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Changes in Chlorophyll and Nitrogen Content in Blackgram Mutant Lines Induced by Different Mutagenic Treatments towards Root-Knot Nematode Infection

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Abstract

A replicated pot culture experiment was designed to investigate the changes of nitrogen and chlorophyll content in superior mutant lines (M_5) developed from three morphologically different blackgram varieties (Pant U-30, PDU-I and Sarala) towards *M. incognita* infection. Nematode inoculation reduced chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, nitrogen content in shoot of blackgram by 18.99, 22.5, 21.24 and 11.41% respectively, but inoculated plant roots recorded 1.761% nitrogen which was 10.06% increase over the uninoculated plants. Sarala variety recorded higher quantities of chlorophyll-*a* (1.794 mg/g), chlorophyll-*b* (1.350 mg/g) total chlorophyll (3.152 mg/g), nitrogen in shoot (3.053%) and nitrogen in root (1.815%) as compared to other two parental varieties on these biochemical characters. EMS (0.3%) treatment induced three characters such as chlorophyll-*a*, chlorophyll-*b* and total chlorophyll by 20.5, 88.4 and 46.4% increase over control respectively. Gamma ray (30 kR) induced higher percentage of nitrogen in shoot (48.4) and root (22.5). NG (0.03%) was superior to gamma ray treatment for chlorophyll-*a*, chlorophyll-*b* and total chlorophyll.

Key words : Blackgram, Gamma ray, Chlorophyll, Nitrogen content, Root-knot nematode infection.

Pulses, a grain legume and protein rich crops are grown all over the world since time immemorial. Pulses occupy an important position in agriculture scenario in India. Root knot nematode, *Meloidogyne incognita* (1) is the most important nematode species limiting crop production. Race-1 of *M. incognita* is common in Orissa (2). Control of this nematode by chemical means is limited because of several factors, such as high cost of nematicides and non-availability in market, pollution problems, health hazards. The possibilities of controlling this nematode by using resistant, high yielding cultivars have been realized. Now a day plant breeding as useful tool for controlling root knot nematode. Mutations, spontaneous or induced bring about hereditary changes. Inductions of mutation by physical and chemical mutagenic treatments have been proved as an effective methodology for creating variability in both quantitative and qualitative characters in various crop plants. The potential of induced mutagens to contribute to improved disease resistance in crops are considered important including biochemical changes. Different mutagens also changed the biochemical properties in the mutants of mung bean (3, 4). Considering the economic importance of blackgram and seriousness of root-knot nematode, *M. incognita* infestation, attempts were made to find out the changes in chlorophyll and nitrogen content in blackgram mutant lines induced by different mutagenic treatments towards *M. incognita* infection.

Methods

The pot culture experiment was carried out in the wire-net house of Department of Nematology, College of Agriculture, OUAT, Bhubaneswar, Orissa during August-October, 2005.

Selection of Experimental Materials

Host. Blackgram is selected for the present

Table 1. Chlorophyll and nitrogen content in mutant lines of blackgram as influenced by *M. incognita.*

Treatment factors	Chlorophyll-*a* (mg/g tissue)	Chlorophyll-*b* (mg/g tissue)	Total chlorophyll (mg/g tissue)	Nitrogen content in shoot (%)	Nitrogen content in root (%)
		Inoculation (I)			
Un-inoculated	1.895	1.404	3.304	3.189	1.600
Inoculated	1.535	1.088	2.602	2.825	1.761
CD (0.05%)	0.080	0.055	0.115	0.075	0.095
		Varieties (V)			
Pant U–30	1.627	1.123	2.719	3.020	1.565
PDU–1	1.725	1.264	2.990	2.949	1.662
Sarala	1.794	1.350	3.152	3.053	1.815
CD (0.05%)	0.099	0.068	0.142	NS	0.117
		Treatments (T)			
Gamma ray (30 kR)	1.669	1.168	2.753	3.414	1.844
EMS (0.3%)	1.869	1.589	3.499	3.216	1.710
NG (0.03%)	1.773	1.382	3.171	3.098	1.664
Control	1.551	0.843	2.390	2.300	1.505
CD (0.05%)	0.114	0.079	0.164	1.074	0.134
		CD (0.05%) for Interaction			
I × V	NS	NS	NS	NS	NS
I × T	NS	NS	NS	NS	NS
V × T	NS	NS	NS	NS	NS
I × V × T	NS	NS	NS	NS	NS

investigation as this crop species is infested by the nematode *Meloidogyne incognita.* This is one of the important protein-rich and an important pulse crop ranking second in Orissa. The best mutant progenies of each of the three treatments gamma ray at 30 kR, EMS at 0.3% and NG at 0.03% of three commonly blackgram varieties Pant U-30, PDU-1 and Sarala along with control were taken to grow M_5 generation for the present investigation.

Preparation of Soil, Pot and Raising of Crop. With a view to facilitate movement, survival of nematode and good growth of blackgram plants sufficient quantity of sandy-loam soil was collected from the university farm, Bhubaneswar, Orissa. The soil was autoclaved at a pressure of 1.1 kg/cm^2 for 30 minutes and later, was air dried. Recommended dose of fertilizer in the form of CAN, single super phosphate and murate of potash were added to the soil. Required number of earthen pots of 15 cm diameter size were surface sterilized with formaldehyde solution (1 : 100 vol/vol) and were dried under shade after which 1 kg of processed soil was kept in each pot and were arranged in CRD factorial experimental design. Seeds treated separately with freshly prepared aqueous mercuric chloride ($HgCl_2$) solution for 30 minutes were removed and washed three times in sterile water. Seeds were sown at 4 seeds per pot at about 1 cm soil depth on 28 August 2005. Thinning was done 5 days after sowing and one healthy seedling was allowed to remain in each pot. Watering and intercultural operation were given as and when necessary.

Culturing, Extraction and Inoculation of Nematodes. To get a large number of second stage juveniles of *M. incognita* (Race-I) for the experiment, the population of the test nematode species was developed from a single egg mass on tomato plants grown in cemented beds containing autoclaved soil well ahead of the experiment. The required numbers of freshly hatched second stage juveniles were col-

Table 2. Chlorophyll and nitrogen content of blackgram varieties influenced by mutagenesis and *M. incognita* infection.

Treatments	Chlorophyll-*a* (mg/g tissue)	Chlorophyll-*b* (mg/g tissue)	Total chlorophyll (mg/g tissue)	Nitrogen content in shoot (%)	Nitrogen content in root (%)
		Un-Inoculated			
Pant U-30					
Gamma ray (30 kR)	1.748	1.155	2.939	3.570	1.676
EMS (0.3%)	2.023	1.657	3.680	3.422	1.596
NG (0.03%)	1.794	1.339	1.133	3.265	1.465
Control	1.762	0.884	2.647	2.489	1.301
		PDU-1			
Gamma ray (30kR)	1.847	1.369	3.216	3.475	1.710
EMS (0.3%)	2.070	1.794	3.865	3.305	1.629
NG (0.03%)	1.866	1.518	3.384	3.188	1.459
Control	1.780	0.964	2.744	2.410	1.414
		Sarala			
Gamma ray (30 kR)	1.877	1.489	3.366	3.637	1.868
EMS (0.3%)	2.141	1.936	4.077	3.553	1.816
NG (0.03%)	1.997	1.685	3.776	3.478	1.761
Control	1.801	1.052	2.827	2.476	1.509
		Inoculated			
Pant U-30					
Gamma ray (30 kR)	1.363	0.880	1.742	3.201	1.767
EMS (0.3%)	1.404	1.295	2.947	3.070	1.634
NG (0.03%)	1.615	1.144	2.759	2.940	1.588
Control	1.272	0.631	1.902	2.200	1.490
		PDU-1			
Gamma ray (30 kR)	1.569	1.026	2.595	3.290	1.806
EMS (0.3%)	1.74	1.400	3.139	3.029	1.738
NG (0.03%)	1.63	1.310	2.913	2.859	1.939
Control	1.326	0.735	2.062	2.114	1.606
		Sarala			
Gamma ray (30 kR)	1.572	1.089	2.661	3.392	2.239
EMS (0.3%)	1.834	1.454	3.288	2.916	1.845
NG (0.03%)	1.764	1.297	3.061	2.861	1.774
Control	1.365	0.794	2.160	2.109	1.710

lected from this pure culture based on requirement. To get rid of any bacterial or fungal contamination of the inoculums, collected nematodes (J_2) were surface sterilized by treating with 1% mercurochrome solution for 30 minutes and then washed three times with sterile distilled water, each time centrifuging and discarding the supernatant liquid until it was almost free of red color.

Inoculation of nematode was done 10 days after sowing. Surface sterilized nematode suspension (10 ml) counting 1000 J_2 thoroughly mixed and poured with the help of a graduated pipette around the base of the blackgram plant through 3 holes made to a depth of about 2 cm with the help of a clean sterile glass rod. Equal volume of distilled water was also added to the control pots. The holes were closed by

pressing the soil into it. Watering was done carefully so that there was no splashing or over watering.

Treatments and Experimental Design

CRD-factorial design was followed to carry out the experiment to investigate the effect of nematode, *M. incognita* on the best mutant progenies and the control and also to determine the effectiveness of different mutagens on blackgram and nematode activities. There were 24 treatment combinations and four replications.

Recording Observations

The experiment was terminated at 40 days after inoculation of nematode. Observation on chlorophyll content, nitrogen content of shoot and root were recorded.

Chlorophyll Content of Leaves. Chlorophyll content of leaves was estimated on day 40 of sowing following the procedure as laid down by Singh (5). Absorbance of the chlorophyll extract was recorded at 645 nm and 663 nm using systronic colorimeter. The quantity of chlorophyll-*a*, chlorophyll-*b* and total chlorophyll was calculated in mg/g tissue by the following equation.

$$\text{chlorophyll-}a\text{ (mg/g tissue)} = [12.7\,(D_{663}) - 2.69\,(D_{645})] \times \frac{\text{Vol}}{1000 \times \text{Wt}}$$

$$\text{chlorophyll-}b\text{ (mg/g tissue)} = [22.9\,(D_{645}) - 4.68\,(D_{663})] \times \frac{\text{Vol}}{1000 \times \text{Wt}}$$

$$\text{Total chlorophyll (mg/g tissue)} = [20.2\,(D_{645}) + 8.02\,(D_{663})] \times \frac{\text{Vol}}{1000 \times \text{Wt}}$$

Where, D = Optical density at the specific indicated wave length, Vol = Final volume of 80% acetone chlorophyll extract in ml, Wt = Fresh weight of leaf in g.

Total Nitrogen Content. Nitrogen content of stem and root was estimated by following the procedure laid down by Vashishth et al. (6). The quantity of nitrogen was calculated by the following equation.

$$\text{Percent N} = \frac{(\text{ST}-\text{BT}) \times \text{Normality of HCl} \times 14 \times 100 \times \text{DF (i.e. 2.5)}}{\text{Sample weight (mg)} \times 1000}$$

Where, ST = Sample titer value, BT = Blank titer value, DF = Dilution factor.

Statistical Analysis

Analysis of variance for each character was carried out with plant means for partitioning of the total variance into components ascribable to inoculation replications, varieties, treatments and error. The test of significance of difference of different characters was studied by *F*-test. The significant difference between means of any two treatments was tested by *t* test. The ANOVA, standard error of treatment means SE (m) and critical difference (CD-0.05) were calculated. The plant characters (chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, nitrogen content in shoot and root) were analyzed by three factor completely randomized design.

Results and Discussion

Chlorophyll Content

The chlorophyll content (chlorophyll-*a*, chlorophyll-*b* and total chlorophyll) of different mutant lines along with their parents under uninoculated and inoculated conditions are presented in Tables 1 and 2. In general, the chlorophyll-*a* content of leaves was noticed to be higher than the chlorophyll-*b*. Inoculation of *M. incognita* was recorded as 1.535 mg/g tissue of chlorophyll-*a* as compared to uninoculated plant (1.895 mg/g tissue) showing a reduction of (19%) and differed significantly. Chlorophyll-*b* was reduced by 2.5% due to inoculation of *M. incognita* over uninoculated condition, which is statistically significant. The variety, Sarala also recorded maximum quantity of chlorophyll-*b* (1.589 mg/g tissue) followed by PDU-1 (1.264 mg/g tissue) and Pant U-30 (1.123 mg/g tissue) in descending order and each variety showed a significant difference in this character. Similar trend in total chlorophyll on parent varieties of blackgram was also recorded. Inoculation of *M. incognita* also reduced total chlorophyll con-

tent of leaves by (21.24%) over uninoculated condition.

The EMS was found to be superior in producing more chlorophyll-*a* (1.869 mg/g tissue) and chlorophyll-*b* (1.589 mg/g tissue) followed by NG and gamma rays. Minimum total chlorophyll (2.390 mg/g tissue) was recorded in control plants which is significantly different from other treatments. EMS induced (46.4%) total chlorophyll over control followed by NG (32.6%) and gamma ray (15.18%). The interaction between nematode inoculation, variety and treatment was found to be statistically non-significant.

Nitrogen Content in Shoot

The nitrogen content of different mutant lines along with their parents under uninoculated and inoculated conditions are presented in Table 2 and pool data is presented in Table 1. Maximum nitrogen content (3.189%) was recorded in shoots under uninoculated condition as compared to 2.825% in *M.. incognita* inoculated plants. The nematode reduced 11.4% nitrogen content of the shoot over uninoculated plants and differed significantly. Nitrogen content of shoot of variety, Sarala was recorded to be 3.053% followed by Pant U-30 (3.02%) and PDU-1 (2.949%), showing statistically non-significant difference. Nitrogen content in shoot of mutant lines developed from gamma ray was found to be maximum (3.414%) followed by EMS (3.216%), NG (3.098%) and control (2.3%) which showed statistically significant difference. Gamma ray induced (48.4%) nitrogen increase in shoot being the highest followed by EMS (39.8%) and NG (34.7%). There was non-significant difference in the interaction between inoculation, variety and treatment on this character.

Nitrogen Content in Root

M. incognita inoculated plants showed higher nitrogen content (1.76%) in roots compared to (1.6%) in uninoculated plants showing about 10% increase of nitrogen content in infected roots. The recorded data are significantly different (Table 1). *M. incognita* infected roots of blackgram showed higher nitrogen content (6). Higher concentration of nitrogen in roots is possibly due to impaired absorption and translocation of minerals and blocking metabolic at the site of infection and also mobilization of nutrient from shoot to infected root. This imbalanced translocation in minerals sufficiently disturbed in plants metabolic system.

The mutants developed through irradiation of gamma rays showed highest nitrogen content is root (1.844%) followed by EMS (1.710%) which are at par. Mutants of NG origin also recorded 1.664% nitrogen compared to 1.505% in control plants. The interactions between inoculation, variety and treatments computed were found to non-significant at each level.

Conclusion

Sarala variety recorded higher quantities of chlorophyll-*a* (1.794 mg/g), chlorophyll-*b* ((1.350 mg/g), total chlorophyll (3.152 mg/g), nitrogen in shoot (3.053%) and nitrogen in root (1.815%) as compared to other two parental varieties. EMS (0.3%) treatment induced three characters such as chlorophyll-*a*, chlorophyll-*b* and total chlorophyll by 20.5, 88.4 and 46.4% increase over control respectively. Gamma ray (30 kR) induced higher percentage of nitrogen in shoot (48.4) and root (22.5). NG (0.03%) was least effective in inducing positive characters, but superior to gamma ray treatment for chlorophyll-*a*, chlorophyll-*b* and total chlorophyll.

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