20 days) to root induction were recorded in control followed by 1.0 mg/l (18.80 days) and 5 mg/l (18.00 days). On the other hand, minimum (14.40 days) was required in 3.0 mg/l IBA followed by 2.0 mg/l (15.80 days) and 4.0 mg/l (16.20 days) (Figure 02). Abbasi et al. (2016) described that *in vitro* regenerated shoots were shifted to MS medium augmented with 5.0 mg/l indole acetic acid (IAA) for rooting after 4 weeks of sub-culturing. This result may be varied due to the differences between genotype and culture environments (Sen et al., 2002).

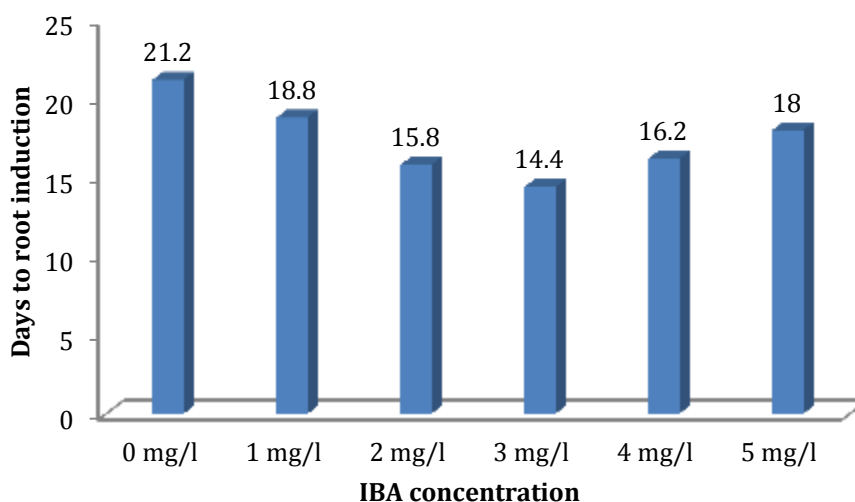


Figure 02. Effect of IBA on days to root induction in Rucola

Number of root per shoot

IBA 4.0 mg/l gave the highest number of root (2.60, 3.60 and 4.60) at 14, 21 and 28 DAI and the control treatment was found with the lowest number of root (1.00, 1.20 and 1.40) at 14, 21 and 28 DAI (Table 02). Sharma et al. (2012) stated that shoots were separated carefully and were transferred to the fresh half strength MS solid medium with indole-3-butyric acid (4.90 μ M) for the development of the roots.

Table 02. Effect of different concentration of IBA on number of root at different days after induction (DAI)

IBA (mg/l)	Number of root/shoot		
	14 DAI	21 DAI	28 DAI
0	1.00d	1.20c	1.40e
1.0	1.40c	1.60bc	2.60cd
2.0	2.20b	3.00a	3.60b
3.0	1.40c	2.20b	2.80c
4.0	2.60a	3.60a	4.60a
5.0	1.00d	1.60bc	2.00de
CV (%)	26.76	24.90	19.86
LSD (0.05)	0.258	0.715	0.734

In a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT.

The combined effect of BA and IBA on shoot and root induction potentiality in Rucola

Days to shoot induction: Significant variations were observed among different concentrations of BA and IBA on days to shoot induction. The minimum duration (6.00 days) was obtained in BA 4.0 mg/l+ IBA 3.0 mg/l than the rest of the treatments. On the other hand, the maximum days (18.80 days) to shoot induction was recorded in control (Table 03). Daud et al. (2015) showed that the new plantlets were raised in a short period when explants were cultured on MS medium containing 1.0 mg/l BAP and 0.5 mg/l NAA. The variation may be due to influence of genetic and environmental factors (Sen et al., 2002).

Number of shoot: The highest number of shoot (2.40, 3.00 and 3.40) was noticed from the BA 4.0 mg/l + IBA 3.0 mg/l and second highest number (1.80, 2.80 and 3.00) at 14, 21 and 28 DAI, respectively, were observed in 4.0 mg/l BA + 2.0 mg/l IBA. Whereas the lowest average number of shoot (1.00, 1.40 and 1.40) at 14 DAI, 21 DAI and 28 DAI, respectively were noticed in the control treatment (Table 03). These findings are completely opposed to the results of (Sharma et al., 2012). They found that each inoculated explant produced 18.10 ± 0.66 shoots within 2 to 3 weeks from *in vitro* grown plantlets inoculated on the Murashige and Skoog (MS) medium supplemented with 4.44 μ M 6-benzyl amino purine (BAP) in combination with 2.85 μ M indole-3-acetic acid (IAA). This variation is due to the age, nature, origin and the physiological state of the explant and environmental factors can play a crucial role in the establishment of cultures and subsequent plant regeneration (Bajaj et al., 1991).

Number of leaves per shoot: The number of leaves per shoot showed a significant difference with combined concentrations of BA and IBA. The treatment BA 4.0 mg/l+ IBA 4.0 mg/l gave the highest number of leaves (3.20, 7.20 and 11.00) at 14, 21 and 28 DAI, respectively whereas the lowest number of leaves (1.20, 1.20 and 1.40) at 14, 21 and 28 DAI respectively were found with hormone free media (Table 03).

Days to root induction: Significant variation was observed among different concentrations of BA and IBA on days to root induction. The maximum (26.60 days) to root induction was recorded in control treatment and minimum (16.20 days) was required in BA 4.0 mg/l + IBA 2.0 mg/l concentration (Table 04).

Number of root per shoot: There was a significant influence of different concentrations of BA and IBA on the number of root per shoot. The treatment BA 4.0 mg/l+ IBA 4.0 mg/l gave the highest number of root (1.80, 2.60 and 3.60) and second best result (1.60, 2.40 and 3.40) was noticed from BA

4.0 mg/l + IBA 3.0 mg/l at 14, 21 and 28 DAI whereas the lowest number of root (1.00, 1.00 and 1.40) at 14, 21 and 28 DAI were found with hormone free media (Table 04).

Table 03. Combined effect of BA and IBA on shoot and root induction potentiality in Rucola at different days after induction (DAI)

Treatments BA+IAA (mg/l)	Days to shoot	No. of shoot			No. of leaves		
		14 DAI	21 DAI	28 DAI	14 DAI	21 DAI	28 DAI
Control	18.80a	1.00c	1.40d	1.40d	1.20e	1.20o	1.40l
1.0+0.5	13.80de	1.60bc	1.60cd	2.20b-d	1.60de	2.00kl	2.80jk
1.0+1.0	14.20d	1.60bc	1.60cd	2.20b-d	1.40e	1.60mn	2.40kl
1.0+1.5	15.40c	1.40bc	1.60cd	1.80c-e	1.60de	1.80lm	2.40kl
1.0+2.0	15.40c	1.20bc	1.40d	1.60de	1.20e	1.60mn	2.20kl
1.0+2.5	16.20b	1.20bc	1.40d	1.80c-e	1.20e	1.40no	2.20kl
2.0+0.5	13.00f	1.60bc	2.00b-d	2.20b-e	1.40e	2.40ij	3.80h-j
2.0+1.0	12.00g	1.40bc	1.60cd	2.00c-e	1.40e	1.80lm	3.80h-j
2.0+1.5	12.40fg	1.60bc	2.00b-d	2.40b-d	1.60de	2.60hi	3.80h-j
2.0+2.0	13.20ef	1.40bc	2.20a-d	2.60a-c	1.40e	2.00kl	3.00jk
2.0+2.5	12.80f	1.40bc	1.60cd	2.40b-d	1.60de	2.20jk	3.40i-k
3.0+0.5	10.20jk	1.40bc	1.60cd	1.80a-c	1.60de	3.20g	4.40g-i
3.0+1.0	10.60ij	1.60bc	2.00b-d	2.20b-e	1.40e	3.20g	5.00f-h
3.0+1.5	10.00jk	1.60bc	1.60cd	2.00c-e	1.80c-e	3.40g	5.00f-h
3.0+2.0	11.80gh	1.60bc	1.80cd	2.00c-e	1.80c-e	3.40g	5.20fg
3.0+2.5	11.20hi	1.60bc	2.20a-d	2.60a-c	1.40e	2.80h	3.80h-j
4.0+0.5	7.80no	1.80ab	2.00b-d	2.60bc	2.40bc	4.40de	8.00c
4.0+1.0	6.60pq	1.80ab	2.80ab	3.00ac	1.80c-e	4.40de	7.20cd
4.0+1.5	6.00q	2.40a	3.00a	3.40a	2.80b	6.80b	9.40b
4.0+2.0	8.60l-n	1.60bc	2.40a-c	2.60bc	3.20a	7.20a	11.00a
4.0+2.5	7.20op	1.60bc	2.20a-d	2.60bc	2.40bc	4.80c	8.20c
5.0+0.5	8.60l-n	1.40bc	2.20a-d	2.40b-d	1.80c-e	4.60cd	7.60cd
5.0+1.0	8.60mn	1.40bc	1.60cd	2.00c-e	2.60ab	4.60cd	5.80ef
5.0+1.5	8.80lm	1.40bc	2.00b-d	2.00c-e	2.20b-d	4.00f	5.80ef
5.0+2.0	9.60k	1.80ab	2.40a-c	2.60a-c	1.80c-e	4.20ef	6.60dg
5.0+2.5	9.40kl	1.60bc	1.80cd	2.40b-d	1.80c-e	4.00f	5.20fg
CV (%)	5.35	16.06	9.61	15.72	31.95	22.38	18.27
LSD (0.05)	0.754	0.523	0.716	0.729	0.583	0.302	1.141

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT.

Table 04. Combined effect of BA and IBA on days to root and number of root induction potentiality in Rucola

Treatments	Days to root	No. of root		
		14 DAI	21 DAI	28 DAI
Control	26.60a	1.00b	1.00e	1.40g
1.0+0.5	22.00cd	1.20b	1.40c-e	1.60fg
1.0+1.0	21.80cd	1.40ab	1.60c-e	2.20d-g
1.0+1.5	22.20cd	1.20ab	1.40c-e	1.80e-g
1.0+2.0	22.60bc	1.20ab	1.20c-e	1.60fg
1.0+2.5	23.80b	1.00b	1.40de	1.60fg
2.0+0.5	19.60f-i	1.20ab	1.40c-e	2.00d-g
2.0+1.0	20.80d-f	1.20ab	1.40c-e	2.00d-g
2.0+1.5	21.20c-e	1.40ab	1.60c-e	2.40c-f
2.0+2.0	21.20de	1.20ab	1.60c-e	2.20d-g
2.0+2.5	20.80d-f	1.20ab	1.60c-e	2.00d-g
3.0+0.5	20.80d-f	1.20ab	1.40c-e	2.00d-g
3.0+1.0	18.60h-j	1.20ab	1.40c-e	2.00d-g
3.0+1.5	18.00jk	1.20ab	1.60c-e	2.00d-g
3.0+2.0	19.60f-i	1.20ab	1.40c-e	1.60fg

Treatments	Days to root	No. of root		
		14 DAI	21 DAI	28 DAI
3.0+2.5	19.40f-j	1.20ab	1.40c-e	2.20d-g
4.0+0.5	17.00kl	1.40ab	2.40ab	3.20a-c
4.0+1.0	16.20l	1.40ab	2.00a-c	2.80a-d
4.0+1.5	16.60l	1.60ab	2.40ab	3.40ab
4.0+2.0	17.20kl	1.80a	2.60a	3.60a
4.0+2.5	18.00jk	1.20ab	1.60c-e	2.60b-e
5.0+0.5	18.80g-j	1.20ab	1.60c-e	2.60b-e
5.0+1.0	18.20i-k	1.20ab	1.80b-d	2.00d-g
5.0+1.5	19.80e-h	1.20ab	1.80b-d	2.00d-g
5.0+2.0	19.20g-j	1.20ab	1.60c-e	2.20d-g
5.0+2.5	20.20e-g	1.20ab	1.60c-e	2.00d-g
CV (%)	5.01	16.01	32.87	30.20
LSD (0.05)	1.257	0.519	0.613	0.783

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT.

Ex Vitro acclimatization and establishment of plantlets on soil

After two and a half months, a good number of root and shoot were found and plants were acclimatized under shade condition and in natural condition. 20 plants were transplanted in shade area after three weeks, 16 plantlets were survived and the survival rate was 80% (Table 05). In natural conditions, 16 plants were transplanted, 12 survived (Plate 02) and the survival rate was 75%. (Sharma et al., 2012) found that plantlets of Rucola were successfully transferred to the soil where they grew well for 8 to 10 weeks with 80% survivability. So considering the survival rate it can be said that acclimatization potentiality of Rucola is satisfactory.

Table 05. Survival rate of *in vitro* regenerated plantlets of Rucola

Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate
In shade area with Controlled atmosphere	20	16	80
In natural condition	16	12	75

IV. Conclusion

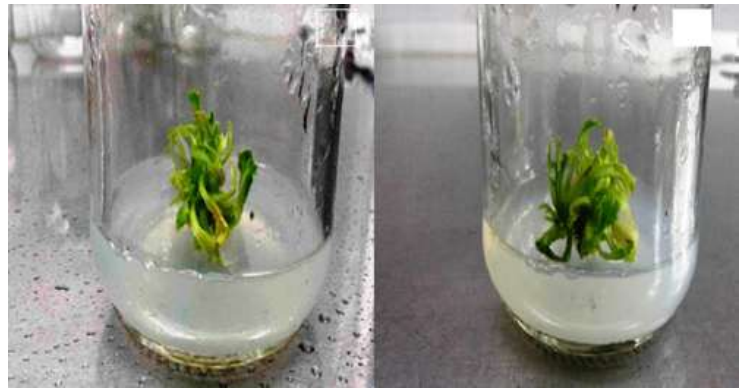
From the above summary, it is concluded that the moderate dose of BA 4.0 mg/l gave the best results for shoot regeneration in comparison with combine dose of BA 4.0 mg/l with IBA 2.0 mg/l and 3.0 mg/l. Besides, BA 4.0 mg/l with NAA (0.5, 1.0, 1.5, 2.0 mg/l) showed good performance for callus and shoot induction and IBA 4.0 mg/l gave the best performance in case of root. Finally, a convenient protocol of *in vitro* rapid regeneration of Rucola has been established which may contribute to breeding programs and large scale virus free seedlings production throughout the year is possible.

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Inoculation of explants



Shoot induction from single dose of BA



Leaf regeneration from BA



Root formation in rucola by IBA



Leaf from combined dose of BA and IBA



Root initiation from combined dose of BA and IBA

Plate 01. Overview of rucola plantlet regeneration treated by different hormonal combination



Plate 02. Hardening of Rucola plantlet in shade condition (a) and in natural condition (b) & (c)

V. References

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MLA

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