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RESEARCH ARTICLE

# Effect of triacontanol on the growth of waterlogged green gram cultivar ARM-1

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## SUMMARY

Seeds of green gram [*Vigna radiata* (Linn.) Wilczek cv. ARM-1] were grown in big plastic containers for waterlogging treatment. Two types of experiments were performed. In experiment I, ten – days old green gram plants were put to waterlogging condition for different periods *viz.,* 3 or 7 days and then analyzed at 15 and 20 DAS, respectively. Control plants were maintained along with the other experimental plants. Various morphometric measurements and biochemical estimations were made at different stages. This experiment confirmed that the green gram plant was able to tolerate 3 days waterlogging period when compared to 7 days period. With this result, another experiment was conducted in 3 days waterlogged plants. These waterlogged plants were sprayed with different concentrations of triacontanol. The results of the first experiment clearly showed a significant increase in pH, conductivity, TDS and salinity levels of the stagnant water when compared to control. The conductivity levels were almost doubled in 7 days stagnant water compared to 3 days. In 7 days of waterlogged plants, the root, shoot and the plant length was significantly increased over control plants. Waterlogging stress promoted the formation of adventitious roots and aerenchymatous tissue. Longer duration of waterlogging period significantly promoted the production of aerial roots. There was a decrease in the number of nodules, total leaf area in waterlogged plant. The root, shoot and plant biomass was significantly decreased in 7 days waterlogged cultivar. The photosynthetic pigments, leaf protein, sugars, starch and phenol contents were significantly lowered by water treatment in 7 days old plants.

***Key Words*** **:** Green gram, Waterlogging, Flooding, TRIA

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Waterlogging generally decreases growth and yield of legume crops. Water stress in *Asplenium nidus* decreased all growth parameters and some physiological responses. Under water stress condition growth of root increased when compared to normal optimum condition (Ainuddin and Nur Najwa, 2009). Jackson *et al.* (2009) confirmed the recovery of the plants during waterlogging seems to be associated with morphological alterations, such as development of adventitious roots and aerenchyma tissue, and with the maintenance of neutral amino acids in roots. Waterlogging had significant effect on plant height, stem diameter, leaf area, biomass production and root growth (Changdee *et al.,* 2009). Viviane *et al.* (2010) found that flood stress induced changes in pigment and protein contents and in photochemical efficiency of thylakoid membranes of chloroplasts and induce changes in genetic, morphological and physiological processes, altering the growth and development of plants. The Foliar application of TRIA at 0.2 per cent -0.5 per cent significantly promoted the plant height, fresh mass, and contents of chlorophylls, saccharides, starch, soluble proteins, amino acid and phenols was confirmed by Muthuchelian *et al.* (1995), Kumaravelu *et al.* (2000),

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Chowdhury *et al.* (2009), Vijayakumari (2010).

## MATERIALS AND METHODS

**Experimental plant :**

Green gram [*Vigna radiata* (L.) Wilczek] cultivar ARM-1 was selected for the experiment. The seeds were obtained from oil seed farm and research centre, Tindivanam, Tamilnadu. Viable seeds of uniform size and colour were selected for the study.

For the present study, treatment solutions of 0.1, 0.2, 0.5 and 1.0 per cent were prepared from pure triacontanol. To this preparation, tween-20 (0.1%w/v) was added as surfactant. Glass distilled water was used for control experiments.

The surface sterilized seeds were sown in large plastic cups for the experimental study. Two types of experiments were performed.

**Experiment – I :**

Ten – days old green gram plants were put to waterlogging condition for different periods *viz.,* 3 or 7 days and then analyzed at 15 and 20 DAS, respectively. Control plants were maintained along with the experimental plants. Various morphometric measurements and biochemical estimations were made at different stages.

The following parameters were studied. Quality of flooded water, root, shoot and plant length, R/S ratio, number of lateral roots and nodules, number of adventitious roots, plant biomass - fresh and dry mass of plant parts; S/R ratio, total leaf area, photosynthetic pigments – Chl. 'a', 'b', a/b ratio, total chlorophylls and carotenoids, total soluble foliar proteins, sugars, starch and phenols.

**Experiment II :**

The effect of triacontanol on the subsequent growth of the waterlogged green gram cultivar was studied. Ten – days old green gram plants were put to waterlogging condition for 3 days and then they were sprayed with different concentrations of TRIA *viz.,* 0.1, 0.2, 0.5, and 1.0 per cent at 15, 25 and 35 DAS. They were then analyzed for various growth parameters at 20, 30 and 40 DAS as was done in experiment I.

**Pot culture studies :**

Viable seeds were selected and surface sterilized in 0.1 per cent (w/v) mercuric chloride and washed in glass distilled water several times. Plastic containers (12 cm x 12 cm) filled with a mixture of sand, red soil and farmyard manure (2:1:1 v/v) were used for raising the plants. Fifteen healthy seeds were selected and sown at equal distance at a depth of 2cm in each container. The arrangements of containers were in the form of randomized block design for different treatments and these were replicated three times. The containers were kept under natural green house conditions (day- temperature maximum 38 ± $2^0$C, minimum night – temperature 18±$2^0$C, relative humidity 60±$5^0$C; maximum irradiance (PAR0 1,400 µ mol/$m^{-2}s^{-1}$; photoperiod (12-14h). TRIA was sprayed through a glass sprayer containing nozzle of pore size 0.35mm. With the help of a compressor, TRIA was showered on the foliage for 5min. about 100ml of TRIA concentration was used in each shower. Care was taken to wet the foliage completely. Control plants were sprayed with glass distilled water. Different concentrations of TRIA *viz.,* 0.1, 0.2, 0.5, and 1.0 per cent at 15, 25 and 35 DAS were sprayed. The measurements for growth parameters *viz.,* root and shoot length and plant height were made. The plant parts were weighted in Anamed Digital balance for fresh weight. They were dried in an oven at $80^0$C for 48h and dry mass measurements were recorded. Besides these, the R/S ratio, S/R ratio (Racey *et al.,* 1983), number of lateral roots, nodules were also monitored at 20, 30 and 40 DAS. Total leaf area, potosynthetic pigments – Chl. 'a', 'b', a/b ratio, total chlorophylls and carotenoids, total soluble foliar proteins, sugars, starch and phenols were also examined. The results of the experiment were tested by a multiple range testing programme. Tukey's Multiple Range Test (TMRT) was applied for the experimental data at 5 per cent level of significance (Zar, 1984).

## RESULTS AND DISCUSSION

The results clearly showed significant changes in the pH, conductivity, TDS and salinity levels of stagnant water. A significant increase in the conductivity, TDS and salinity levels of 7-days stagnant water was noticed when compared to 3-days flooded water. In flooded condition the water became alkaline *i.e.* approximately 9.5. The conductivity and TDS levels were almost doubled when compared to the control (Table 1).

**Table 1 : Changes in the quality of water after flooding of green gram cultivar ARM-1**

| Parameters | Stages | | | |
|---|---|---|---|---|
| | 15 DAS | | 20 DAS | |
| | I | II | III | IV |
| pH | 7.87 a | 9.56 b | 7.56 a | 9.41 b |
| Temperature | 26.2°C a | 26.3°C a | 28.4°C a | 30.2°C a |
| Conductivity(mS) | 1.35 a | 2.17 b | 1.41 a | 2.68 b |
| TDS(mS) | 0.91 a | 1.44 b | 0.93 a | 1.77 b |
| Salinity(ppt) | 1.0 a | 1.2 b | 1.5 a | 2.0 b |

Ten days-old green gram plants were subjected to waterlogging. I and II correspond to control and 3 days waterlogged condition. III and IV correspond to control and 7 days waterlogged condition. Within a row, values followed by different letters are significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

Flooding stress promoted the formation of aerial adventitious roots that contain aerenchyma. These roots of flooded plants tend to become negatively geotropic. An average of 10 and 16 adventitious roots was recorded at 3 and 7- days, respectively in waterlogged plants. Very few adventitious roots were found in the control. Waterlogged condition significantly inhibited the formation of nodules. In the initial stage, nearly 50 per cent reduction in the formation of nodules was evident. The total leaf area was reduced only marginally over control in 3-days waterlogged plants. When the waterlogging period was increased to 7-days, there was a significant decrease in the total leaf area by 24 per cent over control (Table 2).

**Table 2 : Changes in the growth characteristics, number of roots and total leaf area ($cm^2$) of green gram cv. ARM-1 due to waterlogging stress**

| Parameters | Stages | | | |
|---|---|---|---|---|
| | 15 DAS | | 20 DAS | |
| | I | II | III | IV |
| Root length | 8.75 a | 10.65 b | 9.05 a | 8.82 a |
| Shoot length | 10.21 a | 10.93 a | 12.66 a | 13.90 a |
| Plant height | 18.96 a | 21.58 a | 21.70 a | 21.72 a |
| R/S ratio | 0.85 a | 0.97 a | 0.71 a | 0.63 a |
| Aerial root | 1.00 a | 9.50 b | 2.5 a | 16.20 b |
| Nodules | 2.50 a | 1.25 b | 4.00 a | 3.20 b |
| Total leaf area | 14.42 a | 14.19 a | 25.43 a | 19.30 b |

Ten days-old green gram plants were subjected to waterlogging. I and II correspond to control and 3 days waterlogged condition. III and IV correspond to control and 7 days waterlogged condition. Within a row, values followed by different letters are significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

The root, shoot and the plant fresh biomass of 3 and 7 days waterlogged plants were phenomenally increased when compared to control. The fresh biomass of all the parts of waterlogged plants was almost doubled over control (Table 3). The dry weight of the root, shoot and whole plant in 3 days waterlogged plants were almost similar to that of control, whereas in 7 days waterlogged plants, the root dry mass was increased significantly by 32 per cent over control. In shoot, increased waterlogging period reduced the shoot dry mass by 23 per cent over control plants. The plant dry mass was decreased by 10 per cent over control under increased waterlogging condition. Compared to the control plants, the S/R ratio was significantly decreased by 17 per cent over control in 3-days of waterlogging treatment. A significant reduction by 41 per cent over control was observed in 7-days waterlogged plants.

In addition to the morphometric measurements,

**Table 3 : Effect of waterlogging on the biomass contents of green gram cv. ARM-1**

| Biomass (g) | Stages | | | |
|---|---|---|---|---|
| | 15 DAS | | 20 DAS | |
| | I | II | III | IV |
| Root (fw) | 0.15 a | 0.37 b | 0.26 a | 0.57 b |
| Shoot (fw) | 0.16 a | 0.38 b | 0.38 a | 0.58 b |
| Plant (fw) | 0 .31 a | 0.75 b | 0.64 a | 1.15 b |
| Root (dw) | 0.030 a | 0.032 a | 0.034 a | 0.045 b |
| Shoot (dw) | 0.063 a | 0.056 a | 0.104 a | 0.080 b |
| Plant (dw) | 0.093 a | 0.088 a | 0.138 a | 0.125 a |
| S/R ratio | 2.10 a | 1.75 b | 3.06 a | 1.8 b |

Ten days-old green gram plants were subjected to waterlogging. I and II correspond to control and 3 days waterlogged condition. III and IV correspond to control and 7 days waterlogged condition. Within a row, values followed by different letters are significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

biochemical analysis was done in both types of waterlogged plants. The photosynthetic pigment content of the first trifoliate leaf was estimated at 15 DAS in 3-days waterlogged plants and at 20 DAS in 7-days waterlogged plants. Generally, the photosynthetic green pigment contents of the leaf were significantly decreased due to soil waterlogging. The Chl 'a 'contents of the leaf in 3-days waterlogged plants was marginally decreased compared to control. The Chl 'b' and total chlorophyll levels were significantly decreased by 39 and 19 per cent , respectively over control plants in 3-days waterlogged condition. Increased number of days of flooding did not affect the synthesis of pigments significantly. In contrast to the green pigments, the carotenoid pigments were insignificantly reduced by waterlogging treatment. Nearly 10 per cent decrease was noted in both types of waterlogged plants.

The foliar protein content of the trifoliate leaves was significantly reduced by 23 per cent over control plants in 3-days waterlogged plants. When the flooding period was increased to 7-days, only 11 per cent decrease in protein level was found. Flooding stress induced changes in the protein content of the leaves. The reducing sugars in the trifoliate leaves were almost similar to the control. Insignificant increases or decreases were recorded in both types of waterlogged plants. The total sugars were significantly lowered by 37 and 38 per cent in 3 and 7-days waterlogged plants, respectively over control plants. The starch contents of the leaf were significantly increased by 46 per cent over control in 3-days of waterlogging. But when the waterlogging period was increased to 7 days, there was a significant decrease by 30 per cent over control. Waterlogging stress induced more production of O.D. phenols. There was a significant increase in the level of O.D. phenols due to stress factor. In 7

days waterlogged plants the total phenols were almost doubled when compared to the control plants (Table 4). The results of the first experiment confirmed that the green gram cultivar ARM-1 was more sensitive to 7 days of waterlogged condition. Waterlogging for 3 days did not significantly affect the growth of the cultivar.

**Table 4 : Changes in protein (mg $g^{-1}$ fw), sugar, starch, phenol contents (mg $g^{-1}$ dw) and total chlorophyll and carotenoid contents of the trifoliate leaves of green gram cv. ARM-1 due to waterlogging stress**

| Parameters | Stages | | | |
|---|---|---|---|---|
| | 15 DAS | | 20 DAS | |
| | I | II | III | IV |
| Protein | 2.09 a | 1.61 b | 6.49 a | 5.75 a |
| Reducing sugar | 16.87 a | 16.37 a | 14.88 a | 16.11 a |
| Starch | 62.76 a | 91.84 b | 102.92 a | 72.46 b |
| O. D. Phenol | 1.48 a | 1.85 b | 1.70 a | 3.85 b |
| Total Phenol | 3.55 a | 3.74 a | 3.06 a | 6.02 b |
| Total Chlorophyll | 0.97 a | 0.79 b | 1.01 a | 1.06 a |
| Carotenoids | 1.10 a | 1.05 a | 1.17 a | 1.06 a |

Ten days-old green gram plants were subjected to waterlogging. I and II correspond to control and 3 days waterlogged condition. III and IV correspond to control and 7 days waterlogged condition. Within arow, values followed by different letters are significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

**Experiments II :**

The results of the first experiment indicated that the green gram cultivar ARM-1 was less sensitive to short duration of flooding stress. Three days of waterlogged green gram cultivar was chosen for the second experiment where different concentrations of TRIA was foliar sprayed to see the ameliorating effect of the plant growth regulator on this cultivar.

In this experiment the green gram cultivar ARM-1 was allowed to grow in plastic cups for a period of 10 days and then they were subjected to flooding stress for a period of 3 days. These waterlogged plants were allowed for air adaptation and they were sprayed with different concentrations of TRIA *viz.,* 0.1, 0.2, 0.5 and 1.0 per cent at 15, 25 and 35 DAS. To see the effect of TRIA spray on these waterlogged plants, various morphometric measurements and biochemical changes were monitored at 20, 30 and 40 DAS. Waterlogged plants without TRIA application was maintained as control.

TRIA treatment insignificantly increased the root length in all the concentrations except at 0.5 per cent, where a significant increase by 20 per cent in the length of the root over control was noted at stage I and II (Table 5). TRIA of 1.0 per cent did not promote the length of the root. Compared to root growth, the shoot growth was significantly increased by 23 and 22 per cent at 0.2 and 0.5 per cent TRIA respectively at stage I. More or less the same trend was observed in subsequent stages also. TRIA of 0.1 and 1.0 per cent did not influence shoot growth significantly. Due to TRIA applications the plant length was increased either significantly or insignificantly when compared to control. Plant height was significantly higher at 0.2 and 0.5 per cent TRIA treatment during the early stage but in the later stages the plant height was similar to the control. The R/S ratios were almost comparable to control waterlogged plants. TRIA-treatment did not change the R/S ratios.

**Table 5 : Changes in growth characteristics of green gram cv. ARM-1 due to waterlogging stress**

| Growth parameters (cm) | Stages | | | |
|---|---|---|---|---|
| | Treatment | I | II | III |
| Root length | C | 7.4 a | 7.8 a | 8.9 a |
| | 0.1% | 7.8 a | 8.3 a | 9.1 a |
| | 0.2% | 8.5 ab | 9.1 ab | 9.5 a |
| | 0.5% | 8.9 b | 9.3 b | 9.6 a |
| | 1.0% | 7.7 a | 7.9 a | 8.2 a |
| Shoot length | C | 11.2 a | 12.3 a | 13.3 a |
| | 0.1% | 12.6 ab | 13.2 a | 13.7 ab |
| | 0.2% | 13.8 b | 14.1 ab | 15.1 ab |
| | 0.5% | 13.7 b | 14.4 b | 15.6 b |
| | 1.0% | 11.6 a | 12.6 a | 14.2 ab |
| Plant height | C | 18.6 a | 20.1 a | 22.2 a |
| | 0.1% | 20.4 ab | 21.5 ab | 22.8 a |
| | 0.2% | 22.3 b | 23.2 ab | 24.6 a |
| | 0.5% | 22.6 b | 23.7 b | 25.2 a |
| | 1.0% | 19.3 a | 20.5 a | 22.4 a |
| R/S ratio | C | 0.66 a | 0.59 a | 0.67 a |
| | 0.1% | 0.62 a | 0.59 a | 0.68 a |
| | 0.2% | 0.62 a | 0.63 a | 0.63 a |
| | 0.5% | 0.65 a | 0.65 a | 0.62 a |
| | 1.0% | 0.66 a | 0.63 a | 0.58 a |

Ten days old green gram cultivar ARM-1 was subjected to waterlogging stress. TRIA of different concentrations was sprayed at 15, 25 and 35 DAS. Stages I, II and III correspond to 20, 30 and 40 days, respectively. 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).

The influence of TRIA application on the initiation of aerial roots, lateral roots and nodules in waterlogged plants was recorded at three stages. TRIA application with different concentrations significantly promoted the production of lateral roots. At 0.5 per cent TRIA – treatment, maximum increase in the number of lateral roots was noticed. Termination of flooding treatment and subsequent application with different concentrations of TRIA significantly promoted the initiation of nodules. A maximum of 36 per cent increase in the number of nodules over control waterlogged plants was observed in all the stages. At 0.1 and 1.0 per cent TRIA application, the number was comparable to control.

The fresh biomass of the waterlogged plants due to TRIA

application was significantly increased at 0.5 per cent . In the initial stage, the first TRIA application promoted the root, shoot and the plant fresh biomass. In the last stage observed at 40 DAS, by the combined effect of three applications of TRIA, the fresh biomass of the shoot and the whole plant increased significantly at 0.2 and 0.5 per cent (Table 6). The dry mass of the root, shoot and plant was significantly increased by 35, 15 and 19 per cent , respectively over control plants at stage I. At stage III, three applications of TRIA of 0.5 per cent significantly promoted the accumulation of dry matter in all the plant parts. TRIA of 0.2 per cent promoted the dry mass in the shoot and the whole plant (Table 6). A slight inhibition in the growth and dry mass content was noticed in waterlogged plants treated with TRIA of 1.0 per cent . The S/R ratios were almost comparable to control at stage I and II in 0.1, 0.2, and 0.5 per cent TRIA-treated plants. At stage III, the R/S ratios were significantly higher by 46 and 62 per cent than control at 0.2 and 0.5 per cent, respectively.

**Table 6 : Effect of TRIA of different concentrations on the the biomass content (fw) of waterlogged green gram cv. ARM-1**

| Biomass (g) | | Stages | | |
|---|---|---|---|---|
| | Treatments | I | II | III |
| Plant (fw) | C | 0.61 a | 0.71 a | 0.93 a |
| | 0.1% | 0.67 ab | 0.70 a | 0.94 a |
| | 0.2% | 0.65 ab | 0.79 a | 1.35 b |
| | 0.5% | 0.72 b | 0.73 a | 1.59 b |
| | 1.0% | 0.57 a | 0.60 b | 0.94 a |
| Plant (dw) | C | 0.098 ac | 0.116 a | 0.156 a |
| | 0.1% | 0.108 ab | 0.121 ab | 0.155 a |
| | 0.2% | 0.114 b | 0.134 b | 0.223 b |
| | 0.5% | 0.117 b | 0.132 ab | 0.265 b |
| | 1.0% | 0.085 c | 0.098 c | 0.148 a |
| Shoot / root | C | 3.26 a | 2.31 a | 2.25 a |
| ratio | 0.1% | 3.00 a | 2.36 a | 2.16 a |
| | 0.2% | 2.93 a | 2.27 ab | 3.29 b |
| | 0.5% | 2.78 a | 2.14 ab | 3.64 b |
| | 1.0% | 3.90 b | 1.97 b | 2.52 a |

Ten days old green gram cultivar ARM-1 was subjected to waterlogging stress. TRIA of different concentrations was sprayed at 15, 25 and 35 DAS. Stages I, II and III correspond to 20, 30 and 40 days, respectively. Within acolumn, values followed by different letters are significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

respectively in 0.2 and 0.5 per cent TRIA – treated plants. In 1.0 per cent TRIA-sprayed plants, the starch contents of the leaves were almost comparable to control.

The phenol contents of the leaves of the experimental

At stage I, the Chl 'a' and 'b' levels were significantly higher than the control plants almost in all the TRIA concentrations except at 1.0 per cent . The Chl 'a' contents in the leaves of green gram cultivar was significantly higher by 21, 31 and 30 per cent at 0.1, 0.2 and 0.5 per cent TRIA-treatments. Similarly at stage I, the Chl 'b" contents were significantly increased by 18, 23 and 25 per cent over control at 0.1, 0.2 and 0.5 per cent , respectively. At 1.0 per cent TRIA treatment, the values are comparable to control. A similar trend was observed at stage III. At 40 DAS, TRIA of 1.0 per cent decreased the content of chlorophylls in leaves. The chl 'a' content was decreased significantly by 22 per cent over control at 1.0 per cent TRIA-treatment. The chl a/b ratios were comparable to control plants at stage I. However significant increases in the chl a/b ratio was noted at 0.5 per cent TRIA- treatment at stage II and III.

The total chlorophyll levels in the leaf of TRIA-treated plants were significantly increased at stage I and III. TRIA of 0.5 per cent significantly promoted the total chlorophyll contents by 28, 61 and 41 per cent at stage I, II and III, respectively over control plants (Table 7). A maximum decrease by 18 per cent over control was noted at 1.0 per cent TRIA-treatment in stage III. The carotenoid contents of the leaves of waterlogged plants treated with TRIA of 0.2 and 0.5 per cent were significantly higher than the control. At 0.5 per cent a maximum increase by 43, 88 and 62 per cent over control was recorded at stage I, II and III, respectively. At 0.2 per cent , the carotenoid levels were increased by 40, 29 and 36 per cent at stage I, II and III, respectively. At 1.0 per cent, the carotenoid contents of the leaves were comparable to control.

At stage I, the reducing sugars in all the treated plants were comparable to the waterlogged control. In the subsequent stages, due to additional TRIA spray the level of reducing sugars increased significantly at 0.2 and 0.5 per cent . An increase in the level of reducing sugars by 59 and 39 per cent at stage II in 0.2 and 0.5 per cent TRIA, respectively over control was evident. In these concentrations the reducing sugar contents of the leaves were almost doubled when compared to waterlogged control plants. In all the TRIA-sprayed plants, the reducing sugars were significantly higher than the control. A reverse trend was noted in the case of non-reducing sugars. TRIA-treated plants showed a significant increase in the content of total sugars in the leaves compared to control. An increase of 25, 58, 75 and 48 per cent over control was observed at 0.1, 0.2. 0.5 and 1.0 per cent, respectively, in 20-days old plants. In the subsequent stages a significant increase in total sugars was evident only at 0.5 per cent TRIA-sprayed plants.

The level of starches in 0.2 and 0.5 per cent TRIA-sprayed plants were significantly higher than control in all the stages. Increased number of TRIA spray promoted more synthesis of starch. The starch levels were increased by 58 and 44 per cent at stage II and 30 and 27 per cent at stage III,

plants were estimated at 20, 30 and 40 DAS. The results are presented in Table 7.

**Table 7 : Changes in protein (mgg$^{-1}$ fw), sugar and starch (mgg$^{-1}$ dw) chlorophylls and carotenoid contents (mg g$^{-1}$ fw) of green gram cv. ARM-1 due to waterlogging stress after applying TRIA of various concentrations**

| Pigments | | Stages | | |
|---|---|---|---|---|
| | Treatments | I | II | III |
| Protein | C | 1.11 a | 1.95 a | 1.12 a |
| | 0.1% | 2.27 b | 2.42 b | 1.42 b |
| | 0.2% | 2.58 b | 2.02 a | 1.50 b |
| | 0.5% | 3.03 c | 2.71 b | 2.21 c |
| | 1.0% | 2.09 b | 1.04 c | 1.15 a |
| Starch | C | 72.46 a | 69.07 a | 85.84 a |
| | 0.1% | 72.00 a | 91.85 b | 97.85 ac |
| | 0.2% | 85.85 bc | 109.15 b | 111.23 b |
| | 0.5% | 95.54 b | 99.46 b | 109.15 bc |
| | 1.0% | 80.46 ac | 87.23 b | 90.46 a |
| Reducing sugar | C | 17.86 a | 16.87 a | 16.38 a |
| | 0.1% | 16.38 a | 16.15 a | 20.08 b |
| | 0.2% | 18.85 a | 26.87 b | 34.06 c |
| | 0.5% | 19.10 a | 23.39 b | 29.57 c |
| | 1.0% | 17.61 a | 19.15 a | 21.78 b |
| Chlorophyll 'a' | C | 0.67 a | 0.75 a | 1.02 a |
| | 0.1% | 0.81 b | 0.80 a | 1.08 a |
| | 0.2% | 0.88 b | 0.85 a | 1.47 b |
| | 0.5% | 0.87 b | 1.34 b | 1.52 b |
| | 1.0% | 0.71 a | 0.81 a | 0.80 c |
| Chlorophyll 'b' | C | 0.40 a | 0.50 a | 0.49 a |
| | 0.1% | 0.47 b | 0.42 b | 0.45 a |
| | 0.2% | 0.49 b | 0.53 a | 0.59 b |
| | 0.5% | 0.50 b | 0.67 c | 0.61 b |
| | 1.0% | 0.37 a | 0.45 ab | 0.44 a |
| Tatal chlorophyll | C | 1.07 a | 1.25 a | 1.51 a |
| | 0.1% | 1.28 b | 1.22 a | 1.53 a |
| | 0.2% | 1.37 b | 1.38 a | 2.06 b |
| | 0.5% | 1.37 b | 2.01 b | 2.13 b |
| | 1.0% | 1.08 a | 1.26 a | 1.24 c |
| Carotenoids | C | 0.083 a | 0.080 a | 0.074 a |
| | 0.1% | 0.081 a | 0.086 ab | 0.067 a |
| | 0.2% | 0.116 b | 0.103 b | 0.101 b |
| | 0.5% | 0.119 b | 0.150 c | 0.120 c |
| | 1.0% | 0.086 a | 0.082 a | 0.073 a |

Ten days old green gram cultivar ARM-1 was subjected to waterlogging stress. TRIA of different concentrations was sprayed at 15, 25 and 35 DAS. Stages I, II and III correspond to 20, 30 and 40 days, respectively. 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).

At stage I, the O.D phenols in the TRIA-treated plants were almost comparable to the control plants. A significant increase in the level of O.D. phenols was observed at stage II. In the last stage the O.D. phenols were decreased by 18, 19, 15 and 21 per cent over control at 0.1, 0.2, 0.5 and 1.0 per cent , respectively. The total phenols were significantly increased at 0.5 per cent TRIA by 39, 27 and 41 per cent at stage I, II and III, respectively over control. At stage II, the total phenols were significantly increased in all TRIA- treated plants except at 0.1 per cent. A similar trend was observed at stage III.

Changes in the content of nucleic acids were observed in all the three stages. The results are presented in Table 8. The DNA levels in the trifoliate leaves of green gram at stage I were significantly low when compared to control plants. In the subsequent stages, the DNA contents of the leaves were significantly increased due to TRIA-treatment. A maximum increase by 28 and 24 per cent over control was evident at 0.5 per cent TRIA-treatment. In contrast to the DNA levels, the RNA contents were significantly higher than control in all the TRIA-treatments. TRIA of 0.5 per cent significantly promoted the RNA synthesis when compared to other treatments.

The effect of TRIA on the leaf protein content of waterlogged plants was observed in all the stages and the results are presented in Table 8. At stage I, different concentrations of TRIA-treatment significantly increased the protein content of the leaves. The leaf proteins were almost doubled when compared to control. When the frequency of TRIA spray was increased, higher leaf protein was evident at 0.1, 0.2 and 0.5 per cent. Generally the leaf protein was decreased at 1.0 per cent TRIA spray. Out of the four TRIA concentrations used, maximum leaf protein content was observed at 0.5 per cent.

**Table 8 : Changes in nucleic acid content (µg$^{-1}$ fw) of green gram cv. ARM-1 due to waterlogging stress after application of TRIA of various concentrations**

| Parameters | | Stages | | |
|---|---|---|---|---|
| | Treatments | I | II | III |
| DNA | C | 52.30 a | 46.90 a | 68.60 a |
| | 0.1% | 40.08 b | 55.40 bc | 84.60 b |
| | 0.2% | 45.33 b | 59.12 b | 82.40 b |
| | 0.5% | 52.30 a | 60.12 b | 85.00 b |
| | 1.0% | 42.50 b | 50.40 ac | 71.56 a |
| RNA | C | 276.79 a | 306.79 a | 321.24 a |
| | 0.1% | 324.51 b | 309.46 a | 382.05 a |
| | 0.2% | 371.36 b | 433.83 b | 440.00 b |
| | 0.5% | 373.08 b | 483.56 b | 496.75 b |
| | 1.0% | 282.93 a | 290.12 a | 303.40 a |

Ten days old green gram cultivar ARM-1 was subjected to waterlogging stress. TRIA of different concentrations was sprayed at 15, 25 and 35 DAS. Stages I, II and III correspond to 20, 30 and 40 days, respectively. Within a column, values followed by different lettersare significantly different according to Tukey's HSD Multiple Range Test (TMRT) at 5% level of significance (n=15).

From this study, we concluded that the green gram cultivar ARM-1 was able to tolerate 3 days waterlogged condition. The morphological and physiological traits were lowered due to water stress. Amelioration was observed mostly

at 0.2 and 0.5 per cent TRIA spray. Application of three sprays improved the performance of the plant growth.

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