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A STUDY ON THE BIOCHEMICAL CHANGES IN THE BLACK GRAM CULTIVAR GROWN IN TSUNAMI AFFECTED SOIL

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Experiments were conducted with tsunami affected soils to evaluate the biochemical changes during the growth of the black gram cultivar TAU-1.Three types of soils were selected for the experiments by modifying the nature of the affected soil – the tsunami affected soil (A), the top soil (T) and the top soil washed with water five times to reduce the salinity (TT). The plants were allowed to grow for 40 days in pots and the estimations were done at 10, 20, 30 and 40 DAS in the trifoliate leaves. Compared to Chl 'b', Chl 'a' content decreased significantly in top soil. The carotenoid level increased in all plants grown in affected soils. A general reduction in the level of sugars and starch was evident in all the stressed plants. Salinity of the soil promoted the accumulation of O.D. and total phenols. The nitrate and foliar protein levels were very low in top soil, whereas in others reduction was not significant. In TT soil, at 40 DAS, the protein levels were significantly higher than control plants. The nitrate reductase acitivity was generally suppressed in all stressed plants.

Keywords: Biochemical changes; Black gram; Modified soil; Tsunami.

Introduction

The 2004 Indian ocean earthquake that had a magnitude of around 8.9 on the Richter scale, triggered a series of lethal tsunamis on December 26, 2004 that killed over 3,10,000 people, making it the worst tsunami in Indian History. A tsunami is a natural phenomenon caused by a powerful earthquake in the seabed. On December 26, 2004, the earthquake in the eastern Indian Ocean sent massive waves in all directions. Upon reaching the coasts of Southern Asia, the waging waters killed at least 2,20,000 people. The term tsunami comes from the Japanese language meaning harbour waves. There are many causes of tsunami but the most prevalent one is earthquake. In addition, landslides, volcanic eruptions, explosion, and even the impact of cosmic bodies, such as meteorites, can generate tsunamis. The Sumatra, Sri Lanka and Indian coasts underwent significant modification by the tsunami. Within a few hours of the initial tsunami impact, the shoreline eroded, beach sand was carried inland and the coastal areas were flooded. The tsunami killed people over an area ranging from the immediate vicinity of the quake in Indonesia, Thailand and the north-western coast of Malaysia to thousands of kilometers away in Bangladesh, India, SriLanka, the Maldives and even as far as Somalia, Kenya and Tanzania in Eastern Africa. The giant waves intruded all the coastal villages including Pondicherry. Nearly 19 coastal villages in and around Pondicherry were affected by the floods. Several hectares of agricultural lands were inundated with saline water. In the study area i.e. near Thavalakuppam, Pondicherry, most of the groundnut and other pulse crops dried out because of salinity and the field became a barren one within a few days after the tsunami struck the coastal regions. With this background, a study was undertaken to find out the response of the black gram cultivar TAU-1 to the tsunami affected soils.

Material and Methods

Seeds of black gram [*Vigna mungo* (L.) Hepper] cultivar TAU-1 were procured from seed farm in Pondicherry for the experimental study. Seeds were surface sterilized in 0.1% w/v mercuric chloride and washed several times with glass distilled water. Uniform sized healthy seeds were selected for the study. The tsunami affected soil samples were collected from Thavalakuppam, Pondicherry. The soil analysis showed 0.75 (Ec) for tsunami affected soil as against 0.13 in normal soil. The pH of the affected soil was also high due to the salinity. The soil texture also changed, became clayey by tsunami flooding. Phosphate and potassium contents were low in the affected soil. The experiment was conducted in big earthenware pots (25 x 25 cm) filled with different soil samples, sand, DAP (50 mg) and garden manure. The soil sample, sand and manures were mixed in the ratio of 4 : 2 : 1. For control experiment, the unaffected soil (N) from the adjacent

Table 1. Effect of stressed soils on the photosynthetic pigment contents (mg g^{-1} f.w.) in the trifoliate leaves of black gram cultivar TAU-1.

Pigments	Soil type	STAGES I	II	III	IV
Chl. a	N	0.96 a	1.33 a	1.35 a	0.72 a
	A	0.85 ab	1.22 ab	1.24 ab	0.76 ab
	T	0.76 b	0.99 b	1.13 b	0.67 a
	TT	0.91 a	1.26 a	1.39 a	0.85 b
Chl. b	N	0.71 a	0.60 a	1.06 a	0.60 a
	A	0.76 a	0.72 b	0.91 a	0.59 a
	T	0.74 a	0.72 b	0.91 a	0.69 b
	TT	0.67 a	0.82 b	0.92 a	0.68 ab
Chl. a/b ratio	N	1.35 a	2.22 a	1.27 a	1.20 a
	A	1.12 b	1.69 b	1.36 ab	1.29 a
	T	1.03 b	1.38 b	1.24 a	0.97 b
	TT	1.36 a	1.54 b	1.51 b	1.25 a
Total Chlorophyll	N	1.67 a	1.93 ab	2.41 a	1.32 a
	A	1.61 a	1.94 ab	2.15 a	1.35 ab
	T	1.50 a	1.71 a	2.04 a	1.36 ab
	TT	1.58 a	2.08 b	2.31 a	1.53 b
Carotenoids	N	0.89 a	1.09 a	1.33 ab	0.60 a
	A	0.84 a	1.23 a	1.17 a	0.64 ac
	T	0.92 a	1.00 a	1.51 b	0.74 b
	TT	0.91 a	1.21 a	1.32 ab	0.69 bc

Stages I, II, III and IV correspond to 10, 20, 30 and 40 DAS (Days After Sowing). Within a column, values followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=15).
(N = Normal soil, A = Affected soil, T = Top soil, TT = Top soil washed with water for five times)

groundnut field was chosen. The tsunami affected soil was dug out from the field (12" x 12" x 12') and used as affected (A) soil for the experiment. The top soil (T) in the affected field up to a depth of 2" was also chosen. In another pot, the top soil washed with water for five times (TT) was selected. Thus, four different types of soils (N, A, T and TT) were used for the study. Fifteen healthy seeds were selected and sown equidistantly at a depth of 2 cm in each pot. The pots were maintained under natural green house conditions and they were arranged in the form of randomized block design for the different treatments and these were replicated thrice. Ten day – old seedlings were inoculated with 200 mg of the commercial preparation of *Rhizobium* inoculum suspended in 10 ml of water and poured on the surface of the soil.

Biochemical changes were observed at four stages viz. 10, 20, 30 and 40 DAS in the trifoliate leaves belonging to the second node from the top of the shoot. The chlorophyll[1,2] and carotenoid[3] pigments were estimated by extracting the pigments into N,n-dimethyl formamide (DMF). The reducing[4] and non-reducing sugars[5], total sugars[6], starch[7], O.D. phenol [8], total phenol[9], nitrate [10] and nitrite [10]contents were estimated in the leaves at all stages. Fresh leaf samples were used for the

Table 2. Effect of stressed soils on the sugars, starch and phenol contents (mg g^{-1} d.w.) in the trifoliate leaves of black gram cultivar TAU-1.

Parameter	Soil type	STAGES I	II	III	IV
Reducing sugars	N	43.92 a	39.08 a	22.46 a	22.46 a
	A	27.92 b	21.09 b	19.73 a	20.35 ac
	T	37.84 a	21.96 b	20.79 a	19.04 bc
	TT	38.09 a	29.88 c	21.47 a	20.10 ac
Non-reducing sugars	N	10.84 a	10.41 a	11.18 a	13.57 a
	A	21.99 b	18.35 b	7.95 b	10.14 b
	T	9.70 a	14.81 b	7.85 b	10.91 b
	TT	15.53 b	16.61 b	14.22 c	11.34 b
Total sugars	N	55.33 a	50.04 a	34.23 a	36.74 a
	A	51.07 a	40.41 b	28.08 b	31.02 b
	T	48.05 a	37.55 b	29.05 b	30.52 b
	TT	54.44 a	47.36 a	36.44 a	32.04 ab
Starch	N	136.66 a	135.83 a	125.98 a	116.73 a
	A	125.34 a	154.73 a	115.27 a	120.67 a
	T	107.79 b	102.39 b	94.71 b	106.45 a
	TT	138.76 a	150.58 a	117.83 a	112.60 a
O.D.Phenols	N	4.45 a	4.01 a	2.56 a	2.07 a
	A	4.59 a	3.41 b	2.48 a	2.59 b
	T	4.85 a	5.78 c	3.69 b	2.70 b
	TT	4.82 a	4.85 c	2.52 a	3.04 b
Total Phenols	N	6.39 a	8.21 a	6.94 a	6.01 a
	A	5.55 a	8.45 a	5.58 b	5.95 a
	T	7.06 a	9.28 a	7.54 a	7.66 b
	TT	8.25 b	8.79 a	6.57 a	6.62 a

Stages I, II, III and IV correspond to 10, 20, 30 and 40 DAS (Days After Sowing). Within a column, values followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=15).(N = Normal soil, A = Affected soil, T = Top soil, T.T = Top soil washed with water for five times)

estimation of protein[11] content and the nitrate reductase enzyme activity was determined using the standard method[12]. Data were statistically analyzed at 5% level of significance using Tukey's Multiple Range Test (TMRT)[13].

Results and Discussion

Among the stressed soils, a notable decrease in the chlorophyll 'a' pigment content of the leaf was noticed in top soil. The decreases were 21, 26 and 16% at 10, 20, and 30 DAS, respectively. In other treatments, either insignificant increases or decreases were evident (Table 1). A significant increase in the content of chlorophyll 'b' pigment was noted in stressed soils in the early stages. The chl a/b ratio was generally low in stressed soils. Similar to the total chlorophylls, the carotenoid levels remained more or less unchanged in the early stages. At stage III and IV, the carotenoid levels increased in T soil. Salinity of the top soil promoted the synthesis of chl 'b' and the carotenoid levels. Decreased chlorophyll content in the

Table 3. Effect of stressed soils on the nitrate, nitrite (mg g^{-1} d.w.), protein contents (mg g^{-1} f.w.) and NR activity (nm NO_2 h^{-1} g^{-1} f.w.) in the trifoliate leaves of black gram cultivar TAU-1.

Parameters	Soil type	STAGES I	II	III	IV
Nitrate	N	1.78 a	1.36 a	3.51 a	3.17 a
	A	1.56 a	1.17 b	3.06 b	3.01 a
	T	1.37 b	1.04 b	2.57 b	2.19 b
	TT	1.62 a	1.08 b	2.93 b	2.84 a
Nitrite	N	0.05 a	0.03 ab	0.05 a	0.03 a
	A	0.05 a	0.05 b	0.08 b	0.04 a
	T	0.02 a	0.04 a	0.07 bc	0.02 a
	TT	0.05 a	0.02 a	0.06 ac	0,04 a
Protein	N	1.94 a	2.16 a	5.81 ac	3.53 a
	A	1.83 a	1.26 b	4.35 b	3.08 ab
	T	1.36 b	0.96 b	3.68 b	2.77 b
	TT	1.21 b	1.31 b	5.03 bc	4.29 c
NR activity	N	3540 a	3200 a	3080 a	3540 a
	A	3460 a	2810 ab	2620 bc	3020 b
	T	3620 a	2720 b	2840 ac	2980 b
	TT	3440 a	2960 ab	3120 a	3620 a

Stages I, II, III and IV correspond to 10, 20, 30 and 40 DAS (Days After Sowing). Within a column, values followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=15).
(N = Normal soil, A = Affected soil, T = Top soil, TT = Top soil washed with water for five times)

leaves of salinity affected plants was also observed by several workers[14, 15] in groundnut cultivars. The results reported in this study are in close agreement with the findings in green gram plants grown in tsunami affected soil[16]. Reduced level of Chl 'a'pigment in the leaves of 15 day-old black gram plants was also reported in an earlier work[17]. In the present work, the study has been extended up to 40 days to find whether the affected plants recover as the plant grows older. The reason for the reduced synthesis of the pigments may be due to the blockage in the synthesis of photosynthetic pigments which in turn decreased the level of pigments in the leaf [17].

The sugar, starch and phenol contents of the trifoliate leaves were estimated at four stages. A general reduction in the level of reducing and total sugars was observed in all the affected soils. In top soil, a significant reduction by 45% in reducing sugars over control was evident at 20 DAS (Stage II). Significant decrease in the level of total sugars was observed at 20, 30 and 40 DAS in A and T soils. The reductions observed in TT soil-grown plants were not significant. The reduced sugar level in the leaves of salinity grown plants indicates the possibility of high rate of utilization of sugars for increased growth. In TT soils, washing of the top soil removed the salinity to some extent and hence, an insignificant decrease in the level of sugars was noticed (Table 2). Similar to the sugars, the starch contents were significantly decreased in T soils. In affected and top-treated soils, a minimal increase or decrease was observed at all stages. With increasing age, the plants grown in affected soils had recovered and this is quite evident from the data. A similar retarding effect in sugar and starch contents of *Pisum sativum* due to salinity was reported[18].

Salinity of the tsunami affected soil increased the phenol content of the leaves. Under stressed condition, the O.D. phenols increased significantly after 20 days of plant growth. The highest O.D. phenol level was observed at 40 DAS in all the stressed plants. Compared to O.D.

phenol, the total phenols were significantly higher in the leaves of T and TT soil-grown plants.

Since, leaves are the major sites of nitrate utilization, the nitrate contents of the trifoliate leaves were estimated. The nitrate levels were significantly lowered by 23, 24, 27 and 31% at 10, 20, 30 and 40 DAS, respectively, over control (Table 3) in T soil. The magnitude of reduction in the nitrate level was less in other saline soils. The present study substantiated the results of several workers who observed similar reduction in nitrogenous compounds under salinity stress. The nitrite levels were low in all stressed plants. The foliar protein levels were generally very low in all stressed plants at the early stage. Compared to top soil, in affected soil, the toxicity was low and so the protein levels were more or less comparable to that of control at 30 and 40 DAS. At 40 DAS, the protein contents of the trifoliate leaves in TT soils were significantly higher than control. Similar to the present study, a decrease in foliar protein level was observed by several workers[16, 18, 19].

In both A and TT soils, the NR (Nitrate reductase) activity was inhibited at all stages and this reflected a corresponding reduction in the level of soluble proteins (Table 2). The decreased NR activity in A and TT soils may be attributed to reduced nitrate uptake from the soil. Since the synthesis of NR is substrate regulated, reduced uptake of nitrates from the stressed soil affected the synthesis of NR[20].

On the basis of the present investigation, it may be concluded that because of higher salinity in the top soil (T) poor growth and decreased metabolites were evident. The data also revealed that by modifying the soil nature, the performance of the plant can be improved. With increasing age, the plant tries to overcome the unfavourable conditions.

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