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EFFECT OF CHROMIUM (VI) ON GROWTH AND LIPID COMPONENTS IN DEVELOPING SEEDS OF *BRASSICA JUNCEA*

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SUMMARY

The present investigaton was aimed at studying the effect of chromium (Cr) (vi) on plant growth and lipid profile in *Brassica juncea* (cv. RH-30). *Brassica juncea* plants when grown in sandy soil treated with different concentration of Cr (viz. 0.5, 1.0, 2.0, 4.0, 5.0, 7.5 and 10.0 ppm), there was delayed germination and flowering of plants. At 7.5 and 10.0 ppm concentrations, plants could not survive and died within a week. With the increasing concentration of Cr from 0.5 –5.0 ppm, there was continuous inhibition in plant height (4.2-24.6%), number of siliqua /plant (23.7-54.9%), number of seeds /siliqua (1.0-10.9%), seed yield /plant (9.8-62.3%) except at 0.5 ppm. The lowest concentration (0.5ppm) of Cr was found to be stimulatory. Oil yield and lipid profile (non-polar, polar, phospholipid and glycolipid) was inhibited at higher concentration of Cr. During early stages of seed development (15 days after flowering, DAF) there was no erucic acid content in seeds. At all the stages of seed development, with the increasing concentration of Cr (vi) application, oleic, linoleic and linolenic acid contents of lipid decreased, while, erucic content increased. Cr application caused the deterioration of oil quality i.e. increased in erucic acid contents with increasing concentration of Cr Application.

***Key words* : *Brassica juncea*, chromium (vi), fatty acid composition, lipid profile, plant growth, seed yield.**

INTRODUCTION

Application of wastewater for irrigation purposes has increased over the past years. This wastewater contains high amount of trace elements like lead (Pb), cadmium (Cd), nickel (Ni), mercury (Hg), uranium (U), copper (Cu), zinc (Zn), boron (B), cobalt (Co), chromium (Cr), arsenic (As), molybdenum (Mo), manganese (Mn), etc. and heavy metals. Many of these are non-essential and toxic to plants, animals and human beings (Kanwar and Sandha 2000). The use of sewage and industrial sludge for agriculture caused a significant reduction in the yield of *Brassica chinensis* compared to domestic sludge (Wong *et al.* 2001). Edible plants enriched with minerals such as Cr, Fe, Mn, Se and Zn have been tested to determine whether these plants could be used as a new source of mineral-dietary supplements to provide essential minerals in a more available form than current inorganic based mineral supplements (Elless *et al.* 2000). Chromium is essential for normal glucose metabolism in humans and animals (Hamid and Hossner 2000), but its contamination and recovery from soils is of environmental concern. Cr (vi) being toxic to plants impeded growth of *Helianthus annuus* and *Brassica juncea*. The content of free proline, permeability of cell membrane increased in the leaves of *B.chinensis* and chlorophyll a/b ratio gradually decreased in *B. chinensis* and cauliflower with increasing Cr (vi) concentration (Chatterjee and Chatterjee 2000, Ren *et al.* 2000). Trivalent chromium (Cr^{+3}) is essential for human and animal health, whereas

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hexavalent Cr (Cr O_4) is a potent carcinogen and extremely toxic to humans and animals. Various vegetable crops absorb and accumulate Cr^{+3} and Cr (vi) in roots and shoots. X-ray absorption spectroscopy (XAS) analysis indicated that Cr (vi) is converted to Cr^{+3} in roots by all plants (Zayed *et al.* 1998).

Brassica is an important source of edible oil and *Brassica* leaves and stem are used as vegetables, cooked or raw (Gunstone *et al.* 1994). Edible oil of *Brassica* differs from other vegetable oil due to presence of erucic acid (Ahuja *et al.* 1998). Low erucate and oleate rich varieties are recommended for edible purposes (Ackman 1983, Miquel and Browse 1994). Chromium (vi) caused a dramatic increase in erucic and linolenic acid content and a decrease in linoleic and palmitic acid content in the storage tissue of *Brassica campestris* L (Archana *et al.* 1999). It is therefore, of great interest to understand the effect of chromium on lipid biochemistry of oil crops.

MATERIALS AND METHODS

Brassica juncea cv. RH-30 plants were raised in earthenware pots (28x26x13 cm.) lined with polyethylene bags filled with 5 kg.of sandy soils in a naturally lit net house. The soil was treated with requisite amount of Cr (vi), viz. 0, 0.5, 1.0, 2.0, 4.0, 5.0, 7.5 and 10.0 ppm (ug g^{-1} soil) in the form of $K_2Cr_2O_7$. Basal doses of micro and macronutrients were applied as per recommended package of practices. After emergence of seedlings, the number of plants were thinned to two per pot and irrigated with equal quantities of tap water as and when required. Hoagland's nutrient solution was given weekly. Siliqua were collected at 15 days interval from anthesis (flowering) upto maturity. Immediately after harvest, plant height, siliqua length, number of seeds per siliqua, number of siliqua per plant and seed yield per plant were recorded. Dried seeds were used for the estimation of fat and fat profile.

Total lipid, polar and non-polar lipid fractions (Nichols 1954) were determined as described in AOAC (1970). Glycolipids were determined on the basis of galactose content multiplied by 4.55 (Joseph 1954, Trevelyan 1954). Phospholipids were determined by measuring lipid phosphorus and multiplying with a factor of 25 (Fiske and Subbarow 1925). For determing the fatty and composition, lipids were extracted (Folch *et al.* 1957) and methyl esters prepared (Luddy *et al.* 1968). Fatty acids were separated in Hewlett Packard gas chromatography (GLC) model No. 5730, equipped with flame ionization detector (FID), using diethylene glycol succinate (DEGS) column, maintaining at 250 ^{0}C (injector temperature) and 300 ^{0}C (detector temperature). Using authentic standards (from Sigma) the peaks were identified, individual peak area calculated and converted directly into relative fatty acid percentage.

RESULTS AND DISCUSSION

Visual observations on the effect of Cr on seed germination and plant growth indicated that seeds germinated at almost the same time upto 1.0 ppm of Cr. Subsequently, there was delayed germination as compared to that of control (0 ppm). At 7.5 and 10.0 ppm, of Cr, plants could not survive and died within a week. Plants exhibited poor and stunted growth as compared to that of control after 1.0 ppm Cr treatment. Higher doses of Cr induced interveinal chlorosis in young leaves, which turned to necrosis at later stages of growth. Plants treated with higher Cr concentration showed delayed flowering. Marked depression in growth and delayed emergence of inflorescence in maize at 0.25, 0.50 and 1.0mM Cr (vi) has been reported earlier by Sharma and Sharma (1993).

The stimulatory effect of 0.5 ppm of Cr on siliqua length, number of siliqua/plant, number of seeds /siliqua, seed yield /plant and total oil in seeds is shown in Table 1. However, with increasing Cr concentration, a progressive decrease in plant height, siliqua length, number of siliqua/plant, number of seeds/siliqua, seed yield /plant, 100 seed weight and total oil content was observed. Sharma and Sharma (1993) reported that excess supply of Cr (vi) had inhibitory effect on growth, biomass production and reproductive yield of maize. Seed yield/plant decreased by 62.3% at 5.0 ppm Cr (vi) treated plants. Chromium (iii) is essential for plants and normal glucose metabolism in animals (Hamid and Hossner 2000). At 0.5 ppm Cr (vi) concentration, seed yield and total oil in seeds increased by 18.0 and 1.87 % respectively, while at 5.0 ppm, oil content decreased by 20.4% in seeds. It proves that Cr (vi) is essential for plant metabolism in lower doses.

Table 1. Effect of Cr (vi) on plant height, number of siliqua, number of seeds per siliqua, siliqua length, seed yield and oil content at maturity in *Brassica juncea* L. (cv. RH-30)

Cr (ppm)	Plant height (cm)	Siliqua length (cm)	No. of siliqua per plant	Dry weight of siliqua per plant (g)	No. of seeds per siliqua	Seed yield per plant (g)	100-seed weight (g)	Total lipids (mg/g dw)
Control	132.90 ± 6.10	4.38 ± 0.21	37.20 ± 1.76	1.14 ± 0.08	9.30 ± 0.46	1.22 ± 0.03	0.59± 0.04	399.00 ± 3.81
0.5	127.30 ± 7.15 (- 4.2)	4.52 ±0.30 (+ 3.2)	45.80 ± 1.50 (+ 23.1)	1.34 ± 0.08 (+17.5)	9.38 ± 0.31 (+0.86)	1.44 ± 0.04 (+18.0)	0.52 ± 0.03 (-11.9)	406.50±4.16 (+ 1.87)
1.0	122.60 ± 6.85 (-7.7)	4.80 ± 0.29 (+ 9.6)	28.40 ± 2.54 (- 23.7)	1.15 ± 0.09 (+ 0.8)	9.21 ± 0.42 (-1.0)	1.10 ± 0.06 (- 9.8)	0.51 ± 0.02 (-13.6)	428.50 ± 7.83 (+7.4)
2.0	117.80± 5.30 (-11.4)	4.70 ± 0.36 (+ 7.3)	21.60 ± 2.78 (- 42.0)	0.74 ± 0.10 (-35.0)	8.99 ± 0.47 (-3.4)	0.73 ± 0.04 (-40.1)	0.45 ± 0.04 (-23.8)	361.00 ± 6.81 (- 9.6)
4.0	100.80 ± 5.89 (-24.1)	4.12 ± 0.38 (- 5.9)	18.00 ± 1.95 (-51.7)	0.55 ± 0.06 (-51.8)	8.36 ± 0.35 (-10.2)	0.62 ± 0.08 (-49.2)	0.40 ± 0.04 (-32.6)	338.00 ± 1.81 (-15.3)
5.0	100.20 ± 5.97 (-24.6)	4.08 ± 0.35 (- 6.9)	16.18 ± 1.36 (- 54.9)	0.53 ± 0.06 (-53.5)	8.29 ± 0.39 (-10.9)	0.46 ± 0.09 (-62.3)	0.38 ± 0.05 (-35.6)	318.00 ± 2.16 (-20.4)

Values in parentheses indicate per cent inhibition / stimulation w.r.t. control

With the advancement of seed development, total lipids and non- polar lipid fraction increased and polar lipid fraction decreased (Table 2). During early stages of seed development 15 days after flowering (DAF), polar lipids were the major lipid fraction while at later stages, non-polar lipids were the most abundant lipid fraction. Storage lipids were relatively more important at later stages of seed development and relative importance of membrane lipids was more obvious (Perry and Harwood 1993, Archana *et al*. 1999).

Data in Table 2 revealed that during the early stages of seed deveopment (15DAF) synthesis of lipids was slow but became faster as plant matured (60DAF). At 15DAF total lipid, non-polar and polar fraction of lipids decreased with increasing concentration of Cr except at 0.5 ppm, where amount of polar lipid (39.99 mg/g dry wt.) was maximum. At 30 DAF, total lipids in seeds decreased upto 2.0 ppm except at 0.5 ppm (152 mg/g dw), where as it increased in 4.0 ppm (190 mg/g dw) and 5.0 ppm (215 mg/g dw) treated plants as compared

Table 2. Effect of Cr (vi) on total lipids and lipid components (mg/g dry weight) in the developing seeds of *Brassica juncea* L. (cv. RH-30)

Stage of seed development of Cr (ppm)	Total lipids	Non polar lipids	Polar lipids	Phospho lipids	Glycolipids
15 DAF					
Control	72.00 ±1.98	30.07 ±1.89	38.28±2.71	23.52 ±1.34	14.98±1.28
0.5	68.57 ±2.31	26.81 ±2.07	39.99±1.75	24.08 ±1.76	15.76 ±1.34
1.0	40.00 ±1.08	11.03 ±0.43	27.50±1.09	17.12 ±0.82	10.08 ±1.08
2.0	26.60 ±1.25	11.95 ±0.38	13.30±0.74	7.08 ±0.46	6.19 ±0.53
30 DAF					
Control	146.00±5.62	111.48±7.76	32.52±1.96	19.86±1.12	12.16±1.26
0.5	152.00±3.78	117.61±3.79	32.98±1.84	20.27±2.11	11.97±0.98
1.0	130.00±9.05	97.67±4.86	31.24±1.92	19.43±0.98	11.03±1.11
2.0	126.00±4.67	94.93 ±6.97	30.61±1.17	19.46±1.23	10.01±0.99
4.0	190.00±3.99	151.78±5.39	37.00±2.71	22.19±1.75	13.83±1.01
5.0	215.00±8.14	174.78±7.09	38.53±1.83	21.71±0.82	15.81±0.99
45 DAF					
Control	340.00±11.1	311.68±17.53	26.32±1.43	16.23±1.31	9.87±0.68
0.5	348.00±12.7	318.81±14.37	27.12±2.02	17.08±1.21	9.83±0.79
1.0	354.00±20.3	326.69±13.29	25.69±1.76	16.13±1.09	9.03±0.81
2.0	351.00±14.9	325.92±16.76	23.81±1.92	14.97±0.75	8.74±0.69
4.0	349.00±18.3	319.71±9.91	26.83±0.95	15.52±0.53	10.93±0.91
5.0	348.00±20.7	316.86±18.71	27.14±1.08	16.83±0.68	10.23±1.00
60 DAF					
Control	386.00±16.3	359.82±19.7	24.04±1.22	14.86±1.04	8.93±0.69
0. 5	390.00±12.5	361.94±20.9	25.61±0.93	15.64±1.33	9.02±0.76
1.0	404.00±9.7	378.91±11.4	23.84±0.88	14.97±1.52	8.71±0.39
2.0	364.00±20.3	335.92±9.8	24.81±1.78	13.34±0.48	7.78±0.78
4.0	339.00±10.6	309.71±6.9	25.03±0.92	15.87±1.87	8.43±0.46
5.0	324.00±11.9	286.85±13.6	26.09±1.41	17.10±1.42	9.56±0.75

to controls. Similar trend was observed at 45 and 60 DAF. At 30 DAF, amount of non-polar lipids decreased from 117.61 (0.5 ppm Cr) to 94.93mg/g dw (2.0 ppm Cr). Contents of non-polar lipids, phospholipids and glycolipids (at 4.0 ppm) were 151.78, 22.19 and 13.83 mg/g dw respectively. Total lipid contents increased from 340 (control) to 354 mg/g dw (at 1.0 ppm) at 45 DAF and after that lipid content decreased. With the advancement of seed development, amount of non-polar lipid fraction increased while polar lipid fraction decreased. As the neutral lipids increased, proportion of polar or structural lipids in the lipid fraction decreased. This was mainly because of increase of storage lipids during this period (Norton and Harris 1975, Perry and Harwood 1993, Archana *et al*. 1999).

Effect of varying levels of Cr (vi) on fatty acid composition of total lipids in developing *Brassica juncea*.

Table 3. Effect of Cr (vi) on fatty acid composition (relative fatty acid percentage) in developing seeds of *Brassica juncea* L. (cv. RH-30)

Stage of seed development/ Cr (ppm)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic + Eicosenoic acid	Erucic acid	Total unsaturated fatty acids
15 DAF							
Control	8.40	20.06	27.83	23.04	20.67	-	71.54
0.5	20.06	16.10	24.36	24.01	15.47	-	63.84
1.0	36.46	13.20	19.70	25.30	5.33	-	50.33
2.0	42.63	11.19	14.83	26.77	4.56	-	46.16
30 DAF							
Control	7.73	1.81	22.71	22.75	20.32	24.65	90.43
0.5	8.28	2.27	25.87	20.85	20.34	22.43	89.49
1.0	12.57	2.94	27.28	20.27	21.37	15.53	84.45
2.0	7.93	1.62	14.21	15.01	16.21	45.02	90.45
4.0	6.57	1.30	13.73	14.95	15.53	47.91	93.39
5.0	5.20	1.21	12.14	11.49	13.71	56.24	91.46
45 DAF							
Control	2.43	0.76	11.05	16.96	20.33	48.46	96.80
0.5	2.97	0.70	10.97	18.87	19.82	46.66	96.32
1.0	3.46	0.66	10.58	18.97	19.36	46.42	95.33
2.0	2.53	0.52	10.45	17.80	18.52	50.18	96.95
4.0	3.10	0.37	10.20	16.23	14.68	55.33	96.44
5.0	3.58	0.22	9.48	15.00	13.69	58.02	96.19
60 DAF							
Control	2.41	0.81	10.53	17.30	19.57	49.37	96.77
0. 5	3.79	0.71	11.26	16.52	18.48	49.18	95.44
1.0	3.08	0.48	11.17	15.68	17.60	51.98	96.43
2.0	5.60	0.28	8.38	15.68	17.60	52.45	94.11
4.0	5.94	0.19	7.92	15.23	15.69	55.02	93.86
5.0	7.16	0.14	7.68	15.00	14.68	55.33	92.69

L (cv RH-30) seeds are shown in (Table 3). At 15 DAF, erucic acid could not be detected in young seeds. At this stage, young seeds contained approximately 21% linolenic acid + eicosenoic, 23% linoleic acid, 28% oleic acid, 20% stearic acid and 8% palmitic acid of total lipids in control. In 2.0 ppm Cr treated plants, palmitic acid increased approximately upto 43% and linoleic upto 27% while stearic, oleic and linolenic decreased to 11, 15 and 4% respectively.

At 30 DAF, up to 1.0 ppm Cr, palmitic, stearic and oleic acids in seeds increased from 7.7 to 12.5, 1.8 to 2.9 and 22.7 to 27.3 % respectively, while content of linoleic and erucic acid decreased from 22.8 to 20.2 and 24.7 to 15.5 % respectively in comparison to that of control. Beyond 1.0 ppm, palmitic, stearic, oleic, linoleic and linolenic + eicosenoic decreased, while erucic acid content increased dramatically. At this stage the fatty acid composition was typical of storage triglycerides (erucic acid appeared while other fatty acids declined). Upto 1.0 ppm Cr, the fatty acid composition of lipids resembled that of photosynthetic tissue.

At 45 DAF, in control seeds contents of linoleic, linolenic + eicosenoic and erucic acid contents were 17.0, 20.3 and 48.5 % respectively, while palmitic, stearic, and oleic were 2.4, 0.8 and 11.0% respectively. However, the content of erucic acid continued to rise until maturity, where it accounted for almost 50% of the total fatty lipids, while the proportion of other fatty acids remained constant. With increasing concentration of Cr, erucic acid in seeds increased drastically from 46.4 to 58.0 % with concomitant decrease in other fatty acids. Similar trend of these fatty acids was also observed at 60 DAF.An inverse relationship between linoleic and erucic acid in *Brassica campestris* was earlier observed (Ahuja *et al.* 1998, Archana *et al*. 1999). The changes in fatty acids composition are mainly due to differential rates of net synthesis of each of the fatty acids (Zuniga *et al.* 1994, Kim *et al.* 1996). These results suggest an improvement of oil quality due to decrease in erucic acid in seeds at 1 ppm Cr treatment (45 DAF) and at 0.5 ppm Cr treatment (60DAF) in comparison to that of control and deterioration in oil quality at higher Cr doses after 1.0 ppm. Erucate rich triglycerols could be used for industrial purposes (Murphy 1996). For nutritional purposes, seeds (0.5 to 1.0 ppm) should be harvested little bit earlier to maturity.

The above study indicated that Cr (vi) is essential for plant metabolism in lower doses (0.5-1.0 ppm). At 1.0 ppm, the lipid contents in matured seeds increased by 7.4 % at maturity and lipid quality was comparable with that of control.

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