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EFFECT OF COMMERCIAL TRIACONTANOL SPRAY ON NITROGEN METABOLISM AND OTHER CELL METABOLITES IN BLACK GRAM (*VIGNA MUNGO* (L.) HEPPER) CULTIVAR T-9

R. SOURECHE @ VENGUIDARAGAVANE* and G. KUMARAVELU

Botany Laboratory, K. M. Centre for P. G. Studies. Pondicherry-605 008, India.
*School of Life Sciences, Pondicherry University, Pondicherry-605 014, India.

Fifteen days-old black gram seedlings (*Vigna mungo* (L.) Hepper) grown in pots were exposed to a single foliar spray of triacontanol (TRIA-commercial formulation **'vipul'**) of different concentrations (0.1, 0.5, 1.0 and 2.0%). The control plants were sprayed with glass distilled water. The effect of TRIA-spray on cellular metabolites was studied at 20,30 and 40 days after sowing (DAS). Tria-treatment did not promote any significant increase in the level of chlorophyll and carotenoid during the early stages of growth of the plant. However at 40 DAS both the photosynthetic pigments were increased at 0.5% concentration. The contents of sugars and starch were also more in 0.5% TRIA-treated plants. The phenol levels were increased prominently at 2.0% TRIA concentration. The amino acid, protein and foliar NR activity in 0.5% TRIA-treatments were consistently more at all stages. The nodule initiation was affected by 1.0 and 2.0% TRIA spray. The NR activity in the nodules showed a decreasing trend with increasing levels of TRIA concentration.

Keywords : Black gram; Cell metabolite changes; Nitrogen metabolism; Triacontanol.

Introduction

The growth of a plant is controlled by several factors including phytohormones. Many synthetic phytohormones have emerged as significant plant growth regulators that can be exploited suitably to overcome physiological constraints. Recently it has come to light that there are some plant constituents with hormone-like activity and they are mainly aliphatic alcohols and aromatics. Triacontanol (TRIA), a saturated straight chain 30-carbon aliphatic alcohol, was first identified as a plant growth regulator[1]. Triacontanol as foliar spray is shown to regulate growth, physiological and biochemical processes in vegetables and cereals, both in natural and synthetic forms and causes an increase in crop yield[2].

Materials and Methods

Viable seeds of black gram (*Vigna mungo* (L.) Hepper) cv. T-9 with uniform size and shape were selected and surface sterilized in 0.1% (w/v) mercuric chloride and washed several times in glass distilled water. Fifteen healthy seeds were sown per pot. For each concentration, triplicates were maintained and the pots were arranged in randomized block design. The plants were maintained under natural greenhouse conditions and irrigated with bore-well water on alternate days.

The plants were exposed to a single spray of 200 ml of TRIA concentrations (0.1, 0.5, 1.0 and 2.0 %) at 15 DAS. Plants sprayed with glass distilled water of same volume were kept as control. The chlorophyll pigments were estimated by using DMSO reagent[3,4] and the carotenoids were determined using standard method[5]. The total sugar contents[6], starch[7], reducing sugar content[8], O.D. Phenol[9], total phenol content[10], amino caid[11] contents were estimated in three stages. Nitrate and Nitrite[12] were estimated from the nodules (40 DAS) as well as in the dried trifoliate leaf sample (5, 15 and 25 DAT) belonging to the second node from the top of the shoot. Fresh leaf samples were used for the estimation of protein content[13] and the activity of NR[14] was determined using standard protocol[15]. The number of nodules was assessed at 35 and 40 DAS.

The statistical anaylsis was done using the Tukey's HSD multiple range test (TMRT) at 5% level of significance[16].

Results and Discussion

The data on photosynthetic pigments (Table 1) reveal that the total chlorophyll and carotenoid contents in the TRIA - treated leaves were not significantly changed at 20 and 30 DAS. An insignificant increase by

Table 1. Effect of TRIA on the total chlorophyll and carotenoid contents of the trifoliate leaves of black gram cv. T-9.

Pigments (mg g-1 fw)	TRIA (% v/v)	DAS		
		20	30	40
Total chlorophyll	control	2.45 a	2.78 a	2.89 ab
	0.1	2.52 a	2.80 a	3.06 a
	0.5	2.67 a	2.99 a	3.28 a
	1.0	2.45 a	2.57 a	2.89 ab
	2.0	2.39 a	2.42 a	2.53 b
Carotenoids	control	0.81 a	0.93 a	0.95 a
	0.1	0.84 a	0.96 a	1.13 b
	0.5	0.86 a	0.99 a	1.16 b
	1.0	0.82 a	0.91 a	1.03 ab
	2.0	0.79 a	0.87 a	0.88 ac

Table 2. Changes in the sugar, starch and phenol contents of the trifoliate leaves of black gram cv T-9 exposed to a single TRIA spray.

Parameter (mg g-1 dw.)	TRIA (% v/v)	DAS		
		20	30	40
Reducing sugar	control	25.38 a	19.33 a	18.63 a
	0.1	25.85 a	18.61 a	17.22 a
	0.5	21.89 b	17.89 a	11.06 b
	1.0	24.81 ab	13.69 b	13.79 c
	2.0	20.81 b	12.18 b	14.31 c
Non-reducing sugar	control	19.39 a	12.82 a	11.58 a
	0.1	23.84 b	21.86 b	28.64 b
	0.5	25.32 b	24.58 b	31.91 b
	1.0	12.86 c	16.59 c	18.51 c
	2.0	16.78 d	15.64 c	13.65 d
Total sugar	control	44.77 a	32.15 a	30.21 a
	0.1	49.69 a	40.47 b	45.86 b
	0.5	47.21 a	42.47 b	42.97 b
	1.0	37.67 b	30.28 a	32.30 a
	2.0	37.59 b	27.82 a	27.96 a
Starch	control	87.56 a	94.66 a	75.38 a
	0.1	94.66 ab	116.39 b	87.31 b
	0.5	97.86 b	125.01 b	96.92 b
	1.0	94.23 ab	81.57 c	66.15 c
	2.0	92.44 ab	78.28 c	59.51 c
O.D Phenols	control	2.71 a	2.36 a	2.77 ab
	0.1	3.22 b	3.19 b	2.71 ab
	0.5	2.83 ab	3.84 c	2.64 a
	1.0	2.88 ab	3.27 b	3.02 ab
	2.0	2.98 ab	3.03 b	3.06 b
Total Phenol	control	6.37 a	7.52 a	4.36 a
	0.1	6.40 a	7.75 a	3.97 ab
	0.5	5.39 b	7.24 a	3.74 b
	1.0	5.09 b	7.41 a	3.83 b
	2.0	8.28 c	10.15 b	6.86 c

Plants were exposed to a single TRIA spray at 15 DAS. 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=10).

Table 3. Changes in the protein, amino acid, nitrate, nitrite content and NR activity in the trifoliate leaves of black gram cv. T-9 exposed to TRIA spray.

Parameter	TRIA (% v/v)	DAS		
		20	30	40
Protein (mg g-1 fw)	control	6.67 a	4.24 a	3.31 a
	0.1	8.02 b	5.17 b	4.49 b
	0.5	9.46 b	6.04 b	4.79 b
	1.0	6.69 a	4.73 a	3.99 c
	2.0	6.74 a	3.12 c	2.74 c
Amino acid (mg g-1 dw)	control	2.26 a	2.47 a	1.86 a
	0.1	2.48 ab	2.72 a	2.14 a
	0.5	2.91 b	3.57 b	2.60 b
	1.0	1.98 a	2.35 a	1.67 a
	2.0	1.97 a	1.85 c	1.40 c
Nitrate (mg g-1 dw)	control	8.69 a	4.64 a	4.51 a
	0.1	7.77 a	2.80 b	2.60 b
	0.5	6.03 b	2.92 b	2.10 b
	1.0	8.81 a	2.85 b	3.60 c
	2.0	8.96 a	4.76 a	4.15 a
Nitrite (mg g-1 dw)	control	1.12 a	0.48 a	0.18 a
	0.1	1.02 a	0.34 b	0.13 b
	0.5	1.16 a	0.32 b	0.17 a
	1.0	1.12 a	0.55 a	0.14 b
	2.0	1.06 a	0.43 a	0.15 b
NR activity (nM NO2 h-1 g-1 fw)	control	923 ac	837 a	633 a
	0.1	1069 b	916 a	656 ab
	0.5	1192 b	1148 b	760 b
	1.0	1073 a	728 a	495 c
	2.0	884 c	483 c	386 d

Values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n-10).

Table 4. Influence of TRIA spray on the nodule number, nitrate, nitrite (mg g-1 dw) and NR activity (nM NO2 h-1 g-1 fw) in black gram cv. T-9.

Parameter	DAS	TRIA (% v/v)				
		control	0.1	0.5	1	2
Nodule number per root system	35	16.79 a	17.14 a	17.5 a	12.59 b	11.85 b
	40	23.60 ab	25.28 ab	26.59 a	22.33 bc	20.05 c
Nitrate	40	3.34 a	2.11 b	2.42 bc	2.69 c	3.46 a
Nitrite	40	0.24 a	0.25 a	0.23 a	0.20 a	0.18 a
NR activity	40	1109 a	863 b	794 b	516 c	352 d

Values within a row followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=10).

8% at 0.5% TRIA and a decrease by 13% in chlorophyll content was observed in 2.0% TRIA treatment at 30 DAS. Similar trend was noted at 40 DAS also. Compared to chlorophyll, the carotenoid contents were not significantly changed in the treated leaves at 20 and 30 DAS. An increase of 19% and 22% was prominent in 0.1 and 0.5% TRIA-treated plants at 40 DAS. Increased chlorophyll content due to TRIA spray was also reported in lemon grass[17], in three leguminous crops[18], in green gram[19] and in *Erythrina*[20,21]. Similar to our study, a no-effect situation on the carotenoid contents immediately after TRIA application was also observed in tree seedlings[22].

Compared to the control plants, the amount of reducing sugars decreased in the TRIA-treated plants in all the stages (Table 2). At 40 DAS, a significant reduction by 41,26 and 33% was observed in 0.5, 1.0 and 2.0% TRIA at 40 DAS. The amount of non-reducing sugars was generally higher in all the TRIA-treated plants except at 20 DAS in 1.0 and 2.0% TRIA. The total sugar contents were increased by 26 and 32% at 30 DAS and by 52 and 42% at 40 DAS respectively in 0.1 and 0.5% TRIA-treated plants. The starch contents were increased significantly by 32 and 29% at 30 and 40 DAS, respectively in 0.5% TRIA sprayed plants. Similar to the results obtained in this study, increased total sugar and starch contents in the lower concentration of TRIA was also reported by several workers[23-26]. An inhibition of starch synthesis was noted in the plants treated with higher concentration of TRIA (Table 2).

The O.D. phenol contents (Table 2) generally increased due to exogenous application of TRIA. At 30 DAS an increase of 35, 63, 39 and 28% was evident in plants sprayed with 0.1, 0.5, 1.0 and 2.0% TRIA respectively. At 40 DAS the O.D. levels were comparable to control in all the treated plants. The total phenol contents (Table 2) were higher in 2.0% TRIA treated plants. Higher phenol contents were also recorded at higher concentrations of TRIA-treated green gram plants[19].

The leaf nitrate contents (Table 3) were significantly decreased in 0.5% TRIA plants by 37 and 53% at 30 and 40 DAS respectively. The leaf nitrite contents (Table 3) were much lowered in 30 and 40 days old plants. Similar trend in the nitrite content was also reported in mangrove seedlings[27] treated with TRIA. In this study, a negative correlation was observed between NR activity and leaf nitrate contents. This kind of correlation was also reported in *Pennisetum*[15] and in green gram[19]. NR activity was suppressed in 2.0% TRIA-treatment in all the stages. The reduced NRA may be responsible for the impairment of nitrogen metabolism and that in turn might affect the growth and development of plants[28].

Among the treatments, only in 0.5% TRIA-treatment, the foliar protein levels were significantly higher by 41, 42 and 45% over control and the amono acids by 29, 45 and 40% in 20, 30 and 40 DAS respectively. Application of 2.0% TRIA considerably reduced the foliar protein content by 26 and 17% at 30 and 40 DAS, respectively and amino acid levels by 25% both at 30 and 40 DAS.

Since the nitrogen fixing efficiency of a legume lies in its nodules, various nodule characteristics were also assessed apart from the leaf estimations. The number of nodules (Table 4) increased in the plants treated with 0.5% TRIA. The initiation of nodules was inhibited by 29 and 15% at 35 and 40 DAS in 2.0% TRIA-treatment. The trend in the nitrate and nitrite levels of the nodules (Table 4) at 40 DAS were similar to those found in leaves. In contrast to the foliar NRA, the nodular NR activity (Table 4) was significantly low in all TRIA-treatments. The above condition is due to the molecular dinitrogen fixation by the nodules which eventually satisfies the nitrogen requirements of the plant without the help of NR enzyme, and hence the nodules show a decreased NRA. This fact about the regulation of nodular NR activity by growth hormones was also

reported earlier[29].

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