A Study of the Effect of Pyrites and Rhizobium Inoculation on Chlorophyll and Sugar Content in Black Gram under Sodicity Stress Condition

Jolly Singh1, Shashank Sharma*2, Khanna SK3, Bhavtosh Sharma2 and Prasad FM1
1Department of Chemistry, St. John’s College, Dr. BR Ambedkar University, Agra, Uttar Pradesh, India
2Department of Applied Sciences, JMIETI (Kurukshetra University), Yamuna Nagar, Haryana, India
3Department of Science and Technology, Uttarakhand Science Education and Research Centre (USERC), Uttarakhand, India

Abstract
The study reveals that sodic conditions resulted in poor bio-synthesis in black gram plants and hence both chlorophyll 'a', 'b' and reducing and non-reducing sugar contents decreased with increasing levels of RSC water treatments. Incorporation of iron pyrites as an ameliorating agent significantly improved the availability of nutrients and enhanced the chlorophyll and sugar content. The Rhizobium inoculated treatments supplemented with 50 g iron pyrites pot-1 adjusted best among all the treatments and plants could withstand irrigation with 2.5 meL-1 RSC water without being adversely affected. Further, 7.5 meL-1 proved detrimental for plant growth and recorded maximum reduction in sugar and chlorophyll contents in the treatments which were not supplied with pyrites.

Keywords: RSC water; Black gram; Iron pyrites; Reducing and non-reducing sugar; Chlorophyll

Introduction
Calcereous soils have resulted in precipitation of CaCO₃ from water of some rivers which contain high amount of Ca(HCO₃)₂. The tropical aridity creates reaction resulting in accumulation of salts. Most of the water of uplands is lost due to runoff and transpiration reducing effective leaching. Precipitation of calcium increases sodium concentration which is further augmented by high RSC of the available water resources. Salinity is the major yield reducing factor in legume cultivation as legumes are salt sensitive crop, especially in the seedling stages where they are most susceptible to damage due to water stress and ion-imbalance. In saline soils, many environmental factors such as soil pH, water deficiency and nutrient deficiency are reported in Ref. [1-3] that the seedling stage suffers most damage due to ion-imbalance and thus, other physiological processes are negatively affected during the growth of the plant especially photosynthesis. Ref. [4] reported that during long term exposure to salinity, plants experience premature senescence of adult leaves and root nodules which reduce the net photosynthetic area available to support continued growth of the plant. Thus, bio-synthesis of sugar and nitrogen fixation are negatively affected.

In order to overcome this environmental abuse and to raise the plants with better suitability towards changing environmental inputs, various physico-chemical and bio-chemical tools are employed. One such agent that helps in reclamation of saline soil is the use of iron pyrites. Ref. [5,6] suggested that iron pyrites is a good reclaiming agent for calcareous alkali soils.

Application of pyrites has no adverse effects on the soil bacterial and fungal population. Therefore, naturally occurring populations and inoculated strains of rhizobium are not affected. Volatilization losses from ammonium sulphate and urea are considerably reduced by application of pyrites. Ref. [7] reported that Pyrites application increases availability of phosphorus, iron and zinc in soil and hence, responses of cereals and pyrites application in calcareous soils are significant. Inoculation of seeds with rhizobium helps in increasing effective nodulation, especially in those soils that have low population of naturally occurring Rhizobia.

Materials and Methods
In order to study the relative effect of the RSC water and pyrite application on chlorophyll and sugar content, a pot experiment was laid down in the School of Chemical Sciences, Chemistry Dept., St. John’s College, Agra, using factorial randomized block design and replicated thrice using black gram (Vigna mungo L.) C. V. Pant-19 as the test crop. The experiment consisted of four levels of RSC (Residual Sodium Carbonate) water viz. R₀ (0 meL⁻¹), R₁ (2.5 meL⁻¹), R₂ (5.0 meL⁻¹) and R₃ (7.5 meL⁻¹), three levels of iron pyrites viz. S₀ (0 gm pot⁻¹), S₁ (25 gm pot⁻¹) and S₂ (50 gm pot⁻¹) and two levels of Rhizobium inoculation, I₀ (uninoculated) and I₁ (inoculated). The essential nutrients were supplied through basal application in the soil before sowing the test crop by applying urea, single super phosphate and muriate of potash at 20, 40 and 60 g pot⁻¹ containing 10 kg of soil each. Pyrite was incorporated through basal application before sowing as treatment. Inoculation of seeds was done and dropped at depth of about m equally in all the pots. RSC water was prepared by dissolving the sodium salts containing carbonate and bicarbonate in the best available water (bore well water). The pots were irrigated with RSC water after every 15 days. Control sets were irrigated with best available water.

Plant material samples were collected from each pot after harvest after 45 DAS (Days after sowing) for chlorophyll 'a', 'b' and total chlorophyll analysis by Ref. [8] method and after harvest for sugar analysis. Reducing sugar was estimated by method using dinitrosalicilic acid reagent method by Ref. [9] and total sugar was estimated by Ref. [10] phenol reagent method. Non-reducing sugar was calculated by subtracting reducing sugar from total sugar. The chlorophyll content

*Corresponding author: Shashank Sharma, Department of Applied Sciences, JMIETI (Kurukshetra University), Radaur, Yamuna Nagar, Haryana, India, Tel: +919760230620; E-mail: shashank.enviro@gmail.com

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was expressed as mg g⁻¹ sugar contents were expressed as mg/ 100 g of sample. The results obtained were subjected to statistical analysis with the help of variance, using SIGMA STAT 3.5.

**Results and Discussion**

It is revealed from the data in the table that chlorophyll 'a' and 'b' contents at 45 DAS (Days after sowing) significantly increased over control with the rise in pyrite application and rhizobium inoculation but decreased with increasing RSC (Residual Sodium Carbonate) levels. Highest value of chlorophyll 'a' was recorded at R₁ which was 3.63% and 3.03% higher over the control respectively for the years '07 and '08 whereas the lowest chlorophyll 'a' content recorded at R₅ was 20.45% and 21.81% lower than the control respectively for both the years. And chlorophyll 'b' was recorded a highest at R₁, with values of 15.2% and 25% over the control and lowest values recorded at R₅ were 28.4% and 25.4% respectively for both the years. Comparing the treatments, it is obvious that the application of pyrites increased the leaf chlorophyll due to sulphur availability. Similar results were reported by Ref. [11].

The highest content of reducing and non-reducing sugar was reported in the treatment R₁. Reducing sugar was 1.51% and 2.41% higher over the control for the year '07 and '08 respectively and non-reducing sugar was 3.32% and 2.92% higher over control respectively for both the years. Reducing sugar was decreased to the extent of 6.04% and 10.26% and non-reducing sugar was reduced to the extent of 17.49% and 15.57% over the control respectively for the years '07 and '08 in the treatment R₅. Further, it was also seen that there was a decreasing trend in the values of the sugar content in the second year crop. The decrease in the values in the second year was probably due to the adsorption of the carbonate and bicarbonate ions on the soil surface due to continuous irrigation in the two consecutive years (Tables 1 and 2).

Due to the sodic ion toxicity, the availability of soil nutrients is affected and the bio-synthesis of starch is reduced due to reduction in the rate of photosynthesis because it is closely related to chlorophyll content of the plant. Incorporation of pyrites as an ameliorating agent increases the sulphur content causing an increased accumulation of the sugar in pulse crop. Reducing and non-reducing sugar content successively increased over control with pyrite application at RSC level of 2.5 meL⁻¹, Ref. [12,13] explained that the increased accumulation of sugar at elevated salinity levels is one of the ways to combat salinity with an osmotic adjustment. Ref. [14] suggested that it allows the plants to maximize sufficient storage reserves to support basal metabolism under stressed conditions. But, there was a decline in the values of sugar accumulation at RSC level of 5-7.5 meL⁻¹ probably due to metabolic alterations and mineral deficiency that lead to decrease in growth parameters, premature leaf senescence, reduced chlorophyll content of the leaves and therefore, reduction in the net photosynthetic activity.

| Treatments  | Chl'a 2007 | Chl'a 2008 | Chl'b 2007 | Chl'b 2008 |
|-------------|------------|------------|------------|------------|
| R₁S₁I₁      | 0.660      | 0.660      | 0.250      | 0.228      |
| R₁S₁I₂      | 0.673      | 0.670      | 0.259      | 0.248      |
| R₁S₁I₃      | 0.679      | 0.675      | 0.265      | 0.263      |
| R₁S₁I₄      | 0.660      | 0.655      | 0.270      | 0.264      |
| R₁S₁I₅      | 0.674      | 0.670      | 0.283      | 0.278      |
| R₂S₁I₁      | 0.684      | 0.680      | 0.288      | 0.285      |
| R₂S₁I₂      | 0.620      | 0.615      | 0.254      | 0.224      |
| R₂S₁I₃      | 0.632      | 0.628      | 0.247      | 0.222      |
| R₂S₁I₄      | 0.648      | 0.635      | 0.235      | 0.220      |
| R₂S₁I₅      | 0.525      | 0.516      | 0.179      | 0.170      |
| R₃S₁I₁      | 0.576      | 0.560      | 0.205      | 0.204      |
| R₃S₁I₂      | 0.589      | 0.573      | 0.220      | 0.214      |
| CD at 5%     | 0.033      | 0.040      | 0.035      | 0.035      |
| SEM ±        | 0.011      | 0.014      | 0.012      | 0.012      |

Table 1: Effect of varying levels of pyrite and RSC water levels on the chlorophyll a' and b' content (mg/g) of the leaves at 45 DAS in rhizobium treated and untreated black gram.

| Treatments  | Reducing sugar '07 | Reducing sugar '08 | Non-reducing sugar '07 | Non-reducing sugar '08 |
|-------------|--------------------|--------------------|------------------------|------------------------|
| R₁S₁I₁      | 31.60              | 31.09              | 27.64                  | 27.03                  |
| R₁S₁I₂      | 31.77              | 31.12              | 27.92                  | 27.09                  |
| R₁S₁I₃      | 32.01              | 31.17              | 28.45                  | 27.62                  |
| R₁S₁I₄      | 31.56              | 30.60              | 27.85                  | 27.53                  |
| R₁S₁I₅      | 32.03              | 31.71              | 28.16                  | 27.62                  |
| R₂S₁I₁      | 31.08              | 31.84              | 28.56                  | 27.82                  |
| R₂S₁I₂      | 31.53              | 29.79              | 26.79                  | 25.87                  |
| R₂S₁I₃      | 31.79              | 29.99              | 26.92                  | 26.00                  |
| R₂S₁I₄      | 31.84              | 30.05              | 27.42                  | 26.63                  |
| R₂S₁I₅      | 29.69              | 27.90              | 22.83                  | 22.62                  |
| R₃S₁I₁      | 30.46              | 28.00              | 25.94                  | 24.66                  |
| R₃S₁I₂      | 31.26              | 29.30              | 26.61                  | 25.15                  |
| CD at 5%     | 1.098              | 2.232              | 0.958                  | 1.578                  |
| SEM ±        | 0.374              | 0.791              | 0.327                  | 0.538                  |

Table 2: Effect of varying levels of pyrite and RSC water levels on the reducing and non-reducing sugar in mg/g content of the grain in rhizobium treated and untreated black gram.
Conclusion

It is evident from the foregoing discussions that there was depressive effect on the chlorophyll 'a' and 'b' and sugar content due to irrigation with RSC water and the values reduced significantly over control in the treatments supplied with RSC levels of 5.0 and 7.0 eL⁻¹. The values enhanced over control with pyrite application from 0-50 gm pot⁻¹ in all rhizobium treated and untreated plants. In inoculated plants, combination of 2.5 meL⁻¹ RSC water supplemented with 50 g pyrite per pot provided most suitable conditions for plant’s growth whereas 7.5 meL⁻¹ RSC water without pyrite application was detrimental for plant growth.

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References

1. James JJ, Tiller RL, Richards RH (2005) Multiple resources limit plant growth and function in a saline-alkaline desert community. J Ecol 93: 113-126.
2. Kopittke P, Menzies N (2005) Effect of pH on Na induced Ca deficiency. Plant and Soil 269: 119-129.
3. Moradi F, Ismail AM (2007) Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann Bot 99: 1161-1173.
4. Cramer GR, Nowak RS (1992) Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. Physiol Plant 84: 600-605.
5. Sharma SK, Manchanda HR (1989) Effect of irrigation with sodic water of increasing RSC levels on yield and NaCl content of chick pea. J Indian Soc Soil Sci 37: 147-151.
6. Singh H, Singh J, Kumar A (2005) Effect of phosphorus and sulfur on the grain and straw yield of black gram. Annal of Plant Soil Res 7: 99-100.
7. Rai R, Ashraf MH, Chaudhary SN (1977) Pyrite as an amendment of soil. Ferti Tech 14: 370-374.
8. Jayaraman J (1981) Laboratory manual of bio-chemistry. Willey Eastern Ltd., New Delhi, India.
9. Miller GL (1956) Use of dinitro-salicylic acid reagent for the determination of reducing sugar. An Chem 31: 426-428.
10. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356.
11. Sinha RB, Sakal P (1993) Effect of pyrite and organic manures on sulphur nutrition of crops in calcareous soil. J Indian Soc Soil Sci 41: 312-315.
12. Dubey RS, Singh AK (1999) Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. Biol Plantarum 42: 233-239.
13. Flowers TJ, Yeo AR (1989) Effects of Salinity on Plant Growth and Crop Yield. In: Environmental Stress in Plants. Cherry JH (ed.), Springer Verlag, Berlin, pp: 101-119.
14. Pattanagul W, Thitisaksakul M (2008) Effect of salinity stress on growth and carbohydrate metabolism in tree rice (Oryza sativa L.) cultivars differing in salinity tolerance. Indian J Exp Biol 46: 736-742.