Response of Culture Media and Auxin on Growth and Glucosinolate Accumulation in the Hairy Root Cultures of Rocket (*Eruca sativa*)

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Abstract

Rocket (*Eruca sativa*) is a domesticated plant species that is commonly eaten in salads and known to provide health benefits because of the high levels of glucosinolates, flavonols, and other compounds. Hairy root cultures (HRCs) are effective biotechnological tools for biosynthesis of secondary metabolites under various growing conditions. HRCs of rocket were treated with growth media of half-strength and full-strength Murashige-Skoog (MS) media, Gamborg's B5 medium, and Schenk and Hildebrand (SH) medium and auxins to evaluate the growth response and the accretion of glucosinolate. The growth pattern of the hairy roots varied extensively under the different media and auxin treatments; the highest and the lowest fresh weights were recorded in HRCs grown under full-strength SH and half-strength MS media, respectively. Treatment with NAA at 1.0 mg/l produced the highest hairy root fresh weight, followed by the treatments with IAA 0.1 mg/l and IBA 1.0 mg/l. The MS media induced the highest glucosinolate accumulation, followed by B5; all media enhanced the production of glucosinolates but auxins treatments (exception for glucoerucin) did not positively enhance the production of glucosinolates. Total glucosinolate levels were increased 1.7, 1.68, 1.33, and 1.26 fold in full-strength MS, half-strength MS, half-strength B5, and B5, respectively. These findings indicated that hairy root production and glucosinolate accumulation did not follow the same trend. Although SH media and NAA 1.0 slightly enhanced hairy root production, full-strength MS media induced higher amounts of glucosinolate, and auxin treatments did not increase the accumulation of glucosinolate. We therefore propose that MS media, regardless of additional treatment, provides a valuable alternative approach for the mass production of hairy root cultures and glucosinolates in rocket.

Keywords: glucosinolates; growth media; auxin; hairy root; rocket salad
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1. Introduction

Eruca sativa is commonly known as 'salad' or 'cultivated' rocket and is well-known for its hot, peppery, and bitter flavor profile [1]. The crops jointly known as rockets are all members of the family *Brassicaceae* and are native to the regions neighboring the Mediterranean Sea [2]. *Eruca sativa* is cultivated commercially worldwide, particularly in the USA, the UK, Italy, Spain, Morocco, Israel, and Australia [3]. It is also cultivated in some parts of Asia, including Pakistan, Iran, and India, and the leaves are used in salad and as vegetables, or livestock feed [2]. Rocket salad leaves contain glucosinolates, the most abundant of which are glucoerucin, glucoraphanin, and glucosativin [4]. Rocket salad has been reported to have astringent, antiphlogistic, antacid, diuretic, digestive, laxative, stimulative, anti-inflammatory (for colitis), and blood circulation properties [5]. Other studies have shown that rocket salad inhibits cancer [6, 7] and has antioxidant [8, 9], antimicrobial [10], and anti-inflammatory properties [11].

Approximately 200 different glucosinolates occur naturally in plants [12, 13]. Regular intake of vegetables from the family *Brassicaceae* provides benefits to human health [14, 15]. Consumption of vegetables containing glucosinolates (GSLs) and flavonols is associated with reduced risk of numerous cancers [16-18] and with improved cardiovascular health [19]. However, much of the world's populations do not eat sufficient quantities of these vegetables to obtain these benefits [20].

Auxins are a class of phyto-hormones that play a major role in the processes of growth and development in plant life cycles. Auxins influence wound response, axial elongation in the shoot, fruit growth and development, coleoptile growth, initiation of flowering, development of reproductive organs, and ethylene biosynthesis. Auxins are also involved in root growth and development, including the formation of adventitious roots and root re-growth [21]. When the concentration of auxins is decreased by removing the apical meristem, the stimulation of roots is decreased and stem growth is increased. The most common phytohormones, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalic acetic acid (NAA) are transported from the stem to the root, and the overall root development is stimulated. These plant hormones, particularly IBA, IAA, and NAA are generally used to stimulate root growth and development in plant cutting propagation. However, if the concentration of auxin is too high, the elongation of roots is inhibited and the formation of adventitious roots increases. The optimal concentration of auxin is therefore essential.

Plant tissue culture media are used for plant growth and propagation to provide controlled conditions that include the necessary inorganic compounds, adequate pH levels, and sources of carbon [22, 23]. Previous studies investigating the selection of the appropriate basal media for hairy root initiation and growth and the accumulation of desirable natural products have shown that the ideal medium is dependent on the plant species in question [23-27]. HRC is promising biotechnological tool because of the fast growth rate and secondary metabolite production that it induces under controlled conditions [28]. Because of these advantages, HRC has been broadly investigated in many plant species for the mass collection of secondary metabolites used for food additives and by the pharmaceutical industry. Studies have investigated glucosinolate accumulation in response to different treatments in the hairy roots of the plants of Brassicaceae family, including kale [29], watercress [30], mustard [31], Chinese cabbage [32], and broccoli [33].

Few studies have investigated HRC for glucosinolate accumulation in rocket salad [34, 35]. Auxin and media played a vital role in the accumulation of secondary metabolites. However, the use of HRC according to media and auxin concentration in rocket salad has never been studied. Therefore in this study, we investigated the growth and glucosinolate content in rocket salad hairy roots in various culture media and auxin concentrations to determine the optimal culture environment and thereby enhance the production of glucosinolates.

2. Materials and Methods

2.1 Plant Materials

Rocket salad seeds were obtained from a seed company (Asia Seeds Co., Ltd, Seoul, Korea). The seeds were sterilized by immersion in 70 % (v/v) ethanol for 30 s and 2% sodium hypochlorite for 10 min. The seeds were then rinsed five times with sterilized water and maintained on Petri dishes with MS medium consisting of 30 g/l sugar and 0.8 % agar at pH 5.7 with no additional hormones for germination.

2.2 Establishment of hairy roots

Young leaves of 10-day-old old rocket salad were wounded using a scalpel and dipped in a suspended liquid medium of *Agrobacterium rhizogenes* (strain R1000) for 10 min. The seedlings were cleaned with sterilized paper and incubated on antibiotic-free MS (Murashige and Skoog) medium [36], for 2 days under dark conditions at 25°C. Infected leaves were co-cultured for 2 days and then washed using sterilized water and dried with sterilized paper. These were then transferred to 1/2 MS solid medium for 2 to 3 weeks after addition of 250 mg/l cefotaxime and then incubated under dark conditions. The hairy roots that emerged from the wounded parts were separated from the leaves and cultured under the same conditions. One fast-growing clone was selected for the experiment and cultured further.

2.3 Treatment of culture medium and auxins

Liquid growth media of both half-strength and full-strength MS, SH (Schenk and Hildebrandt medium) [37], and B5 (Gamborg B5 medium) [38] were selected as the culture media for the hairy root cultures. To allocate equal weight (2g fresh weight), hairy roots were measured and transferred to each liquid culture medium where 30 ml was maintained in each flask. Treated samples of the different media were collected after a culture period of 4 weeks and kept in liquid nitrogen for glucosinolate analysis. The samples were then ground finely using a pestle and lyophilized for 72 h at -80°C for HPLC analysis of GSL compounds. In addition, the auxins IAA, IBA, and NAA were used in concentrations of 0.1, 0.5, and 1 mg/l using half-strength of MS media as basal media to compare the effects of auxin type and concentration on hairy root cultures. Hairy roots were measured and transferred to each liquid culture medium in equal weight (2g fresh weight). Auxin-treated samples were collected after 4 weeks of culture and immediately frozen in liquid nitrogen for analysis of glucosinolates. The samples were lyophilized for 72 h at -80°C for HPLC analysis of GSL compounds and ground finely using a pestle. All samples were prepared in triplicates.

2.4 Extraction of DS-GSLs and HPLC analysis

Glucosinolates were extracted and desulfated according to the procedure of International Organization for Standardization (ISO) 9167-1 [39] and Kim *et al.* [40]. Glucosinolates were extracted with 70 % MeOH (v/v) and incubated at 70°C for 5 min in a water bath. The extracted samples were centrifuged at 12,000 rpm for 10 min at 4°C. The resulting supernatant was placed into a mini-column packed with DEAE-Sephadex A 25 (40 mg dry weight) and desulfated by the addition of 75 µl aryl sulfatase solution (23 mg/ml). Ultrapure water (1.5 ml) was added to elute the desulfo-glucosinolates samples. An Agilent Technologies 1200 series HPLC system (Palo Alto, CA, USA) was used to analyze the desulfated extracts, and reversed-phase chromatography was performed with an Inertsil ODS-3 column (150 × 3.0 mm i.d., particle size 3 µm; GL Sciences,

Tokyo, Japan) equipped with an E-type cartridge guard column (10 × 2.0 mm i.d., 5 µm). The wavelength for UV detection of glucosinolate was 227 nm, and the column oven temperature and flow rate were 40°C and 0.4 ml/min, respectively. The mobile phase was composed of (A) ultrapure H2O and (B) acetonitrile (HPLC grade). The gradient was as follows: initiation 7% solvent B followed by 7-24% solvent B (18 min), 24% solvent B (14 min), 7% solvent B (0.1 min), and 7 % solvent B for 8 min (total 40 min). The individual desulfo-glucosinolates were quantified based on their HPLC peak area ratios, reference to a desulfo-sinigrin external standard, and response factors (ISO 9167-1, 1992).

2.5 Statistical analysis

All data were measured as the means of three replicates. Three subsamples were randomly generated from each treated sample of rocket salad, and each replicate sample was separately subjected to extraction. Data were analyzed by analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects, using SAS Software (release 9.2; SAS Institute Inc., Cary, NC, USA), and the means were separated via Duncan's multiple range test($P < 0.05$).

3. Results and Discussion

3.1 Production of hairy root cultures in rocket salad grown with different media

Rocket salad hairy roots were treated in full-strength and half-strength MS medium, B5 medium, and SH medium to investigate the effect of culture medium on hairy root growth and glucosinolate production. The growth pattern of the hairy roots of rocket salad varied greatly under different media conditions (Figure 1). The highest dry weight (309 mg/flask) and the lowest dry weight (185 mg/flask) were recorded from full-strength SH and ½ MS media, respectively. The dry weights of the hairy roots of rocket salad were 67.03%, 57.48%, 49.73%, 43.78%, and 20.90% higher when cultured with SH, B5, ½ SH, 1/ B5, and MS, respectively, as compared to the lowest dry weight producer (½ MS medium).

Several factors increase secondary metabolite production and biomass production, including the type and concentration of salt in the medium, the type and quantity of carbohydrates, nitrate and phosphate, and the levels of growth regulator [22, 23-27]. Different media formulations (including MS, SH, B5, and Linsmaier and Skoog (LS)), have been developed and widely implemented for plant cultures [41]. In this study, different media showed significant effects on hairy root cultures (HRCs) of rocket salad, where the highest and the lowest fresh weights were observed when cultured in full-strength SH and ½ MS media, respectively. Numerous studies reported that different media affect the production of hairy root cultures. For instance, SH medium was reported to be optimal for HRC growth in watercress [42] and Chinese skullcap [27]; our results are consistent with these previous studies. In contrast, the hairy roots of hybrid ginseng [24], gamhar [25], and potato [26] exhibited the greatest growth in B5 media, and MS medium positively affected the growth of hairy root cultures of ginseng [43], cell suspension cultures of *Gymnema sylvestre* [44] and the hairy roots of broccoli [33].

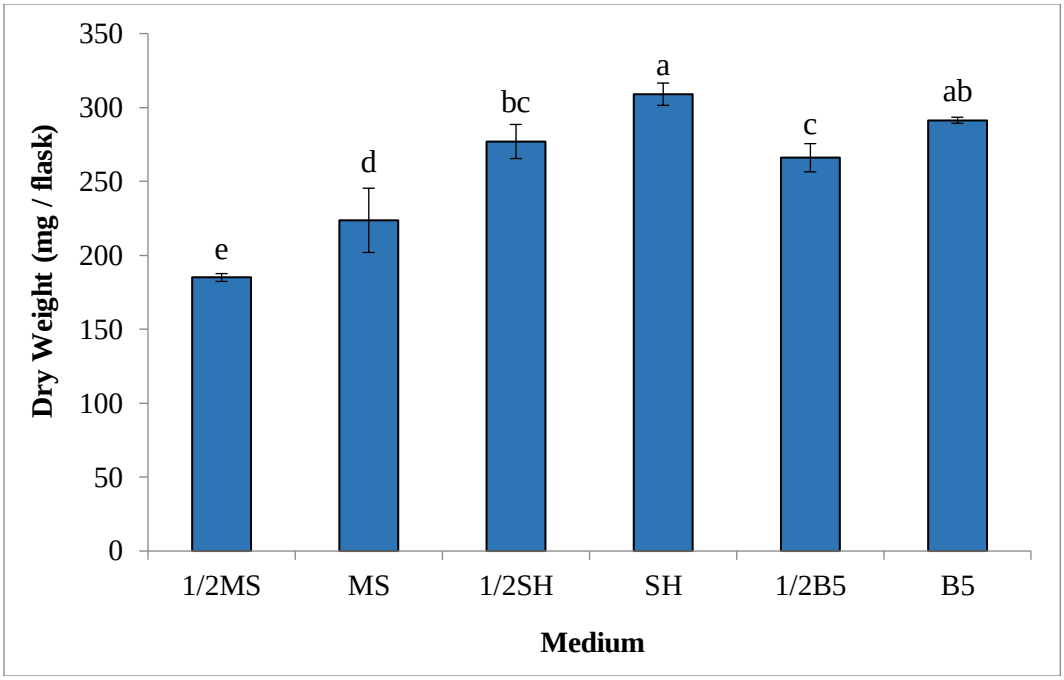

Figure 1. Effects of different media on the dry weight of rocket salad hairy root cultures. Values are means of three independent replicate results. Mean values with different letters were significantly different ($p < 0.05$, ANOVA, DMRT). DMRT: Duncan's multiple range test, MS= Murashige and Skoog, SH=Schenk and Hildebrandt, B5= Gamborg

3.2 Production of hairy root culture in rocket salad grown under conditions of different auxins

The hairy roots of rocket salad were treated with different concentrations of auxins. Three concentrations of the auxins, IAA, IBA, and NAA, were used to determine the effects on hairy root growth in rocket salad. The highest dry weight (331.33 mg/flask) and the lowest dry weight (270.67 mg/flask) were recorded from cultures in NAA 1.0 and from the control (only half-strength MS media), respectively (Figure 2). The dry weights of the hairy roots of rocket salad were not significantly different among the auxin treatments. The dry weights of the hairy roots of rocket salad were 22.41%, 21.18%, 20.44%, 18.59%, 16.75%, 15.76%, 15.15%, 14.16%, and 4.43% higher under treatments of NAA 1.0, IAA 0.1, IBA 1.0, IAA 0.5, IBA 0.5, NAA 0.5, IBA 0.1, IAA 1.0, and NAA 0.1, respectively, compared with the control treatment.

Exogenous growth regulators have been used extensively in plant cell, tissue, and hairy root cultures [41]. Among the various growth enhancers, auxins (particularly IAA, IBA, and NAA) play important roles in root development and promote hairy root induction [45]. Moreover, biomass and metabolite accumulation in media treated with different types and concentrations of auxins differed in plant cell cultures [46]. In this study, the highest fresh weight of HRC of rocket salad was obtained from a medium treated with NAA 1.0, followed by that with IAA 0.1 and IBA 1.0. Our findings support the results of other studies that determined the effects of auxins on higher biomass production in a wide range of plant species, such as tobacco [47], Indian mulberry [48], sorghum [49, 50], broccoli [51], and kale [52].

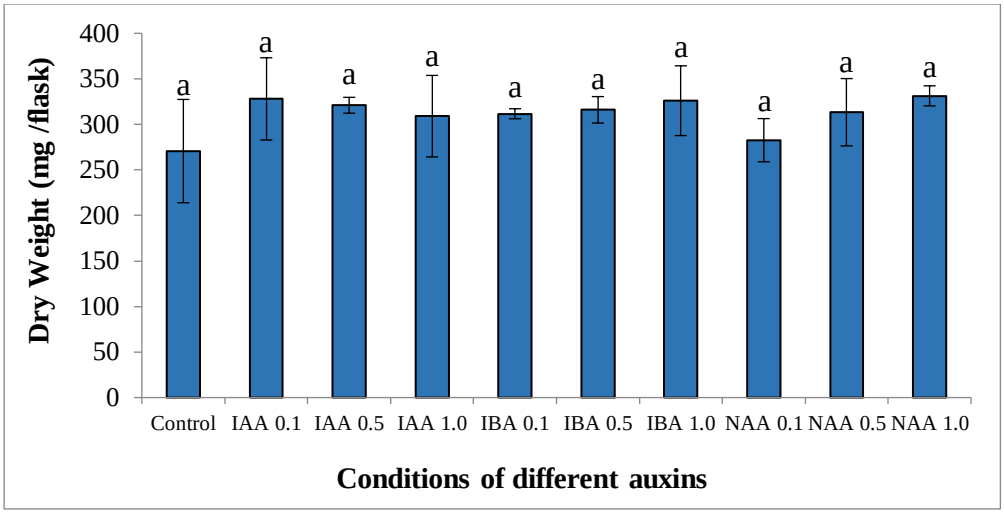

Figure 2. Effects of exogenous auxins on the fresh weight of rocket salad hairy root cultures. Values are means of three independent replicate results. Mean values with different letters were significantly different ($p < 0.05$, ANOVA, DMRT). IAA = indole-3-acetic acid, IBA = indole-3-butyric acid (IBA), and NAA = 1-naphthaleneacetic acid

3.3 Glucosinolates in the hairy root cultures of rocket salad in response to growth media

Based on the analysis of the hairy root cultures of rocket (*Eruca sativa*), the following six glucosinolates: glucoiberin, glucoerucin, glucoraphasatin, glucobrassicin, 4-methoxyglucobrassicin, and glucosativin, were observed at different levels in response to different growth media (Table 1). MS media yielded the greatest total accumulation of glucosinolates, followed by B5, and the lowest total accumulation of glucosinolates was found in the SH media. Total glucosinolate levels were increased by 1.7, 1.68, 1.33, and 1.26 in MS media containing ½ MS, ½ B5, and B5, respectively, compared with the lowest accumulated glucosinolates (from SH medium). The glucosativin level was much higher than that of 4-methoxy glucobrassicin, irrespective of the medium. MS media showed the best results for accumulation of glucoiberin, followed by B5 media, and SH media resulted in the least glucoiberin production. Glucoerucin content also varied widely by medium. MS media at half-strength accumulated the most glucoerucin, which was 2.89 times higher than the lowest (SH medium). Glucoraphasatin content did not vary significantly among the media. Half-strength MS media yielded double the quantity of glucoraphasatin of the least productive medium (MS). Glucobrassicin content was significantly higher in B5 and SH media; for the other media, the accumulation was almost similar. The content of glucobrassicin was 13.33 and 10.67 times higher in B5 and SH media, respectively, compared with the other four media. The quantity of 4-methoxy glucobrassicin was higher than in the control, irrespective of the medium used. Full-strength B5 medium showed the highest accumulation of 4-methoxy glucobrassicin, resulting in levels 4.2, 3.71, 3.58, and 2.56 times greater than those of the lowest (MS medium) for media treated with B5, ½ B5, SH,1/2 B5, and ½ SH, respectively. Glucosativin accumulation was higher at both concentrations of MS media, and the accumulation in other media was also slightly increased. Glucosativin accumulation, as influenced by different media, ranged from 3.48 to 18.85 µmol/g dry wt. Glucosativin accumulation was remarkably higher in MS medium (18.85 µmol/g dry wt) and

Table 1. Glucosinolate content in rocket salad hairy roots in various media

Glucosinolate (μmol/g dry wt)	1/2 MS	MS	1/2 SH	SH	1/2 B5	B5
Glucoiberin	0.15^{a}	0.20^{ab}	0.03^{c}	0.03^{c}	0.09^{b}	0.11^{b}
Glucoerucin	0.52 ^{a}	0.25^{bc}	0.31^{b}	0.18^{c}	0.45^{a}	0.25^{bc}
Glucoraphasatin	0.06 ^{a}	0.03^{b}	0.05^{ab}	0.04^{b}	0.04^{ab}	0.05^{ab}
Glucobrassicin	0.03 ^{c}	0.03^{c}	0.03^{c}	0.32^{b}	0.03^{c}	0.40^{a}
4-Methoxy glucobrassicin	3.24^{d}	2.25^{e}	5.77^{c}	8.35^{b}	8.05^{b}	9.45^{a}
Glucosativin	14.77^{a}	16.00^{a}	4.97 ^{bc}	3.48^{c}	6.14^{b}	3.83^{c}
Total	**18.76^{a}**	**18.85^{a}**	**11.16^{c}**	**12.40^{bc}**	**14.80^{b}**	**14.09^{b}**

Note: The data are presented as means with three replications in each individual sample. Mean values with different letter (s) in the same rows were significantly different ($p < 0.05$, ANOVA, DMRT). B5 = Gamborg B5 medium; 1/2 B5 = half-strength B5; MS = Murashige and Skoog medium; 1/2 MS = half-strength MS; SH = Schenk and Hildebrandt medium; 1/2SH = half-strength SH.

½ MS medium (18.76 μmol/g dry wt). The accumulated glucosativin was 4.6, 4.24, and 1.76 times higher following treatment with MS, ½ MS, and ½ B5 media, respectively.

GSL content in plants varies widely in response to factors including agronomic management, climatic conditions, mineral nutrient availability location, and plant variety [53-55]. In the present study, growth medium significantly influenced the accumulation of glucosinolates. Full-strength MS medium yielded the highest accumulation of total and individual glucosinolates, followed by ½ MS, ½ B5, and B5 media, respectively, whereas the lowest accumulated glucosinolates was found in SH media. These data are supported by a study in which growth medium was shown to significantly influence growth and glucosinolate content in broccoli [33]. In another study, MS basal medium increased the accumulation of glucosinolates over that of tissue grown in 1/2 SH medium [42]. In contrast, half and full-strength B5 and SH media induced the highest accumulations of glucosinolates in the hairy roots of broccoli [33]. Lee *et al*. [52] reported that B5 medium positively affected the production of total glucosinolates in HRCs of kale (*Brassica oleracea* var. *acephala*). B5 medium was optimal for both callus growth and the production of flavonoids. In addition, 1/2 B5 medium supported the highest production of flavones in HRCs of *Scutellaria baicalensis* [27].

3.4 Glucosinolate accumulation in hairy root cultures of rocket (*Eruca sativa*) in response to auxin treatment

The effects of different levels of auxins (IAA, IBA, and NAA) on glucosinolate accumulation in the hairy roots of rocket salad were investigated. The glucosinolates glucoiberin, glucoerucin, glucoraphasatin, glucobrassicin, 4-methoxy glucobrassicin, and glucosativin were observed in different quantities in response to different auxins (Table 2). Treatment with auxins negatively affected the production of glucosinolate in HRCs. The range of total accumulation level of

glucosinolates as influenced by different concentrations of auxins was 6.24 to 11.43 μmol/g dry wt, in which the highest level was found in the control treatment and the lowest in the IBA 1.0 treatment. The accumulation of total glucosinolates in the hairy roots of rocket salad was decreased by 45.41%, 44.88%, and 39.63% by treatment with IBA 1.0, IAA 1.0, and NAA 0.5, respectively, as compared with the control. The decreasing trend for other concentrations was the same but the level of decreasing was lower compared to IBA 1.0, IAA 1.0, and NAA 0.5. The auxin treatment did not increase the level of any glucosinolates, with the exception of glucoerucin. Glucoerucin accumulation level in the hairy roots of rocket salad was increased by 69.57%, 43.48%, and 26.09% on treatments with NAA 1.0, IBA 1.0, and IAA 0.1, respectively, compared to that of the control. The range of glucoiberin accumulation as influenced by different concentrations of auxins was 0.03 to 0.12 μmol/g dry wt, where the highest amount was obtained in the control and the lowest was obtained with IBA 0.5 treatment. Glucoiberin accumulation in the hairy roots of rocket salad was decreased by 75%, 41.67%, and 33.33% on the treatment with IBA 0.5, 0.1 and 0.5 IAA, and NAA 0.5, respectively, compared with the control. Gucoraphasatin content affected by different concentrations of auxins ranged from 0.02 to 0.04 μmol/g dry wt, with the highest value being in the control, and the lowest values were observed with IAA and IBA treatments. Glucoraphasatin accumulation in the hairy roots of rocket salad was decreased by 50%, 50%, and 25% on treatment with all IAA, IBA 1.0, and all NAA, respectively, as compared with the control. The difference in glucobrassicin content was very close among the treatments; the highest value was obtained from the control and IBA 0.05 treatments, and the values were equivalent for the rest of the treatments. The accumulation of 4-methoxyglucobrassicin was the highest, irrespective of auxin treatment. The range of accumulation levels of 4-methoxyglucobrassicin affected by the concentration of auxins was 3.96 to 6.66 μmol/g dry wt, with the highest level being obtained with the control treatment and the lowest level being obtained with the IAA 0.5 treatment. Glucoiberin accumulation in the hairy roots of rocket salad was decreased by 40.54%, 31.98 %, and 27.33% on treatment with IAA 0.5, NAA 0.5, and IBA 1.0, respectively, compared with the control. The second highest accumulated glucosinolate was glucosativin, irrespective of auxin treatment. The highest level was detected in the control treatment and the lowest level was detected in the NAA 1.0 treatment. Glucoiberin accumulation in the hairy roots of rocket salad was decreased by 81.38%, 79.77%, and 78.16% on treatments with NAA 1.0, IAA 1.0, and IBA 1.0, respectively, compared with the control.

Secondary metabolite accumulation in any part of the mother plants or transformed plants is dependent upon the organs or parts of the plants [5]. Accumulation of any product can be affected by external treatments such as phytohormones and elicitors, as well as by environmental factors [47-50]. Auxins are known to play important roles in plant growth, root development, and variation in the accumulation of secondary metabolites. The improvement in hairy root culture observed in this study is consistent with earlier reports on enhanced growth following treatment with exogenous auxin in hairy root cultures of *Lippia dulcis* [48], *Lobelia inflata* [56], *Panax hybrid* [57], and *S. baicalensis* [27].

Auxin treatment did not induce an increase in the level of any glucosinolates in this study, with the exception of glucoerucin. Only glucoerucin accumulation in the hairy roots of rocket salad was increased by 69.57%, 43.48%, and 26.09% on treatments with NAA 1.0, IBA 1.0, and IAA 0.1, respectively. The accumulation of any secondary metabolites in response to any treatment, particularly for auxin, can vary depending upon the plant species. From several previous studies, it was revealed that glucosinolate accumulation was enhanced at 0.5 to 1.0 mg/l of auxins particularly in the hairy roots of broccoli [33, 51], in Chinese cabbage [32], and in kale [52]. In other studies, it was reported that auxins treatments boosted the accumulation of sorgoleone in the root hairs of sorghum [49, 50]. In this study, media composition and auxin enhanced the production of hairy root cultures of rocket salad, although auxin had little effect on the accumulation of glucosinolates. Nevertheless, the effects of auxins can differ across different plant species.

Table 2. Glucosinolate content in rocket salad hairy roots under various auxin concentrations (μmol/g dry wt)

Treatment	Control	IAA 0.1	IAA 0.5	IAA 1.0	IBA 0.1	IBA 0.5	IBA 1.0	NAA 0.1	NAA 0.5	NAA 1.0
Glucoiberin	0.12^{a}	0.07^{b}	0.07^{bc}	0.08^{ab}	0.08^{ab}	0.03^{c}	0.08^{ab}	0.10^{ab}	0.08^{ab}	0.09^{ab}
Glucoerucin	0.23^{cd}	0.29^{bc}	0.28^{bc}	0.20^{d}	0.24^{cd}	0.23^{cd}	0.33^{ab}	0.24^{cd}	0.33^{ab}	0.39^{a}
Glucoraphasatin	0.04^{a}	0.02^{ab}	0.02 ^{ab}	0.02^{b}	0.03 ^{ab}	0.03 ^{ab}	0.02 ^{ab}	0.03 ^{ab}	0.03 ^{ab}	0.03^{ab}
Glucobrassicin	0.03^{a}	0.02^{cd}	0.02 ^{cd}	0.02 ^{cd}	0.02 ^{cd}	0.03 ^{ab}	0.02d	0.02 ^{cd}	0.00^{e}	0.02^{bc}
4-Methoxy Glucobrassicin	6.66^{a}	4.99^{cd}	3.96^{e}	5.10 ^{cd}	5.42^{bcd}	5.62^{bc}	4.84^{cde}	5.01 ^{cd}	4.53^{de}	6.17 ^{ab}
Glucosativin	4.35^{a}	1.33^{def}	2.38^{b}	0.88^{f}	1.05^{ef}	1.57^{cde}	0.95^{f}	2.00^{bc}	1.93^{bcd}	0.81^{f}
Total	**11.43^{a}**	**6.72^{b}**	**6.72 ^{b}**	**6.30 ^{b}**	**6.83 ^{b}**	**7.51 ^{b}**	**6.24 ^{b}**	**7.40 ^{b}**	**6.90 ^{b}**	**7.51 ^{b}**

Note: The data are presented as mean ± SD with three replications in each individual sample. Mean values with different letters in the same rows were significantly different ($p < 0.05$, ANOVA, DMRT). IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, 1-naphthaleneacetic acid

4. Conclusions

The growth of rocket salad hairy roots was increased by treatment with SH media, and the auxin NAA at 1.0 concentration and MS medium induced greater accumulation of glucosinolates. No single auxin treatment enhanced the accumulation of any glucosinolate except for glucoerucin. Our findings indicate that hairy roots are a viable option for obtaining glucosinolate compounds from rocket salad, and that MS medium provides an alternative approach for mass production of hairy roots and glucosinolates in rocket salad, regardless of additional treatment. These findings support our current laboratory endeavors to enhance glucosinolate compound accumulation in hairy root cultures of rocket salad.

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