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Effect of ultra sound on physiological parameters in cultivars of V*igna radiata*

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ABSTRACT

In the present study an attempt has been made to study the influence of ultrasound (25 Kc sec^{-1}) waves of different durations on physiological variables like photosynthetic rate, inter cellular CO_2 concentrations, stomatal conductance, transpiration rate, water use efficiency and total chlorophyll content, a content, b content, a/b ratios, DCPIP, protein content, nitrate and nitrite reductase and glutamin synthetase. Photosynthetic rate, inter cellular CO_2 concentration, transpiration rates, total chlorophyll content, total protein content have increased and DCPIP levels reduced up to 35 days, whereas, water use efficiency decreased and glutamine synthatase activity increased between 25 and 35 days of treatment of ultrasound in all three cultivars.

Keywords: photosynthetic rate, intracellular Co2 concentration, stomatal conductance, transpiration rate, water use efficiency, chlorophyll, DCPIP, reductase.

INTRODUCTION

Green gram (Mungbean) *Vigna radiata* (L.) is one of the most important pulse crops widely grown in almost all parts of the country during rabi, kharif and summer seasons. A large number of high yielding varieties suitable for India are identified through classical breeding programmes and varietal trials and resulted in no improvement of production or productivity because of their location specificity and susceptible to major diseases like powdery mildew, *Cercospora* leaf spot and yellow mosaic virus diseases and also susceptible to water logging. The varieties are photosensitive. If these varieties could not be sown in optimum time, yields will be reduced. High yielding varieties suitable for upland cultivation are not suitable for rise because most of the varieties are of long duration. They are subjected to water stress during maturity period in *Rabi* season when grown under receding moisture condition. During Rabi, green gram has competition from black gram which has higher yield potential. Green gram maturity with the monsoon rains during August and September months and the seed sprout in the pod itself resulting in heavy losses.

Evidence accumulated during the last 25 to 30 years, particularly work carried out in India and abroad tends to show that small radiation doses can be advantageously used to stimulate growth in plants for increasing yield potential. Seeds of many agricultural crops including several varieties of rice, maize, sorghum, pearl millet and groundnut irradiated with X-rays, gamma rays and pile neutrons have been shown to induce such effects as variation in the leaf shape and size, alteration of stomatal frequency, development of lawns and pigments, twin or fused flowers, variation in the morphology of seeds and their colour have been carried out[1]. Irradiation of seeds of many agricultural crops with ultrasound have been shown to cause enhancement of germination, growth and yield[2]. Several major internal and external factors that determine the bioproductivity of plant species among the internal factors photosynthesis and water use efficiency are the most important ones that contribute to the biomass productivity. Since photosynthetic efficiency ultimately determines biomass yields, study of physiological basis of

yield improvement assumes importance Therefore, in the present study an attempt has been made to study the influence of ultrasound (25 Kc sec^{-1}) waves of different durations on physiological variables like Gas Exchange Characteristics including Net photosynthetic rate (P_N), Inter cellular CO_2 concentrations (Ci), Stomatal conductance (GS), Transpiration rate (E), Water use efficiency (WUE); and Total chlorophyll content, a content, b content, a/b ratios, DCPIP Photo reduction, Total protein content, Nitrate reductase, Nitrite reductase. Glutamin Synthetase

MATERIALS AND METHODS

The seeds of three cultivars (LGG 407, LGG 410 and LGG 450) of green gram ***(mungbean) Vigna radiata* (L.)** were obtained from Andhra Pradesh Agricultural Research Station, Tirupati, Andhra Pradesh. A batch of 50 grams seeds of each variety were weighed and transferred into muslin cloth bags. After transferring the seeds into the bags, the open ends of the bags were tied with thread. The bags were then soaked with distilled water for 3 hours. The bags containing seeds were then subjected to treatment with ultrasound (25 Kc sec^{-1}) at intervals of 15, 30 and 60 minutes. The treated and untreated seeds were then sown in field plots of Sri Venkateswara University Botanical garden, Tirupati. Each field plot measured a size of 3 x 2 square meters.

The experimental layout was Randomized Block Design and replicated 4 times for each duration of treatment and control for each variety. At seedlings stage after thinning, the rows spaced 10.5 x 10 cm was maintained as far as possible. All package of agricultural practices of mungbean cultivation were adopted. The crop was raised during the *Kharif – Rabi* and *summer* seasons of the years 1996 to 1998. Results on physiological parameters represent the average data collected for the two years.

Leaf gas exchange measurements were made on fully expanded 5^{th} leaf of 3 green gram cultivars 25, 35, 45 and 55 Days after sowing in control and treatment groups. Plants raised from treated seeds for duration of 15, 30 and 60 minutes. The gas exchange characteristics included net photosynthetic rate (P_N), inter cellular CO_2 concentration (Ci), stomatal conductance (GS), transpiration rate (E) and water use efficiency (WUE). The gas exchange measurements were made on The LCA-3 of Analytical Development Corporation, England, was used for the measurement of transpiration and photosynthesis.

Chlorophyll content of the fully expanded leaves was determined according to the method of Arnon (1949)[3]; DCPIP Photo reduction using spectrophotometer as per Amstrong, 1964[4]; total Protein content following Lowry *et al.*, (1951)[5]; Nitrate Reductase and Nitrite Reductase using spectrophotometer following Lin and Kao, 1980[6] and Losada and Paniqul, 1971[7] and Glutamine Synthetase activity was determined as per the method of Oneal and Joy (1973)[8].

Statistical Analysis:
All quantitative data were expressed in mean and standard deviation.Two way analysis of variance was used to draw inferences.

RESULTS AND DISCUSSION

The photosynthetic rates showed an increase with each increase in durations of treatment than the control plant in all the cultivars on 25-35 DAS. The photosynthetic rate started declining on 45 and 55 DAS both in control and treatments. The differences in photosynthetic rate between the control and plants received seed treatments are marginal. The photosynthetic rates showed an increase in net photosynthetic rate with each increase in the duration of treatment than the control plants in all the cultivars on 25-35 DAS. The decrease after 35 DAS may be attributed to the decrease in stomatal conductance (Gs) and intercellular CO_2 concentration (C_i).

The Intercellular CO_2 concentration values of the leaves in control and treatments on 25 - 35 DAS are consistent with the net photosynthetic values in all the cultivars since net photosynthetic rate and intercellular CO_2 concentration are closely related to each other. The intercellular CO_2 concentration was found to be more at all the treatment durations and cultivars when compared to control. Stomatal conductance is reciprocal of resistance. Ultrasound treatment for different durations caused an increase in stomatal conductance of leaves in all the three cultivars on 25 and 35 DAS. The stomatal conductance showed a gradual decline from 45 DAS both in control and treatments. This decrease finds its relationship with net photosynthetic rate is also decreased both in control and treatments. However, the differences in intercellular CO_2 concentration due to treatments are narrow.

The transpiration rates increased in control and treatments up to 35 DAS like any other gas exchange characteristics and decreased from 45 DAS and 55 DAS both in control and treatments. The decrease is consist with decrease in net photosynthetic rate; inter cellular CO_2 concentration and stomatal conductance both in control and treatments on 45 and 55 DAS after sowing. In conclusion, the differences in the rate of transpiration are marginal.

Water use efficiency (WUE) is predominantly regulated by stomatal conductance (Gs) and or mesophyll efficiency that inturn regulates carbon assimilation. Ultrasound treatment caused decrease in water use efficiency to various degrees in the three cultivars between 25 and 45 DAS. However, the water use efficiency improved and extent of 40% in cv. 407 at 55 DAS when treatment was given for 60 min. Variations in water use efficiency are brought about by stomatal conductance (Gs.) and or intrinsic photosynthetic capacity ($P_{.N.}$). It may be inferred based on the results obtained in the present study that the mean transpiration was found to be inversely related to water use efficiency (Tables 1a-d).

Treatment of seeds with ultrasound for different durations caused an increase in the total chlorophyll content of leaves of different cultivars up to 35 DAS followed by a gradual decline.

The total chlorophyll content was found to be at higher level in cv. 410 and cv. 450 than cv. 407. However, the total chlorophyll content showed variation among the cultivars and duration of treatment. Treatment for 60 min. showed higher level of chlorophyll content at 35 DAS in all the cultivars. Chlorophyll a content was found to be higher than chlorophyll b content in all three cultivars. Chlorophyll a/b ratios did not indicate any definite pattern of change due to treatment at different stages of growth in the cultivars studies. However treatment of seeds for 60 min. in all the cultivars at 25th DAS showed maximum a/b ratios. (Tables 2a-d)

The chloroplasts of the leaves of all the three cultivars are able to reduce the DCPIP effectively up to 35th DAS both in control and treatment for different durations indicating that the chloroplasts of the leaves are active only up to 35 DAS both in control and treatments. The DCPIP photo reduction of the chloroplasts of the leaves was slightly more in cv. 410 and cv. 450 than cv. 407 both in control and at all durations of treatments (Table 3).

The ultrasound treatment for different durations caused an increase in the total protein content in all the cultivars up to 35 DAS followed by a gradual decline. Treatment for 30 and 60 min. resulted in higher protein content on 35 DAS in cultivars 410 and 450 than cv. 407 and respective controls. Treatment for 60 min showed maximum protein content in cv. 450 at 35 DAS (Table 4). One doesn't know anything about the nature of protein synthesis as influenced by ultrasound.

The nitrate reductase activity of the leaves showed an increase up to 45 DAS followed by a decline both in control and treatments. The treatment for different durations caused increase in the level of enzyme activity in a time dependent manner in all the cultivars. Treatment for 60 min duration caused maximum level of nitrate reductase activity on 45th DAS in cv. 450.

The level of activity of the enzyme nitrite reductase increased gradually up to 45 DAS both in control and treatments. Unlike the nitrate reductase, the increase was lower. The level of enzyme activity increased with each duration of treatment. The level of enzyme activity was higher in cv. 450 than cv. 407 and cv. 410 at 45 DAS and 60 min of treatment (Table 5 and 6).

Ultrasound treatment for different durations caused an increase in the glutamine synthetase activity in all the cultivars from 25 to 45 DAS. The treatment for different durations resulted in higher level of enzyme activity in cv. 410 than cv. 407 and cv. 450.

All plant biochemical reactions are governed by enzymes. Which were found to be affected by ultrasound[9]. Thus all observed increases in the rates of metabolism can be explained to some extent by an increase in enzyme activity.[9,10]. Not all enzymes are similarly effected by ultrasound[11][1] , some show a marked (at least two fold) increase in activity, eg: amylase, while in others eg: catalase, there is a decrease in activity[12]. Enzymes are easily destroyed by external factors, including heat, and excess ultrasonic dosage rapidly destroy them. Our understanding about the kinetic activities of large number of enzyme activities as effected by ultrasound needs further in depth study and it appears that ultrasound treatment mimics the action of growth regulators and the hypothesis is to be verified by future research workers.

Table 1(a): Measurement of Gas exchange characteristics of green gram (*vigna radiata* (L.) cultivars subjected to ultrasound (25 Kc. Sec^{-1}) treatment for different durations on 25 Das Net photosynthetic rate (P_N) [µ mol (co_2) m^{-2} s^{-1}], inter cellular co_2 concentration (ci) [µ mol^{-1} mol^{-1}], Stomatal conductance (GS) [mol m^{-2} s^{-1}], Transpiration rate (E) [m mol (H_2O) m^{-2} s^{-1}] and Water Use Efficiency (WUE) [m mol (Co_2) mol^{-1} (H_2O)]. (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	P_N	CI	GS	E	WUE
407	**Control**	22.4 ± 0.14	13.9 ± 0.11	0.44 ± 0.16	3.50 0.15	0.143 ± 0.11
	15	23.4 ± 0.14 (4.46)	143 ± 0.12 (2.87)	0.50 ± 0.11 (13.63)	4.12 ± 0.11 (17.71)	0.121 ± 0.12 (- 15.38)
	30	24.2 ± 0.11 (8.03)	15.7 ± 0.14 (12.94)	0.57 ± 0.12 (29.54)	4.20 ± 0.12 (20.00)	0.100 ± 0.11 (- 30.06)
	60	25.3 ± 0.12 (12.94)	164 ± 0.15 (17.98)	0.64 ± 0.11 (45.45)	4.34 ± 0.14 (24)	0.086 ± 0.13 (- 13.86)
410	**Control**	23.2 ± 0.16	141 ± 0.18	0.48 ± 0.14	3.57 ± 0.12	0.129 ± 0.12
	15	23.9 ± 0.13 (3.01)	147 ± 0.10 (4.25)	0.55 ± 0.15 (14.58)	4.19 ± 0.14 (17.36)	0.104 ± 0.13 (-19.37)
	30	24.8 ± 0.14 (6.89)	162 ±0.15 (14.89)	0.61 ± 0.15 (27.08)	4.28 ± 0.12 (19.88)	0.091 ± 0.13 (-29.45)
	60	25.9 ± 0.14 (11.63)	169 ± 0.13 (19.85)	0.68 ± 0.14 (41.66)	4.42 ± 0.12 (23.80)	0.080 ± 0.11 (- 37.98)
450	**Control**	22.9 ± 0.15	140 ± 0.13	0.46 ± 0.17	3.52 ± 0.14	0.137 ± 0.13
	15	23.6 ± 0.12 (3.05)	145 ± 0.12 (3.57)	0.52 ± 0.11 (13.04)	4.16 ± 0.12 (18.18)	0.114 ± 0.12 (-16.78)
	30	24.4 ± 0.16 (6.55)	159 ± 0.12 (13.57)	0.59 ± 0.14 (28.26)	4.24 ± 0.13 (20.45)	0.095 ± 0.12 (- 30.65)
	60	25.5 ± 0.12 (11.35)	166 ± 0.13 (18.57)	0.66 ± 0.11 (43.47)	4.38 ± 0.14 (24.43)	0.082 ± 0.11 (-40.14)

Values in the parenthesis indicate percent increase over control.

Table 1(b): Measurement of Gas exchange characteristics of green gram (*vigna radiata* (L.) cultivars subjected to ultrasound (25 Kc. Sec^{-1}) treatment for different durations on 35 Das Net photosynthetic rate (P_N) [µ mol (co_2) m^{-2} s^{-1}], Inter cellular co_2 concentration (ci) [µ mol^{-1} mol^{-1}], Stomatal conductance (GS) (mol m^{-2} s^{-1}), Transpiration rate (E) [m mol (H_2O) m^{-2} s^{-1}] and Water Use Efficiency (WUE) [m mol (Co_2) mol^{-1} (H_2O]. (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	P_N	CI	GS	E	WUE
407	**Control**	27.3 ± 0.11	1.44 ± 0.13	0.52 ± 0.14	4.2 ± 0.12	0.153 ± 0.12
	15	28.2 ± 0.11 (3.27)	154 ± 0.15 (6.94)	0.61 ± 0.15 (17.30)	4.30 ± 0.12 (2.38)	0.118 ± 0.11 (-22.87)
	30	29.2 ± 0.14 (6.95)	162 ± 0.13 (12.57)	0.69 ± 0.13 (32.69)	4.52 ± 0.13 (7.61)	0.099 ± 0.13 (-35.29)
	60	30.4 ± 0.15 (11.35)	170 ± 0.12 (18.05)	0.75 ± 0.12 (44.23)	5.12 ± 0.14 (22.33)	0.012 ± 0.11 (-92.15)
410	**Control**	27.9 ± 0.13	149 ± 0.14	0.58 ± 0.14	4.17 ± 0.12	0.128 ± 0.13
	15	28.9 ± 0.11 (3.58)	159 ± 0.14 (6.71)	0.67 ± 0.16 (15.51)	4.37 ± 0.13 (4.79)	0.103 ± 0.12 (-19.53)
	30	29.8 ± 0.14 (6.81)	169 ± 0.14 (13.42)	0.72 ± 0.15 (24.13)	4.57 ± 0.11 (9.59)	0.033 ± 0.11 (-74.21)
	60	30.9 ± 0.14 (10.75)	174 ± 0.15 (16.77)	0.79 ± 0.13 (36.20)	5.17 ± 0.11 (23.98)	0.084 ±013 (-34.37)
450	**Control**	27.6 ± 0.12	147 ± 0.11	0.56 ± 0.15	4.15 ± 0.14	0.134 ± 0.12
	15	28.4 ± 0.15 (2.89)	157 ± 0.15 (6.80)	0.64 ± 0.15 (14.28)	4.34 ± 0.14 (4.57)	0.109 ± 0.14 (-18.65)
	30	29.6 ± 0.11 (7.24)	166 ± 0.12 (12.92)	0.70 ± 0.14 (25)	4.54 ± 0.12 (9.39)	0.099 ± 0.13 (-26.11)
	60	30.6 ± 0.17 (10.86)	172 ± 0.16 (17.00)	0.77 ± 0.16 (3.75)	5.17 ± 0.14 (23.85)	0.087 ± 0.14 (-34.30)

Values in the parenthesis indicate percent increase over control.

Table1 (c): Measurement of Gas exchange characteristics of green gram (*vigna radiata* (L.) cultivars subjected to ultrasound (25 Kc. Sec^{-1}) treatment for different durations on 45 Das Net photosynthetic rate (P_N) [µ mol (co_2) m^{-2} s^{-1}], Inter cellular co_2 concentration (ci) [µ mol^{-1} mol^{-1}], Stomatal conductance (GS) (mol m^{-2} s^{-1}), Transpiration rate (E) [m mol (H_2O) m^{-2} s^{-1}]and Water Use Efficiency (WUE) [m mol (Co_2) mol^{-1} (H_2O]. (Values are the mean of 3 replications ± S.E.).

Variety	Duration of Treatment in minutes	P_N	CI	GS	E	WUE
407	Control	21.5 ± 0.13	1.30 ± 0.12	0.40 ± 0.11	3.12 ± 0.12	0.160 ± 0.14
	15	22.4 ± 0.15 (4.18)	137 ± 0.14 (5.38)	0.46 ± 0.14 (15)	3.25 ± 0.12 (4.16)	0.131 ± 0.12 (-18.12)
	30	23.4 ± 0.15 (8.83)	140 ± 0.12 (7.69)	0.51 ± 0.13 (27.5)	3.40 ± 0.14 (8.97)	0.116 ± 0.13 (-27.5)
	60	24.2 ± 0.14 (12.55)	149 ± 0.011 (14.61)	0.56 ± 0.12 (40.10)	4.20 ± 0.16 (34.61)	0.103 ± 0.12 (-35.62)
410	Control	21.9 ± 0.13	134 ± 0.11	0.43 ± 0.12	3.16 ±0.14	0.144 ± 0.13
	15	22.9 ± 0.11 (4.56)	139 ± 0.16 (3.73)	0.49 ± 0.12 (13.95)	3.29 ± 0.13 (4.11)	0.121 ± 0.13 (-15.97)
	30	23.9 ± 0.17 (9.13)	145 ± 0.14 (8.20)	0.54 ± 0.11 (25.58)	3.46 ± 0.15 (9.49)	0.108 ± 0.13 (-25.12)
	60	24.8 ± 0.16 (13.24)	155 ± 0.16 (15.67)	0.59 ± 0.17 (37.20)	4.25 + 0.13 (34.49)	0.098 ± 0.11 -31.94
450	Control	21.7 ± 0.12	132 ± 0.12	0.42 ± 0.11	3.15 ± 0.14	0.148 ±0.14
	15	22.7 ± 0.15 (4.60)	136 ± 0.14 (3.03)	0.48 ± 0.14 (14.28)	3.20 ± 0.15 (1.58)	0.124 ± 0.11 (-16.21)
	30	23.7 ± 0.13 (9.21)	142 ± 0.17 (7.57)	0.52 ± 0.13 (23.80)	3.42 ± 0.15 (8.57)	0.115 ± 0.12 (-22.29)
	60	24.6 ± 0.17 (13.36)	152 ± 0.14 (15.15)	0.57 ± 0.14 (35.71)	4.22 ± 0.15 (33.96)	0.103 ± 0.10 (-30.40)

Values in the parenthesis indicate percent increase over control.

Table 1(d): Measurement of Gas exchange characteristics of green gram (*vigna radiata* (L.) cultivars subjected to ultrasound (25 Kc. Sec^{-1}) treatment for different durations on 55 Das Net photosynthetic rate (P_N) [µ mol (co_2) m^{-2} s^{-1}], Inter cellular co_2 concentration (ci) [µ mol^{-1} mol^{-1}], Stomatal conductance (GS) [mol m^{-2} s^{-1}], Transpiration rate (E) (m mol (H_2O) m^{-2} s^{-1}) and Water Use Efficiency (WUE) [m mol (Co_2) mol^{-1} (H_2O]. (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	P_N	CI	GS	E	WUE
407	Control	20.1 ± 0.12	125 ± 0.13	0.30 ± 0.14	3.11 ± 0.14	0.033 ±0.11
	15	20.8 ± 0.12 (3.48)	129 ± 0.14 (3.2)	0.34 ± 0.17 (13.33)	3.17 ± 0.14 (2.25)	0.207 ± 0.12 (52.72)
	30	21.4 ± 0.11 (6.46)	138 ± 0.11 (10.4)	0.39 ± 0.12 (30)	3.20 ± 0.16 (3.22)	0.248 ± 0.13 (65.15)
	60	22.4 ± 0.13 (11.44)	144 ± 0.14 (16.53)	0.45 ± 0.11 (50)	4.10 ± 0.12 (32.25)	0.137 ± 0.14 (31.51)
410	Control	20.6 ± 0.16	127 ± 0.14	0.33 ± 0.13	3.12 ± 0.15	0.216 ± 0.13
	15	21.1 ± 0.14 (2.42)	133 ± 0.12 (4.72)	0.37 ± 0.14 (12.13)	3.19 ± 0.17 (2.24)	0.180 ± 0.13 (-16.66)
	30	21.8 ± 0.15 (5.82)	142 ± 0.11 (11.81)	0.42 ± 0.13 (27.27)	3.26 ± 0.14 (4.48)	0.149 ± 0.13 (-31.09)
	60	22.7 ± 0.14 (10.19)	148 ± 0.14 (16.53)	0.49 ± 0.12 (48.48)	4.15 ± 0.11 (33.0)	0.119 ± 0.14 (-44.90)
450	Control	20.3 ± 0.16	124 ± 0.11	0.31 ± 0.14	3.10 ± 0.16	0.238 ± 0.11
	15	20.9 ± 0.13 (2.95)	131 ± 0.14 (5.64)	0.35 ± 0.12 (12.90)	3.18 ± 0.14 (2.58)	0.198 ±0.12 (-16.80)
	30	21.6 ± 0.17 (6.40)	140 ± 0.11 (12.90)	0.40 ± 0.15 (29.03)	3.23 ± 0.13 (4.19)	0.162 ± 0.14 (-31.93)
	60	22.6 ± 0.14 (11.33)	146 ± 0.15 (17.74)	0.47 ± 0.13 (51.61)	4.13 ± 0.12 (33.2)	0.128 ± 13 (-46.21)

values in the parenthesis indicate percent increase or decrease over control

Table 2(a): Effect of ultrasoud (25 kc Sec^{-1}) Treatment on changes in the Total chlorophyll content of green gram *(vigna radiata* L.*)* cultivars. mg gm^{-1} Fr. Wt). (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	1.29 ±0.02	1.34 ±0.01	1.23 ±0.02	1.15 ±0.04
	15	1.59 ±0.01 (22.35)	1.64. ±0.02 (22.38)	1.51 ±0.04 (22.76)	1.43 ±0.05 (24.34)
	30	1.68 ±0.02 (30.23)	1.79 ±0.05 (35.58)	1.60 ±0.04 (30.08)	1.50 ±0.04 (30.43)
	60	1.78 ±0.03 (37.98)	1.92 ±0.07 (43.28)	1.70 ±0.07 (47.82)	1.60 ±0.01 (39.13)
410	Control	1.35 ±0.04	1.39 ±0.03	1.27 ±0.01	1.20 ±0.01
	15	1.64 ±0.02 (21.48)	1.73 ±0.01) (24.46)	1.57 ±0.08 (23.62)	1.49 ±0.03 (24.16)
	30	1.74 ±0.04 (28.88)	1.88 ±0.05 (35.25)	1.64 ±0.02 (29.13)	1.58 ±0.01 (31.66)
	60	1.86 ±0.05 (37.77)	1.98 ±0.05 (42.44)	1.75 ±0.04 (37.79)	1.67 ±0.03 (39.16)
450	Control	1.32 ±0.03	1.36 ±0.04	1.25 ±0.03	1.17 ±0.04
	15	1.61 ±0.04 (21.96)	1.67 ±0.04 (22.79)	1.54 ±0.04 (23.2)	1.47 ±0.04 (25.64)
	30	1.70 ±0.02 (28.78)	1.82 ±0.03 (33.82)	1.62 ±0.05 (29.6)	1.53 ±0.05 (30.76)
	60	1.81 ±0.01 (37.12)	1.95 ±0.02 (43.38)	1.72 ±0.04 (37.6)	1.63 ±0.03 (39.31)

Values in the parenthesis indicate percent increase or decrease over control

Table 2 (b): Effect of ultrasound (25 kc Sec^{-1}) Treatment on changes in the chlorophyll a content of 3 green gram *(vigna radiata* L.*)* cultivars. (mg gm^{-1} $Fr.^{-1}$ Wt) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	0.93 ±0.01	0.96 ±0.03	0.90 ±0.02	0.85 ±0.02
	15	1.19 ±0.03 (27.95)	1.22 ±0.04 (27.08)	1.14 ±0.03 (26.66)	1.10 ±0.02 (29.41)
	30	1.23 ±0.01 (32.25)	1.30 ±0.02 (35.41)	1.20 ±001 (33.33)	1.15 ±0.02 (35.29)
	60	1.28 ±0.03 (37.63)	1.37 ±0.04 (42.70)	1.25 ±0.03 (38.88)	1.20 ±0.01 (41.17)
410	Control	0.97 ±0.02	0.91 ±0.01	0.92 ±0.03	0.88 ±0.03
	15	1.21 ±0.04 (24.74)	1.27 ±0.03 (28.28)	1.17 ±0.04 (27.17)	1.14 ±0.01 (29.54)
	30	1.26 ±0.01 (29.89)	1.35 ±0.04 (36.36)	1.22 ±0.03 (32.60)	1.19 ±0.01 (35.22)
	60	1.32 ±0.02 (36.08)	1.40 ±0.02 (41.41)	1.28 ±0.02 (39.13)	1.24 ±0.02 (40.90)
450	Control	0.95 ±0.02	0.97 ±0.02	0.91 ±0.02	0.86 ±0.03
	15	1.20 ±0.02 (26.31)	1.24 ±0.04 (27.83)	1.15 ±0.02 (26.37)	1.13 ±0.01 (31.39)
	30	1.24 ±0.02 (30.52)	1.32 ±0.01 (36.08)	1.21 ±0.04 (32.96)	1.17 ±0.03 (36.04)
	60	1.30 ±0.01 (36.84)	1.39 ±0.01 (43.29)	1.26 ±0.01 (38.46)	1.22 ±0.01 (41.86)

Values in the parenthesis indicate percent increase or decrease over control

Table 2 (c): Effect of ultrasound (25 kc Sec^{-1}) Treatment on changes in the chlorophyll b content of 3 green gram *(vigna radiata* L.*)* cultivars. (mg gm^{-1} Fr. Wt). (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	0.36 ±0.02	0.38 ±0.01	0.33 ±0.02	0.30 ±0.04
	15	0.040 ±0.02 (11.11)	0.42 ±0.04 (10.52)	0.37 ±0.02 (12.12)	0.33 ±0.01 (10.12)
	30	0.45 ±0.04 (25)	0.49 ±0.03 (28.94)	0.40 ±0.01 (21.21)	0.35 ±0.01 (16.66)
	60	0.50 ±0.03 (38.88)	0.55 ±0.02 (44.73)	0.45 ±0.01 (36.36)	0.40 ±0.04 (33.33)
410	Control	0.38 ±0.01	0.40 ±0.04	0.35 ±0.03	0.32 ±0.03
	15	0.43 ±0.03 (13.15)	0.46 ±0.03 (15)	0.40 ±0.01 (14.28)	0.35 ±0.01 (9.3)
	30	0.43 ±0.04 (26.31)	0.53 ±0.02 (32.05)	0.42 ±0.03 (20)	0.39 ±0.04 (21.87)
	60	0.54 ±0.01 (42.10)	0.58 ±0.01 (45)	0.47 ±0.04 (34.28)	0.43 ±0.01 (34.37)
450	Control	0.37 ±0.03	0.39 ±0.02	0.34 ±0.01	0.31 ±0.02
	15	0.41 ±0.02 (10.81)	0.43 ±0.01 (10.25)	0.39 ±0.01 (14.70)	0.34 ±0.02 (19.67)
	30	0.46 ±0.03 (24.32)	0.50 ±0.01 (28.20)	0.41 ±0.04 ((20.58)	0.36 ±0.04 (16.12)
	60	0.51 ±0.04 (37.83)	0.57 ±0.02 (46.15)	0.46 ±0.01 (35.29)	0.41 ±0.01 (32.25)

Values in the parenthesis indicate percent increase or decrease over control

Table 2 (d): Effect of ultrasound (25 kc Sec^{-1}) Treatment on changes in the chlorophyll a/b ratios of 3 green gram *(vigna radiata* L.*)* cultivars. (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	2.58 ±0.01	2.52 ±0.02	2.72 ±0.01	2.83 ±0.03
	15	2.97 ±0.01 (15.1)	2.90 ±0.02 (15.0)	3.08 ±0.04 (13.23)	3.33 ±0.03 (17.66)
	30	2.73 ±0.04 (5.81)	2.65 ±0.04 (5.15)	3.00 ±0.05 (10.21)	3.28 ±0.04 (15.90)
	60	2.56 ±0.04 (-0.77)	2.49 ±0.03 (-1.19)	2.77 ±0.01 (1.83)	3.00 ±0.04 (6.02)
410	Control	2.55 ±0.02	2.47 ±0.04	2.62 ±0.02	2.75 ±0.04
	15	2.81 ±0.04 (10.19)	2.76 ±0.04 (11.74)	2.92 ±0.03 (11.45)	3.25 ±0.04 (18.18)
	30	2.62 ±0.01 (2.74)	2.54 ±0.04 (6.45)	2.90 ±0.01 (10.6)	3.05 ±0.04 (16.90)
	60	2.44 ±0.03 (-4. 31)	2.41 ±0.04 (-2.42)	2.72 ±0.03 (3.81)	2.88 ±0.03 (4.72)
450	Control	2.56 ±0.04	2.48 ±0.03	2.67 ±0.03	2.77 ±0.03
	15	2.92 ±0.03 (14.06)	2.88 ±0.01 (16.12)	2.94 ±0.04 (10.12)	3.32 ±0.01 (19.85)
	30	2.69 ±0.04 (5.07)	2.64 ±0.05 (6.45)	2.95 ±0.03 (10.48)	3.25 ±0.01 (17.32)
	60	2.54 ±0.04 (-0.78)	2.43 ±0.02 (-2.01)	2.73 ±0.04 (2.24)	2.97 ±0.04 (7.22)

Values in the parenthesis indicate percent increase or decrease over control

Table 3: Effect of ultrasound (25 kc Sec^{-1}) treatment on DCPIP Photo reduction of isolated chloroplasts of 3 of green gram *(Vigna radiata.*L*)* cultivars. (μ moles of DCPIP reduced mg^{-1} Chl hr^{-1}) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	182.1 ±1.16	190.3 ±1.19	170.8 ±1.23	157.2 ±1.20
	15	191.3 ±1.20 (5.05)	205.2 ±1.26 (7.82)	185.2 ±1.24 (8.43)	173.7 ±1.26 (10.46)
	30	197.5 ±1.16 (8.45)	210.2 ±1.29 (10.45)	190.1 ±1.24 (11.29)	179.2 ±1.20 (13.99)
	60	204.3 ±1.12 (12.19)	217.3 ±1.20 (14.18)	195.2 ±1.24 (14.28)	185.2 ±1.24 (17.81)
410	Control	185.2 ±1.17	194.2 ±1.20	178.2 ±1.17	160.1 ±1.19
	15	195.2 ±1.25 (5.39)	209.13 ±1.25 (7.77)	189.3 ±1.24 (6.22)	177.6 ±1.27 (10.93)
	30	201.3 ±1.14 (8.69)	215.1 ±1.25 (10.76)	194.2 ±1.25 (8.97)	183.2 ±1.24 (14.42)
	60	209.1 ±1.14 (12.90)	221.4 ±1.22 (14.00)	199.3 ±1.22 (11.84)	189.2 ±1.20 (18.17)
450	Control	183.1 ±1.18	192.1 ±1.19	172.4 ±1.16	159.2 ±1.15
	15	193.1 ±1.23 (5.96)	207.4 ±1.27 (7.96)	187.2 ±1.22 (8.71)	175.4 ±1.21 (10.17)
	30	199.2 ±1.13 (8.79)	212.4 ±1.22 (10.56)	192.3 ±1.22 (11.54)	181.3 ±1.27 (13.88)
	60	207.2 ±1.16 (13.16)	219.1 ±1.27 (14.05)	197.4 ±1.20 (14.50)	187.3 ±1.19 (17.65)

Values in the parenthesis indicate percent increase or decrease over control

Table 4: Effect of ultrasound (25 kc Sec^{-1}) treatment on Changes in the Total Protein content of 3 of green gram *(Vigna radiata* L.*)* cultivars. (μ moles of reduced mg gm^{-1} dry wt^{1}) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	20.28 ±0.22	24.20 ±0.23	17.23 ±0.27	14.29 ±0.24
	15	24.62 ±0.23 (21.40)	29.59 ±0.29 (22.27)	21.33 ±0.33 (23.79)	18.60 ±0.27 (30.16)
	30	29.49 ±0.27 (45.41)	34.50 ±0.34 (42.56)	26.93 ±0.38 (56.29)	22.86 ±0.20 (59.97)
	60	34.69 ±0.32 (17.63)	39.32 ±0.43 (62.47)	30.28 ±0.43 (75.73)	27.72 ±0.34 (93.98)
410	Control	22.32 ±0.26	28.24 ±0.25	19.27 ±0.39	16.30 ±0.32
	15	27.64 ±0.28 (22.38)	32.60 ±0.24 (15.43)	24.36 ±0.34 (26.41)	21.63 ±0.29 (32.69)
	30	32.50 ±0.29 (45.60)	37.53 ±0.34 (32.89)	29.37 ±0.25 (52.41)	26.89 ±0.24 (64.96)
	60	38.57 ±0.30 (18.67)	41.21 ±0.34 (45.92)	35.30 ±0.32 (83.18)	30.72 ±0.38 (88.46)
450	Control	21.39 ±0.24	26.27 ±0.27	18.33 ±0.33	15.32 ±0.22
	15	25.68 ±0.22 (20.62)	30.64 ±0.31 (16.63)	23.30 ±0.23 (27.11)	19.67 ±0.20 (28.39)
	30	30.52 ±0.24 (41.35)	35.54 ±0.31 (35.28)	27.54 ±0.30 (50.24)	24.92 ±0.24 (62.66)
	60	36.59 ±0.29 (19.88)	40.22 ±0.24 (53.10)	32.42 ±0.26 (76.86)	28.89 ±0.37 (88.57)

Values in the parenthesis indicate percent increase or decrease over control

Table 5: Effect of ultrasound (25 kc Sec^{-1}) treatment on Changes in the level of Nitrate reductase activity in the leaves of 3 of green gram *(Vigna radiata* L.*)* cultivars. (µ moles of Nitrate formed gr^{-1} Fr wt^{1}) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	0.377 ±0.03	0.380 ±0.09	0.392 ±0.09	0.323 ±0.01
	15	0.394 ±0.03 (4.50)	0.424 ±0.07 (11.57)	0.530 ±0.04 (35.20)	0.342 ±0.07 (5.88)
	30	0.392 ±0.04 (3.97)	0.4.35 ±0.02 (14.47)	0.539 ±0.03 (37.50)	0.357 ±0.05 (10.52)
	60	0.401 ±0.03 (6.36)	0.542 ±0.02 (42.63)	0.544 ±0.02 (38.72)	0.366 ±0.08 (13.31)
410	Control	0.382 ±0.09	0.385 ±0.04	0.444 ±0.07	0.329 ±0.08
	15	0.397 ±0.03 (3.14)	0.430 ±0.06 (11.68)	0.537 ±0.07 (20.94)	0.351 ±0.01 (7.59)
	30	0.399 ±0.07 (4.45)	0.470 ±0.09 (22.07)	0.542 ±0.02 (22.07)	0.367 ±0.03 (11.50)
	60	0.417 ±0.02 (9.16)	0.550 ±0.03 (42.85)	0.552 ±0.05 (24.32)	0.72 ±0.09 (13.06)
450	Control	0.379 ±0.02	0.383 ±0.04	0.440 ±0.08	0.325 ±0.09
	15	0.395 ±0.01 (4.22)	0.428 ±0.03 (11.74)	0.535 ±0.06 (21.59)	0.346 ±0.02 (6.46)
	30	0.393 ±0.02 (3.69)	0.426 ±0.03 (11.22)	0.540 ±0.04 (22.72)	0.359 ±0.01 (10.46)
	60	0.410 ±0.06 (8.17)	0.544 ±0.08 (42.03)	0.546 ±0.01 (24.09)	0.369 ±0.08 (13.53)

Values in the parenthesis indicate percent increase or decrease over control

Table 6: Effect of ultrasound (25 kc Sec^{-1}) treatment on Changes in the Nitrite reductase activity in the leaves of 3 of green gram of *(Vigna radiata* L.*)* cultivars. (µ moles of N_2O reduced gr^{-1} Fr. wt. hr^{-1}) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	3.202 ±0.04	3.230 ±0.02	3.340 ±0.03	3.330 ±0.07
	15	3.302 ±0.07 (3.12)	3.310 ±0.18 (2.47)	3.345 ±0.04 (0.14)	3.340 ±0.09 (0.30)
	30	3.314 ±0.02 (3.49)	3.320 ±0.04 (2.78)	3.352 ±0.01 (0.35)	3.347 ±0.02 (0.51)
	60	3.322 ±0.03 (3.74)	3.419 ±0.09 (5.85)	3.540 ±0.04 (5.98)	3,510 ±0.02 (5.40)
410	Control	3.210 ±0.05	3.237 ±0.06	3.343 ±0.04	3.335 ±0.09
	15	3.308 ±0.04 (3.05)	3.318 ±0.07 (2.50)	3.348 ±0.07 (0.14)	3.345 ±0.09 (0.29)
	30	3.318 ±0.01 (3.36)	3.328 ±0.02 (2.81)	3.356 ±0.06 (0.38)	3.349 ±0.04 (0.41)
	60	3.331 ±0.02 (3.76)	3.423 ±0.08 (5.74)	3.549 ±0.02 (6.16)	3.514 ±0.08 (5.36)
450	Control	3.290 ±0.07	3.235 ±0.07	3.341 ±0.01	3.332 ±0.04
	15	3.304 ±0.08 (2.96)	3.315 ±0.09 (2.47)	3.346 ±0.08 (0.14)	3.342 ±0.07 (0.30)
	30	3.316 ±0.04 (3.33)	3.325 ±0.03 (2.81)	3.353 ±0.09 (0.35)	3.348 ±0.08 (1.98)
	60	3.329 ±0.09 (3.73)	3.420 ±0.04 (5.71)	3.543 ±0.07 (6.04)	3.512 ±0.02 (5.40)

Values in the parenthesis indicate percent increase over control

Table 7: Effect of ultrasound (25 kc Sec^{-1}) treatment on changes in the Glutamine Synthetase activity in the leaves of 3 green gram *(Vigna radiata* L.*)* cultivars. (μ moles of α glutamyl hydroxmate formed mg^{-1} protein) (Values are the mean of 3 replications ± S.E.)

Variety	Duration of Treatment in minutes	25 DAS	35 DAS	45 DAS	55 DAS
407	Control	0.420 ±0.02	0.520 ±0.04	0.623 ±0.01	0.425 ±0.06
	15	0.435 ±0.05 (3.57)	0.534 ±0.01 (2.69)	0.640 ±0.02 (2.72)	0.432 ±0.08 (1.64)
	30	0.450 ±0.05 (7.14)	0.549 ±0.09 (5.57)	0.653 ±0.07 (4.81)	0.449 ±0.01 (5.64)
	60	0.472 ±0.03 (12.38)	0.572 ±0.07 (10.12)	0.721 ±0.05 (15.73)	0.460 ±0.04 (8.23)
410	Control	0.428 ±0.07	0.529 ±0.08	0.629 ±0.04	0.427 ±0.09
	15	0.442 ±0.04 (3.27)	0.540 ±0.06 (2.07)	0.650 ±0.05 (3.33)	0.436 ±0.07 (2.10)
	30	0.458 ±0.07 (7.00)	0.558 ±0.06 (5.48)	0.660 ±0.03 (4.92)	0.455 ±0.03 (6.55)
	60	0.480 ±0.03 (12.14)	0.580 ±0.07 (9.64)	0.730 ±0.03 (16.73)	0.467 ±0.01 (9.36)
450	Control	0.423 ±0.08	0.522 ±0.01	0.625 ±0.06	0.423 ±0.04
	15	0.438 ±0.07 (3.54)	0.536 ±0.03 (2.68)	0.643 ±0.02 (2.88)	0.434 ±0.09 (2.60)
	30	0.452 ±0.09 (6.85)	0.553 ±0.01 (5.93)	0.655 ±0.08 (4.81)	0.451 ±0.04 (6.61)
	60	0.474 ±0.02 (12.05)	0.575 ±0.03 (10.15)	0.724 ±0.04 (15.84)	0.463 ±0.03 (9.45)

Values in the parenthesis indicate percent increase over control

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