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RESEARCH ARTICLE

Effect of Chromium studies on germination and Bio chemical content of Black gram (*Vigna mungo* (L.) Hepper.)

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Abstract

The effect of heavy metal- chromium studies on seed germination, growth, root length, lateral length, shoot length, fresh weight, dry weight and bio chemical content analyzed in five varieties of Black gram *viz.,* ADT-3 and T-9. Vamban variety VBN-3, VBN-4 and VBN-5. The heavy metal treatment and different concentration of 10, 25, 50100mg/l was prepared including control. The germination study was carried out in plastic plates lined with filter paper of five varieties of Black gram. They were watered with different concentration of treated water. The germination study was conducted, with tolerant variety (T-9) and susceptible variety (VBN-5) under different concentration of chromium treatment. The germination percentage, growth, and dry weight and biochemical content were analyzed in all the varieties. The maximum seed germination, morphological study and biochemical content in maximum tolerant variety (Tindivanam - 9) and susceptible variety (VBN-5) under different concentration of chromium treatment. The control seeds were also maximum effect in all the parameter and both varieties. Chromium induced in higher concentration treatment minimum effect in all the varieties. In optimum concentration maximum effect in all the variety, especially T-9 variety more effectively in all the parameters. The biochemical studies like chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content protein and reducing sugar were found to be more and more in untreated concentration, (control) the biochemical contents gradually decreased as the concentration increased.

1. Introduction

Black gram is the third important pulse crop in India. In a Black gram (*Vigna mungo* (L.) Hepper.) Popularly known as "urd bean" is one of the important grain legumes and is an excellent source of easily digestible good quality protein. It belongs to the family Leguminaceae under the genus *Vigna*. It is widely cultivated in Indian subcontinent and to a lesser extent in Thailand, Australia and other Asian and South Pacific countries (Poehlman, 1991). The major constrains in achieving higher yield of this crop lack of genetic variability are absence of suitable ideotypes for different cropping system, poor harvest index and susceptibility to diseases. Lack of suitable varieties and genotypes with adaptation to local condition is important among the factors that affect the production (Srivastava *et al*., 2011).

1.1. Production of Block gram

Pulses are the major source of dietary protein in a vegetarian diet in our country. Besides, being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furnishing sustainable agriculture (Kannaiyan, 1999). At present, globally 60 million tonnes of pulses are produced annually from 70 million hectares. Normally the area under pulses in the country is around 24.38 million hectares with a production of 14.52 million tonnes. The average

productivity of the country is about 600 Kg/ha against the average global productivity of 857 Kg/ha.

In Tamil Nadu, the total area under pulses is around 9.5 lakh ha with a production of 4.08 lakh tonnes. The average productivity of pulses in the state is around 430 Kg/ha which is far below the average productivity of the country as well as that of the global productivity. The area under Black gram in the state is around 4.46 lakh ha in the year 1999 with a production of 2.06 lakh tonnes which accounts for an average productivity of 461 Kg/ha (Dixit *et al.*, 2002). The increase in area and production is attributed to the development of high yielding and Yellow Mosaic Virus resistant varieties suitable for cultivation in rabi season in rice fallows. The average of black gram in the state is just above the national average productivity of 448 Kg/ha, however, it is lesser than that recorded in states like Bihar (694 Kg/ha), Maharashtra (631 Kg/ha), Gujarat (601 Kg/ha) and Andhra Pradesh (555 Kg/ha).

1.2. Economic Importance of Block gram

Black gram is a rich protein food. It contains about 26% protein, which is almost three times that of cereals. Black gram supplies a major share of protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. The biological value improves greatly, when wheat or rice is combined with Black gram because of the complementary relationship of the essential amino acids such as arginine, leucine, lysine, isoleucine, valine and phenylalanine etc. It is an annual pulse crop and native to central Asia. It is also extensively grown in West Indies, Japan, and other tropics/subtropical countries. Black gram seeds are highly nutritious containing higher amount of protein (24-26%) and are reported to be rich in vitamin A, B_1, B_3, besides nutritionally rich proteins, important mineral and vitamins. In addition, being an important source of human food and animal feed, it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Being a drought resistant crop, it is suitable for dry land farming and predominantly used as an intercrop with other crops (Tawfik *et al*., 2011).

2. Materials and Methods

The five varieties of Black gram seed (*Vigno mungo* (L.) Hepper.) Black gram was obtained from The National Pulses Research Centre, Vamban of Pudukottai district and Regional Rice Research Institute, Aduthurai of Thanjavur district in TamilNadu. Chromium stock solution was prepared by dissolving 141.4mg of Potassium dichromate ($K_2Cr_2O_7$) in 100ml of distilled water. This stock solution, different concentration of 10, 25, 50, and 100mg/l of chromium solution were prepared freshly at the time of experiments. In control (C) plain distilled water was using.

The seeds of five varieties of Black gram ADT-3, T-9, VBN-3, VBN-4, and VBN-5 of uniform size, color and weight were surface sterilized with 0.1% mercuric chloride $HgCl_2$ solution and washed 5-6 times with distilled water. Fifty seeds of Black gram were placed for each treatment and were placed equispacially in sterilized plastic plates, lined with filter paper soaked with different concentration of chromium dissolved water uniformly. Each treatment was replicated three times. The number of seeds eliminated in each treatment was counted on the 10^{th} day and the total germination percentage was calculated. The emergence of radical was taken as a criterion for germination. The seedlings from each treatment were randomly selected for the measurement of root length and shoot length. The tolerant and susceptible variety was selected based upon the above mentioned characters. The seedlings were separated into root and shoot system. They were kept in a hot air oven at 80°C for 24 hours. After that the dry weight were recorded.

The percentage of phytotoxicity of the effluent was calculated by the formulae suggested by Chou *et al.,* (1978). Vigour index of the seedlings were calculated by using the formulae proposed by Abdul-Baki and Anderson, (1973). The tolerance index of the seedlings can also be calculated by using the formulae proposed by Turner and Marshal, (1972). The biochemical 10^{th} day Black gram seedlings were separated in to root, stem and leaf and they were used for bio chemical analysis chlorophyll, (Arnon, 1949). Protein content were analyzed in (Lowry *et al.,* 1951) and sugar content were analyzed by (Nelson, 1944) using the following methods.

3. Results

3.1. Morphological studies

The germination study was conducted to find out the tolerant and susceptible variety under heavy metal of chromium irrigation. The five varieties of Black gram namely ADT-3, T-9, VBN-3, VBN-4 and VBN-5 were analyzed. The germination percentage was Black gram cultivar as affect by different concentration of heavy metal chromium. The germination percentages of all the Black gram cultivars are found to be maximum at control 90, 100, 96, 92, 80 for ADT-3, T-9, VBN-3, VBN-4 and VBN-5 respectively. The germination percentage of all the cultivars of Black gram decreased gradually with progressive increase in heavy metal chromium concentration. The minimum percentage of

germination was recorded at 100mgl^{-1} in all the cultivar was Black gram 40, 80, 50, 65, and 35 for ADT-3, T-9, VBN-3, VBN-4 and VBN-5 respectively. The root length of Black gram cultivars showed a decreased with increased in heavy metal chromium concentration. Among the cultivars studied theT-9 exhibited maximum root length at C (12.00) and minimum length of root was observed in 100mgl^{-1} heavy metal chromium concentration of (1.00) of VBN-5 cultivars were observed. The number of lateral roots of Black gram cultivar as affected by different concentration of heavy metal chromium The maximum number of lateral roots was recorded at control (5, 9, 6, 7, 4) forADT-3, T-9, VBN-3, VBN-4 and VBN-5 respectively and minimum number of lateral roots was recorded at 100 mgl^{-1} (2, 3, 2, 2, 1) for ADT-3, T-9, VBN-3, VBN-4 and VBN-5 respectively (Table-1).

The shoot length of Black gram variety was affected by different concentration of heavy metal chromium are presented. The Shoot length was found to be high value at C (29.00) of T-9 variety and minimum shoot length was observed in 100mgl^{-1}concentration (8.00) of VBN-5 variety. There was a gradual decline in shoot length with the increase in heavy metal chromium concentration was observed. The fresh weight was Black gram seedlings of different concentration of heavy metal chromium treatment are presented. The maximum fresh weight of seedlings occurred at control (0.40) of T-9 variety. The minimum fresh weight was observed at 100 mgl^{-1} chromium concentration (0.10) of VBN-5 variety. The dry weight was Black gram seedling of different concentration of heavy metal chromium treatment is presented. The maximum dry weight of seedlings occurred at C (0.08) of T-9 variety. The minimum dry weight was observed in 100 mgl^{-1} heavy metal chromium concentration in (0.01) of VBN-5 variety (Table-1).

In the above preliminary study was varietal screening test it is found that the variety T-9 performed better in germination seedling growth, fresh weight and dry matter production when compared to other varieties taken up for the study. The biochemical content also compared with all the variety and highly effected, so the T-9 cultivar was selected as tolerant variety. While VBN-5 variety showed least result among the other varieties, so it was selected as susceptible variety.

3.2. Biochemical analysis

3.2.1.Pigment content (mg/g fresh weight)

The tolerant variety of T-9, chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of 10th day seedlings of black gram are furnished. The maximum chlorophyll content was found at C (0.9410, 0.4890, 1.4300,) respectively. The minimum value were recorded at 100 mgl^{-1} heavy metal chromium concentration (0.7861, 0.2093, 0.9954) respectively. Then susceptible variety of VBN-5, chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of 10thdayseedlings of Black gram are furnished. The maximum chlorophyll content was found at C (0.5320, 0.1952, 0.7272,) respectively. The minimum value was recorded at 100 mgl^{-1} heavy metal chromium concentration (0.3920, 0.1477, and 0.5397) respectively (Table-2).

3.2.2. Protein (mg/g fresh weight)

The tolerant variety of T-9, Protein content of 10th day seedling under heavy metal chromium of black gram was recorded. The maximum amount of protein content of root and shoot are seen in control (6.1320). The minimum amount of protein content is seen in 100 mgl^{-1}concentration was (4.9820). Then susceptible variety of VBN-5 Protein content of 10th day seedling under heavy metal chromium of Black gram was recorded. The maximum amount of protein content of root and shoot are seen in control (4.8110). The minimum amount of protein content is seen in 100 mgl^{-1} concentration was (2.8161).observed in (Table-2).

3.2.3. Reducing Sugar (mg/g fresh weight)

Tolerant variety of T-9, total sugar content of 10th day Black gram seedling under heavy metal chromium is furnished. The maximum amount of sugar content in root and shoot was at C (1.3206). The minimum amount of sugar content in the root and shoot is at 100mgl^{-1}of heavy metal chromium concentration was (0.7340) conducted. Susceptible variety of VBN-5 the total sugar content of 10th day Black gram seedling under heavy metal chromium is furnished. The maximum amount of sugar content in root and shoot was at C (O.8402). The minimum amount of sugar content in the root and shoot at 100 mgl^{-1}ofheavy metal chromium concentration was (0.5361) observed (Table-2).

Table -1: Effect of chromium on seed germination, root length, shoot length, lateral roots, fresh and dry weight in five varieties of Black gram (*Vigna mungo* (L.) Hepper) 10th day's seedlings

Varieties/ Treatments		Seed germination	Root length	Shoot Length	Lateral roots	Fresh weight	Dry weight
ADT-3	C	90	07	24	05	0.33	0.04
	10	80(-11.11)	05(-28.57)	21(-12.05)	04(-20.00)	0.25(-24.24)	0.03(-25.00)
	25	70(-22.22)	03(-57.14)	17(-29.16)	04(-20.00)	0.22(-33.33)	0.02(-50.00)
	50	50(-44.44)	03(-57.14)	14(-41.66)	03(-40.00)	0.20(-39.39)	0.02(-50.00)
	100	40(-55.55)	01(-85.71)	01(-54.16)	02(-60.00)	0.11(-66.66)	0.01(-75.00)
T-9	C	100	12	29	09	0.40	0.08
	10	96(-6.00)	09(-25.00)	26(-10.34)	05(-44.44)	0.28(-30.00)	0.04(-50.00)
	25	90(-10.00)	05(-58.33)	22(-24.13)	05(-44.44)	0.20(-50.00)	0.03(-62.50)
	50	83(-17.00)	03(-75.00)	14(-51.72)	04(-55.55)	0.18(-55.00)	0.02(-75.00)
	100	80(-20.00)	02(-83.33)	10(-65.51)	03(-66.66)	0.14(-65.00)	0.02(-75.00)
VBN-3	C	96	05	24	06	0.37	0.05
	10	90(-6.25)	03(-16.66)	20(-16.66)	05(-16.66)	0.31(-16.21)	0.04(-20.00)
	25	85(-11.45)	02(-60.00)	16(-33.33)	04(-33.33)	0.17(-54.05)	0.03(-40.00)
	50	65(-32.29)	02(-60.00)	12(-50.00)	03(-50.33)	0.14(-62.16)	0.02(-60.00)
	100	50(-47.91)	01(-80.00)	10(-58.33)	02(-66.66)	0.12(-67.56)	0.02(-60.00)
VBN-4	C	92	04	24	07	0.30	0.05
	10	86(-6.52)	02(-50.00)	16(-33.33)	05(-28.57)	0.24(-20.00)	0.04(-20.00)
	25	80(-13.04)	01(-75.00)	12(-50.00)	04(-42.85)	0.21(-30.00)	0.02(-60.00)
	50	72(-21.73)	01(-75.00)	10(-58.33)	03(-57.14)	0.08(-40.40)	0.02(-60.00)
	100	65(-29.34)	01(-75.00)	08(-66.66)	02(-71.42)	0.11(-63.33)	0.01(-80.00)
VBN-5	C	80	03	15	04	0.38	0.03
	10	60(-25.00)	02(-33.33)	12(-20.00)	03(-25.00)	0.25(-34.21)	0.02(-33.33)
	25	50(-37.05)	02(-33.33)	10(-33.33)	03(-25.00)	0.20(-47.36)	0.02(-33.33)
	50	42(-47.05)	02(-33.33)	09(-40.00)	02(-50.00)	0.18(-52.63)	0.02(-33.33)
	100	35(-56.25)	01(-66.66)	08(-46.66)	01(-75.00)	0.10(-73.68)	0.01(-66.66)

Table- 2: Effect of chromium on Chlorophyll content, protein content, and reducing sugar content tolerant and susceptible characters on seedlings of Block gram (*Vigna mungo* (L.) Hepper) variety T-9 and VBN-5.

Varieties / Treatments		Chlorophyll content			Protein content	Reducing sugar content
		Chlorophyll a	Chlorophyll b	Total Chlorophyll		
T-9	C	0.9410	0.4890	1.4300	6.1320	1.3206
	10	0.9389 (-0.22)	0.3480 (-8.83)	1.2869 (-33.27)	5.9301 (-3.29)	1.1174 (15.38)
	25	0.8863 (-5.81)	0.2341 (-52.12)	1.1204 (-21.65)	5.4627 (-10.91)	1.0273 (-22.20)
	50	0.8212 (-12.73)	0.2103 (-56.99)	1.0315 (-27.86)	5.3932 (-12.04)	0.96.47 (-26.94)
	100	0.7861 (-16.46)	0.2093 (-57.19)	0.9954 (-30.39)	4.9820 (-18.75)	0.7340 (-44.41)
VBN-5	C	0.5320	0.1952	0.7272	4.8110	0.8402
	10	0.4511 (-15.20)	0.1731 (-11.32)	0.6242 (-14.16)	3.1310 (-34.91)	0.6691 (-20.36)
	25	0.4490 (-15.60)	0.1642 (-15.88)	0.6132 (-15.67)	3.1250 (-35.04)	0.6196 (-26.25)
	50	0.4131 (-22.34)	0.1587 (-18.69)	0.5718 (-21.36)	3.0682 (-36.22)	0.5820 (-30.73)
	100	0.3920 (-26.31)	0.1477 (-24.33)	0.5397 (-25.78)	2.8161 (-41.46)	0.5361 (-36.19)

4. Discussion

In India, many villages use polluted water as a major source for irrigation of crop plants. The present study was carried out to find out the effects of heavy metal-Chromium on germination and seedling growth of Black gram (*Vigna mungo* (L.) Hepper.) The germination study was conducted in the laboratory to find out the effect of heavy metal-Chromium on seed germination, seedling growth, fresh weight and dry weight of black gram seedlings. In addition to that, biochemical analyses were also conducted in tolerant and susceptible varieties of Black gram under heavy metal- chromium concentration.

In the varietal screening experiment, the seeds of five varieties of Black gram (ADT-3, T-9, VBN-3, VBN-4 and VBN-5) were allowed to germinate in plastic plates lined with filter paper. They were irrigated with different concentrations (C, 10, 25, 50, and 100mgl^{-1}) of heavy metal chromium. The control was irrigated with distilled water. The five varieties of Black gram seeds were screened for tolerance and susceptible varieties for heavy metal chromium. The germination percentage, seedling growth, and dry weight were taken into consideration for varietal screening experiment. The plant basis of data obtained from germination studies, the variety T-9 showed better performance (tolerant) while the variety VBN-5 showed poor performance (susceptible) than the other varieties under heavy metal- chromium treatment. In similar types of varietal screening experiment was carried out in Black gram cultivars under the influence of heavy metal-Lead by (Mumtaz Hussain, 2006), in Black gram under the influence of Zinc and Copper (Dhankar, 2010), in Black gram under the influence of Cobalt (Jayakumar, 2008), in tomato under the influence of mercury (Chandra Sekar, 2011).

In the present study of seed germination was found to be high in control (95%) and gradually decreased on the concentration increased. It was high in control of all varieties, and was very low at 100 mgl^{-1} concentration (35%) the same trend was observed in experiment conducted in tomato under the influence of heavy metal- mercury which showed 95% of germination in control while it was 20% of germination in high concentration (Chandra Sekar, 2011), heavy metal-chromium treatment on Green gram (Abbasi-2012). Tannery effluent irrigation in Maize (Sangeetha, 2012) also showed seedling growth more in control than the treated plants. The presence of metal- chromium in rooting medium significantly reduced the seed germination which might be due to the decreased photosynthetic pigment chromium hexavalent concentration even causes of chromosomal aberrations in roots of black gram (Sundramoorthy, 2009). The reason for reduced seedling growth under heavy metal treatment could

be the reduction in meristamatic cells present in the cotyledons and endosperms. During seedling growth hydrolysis of food reserves takes place which is carried out by hydrolytic enzymes. So the activities of hydrolytic enzymes might be affected and the food did not reach to the radical and plumule leading to the reduction in seedling growth.

4.1.Biochemical studies

In the Chlorophyll estimation, Chlorophyll a and Chlorophyll b was significantly affected under heavy metal- Chromium concentration. The seedling grown in the control of all varieties showed high amount (1.4300 mg/g/fresh weight) when compared to other concentrations which is showed less amount (0.5397 mg/g/fresh weight) similar results were reported by (Mumtaz Hussain 2006) in Black gram under the influence of heavy metal- Lead, chlorosis on older leave degradation of chlorophyll pigment also presented. The increase of Protein content of Black gram seedling was found to be more in control (6.1320mg/g/fresh weight) and low of (2.8161 mg/g/fresh weight). The similar results were obtained in groundnut treated with tannery effluent. The total Sugar content of black gram seedlings was found to be more in control (1.3206 mg/g/fresh weight) and gradually decreased (0.5361 mg/g/fresh weight) as the concentrations increased. The reason may be due to transportation of most of the elements absorbed by the plant as possible. The results were in conformity with possible in Green gram under the influence of tannery effluent.

5. Conclusion

The five varieties of Black gram were obtained from The National Pulses Research Institute Vamban and Aduthurai in Tamilnadu. They were used for varietal screening experiments. The germination percentage, growth, and dry weight of the seedlings were taken into consideration for varietal screening experiment. The plant basis of data obtained from germination studies, the variety T-9 was found to be tolerant and the variety VBN-5 was susceptible variety, among the other varieties for heavy metal chromium treatment. The germination study was conducted with tolerant variety (T-9) and susceptible variety (VBN-5) under different concentration of heavy metal chromium. In the present study in untreated concentration (control) showed more seed germination percentage, seedling growth, fresh and dry weight, while the higher concentrations decreased the above mentioned parameters. The biochemical studies like chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content Protein and Reducing sugars were found to be more in untreated concentration (control) and the contents gradually decreased as the concentrations increased.

Finally from the results obtained the T-9 variety showed better performance than the other varieties and so, it can be advised to the farmers to use the T-9 cultivar of Black gram and to avoid VBN-5 variety for cultivation, where they have no choice of water other than polluted water for agriculture.

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