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Biochemical attributes of Indian mustard (*Brassica juncea*) and rapeseed (*B. napus*) as influenced by salicylic acid and benzothiadiazole

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Abstract: A field experiment was conducted during 2011-12 at Punjab Agricultural University, Ludhiana, India to assess the effect of foliar application of elicitors *viz.,* salicylic acid (SA) and benzothiadiazole (BTH) on biochemical constituents of *Brassica* seeds. Seeds of *Brassica juncea* and *B. napus* were sown in the field during *rabi* season. Four different treatments of elicitors along with a fungicide and control were given to 10 week old plants up to four consecutive weeks. After harvesting, the seeds were analyzed for oil, total soluble protein and glucosinolate content. Results indicated that the elicitor treatments increased the oil, total soluble protein and glucosinolate content in seeds of both the *Brassica* species. In *B. juncea,* BTH (7 ppm) + SA (17 ppm) showed 12.5% higher oil content than control. In *B. napus*, the combinations of elicitors *viz.,* BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) exhibited 15% increase in oil content compared to control. Treatment containing BTH (3 ppm) + SA (33 ppm) showed 18.72 and 15.86% higher total soluble protein content in *B. juncea* and *B. napus* seeds, respectively compared to control. In *B. juncea*, BTH (3 ppm) + SA (33 ppm) exhibited 7.13% higher glucosinolate content compared to control. In *B. napus*, BTH (7 ppm) + SA (17 ppm) showed 11.23% higher glucosinolate content than control. In conclusion, the application of elicitors, i.e., SA and BTH could be a useful tool for improving the nutritive value of *B. juncea* and *B. napus seeds*.

Keywords: Benzothiadiazole, *Brassica*, Glucosinolate, Oil, Total soluble protein, Salicylic acid

INTRODUCTION

Brassica species occupy a unique position in world agriculture as a source of oilseed, vegetable, fodder, green manure and condiment. The oil content of the seed varies from 30-45% depending on the species, the variety and climatic conditions under which it is grown (Mekki, 2013). *Brassica juncea* (Indian mustard or raya) is a major crop of oilseed *Brassica*. It is a good source of protein (28-36%), and phenolic antioxidants like sinapic acid and sinapine. *Brassica napus* (rapeseed) is also an important crop of *Brassica* species. Its oil contains a low content of saturated fatty acids (5-7%) and a high content of polyunsaturated fatty acids with about 7-10% α-linolenic and 17-21% linoleic acids. It is therefore, considered as very healthy edible oil (Baux *et al.*, 2008). After oil extraction, the remaining meal contains different nutritional and anti-nutritional compounds. Oil-free rapeseed meal contains 38-40% crude protein that displays a well-balanced amino acid composition with high levels of sulfur containing amino acids (Nesi *et al.*, 2008). Glucosinolates are the most important anti-nutritional compounds found in rapeseed-mustard. The glucosinolates are nitrogen and sulphur containing natural plant products that have become increasingly important as flavor precursors, cancer prevention agents and crop protectants (Graser *et al.*, 2000).

Rapeseed-mustard encounter a number of foliar diseases, among them Alternaria blight is the most devasting causing yield loss of 47% (Meena *et al.*, 2010). In addition to the direct losses in yield, the disease adversely affects seed quality by reducing seed size, seed discolouration and reduction in oil content (Prasad and Lallu, 2006). The disease also reduces germinability and protein content of seeds. Although, fungicides can provide resistance against the disease but these have poor compliances including health hazards. The most practical way to overcome this situation is through understanding the in built resistance in plants.

Elicitors have been involved in plant defense response and these were also reported to increase the production of nutraceutically important compounds (Al-Tawaha *et al.*, 2005). The elicitors trigger signalling cascades that activate several defense responses, including the synthesis of glucosinolates (Brader *et al.*, 2001;

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Mikkelsen *et al*., 2003). Moreover, the use of elicitors lacks environmental and toxicological side-effects. Salicylic acid (SA) is an important signaling molecule in plants and induces plant tolerance to various biotic and abiotic stresses (Horvath *et al*., 2007). Benzothiadiazole (BTH) [S-methylbenzo-1,2,3-thiadiazole-7 -carbothiate] is a chemical analogue of SA and has successfully been used to induce resistance to a wide range of diseases in field crops (Karthikeyan *et al*., 2009; Abdel-Monaim *et al*., 2011).

Considering the importance of oilseeds, the objective of this study was to study the effect of foliar spray of salicylic acid and benzothiadiazole on seed oil, total soluble protein and glucosinolate content in *B. juncea* and *B. napus* cultivars.

MATERIALS AND METHODS

The seeds of Indian mustard (*Brassica juncea*) cv. 'PBR-91' and rapeseed (*B. napus*) cv. 'GSC-6' were procured from the Oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, India. A field experiment was conducted in *rabi* (winter) season 2011-12 at the Experimental Farm, PAU, Ludhiana (30°54'N latitude and 75°48'E longitude). The seeds were sown in field at plant spacing of 30 × 10 cm in case of *B. juncea* and 45 × 10 cm in case of *B. napus* in plot size of 3 × 4 m^2 for each treatment. The treatments comprised of benzothiadiazole (BTH) @ 10 ppm, salicylic acid (SA) @ 50 ppm, BTH (3 ppm) + SA (33 ppm), BTH (7 ppm) + SA (17 ppm), copper oxychloride 50% WP @ 0.25% (fungicide) and a control (water-spray). The treatments were replicated three times for both the cultivars. The first foliar spray of elicitors/fungicide was given to 10 week old plants no sooner the first symptom of blight appeared in the field. A total of four foliar sprays of elicitors and three sprays of fungicide (as recommended by agronomic practices) were given on consecutive weeks. After harvesting, seed samples from both the species were oven dried at 50-60°C and used for the analysis of different biochemical parameters.

The extraction of oil was done by the method of Folch *et al.* (1957). A suitable aliquot of extract was evaporated to dryness to determine the oil content by weighing. For the estimation of total soluble protein, the defatted seed sample (200 mg) was extracted with 0.1M phosphate buffer (pH 7.5) followed by centrifugation at 8000 rpm for 20 min. The total soluble protein content in the supernatant was determined by the method of Lowry *et al.* (1951). Glucosinolates were extracted and analyzed according to the method of Tholen *et al.* (1989). Defatted seed sample (400 mg) was homogenized with 3 ml hot distilled water followed by the addition of 0.5M barium sulphate (0.5 ml) and 0.5M lead acetate (0.5 ml). After centrifugation at 8000 rpm for 20 min the supernatant was made up to 6 ml with distilled water. Aliquot (1 ml) of the supernatant was applied to DEAE-Sephadex A-25 column. The intact glucosinolates were eluted with 0.3 M potassium sulphate and their absorbance was read at 505 nm. The glucosinolate concentration was expressed as µmol/g defatted meal of *Brassica* seeds. The data was subjected to analysis of variance (ANOVA) using SPSS (version 16). Tukey's test was used to test the significance of difference between the treatment means.

RESULTS AND DISCUSSION

In *B. juncea*, oil content ranged from 31.77-35.74%. Treatment containing BTH (7 ppm) + SA (17 ppm) showed 12.5% higher oil content compared to control. In *B. napus*, oil content ranged from 31.70-36.62%. Maximum oil content was exhibited by the combinations of elicitors *viz*., BTH (7 ppm) + SA (17 ppm) and BTH (3 ppm) + SA (33 ppm). Both the treatments showed 15% higher oil content compared to control (Table 1). The observations for oil content are in agreement with Fatma Abd-El (2007) who reported increased oil content in SA (10^{-4} M and 10^{-3} M) treated basil and marjoram leaves compared to non treated control. They suggested that the increment in oil content might be due to increase in vegetative growth and nutrient uptake.

In *B. juncea*, total solule protein content varied from 24.31-28.86%. Treatment containing BTH (3 ppm) + SA (33 ppm) exhibited 18.72% higher protein content than control. In *B. napus*, total soluble protein content ranged from 24.33-28.19%. BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) showed 15.8% higher total soluble protein content than control (Table 2). Al-Tawaha and Ababneh (2012) observed the effect of foliar application of different concentrations of yeast extract (1, 2, 3 and 4 mg/ml) on growth and chemical composition of soybean. They found that the yeast extract at concentrations 1 and 3 mg/g, increased the protein content in soybean seeds.

Glucosinolates are one of the most important health-promoting compounds in *Brassica* species for the remarkable anticarcinogenic activity of their hydrolysis products, isothiocyanates. In *B. juncea*, glucosinolate content ranged from 91.51-98.04 µmol/g. Maximum glucosinolate content was exhibited by BTH (3 ppm) + SA (33 ppm), which showed 7.13% higher glucosinolate content compared to control. In *B. napus*, glucosinolate content varied from 41.93-46.64 µmol/g. BTH (7 ppm) + SA (17 ppm) exhibited 11.23% higher glucosinolate content compared to control (Table 3). Similar observations have been reported by other researchers. Doughty *et al.* (1995) reported 20-fold increase in concentration of total glucosinolate in *B. napus* leaves after methyl jasmonate spray. Van Dam and Oomen (2008) reported that jasmonic acid (500 µg) treated *B. oleracea* resulted in increased glucosinolate content. Perez-Balibrea *et al.* (2011) observed significant increase in glucosinolate content in broccoli sprouts

Table 1. Effect of foliar spray of elicitors on oil content in seeds of *B. juncea* and *B. napus.*

Elicitor/fungicide sprays	Oil content (%)	
	B. juncea **(cv. PBR-91)**	***B. napus*** **(cv. GSC-6)**
BTH (10 ppm)	34.12 ± 0.76[bc]	35.37 ± 0.93[bc]
SA (50 ppm)	34.02 ± 0.50[bc]	35.70 ± 1.01[bc]
BTH (3 ppm) + SA (33 ppm)	35.30 ± 0.61[c]	36.53 ± 1.04[c]
BTH (7 ppm) + SA (17 ppm)	35.74 ± 0.87[c]	36.62 ± 0.82[c]
Copper oxychloride (0.25%)	32.17 ± 0.86[ab]	33.70 ± 0.96[ab]
Control (water-spray)	31.77 ± 0.91[a]	31.70 ± 0.79[a]

*Data represent mean ± SD of three replicates. Different letters indicate significant difference between treatments at p = 0.05, according to Tukey's test.

Table 2. Effect of foliar spray of elicitors on total soluble protein content in seeds of *B. juncea* and *B. napus.*

Elicitor/fungicide sprays	Total soluble protein content (%)	
	B. juncea **(cv. PBR-91)**	***B. napus*** **(cv. GSC-6)**
BTH (10 ppm)	27.73 ± 0.64[bc]	27.52 ± 0.53[bc]
SA (50 ppm)	26.98 ± 0.97[bc]	26.81 ± 0.73[bc]
BTH (3 ppm) + SA (33 ppm)	28.86 ± 0.67[c]	28.19 ± 0.35[c]
BTH (7 ppm) + SA (17 ppm)	28.19 ± 0.73[bc]	28.18 ± 0.65[c]
Copper oxychloride (0.25%)	25.97 ± 0.95[ab]	25.74 ± 0.89[ab]
Control (water-spray)	24.31 ± 1.25[a]	24.33 ± 1.13[a]

*Data represent mean ± SD of three replicates. Different letters indicate significant difference between treatments at p = 0.05, according to Tukey's test.

Table 3. Effect of foliar spray of elicitors on glucosinolate content in seeds of *B. juncea* and *B. napus.*

Elicitor/fungicide sprays	Glucosinolate content (μmol/g defatted meal)	
	B. juncea **(cv. PBR-91)**	***B. napus*** **(cv. GSC-6)**
BTH (10 ppm)	96.23 ± 0.68[bcd]	45.59 ± 0.41[bc]
SA (50 ppm)	94.90 ± 0.98[bc]	46.20 ± 0.72[bc]
BTH (3 ppm) + SA (33 ppm)	98.04 ± 0.93[d]	46.27 ± 1.02[bc]
BTH (7 ppm) + SA (17 ppm)	96.70 ± 1.13[cd]	46.64 ± 1.32[c]
Copper oxychloride (0.25%)	93.88 ± 0.78[ab]	43.30 ± 1.13[ab]
Control (water-spray)	91.51 ± 1.14[a]	41.93 ± 0.50[a]

*Data represent mean ± SD of three replicates. Different letters indicate significant difference between treatments at p = 0.05, according to Tukey's test.

treated with different concentrations of elicitors *viz.*, methionine, typtophan, chitosan, salicylic acid and methyl jasmonate. Wei *et al.* (2011) observed increased glucosinolate content in *Brassica* sprouts treated with 5% glucose.

The present study revealed that both SA and BTH increased oil, total soluble protein and glucosinolate content in seeds of *B. juncea* and *B. napus* cultivars. The combinations of SA and BTH were highly effective than applied alone. Fungicide also exhibited higher content of oil, total soluble protein and glucosinolate than control, but it was less than elicitor treatments.

Conclusion

Results here obtained indicated that foliar application of elicitors could significantly increase the oil, total soluble protein and glucosinolate content, revealing the use of elicitors as a feasible tool to obtain plant foods with enhanced nutritional quality.

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