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Research Article

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Effect of culture media and auxin on growth and glucosinolate accumulation in the hairy root cultures of mustard (*Brassica juncea*)

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ABSTRACT

Brassica juncea is a vegatable that are rich in glucosinolate (GSL) content. The hairy root (HR) cultures system is one of the most useful tools for secondary metabolites (SM) biosynthesis under various growth conditions. In the past, GSLs were mostly used as biopesticides in agriculture, anti-nutritional factors in fodder, and flavors in condiments. However, in recent days, GLSs have received much attention in human health. To investigate the growth response and variation of GSLs accumulation, HRs of mustard were grown in different growth media and auxins. The HRs growth pattern varied largely under the treatments of growth media and auxin. The full-strength SH media responded greatly for achieving the highest dry weight (DW) followed by the ½ SH media and the lowest DW was obtained in full-strength MS media. In all the auxin treatments the HRs production was higher than that of the control. It was noted that at higher NAA and IBA concentrations HR production was increased than that at the lower concentrations. In addition, different growth mediums significantly influenced the GSLs accumulation in mustard HR. The results revealed that ½ B5 media showed the highest total GSLs content followed by B5 and ½ SH. Treatment of mustard HRs with auxins such as IAA and IBA negatively influenced the accumulation of GSLs except for 4-methoxyglucobrassicin. We, therefore, suggest that HRs are a viable option for improving the GSLs content from the HR culture of mustard and that SH and ½ B5 medium provides an alternative approach for mass production of HRs and GSLs in mustard, respectively.

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INTRODUCTION

Mustard (*Brassica juncea*) has been cultivated in Asia and Europe for thousands of years and it has been used as a source of edible oils, medicine, and spices (Lietzow, 2021). In addition, it is rich in pharmaceutical compounds, such as ascorbic acid, β-carotene, flavonoids GSLs, and phenolics (Ismail & Cheah, 2003; Guo *et al.*, 2005; Antonious *et al.*, 2009; Lin & Harnly, 2010; Lin *et al.*, 2011; Kim *et al.*, 2016).

Glucosinolates (GSLs) belong to a large group of plant SMs. It comprises sulfur and nitrogen in its structure, and they are derived from glucose and amino acids. These compounds have the potential ability to defend against plant pests and diseases (Halkier & Du, 1997). Consistent intake of Brassicaceae vegetables has huge benefits to human health due to the rich content of GSLs and flavonols which will reduce the risk of various types of cancers and with improved cardiovascular health (Marino *et al.*, 2021; Melim *et al.*, 2022).

Culture media generally consist of inorganic elements and organic compounds. The composition and types of media used for the HR culture of plants influence SMs production (George *et al.*, 2008; Saad & Elshahed, 2012). In previous studies, it was proclaimed that the selection of the suitable basal media for HR induction and growth for the accumulation of desirable products is mainly based on the ideal medium and plant species (Washida *et al.*, 1998; Dhakulkar *et al.*, 2005; Kumar *et al.*, 2006).

Plant hormones, mainly, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphtalic acetic acid (NAA) are mostly used to induce the growth and development of root in plant propagation (Woodward & Bartel, 2005). In contrast, high concentrations of these plant hormones inhibited root

elongation and increased adventitious root formation. For this reason, a suitable concentration of auxin is therefore essential (Devi *et al.*, 2021).

HR culture is an encouraging biotechnological tool for faster growth as well as mass production of SMs under controlled conditions (Gantait & Mukherjee, 2021; Hussain *et al.*, 2022). As with many of these advantages, nowadays HR culture has been largely explored in several plant species for the mass production of SMs used as food additives and by the pharmaceutical industries (Gutierrez-Valdes *et al.*, 2020; Roy, 2021).

Previously studies have been undertaken to compare the level of GSL accumulation in the HR culture in mustard (Cuong *et al.*, 2018). It is well reported that media and auxin played an important role in the accumulation of SMs. However, the media and auxin treatment for the accumulation of GSL in the HR culture of mustard has not yet been studied well. Therefore, in this study, we investigated the response of various culture media and concentrations of auxins on growth and GSL content in mustard HRs for determining the suitable culture conditions to enhance GSLs production.

MATERIALS AND METHODS

Plant Materials

Mustard seeds were collected from Asia Seeds Co., Ltd, Seoul, Korea. Seeds were sterilized by soaking in 70 % ethanol (v/v) for 30 s and then with 2% sodium hypochlorite for 10 min. Then the seeds were washed with sterilized water for five times and then placed on the Petri plate containing MS medium consisting of 30 g/L sugar and 0.8 % agar at pH 5.7. No additional hormones were used in the media for germination.

Hairy Roots Induction

Ten-day-old young leaves of mustard were cut into small pieces and immersed in a liquid ½ SH medium containing *Agrobacterium rhizogenes* (strain R1000) for a period of 20 min. The sterilized paper was used to clean the seedlings and then incubated for 2 days under dark conditions at 25 °C on an antibiotic-free MS medium (Murashige & Skoog, 1962). After 2 days, the infected leaves were washed with sterilized water and then dried using sterilized paper. After that, the infected leaves were moved to ½ MS solid medium containing 250 mg/L cefotaxime and incubated under dark conditions for 2 to 3 weeks. After incubation, the HRs emerged from the wounded parts, were detached from the leaves, and then cultured under the same conditions.

Effect of Culture Medium and Auxins

In this study, the liquid MS, SH (Schenk & Hildebrandt, 1972), and B5 (Gamborg *et al.*, 1968) media were applied both in half and full strength for the HR cultures. Two grams of fresh HRs were inoculated in the flask containing 30 mL of each liquid culture medium and allowed to grow for 4 weeks. Then the samples were collected and then measured the fresh weight. After measuring the samples of the different media and the samples were frozen by using liquid nitrogen and then stored at -80 °C for 72 h. Then the samples were lyophilized and then ground into fine powders by using a pestle and mortar for further analysis. For GSL analysis, all the samples were done in triplicate.

The auxins (IAA, IBA, and NAA) with different concentrations of 0.1, 0.5, and 1 mg/L were added with ½ MS media to determine whether auxin type and concentration showed any significant role in the production of HRs and analysis of GSL content. Two grams of fresh HRs were inoculated in the flask containing 30 mL of different types and concentrations of auxins and allowed to grow for 4 weeks. Then the freeze-dried samples were ground into fine powders by using a pestle and mortar for further analysis. For GSL analysis, all the samples were done in triplicate.

Ds-GSLs Extraction of and HPLC Analysis

The extraction procedure for GSLs was done following the procedure of the (Norm, 1992) GSLs were extracted by using 70 % MeOH (v/v) for 5 min at 70 °C. Then the extracted samples were centrifuged at 12,000 rpm for 10 min at 4 °C. The collected supernatant was kept in a mini-column packed with DEAE-SephadexA 25 (40 mg dry weight (DW)) and then desulfated by adding 75 µL aryl sulfatase solution (23 mg/mL). Then 1.5 mL ultrapure water was added to fractionate the desulfo-GSLs samples. The desulfated extracts were analyzed by using an Agilent Technologies 1200 series HPLC system (Palo Alto, CA, USA) coupled 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, oven temperature, flow rate, and mobile phase were similar to the protocol described by (Sathasivam *et al.*, 2021). Each desulfo-GSLs were identified by using the retention times and HPLC peak areas with a desulfo-sinigrin external standard. Analysis was done in triplicate.

Statistical Analysis

All the analyses were done in triplicates. All the analyses were done by using two-way ANOVA, SAS Software (version 9.2; SAS Institute Inc., Cary, NC, USA). In order to determine the significance of the means, Duncan's multiple-range test was performed at $P \leq 0.05$.

RESULT AND DISCUSSION

Induction of HR Cultures in Mustard Grown With Different Media

Different growth media were used to examine the production of the HR culture of mustard. From the treatments, it is revealed that vast differences were observed in the production of HRs. Among the treated growth media, full-strength SH media achieved the highest (272 DW mg/flask) HRs growth followed by the ½ strength SH media (256 DW mg/flask), whereas the lowest (212 DW mg/flask) were recorded from full-strength MS media (Figure 1). Higher amounts of HRs growth were recorded in the

SH media which was 1.28-, 1.14-, 1.08-, 1.08-, 1.06- times higher than that in the MS, ½ B5, MS, B5, and ½ SH, respectively.

The accumulation of SM is highly influenced by several factors like medium salt concentration, the type and quantity of carbohydrates, phosphate, and nitrate, and the growth regulator levels (Washida *et al.*, 1998; Dhakulkar *et al.*, 2005; Kumar *et al.*, 2006; George *et al.*, 2008; Kim *et al.*, 2012; Saad & Elshahed, 2012). It has been reported that different formulations of media such as B5, Linsmaier and Skoog (LS), MS, and SH, have been used for different plant cultures (Murthy *et al.*, 2014). Here in this study, significant variation was denoted in HR cultures of mustard due to the application of different media. From the results of this study, it was found that full-strength SH media responded greatly for achieving the highest DW followed by the ½ SH media, and the lowest DW was recorded from full-strength MS media. From earlier studies, it has been proved that different growth media significantly influenced the production of HR cultures. Our results are consistent with these previous study report that in several plants such as watercress (Park *et al.*, 2019), Chinese skullcap (Kim *et al.*, 2012), and rocket salad (Park *et al.*, 2021), SH medium was the most suitable medium for the growth of HR culture. In contrast, other crops such as hybrid ginseng (Washida *et al.*, 1998), gamhar (Dhakulkar *et al.*, 2005), and potato (Kumar *et al.*, 2006) displayed the highest HRs growth in B5 and MS medium, whereas these media positively affected the growth of HR cultures in ginseng (Sivakumar *et al.*, 2005), cell suspension cultures of *Gymnema sylvestre* (Nagella *et al.*, 2011) and the HRs of broccoli (Kim *et al.*, 2013a). From these results, it is shown that selecting a suitable medium for HRs growth might be plant specific.

Induction of HR by Using Different Types and Various Concentrations of Auxins

To investigate the effects of auxins on the growth of HRs, different types and various concentrations of auxins were analyzed. A distinct growth variation of mustard HR culture was observed among the different types of auxin treatments. All the auxin treatments enhanced the production of HRs when compared with the production in the control treatment. Here in this study, it was noted that all the auxin-treated HRs showed better growth when compared to the control. The maximum was recorded in the treatment of NAA 1.0 (256.0 DW mg/flask) followed by NAA 0.5 (248.67 DW mg/flask), IBA 0.5 (243.33 DW mg/flask), IBA 1.0 (237.33 DW mg/flask) and the lowest was recorded in the control (192.0 DW mg/flask) (Figure 2). As compared to the control, the DW of mustard HRs was 1.33-, 1.30-, 1.27-, 1.24-, 1.22-, 1.16-, 1.16-, 1.14-, and 1.08- times higher than that in the media supplemented with NAA 1.0, NAA 0.5, IBA 0.5, IBA 1.0, IAA 0.1, IAA 1.0, NAA 0.1, IBA 0.1, and IAA 0.5, respectively,

To enhance the growth of HR cultures, plant cells, and exogenous tissue growth regulators have been extensively used (Murthy *et al.*, 2014). Auxins (particularly IAA, IBA, and NAA) have been playing significant roles among the various growth enhancers, in root development and promoting HR induction (Cheruvathur & Thomas, 2014). It is observable that biomass and metabolite accumulation in media supplemented with different types and concentrations of auxins differed based on plant cell cultures (Mantell & Smith, 1983). In this study, the highest fresh weight of mustard HR was achieved in the medium supplemented with NAA 1.0 followed by NAA 0.5, and the lowest DW was recorded from the control. Our findings coincide with the other study's results that governed the effects of auxins on higher biomass production in a wide range of plant species, such as broccoli (Kim *et al.*, 2013b), kale (Lee *et al.*, 2016), Indian mulberry (Sauerwein *et al.*, 1991), rocket salad (Bennett *et al.*, 2006), sorghum (Uddin *et al.*, 2010; Uddin *et al.*, 2011), and tobacco (Sahai & Shuler, 1984). From this study's results, it is proved that the auxins enhance the HRs growth in most of the plant species.

GSLs in the HR Cultures Of Mustard in Response to Growth Media

HPLC analysis of the HR cultures of mustard showed that four GSLs (4-methoxyglucobrassicin, glucobrassicin, gluconasturtiin,

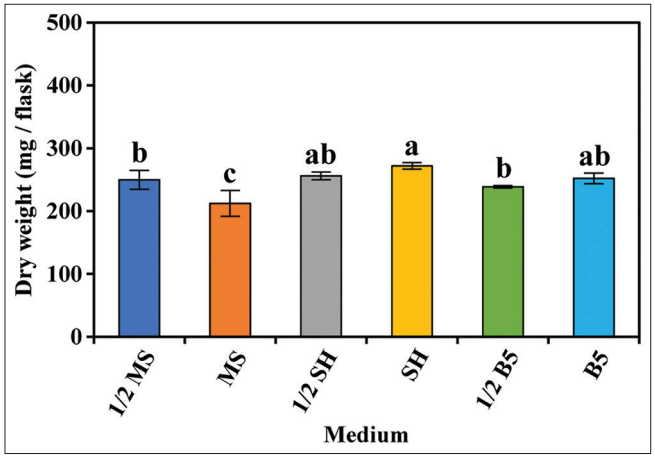

Figure 1: Effects of different media on the DW of mustard HR cultures. MS= Murashige and Skoog, SH=Schenk and Hildebrandt, B5= Gamborg

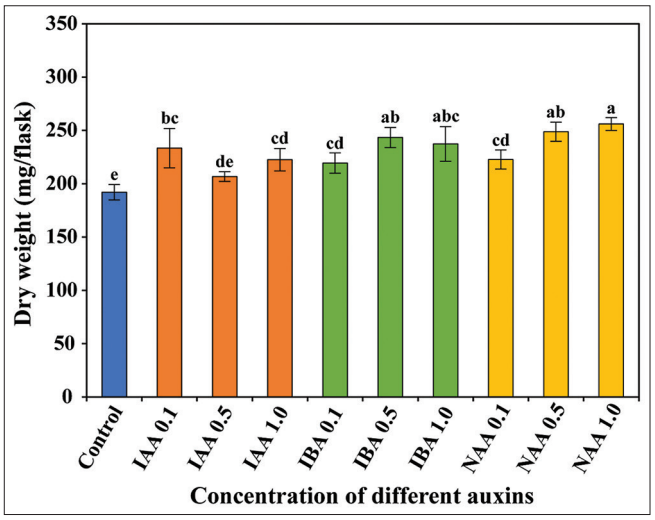

Figure 2: Effects of exogenous auxins on the fresh weight of mustard HR cultures. IAA= indole-3-acetic acid, IBA= indole-3-butyric acid, and NAA= 1-naphthaleneacetic acid

and neoglucobrassicin) and one unknown compound were detected at different levels in response to different growth media (Table 1). Growth media significantly influenced the accumulation of GSLs. From the results of this study, it is revealed that ½ B5 media responded well for the highest total accumulation of detected GSLs (16.37 µmol/g DW) in the HR of mustard which was followed by B5 (15.17 µmol/g DW) and ½ strength SH (14.09 µmol/g DW) whereas the lowest total accumulation of GSLs (10.79 µmol/g DW) was found in the ½ strength MS media. As compared to the lowest total GSLs content (½ MS media), the total GSL content, which was 1.57-, 1.50-, 1.27-, 1.25-, and 1.21- times higher than that in the medium supplemented with ½ B5, B5, ½ SH, MS, and SH, respectively, compared with the lowest accumulated GSLs (from ½ MS medium). The trend of GSLs accumulation due to different growth media detected here in this study in descending order was as follows; glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin, and gluconasturtiin. A higher level of glucobrassicin content was found in the treatment of B5 media exhibiting 2.10-, 1.67-, 1.35-, 1.32-, 1.0- times higher than that in the ½ MS, ½ SH, MS, SH, and ½ B5, respectively. The neoglucobrassicin content was highest in the ½ B5 media, which was 1.54-, 1.37-, 1.28-, 1.12-, and 1.09- times higher than that in the medium supplemented with ½ MS, MS, SH, ½ SH, and B5, respectively. A higher level of 4-methoxyglucobrassicin content was found in ½ B5, which was 1.34-, 1.27-, 1.09-, 1.04-, and 1.02- times higher than that in the SH, ½ MS, MS, B5, and ½ SH, respectively. The SH medium exhibited a higher level of gluconasturtiin content, by 1.31-, 1.26-, 1.21-, 1.11-, and 1.01- times than the ½ MS, MS, B5, ½ B5, and ½ SH medium, respectively.

In plants, GSL content varies extensively in response to factors such as agronomic management, mineral nutrient availability, location, climatic conditions, and plant variety (Brown *et al.*, 2002; Kumar & Andy, 2012; Vallejo *et al.*, 2003). In this study, the highest total GSL levels were highest in the ½ B5 media, followed by B5, ½ SH, MS, SH, and ½ MS medium. Our results were consistent with the previous study that the half and full-strength B5 medium induced the highest accumulations of GSLs content in the HRs of broccoli (Kim *et al.*, 2013b). In addition, it has been reported that in HR cultures of kale, the B5 medium positively affected the total GSLs production (Lee *et al.*, 2016). In another study, it has been reported that ½ B5 medium enhanced the flavones production in HR cultures of *Scutellaria baicalensis* (Kim *et al.*, 2012). From these results, it is shown that B5 media have a significant impact on GSLs accumulation.

GSLs Accumulation in HR Cultures of Mustard in Response to Auxin Treatment

Different types of auxins (IAA, IBA, and NAA) with different concentrations were treated to investigate the levels of GSL accumulation in the HRs of mustard. The GSLs (4-methoxyglucobrassicin, glucobrassicin, gluconasturtiin, and neoglucobrassicin) and one unknown compound were detected at different levels in response to different growth media (Table 2). Treatment of auxin negatively affected the GSLs accumulation except for 4-methoxyglucobrassicin. The level of 4-methoxyglucobrassicin accumulation was enhanced in both the IAA and IBA treatment when compared to control treatments, whereas it was decreased in the NAA treatment. The 4-methoxyglucobrassicin accumulation in IAA-treated HR culture ranges from 4.24-4.65 µmol/g DW, whereas the IBA treatment ranges from 4.27 to 4.42 µmol/g DW. Among these auxin concentrations, the highest level was found in the IAA 0.1 treatment. The 4-methoxyglucobrassicin level in the IAA 0.1, IAA 1.0, IBA 0.1, IBA 1.0, IBA 0.5, and IAA 0.5 treated HRs were increased by 1.21-, 1.11-, 1.19-, 1.15-, 1.11-, 1.12- times higher than that in the control, respectively. The highest total GSLs were achieved in the IAA 1.0 treatment (10.82 µmol/g DW), whereas in the control it showed decreased accumulation (10.79 µmol/g DW). Except for the IAA 1.0 treatment, the accumulation of total GSLs in all other auxins treatments was decreased. Among the auxin treatments, the NAA showed a negative response when compared to other auxin treatments. In detail, when compared to the control, the total GSLs level was decreased in all the NAA auxin treatments. In addition, the range of glucobrassicin accumulation as influenced by different concentrations of auxins was 1.45 to 2.54 µmol/g DW, where the highest amount was obtained in the control and the lowest was obtained with NAA 0.1 treatment. Similarly, the level of gluconasturtiin accumulation was decreased in all the auxins exposed HRs, whereas the highest accumulation was achieved in the control, and the lowest value was observed with IAA 0.1 treatment. Like other GSL, neoglucobrassicin content also decreased in all the auxins treatments. The variation of neoglucobrassicin accumulation was closer to that of the other auxins exposure. The neoglucobrassicin level after exposure to different concentrations of auxins ranged from 1.25 to 1.97 µmol/g DW. The highest level was obtained in the control treatment, whereas the lowest level was achieved in the IAA 0.1 treatment.

Accumulation of SM in any part of the mother plants or transformed plants varied on the organs or parts of the plants

Table 1: GSL content in mustard HRs in various media

GSL (µmol/g DW)	½ MS	MS	½ SH	SH	½ B5	B5
4-Methoxy glucobrassicin	3.83 ± 0.08^{c}	4.47 ± 0.22^{b}	4.76 ± 0.09^{a}	3.63 ± 0.04^{c}	4.85 ± 0.18^{a}	4.69 ± 0.15^{ab}
Glucobrassicin	2.54 ± 0.03^{d}	3.94 ± 0.18^{b}	3.20 ± 0.06^{c}	4.06 ± 0.12^{b}	5.32 ± 0.05^{a}	5.34 ± 0.33^{a}
Gluconasturtiin	0.93 ± 0.16^{b}	0.96 ± 0.04^{b}	1.20 ± 0.02^{a}	1.21 ± 0.18^{a}	1.10 ± 0.04^{ab}	1.01 ± 0.03^{b}
Neoglucobrassicin	1.97 ± 0.18^{d}	2.15 ± 0.03^{cd}	2.52 ± 0.07^{b}	2.2 ± 0.08^{c}	3.03 ± 0.11^{a}	2.7 ± 0.07^{b}
Unknown	1.52 ± 0.16^{c}	1.14 ± 0.07^{d}	2.41 ± 0.11^{a}	0.81 ± 0.02^{e}	2.07 ± 0.13^{b}	1.44 ± 0.03^{c}
Total	10.79 ± 0.61^{e}	12.66 ± 0.54^{d}	14.09 ± 0.34^{c}	11.92 ± 0.43^{d}	16.37 ± 0.50^{a}	15.17 ± 0.62^{b}

B5- Gamborg's B-5 medium; ½ B5 - half strength of B5; MS - Murashige and Skoog medium; ½ MS-half strength of MS; SH -Schenk and Hildebrandt medium; ½ SH - half strength of SH

Table 2: GSL content in mustard HRs under various auxin concentrations (µmol/g DW)

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
Glucobrassicin	2.54 ± 0.03^{a}	1.77 ± 0.03^{c}	2.03 ± 0.07^{bc}	2.26 ± 0.02^{b}	1.80 ± 0.38^{c}	1.78 ± 0.14^{c}	1.81 ± 0.04^{c}	1.45 ± 0.11^{d}	1.53 ± 0.12^{d}	1.49 ± 0.02^{d}
4-Methoxy Glucobrassicin	3.83 ± 0.08^{c}	4.65 ± 0.15^{a}	4.24 ± 0.04^{b}	4.57 ± 0.07^{ab}	4.42 ± 0.09^{ab}	4.27 ± 0.30^{b}	4.30 ± 0.20^{b}	3.06 ± 0.26^{e}	3.78 ± 0.23^{c}	3.38 ± 0.07^{d}
Gluconasturtiin	0.93 ± 0.16^{a}	0.48 ± 0.05^{c}	0.58 ± 0.06^{bc}	0.89 ± 0.09^{a}	0.85 ± 0.12^{a}	0.50 ± 0.15^{c}	0.78 ± 0.08^{a}	0.75 ± 0.15^{ab}	0.80 ± 0.10^{a}	0.54 ± 0.07^{c}
Neoglucobrassicin	1.97 ± 0.18^{a}	1.25 ± 0.03^{e}	1.56 ± 0.01^{cd}	1.84 ± 0.03^{ab}	1.64 ± 0.20^{bcd}	1.40 ± 0.17^{de}	1.75 ± 0.09^{abc}	1.53 ± 0.21^{cd}	1.61 ± 0.16^{bcd}	1.52 ± 0.12^{cd}
Unknown	1.52 ± 0.16^{a}	1.26 ± 0.01^{b}	1.30 ± 0.03^{b}	1.27 ± 0.05^{b}	1.54 ± 0.20^{a}	1.24 ± 0.09^{b}	1.70 ± 0.11^{a}	0.98 ± 0.10^{c}	1.21 ± 0.08^{b}	1.19 ± 0.06^{b}
Total	10.79 ± 0.61^{a}	9.41 ± 0.27^{bc}	9.72 ± 0.21^{abc}	10.82 ± 0.27^{a}	10.25 ± 0.98^{ab}	9.18 ± 0.86^{bcd}	10.34 ± 0.52^{ab}	7.77 ± 0.83^{e}	8.94 ± 0.70^{cd}	8.12 ± 0.34^{de}

Note: IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; NAA - 1-naphthaleneacetic acid

(Bennett *et al.*, 2006). Several studies have reported that the accumulation of any product might be increased or decreased by external treatments such as elicitors and phytohormones, as well as by environmental factors (Sahai & Shuler, 1984; Sauerwein *et al.*, 1991; Uddin *et al.*, 2010; Uddin *et al.*, 2011). In this study, the improvement of HR growth by auxin was observed which was in agreement with the earlier studies' reports that the application of exogenous auxin enhanced the HR growth of *Lippia dulcis* (Sauerwein *et al.*, 1991), *Panax hybrid* (Washida *et al.*, 2004), *Lobelia inflata* (Bálványos *et al.*, 2001), *S. baicalensis* (Kim *et al.*, 2012), and *Eruca sativa* (Park *et al.*, 2021).

Treatment of auxin negatively affected the accumulation of GSLs except for 4-methoxyglucobrassicin in the HR of mustard treated with IAA and IBA. The 4-methoxyglucobrassicin accumulation was enhanced in both IAA and IBA treatment when compared to the control. The 4-methoxyglucobrassicin level is influenced by different concentrations of IAA and IBA which range from 3.83 to 4.65 µmol/g DW, in which the highest level was found in the IAA 0.1 treatment. Similar findings were reported by (Park *et al.*, 2021) that auxin treatments did not increase the GSL accumulation in the HR of rocket salad. Auxins are known to play vital roles in plant growth, root development, and variation in the accumulation of SMs. It is very well known that auxin has a significant effect on any kind of SMs accumulation, but the trend of accumulation is not the same for all plant species. Several previous studies reported that GSL accumulation enhanced by the auxin's treatment ranges from 0.5 to 1.0 mg/L, particularly in the HRs of broccoli (Kim *et al.*, 2013a, 2013b), in Chinese cabbage (Park *et al.*, 2015), and kale (Lee *et al.*, 2016). In other studies, it was revealed that auxins treatments highly influenced for higher accumulation of sorgoleone in the root hairs of sorghum (Uddin *et al.*, 2010; Uddin *et al.*, 2011). In this study, media composition and auxin induce the HR cultures of mustard, although auxin did not respond well to the accumulation of GSLs. However, the effects of auxins might be different among the plant species.

CONCLUSIONS

The growth of mustard HRs were significantly increased by the treatment of SH media, and at the higher concentration of auxins (IBA and NAA). Growth media significantly influenced the GSLs accumulation especially ½ strength B5 media responded well for the highest total GSLs accumulation. Auxin negatively affected the accumulation of GSLs except for 4-methoxyglucobrassicin for the treatment of IAA and IBA in the HR of mustard. Our findings showed that HRs are a feasible choice for obtaining GSL compounds from the HR culture of mustard and that SH and ½ strength B5 medium provide an alternative approach for mass production of HRs and GSLs in mustard, respectively. These findings support our current laboratory deeds to enhance GSL compound accumulation in HR cultures of any plant species.

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