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Research Article

EFFECT OF WATERLOGGING ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS AND SEED YIELD IN GREENGRAM GENOTYPES

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ABSTRACT
A field experiment was conducted at Agricultural College Farm Bapatla, during rabi 2012-13 to study the effect of waterlogging on Physiological and biochemical parameters and seed yield in greengram genotypes. The results revealed that physiological parameters like LAI, CGR, NAR, SLA, LAD and RWC decreased with the waterlogging due to decrease in the leaf area and drymatter production. TM96-2 maintained higher values of all the above parameters followed by LGG460, and LGG407 showed lowered values of all the above parameters. The biochemical parameters indicated that four days waterlogging decreased the SCMR, CSI, total sugars by 35.09, 22.28 and 49.05 percent respectively over the control while MII and proline content was increased by 58.28, 91.04 percent respectively over the control. TM96-2 maintained higher proline content and lower MII followed by LGG 460 and LGG407 showed lowest proline content and higher MII. Water logging for four days decreased the seed yield compared to control. Among the genotypes TM96-2 maintained higher seed yield and its attributes apart from higher physiological and biochemical traits followed by LGG460 and LGG407 recorded lowest values of all the above parameters. Hence, TM96-2 and LGG460 are considered to possess submergence tolerance among the five genotypes studied.

Keywords: *Greengram, Biochemical Traits, Physiological Parameters, SCMR, Waterlogging*

INTRODUCTION
Greengram is an important short duration pulse crop having wider adaptability and low input requirement. In India mungbean occupies an area of 34.4 lacks. ha with annual production of 14 lakhs tons and with a productivity of 406.98kg ha^{-1}. In Andhra Pradesh it occupies an area of 4.4 lakhs ha with the production of 2.17 lakh tons and with the productivity of 493.18 kg ha^{-1}(Agropedia.iit.ac.in 2011-12). Waterlogging is serious problem which effects the crop growth and yield. Waterlogging blocks the oxygen supply to the roots thus inhibiting root respiration resulting in severe decline in energy status of root cells affecting important metabolic processes of plants. Waterlogging often results in yellowing of the greengram crop, if it persists for 7-10 days leads to mortality. Nearly 60% of the crop stand was lost when waterlogging persists for 8 days in 20days old crop (Singh *et al.*, 1986). A negative correlation was found between the duration of waterlogging and total soluble sugars and total soluble proteins (Das *et al.*, 2000). Kumar *et al.*, (2013) reported that waterlogging in greengram resulted in decline RWC, MSI in root and leaf tissue.
Much research work was not done on physiological and biochemical parameters of newly released greengram genotypes. Hence, this study was taken up to study the effect of waterlogging on physiological and biochemical parameters and yield in greengram genotypes.

MATERIALS AND METHODS
A field experiment was conducted at College Farm, Agricultural College, Bapatla during *rabi* 2012-13. The experiment was laid out in sandy clay loam soil in a split plot design with five genotypes, three treatments and replicated thrice. Treatments consist of waterlogging treatments as main plots W_0- Control (No waterlogging), W_1-Waterlogging for 2 days (at vegetative stage 21DAS), W_2-Waterlogging for 4 days (at vegetative stage 21DAS) and genotypes as subplots (LGG460, LGG450, LGG486, TM96-2, LGG407). The plot size was 4mx3m and spacing of 30cmx10cm. Waterlogging stress was imposed at vegetative stage i.e at 21DAS, two days for one treatment from 21-22 DAS and four days for another

treatments from 21to 24 DAS and control plants were maintained under normal irrigation conditions. Waterlogging was administered by applying heavy irrigation to the plots assigned to the waterlogging treatments. Soil was kept saturated with the water above field capacity by continuous flooding, usually every day twice to create an oxygen deficiency environment. The crop was grown following the recommended package of practices and timely plant protection measures were also adapted. Sampling was done at 25, 35,45,55,65 DAS. Five plants from each treatment were dugout along with roots and separated into leaf, stem, root and pods and dried at 80^{oc} temperature in a hot air oven until constant weight was attained. The dry weight of leaf, stem, pods and roots of the plant was recorded separately. Physiological parameters like LAI, CGR, NAR, SLA, LAD and RWC were derived by the formulas given by Radford. The biochemical parameters like CSI (Rajagopal *et al.*, 1990), sugars (Somogyi, 1952), Proline (Bates *et al.*, 1973) was estimated by standard methods at 25, 35,45,55,65 DAS.

RESULTS AND DISCUSSION

Significant differences were observed between waterlogging treatments and genotypes throughout the crop growth for LAI and CGR (Table 1). Imposition of waterlogging for two days and four days significantly reduced the leaf area index (LAI) and crop growth rate (CGR) at all stages of plant growth. At 55 DAS, control plants showed highest LAI (1.90) and CGR (16.45g m-2 d-1), where as four days waterlogging showed lowest LAI (1.45) and CGR (12.28 g m-2 d-1). Waterlogging for two days found less detrimental to LAI and CGR as compared to waterlogging for four days. The leaf area index and CGR was decreased in all the treatments compared to control, which was due to the impairment of water absorbing ability of the plants as indicated by the reduction in leaf turgidity as well as translocation of drymatter from the pods to seeds possibly due to damage caused to the root system. Such inhibition may also be due to adverse effects of waterlogging on water and mineral uptake (Hocking *et al.*, 1987).

The genotypes tested for waterlogging tolerance were also significantly varied for leaf area index and crop growth rate at all stages. Among the genotypes tested, TM96-2 recorded higher LAI and CGR followed by LGG 460 and the lowest LAI and CGR was recorded by LGG407 and remaining genotypes LGG450 and LGG 486 were on par with each other. Higher LAI and CGR in TM96-2 was recorded due to maintaining higher physiological traits under waterlogged conditions like SCMR, higher plant height, total drymatter, leaf area, higher rate of photosynthesis and leaf growth and due to quick recovery of photosynthesis after waterlogging and leaf growth ,higher photosynthetic rate as reflected through the total drymatter (Ahmed *et al.*, 2002). Similar differences in genotypes were also observed in green gram by Yadav and Saxena (1998) and in maize by Saritha and Singh (2002). Interaction between genotypes and duration of waterlogging stress were non significant at all growth stages.

Irrespective of treatments, the NAR and SLA decreased in all the genotypes in control and under waterlogged conditions from 25-35 DAS upto harvest (Table 1 & 2). Significant differences were observed between waterlogged treatments and genotypes upto 45-55DAS in NAR and upto harvest in SLA. Imposition of waterlogging for two days and four days were significantly reduced the NAR and SLA at all stages of plant growth.

At 25DAS, control plants showed highest NAR (0.92 mg cm^{-2} d^{-1}) and SLA (361.01cm^2 g^{-1}) where as four days waterlogging showed lowest NAR (0.58 mg cm^{-2} d^{-1}) and SLA(203.66 cm^2 g^{-1}) and in two days waterlogging NAR was (0.70 mg cm^{-2} d^{-1}) and SLA (261.78 cm^2 g^{-1}). Waterlogging for two days was found less detrimental to the NAR and SLA compared to four days. The NAR and SLA was decreased in all treatments compared to control, which was due to decreased photosynthetic efficiency due to impaired chlorophyll content and assimilatory apparatus and decreased drymatter accumulation at growth stages and the impaired of water absorbing ability of plants. Such inhibition may be due to adverse effects of waterlogging on water and mineral uptake (Hocking *et al.*, 1987). Similar results were also reported in tobacco by Hurng and Kao (1993).

Table 1: Effect of waterlogging on physiological parameters in greengram genotypes

	LAI				CGR(g m^{-2} d^{-1})				NAR(mg $dm^{-2}d^{-1}$)			
Treatments	**25 DAS**	**45 DAS**	**55 DAS**	**65 DAS**	**25-35 DAS**	**35-45 DAS**	**45-55 DAS**	**55-65 DAS**	**25-35 DAS**	**35-45 DAS**	**45-55 DAS**	**55-65 DAS**
Control (W_0)	0.67	1.72	1.90	1.40	3.35	9.74	16.45	6.06	0.92	0.79	0.64	0.14
Two days waterlogging (W_1)	0.61	1.51	1.66	1.29	3.05	7.42	14.21	5.00	0.70	0.44	0.40	0.12
Four days waterlogging (W_2)	0.52	1.39	1.45	1.24	2.22	6.28	12.28	4.25	0.58	0.35	0.31	0.10
CD (P=0.05)	0.06	0.10	0.20	NS	0.46	0.91	0.85	0.85	0.14	0.13	0.05	NS
Genotypes												
LGG 460 (V_1)	0.60	1.59	1.70	1.31	2.93	8.12	15.25	5.38	0.74	0.54	0.48	0.15
LGG 450 (V_2)	0.59	1.49	1.67	1.28	2.72	7.20	14.17	4.53	0.66	0.50	0.45	0.10
LGG 486 (V_3)	0.58	1.51	1.69	1.30	2.87	7.49	14.67	4.80	0.69	0.52	0.47	0.11
TM 96-2 (V_4)	0.70	1.71	1.89	1.40	3.24	9.63	16.78	6.32	0.91	0.66	0.54	0.21
LGG 407 (V_5)	0.55	1.39	1.48	1.23	2.61	6.62	13.38	4.48	0.58	0.42	0.36	0.09
CD (P=0.05)	0.05	0.12	0.18	0.08	0.30	1.45	1.43	0.84	0.15	0.12	0.05	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2: Effect of waterlogging on physiological parameters and seed yield in greengram genotypes

Treatments	SLA($cm^2 g^{-1}$)				LAD($cm^2 d^{-1}$)				RWC (%)			Seed Yield (Kg ha^{-1})
	25 DAS	45 DAS	55 DAS	65 DAS	25-35 DAS	35-45 DAS	45-55 DAS	55-65 DAS	15 DAS	25 DAS	35 DAS	
Control (W_0)	361	149	90	72	8.70	13.95	18.10	16.50	78.96	79.90	84.70	982.71
Two days waterlogging (W_1)	261	130	85	71	8.15	12.65	15.85	14.75	78.99	74.93	77.40	787.36
Four days waterlogging (W_2)	203	114	81	67	6.45	10.80	14.45	13.70	78.85	72.97	74.16	576.31
CD (P=0.05)	20.19	18.25	5.15	4.13	0.30	0.29	0.54	0.63	NS	2.62	4.95	74.77
Genotypes												
LGG 460 (V_1)	270	134	86	72	7.85	12.80	16.45	15.65	79.94	76.04	78.69	821.33
LGG 450 (V_2)	257	133	85	70	7.64	12.15	15.80	14.75	78.76	73.92	76.25	627.52
LGG 486 (V_3)	263	134	86	71	7.66	12.30	16.00	14.95	78.89	75.75	77.89	793.11
TM 96-2 (V_4)	314	142	91	73	9.10	14.15	18.00	16.45	80.46	79.57	83.29	963.85
LGG 407 (V_5)	208	112	81	68	6.60	10.80	14.35	13.55	78.40	72.74	74.60	603.15
CD (P=0.05)	42	7.12	5.10	3.18	0.33	0.87	0.57	1.00	2.31	3.31	3.64	131.70
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

The genotypes also tested for waterlogging tolerance were also significantly varied for NAR and SLA at all stages of plant growth. Among the genotypes tested, TM96-2 recorded higher NAR and SLA followed by LGG 460 and the lowest NAR and SLA was recorded by LGG407 and remaining genotypes LGG450 and LGG 486 were on par with each other.

Higher NAR and SLA in TM96-2 was recorded due to maintaining higher physiological traits under waterlogged conditions like SCMR, higher plant height, total drymatter, leaf area, higher rate of photosynthesis and leaf growth and due to quick recovery of photosynthesis after waterlogging and leaf growth, higher photosynthetic rate as reflected through the total drymatter (Ahmed *et al.*, 2002). Similar differences in genotypes were also observed in green gram by Yadav and Saxena (1998) and in maize by Saritha and Singh (2002). Interaction between genotypes and duration of waterlogging stress were non significant at all growth stages.

Table 3: Effect of waterlogging on Biochemical parameters in greengram

	SCMR			**CSI(%)**			**MII (%)**			**Total Sugars (mg g^{-1} dry weight)**			**proline content (µg g^{-1} fresh wt)**		
Treatments	**15 DAS**	**25 DAS**	**35 DAS**	**15 DAS**	**25 DAS**	**35 DAS**	**15 DAS**	**25 DAS**	**35 DAS**	**15 DAS**	**25 DAS**	**35 DAS**	**15 DAS**	**25 DAS**	**35 DAS**
Control (W_0)	36.94	45.86	59.67	60.44	67.02	84.42	34.41	35.04	35.85	25.78	26.05	27.50	80.47	84.07	87.56
Two days (W_1)	36.74	33.77	46.39	59.23	49.23	73.51	34.21	60.10	54.44	25.82	18.76	20.13	81.63	136.37	124.32
Four days (W_2)	36.70	30.19	44.17	59.45	46.35	69.04	34.33	70.47	59.74	25.98	15.88	18.45	82.81	193.94	167.28
CD (P=0.05)	NS	2.69	2.10	NS	5.32	8.54	NS	6.78	3.55	NS	2.88	1.54	NS	8.34	6.09
Genotypes															
LGG 460 (V_1)	36.56	34.08	47.01	58.17	53.03	78.12	32.59	45.51	41.05	25.28	20.44	23.55	79.28	150.42	147.09
LGG 450 (V_2)	35.24	31.21	46.27	55.35	47.07	76.73	54.10	60.42	53.07	26.20	18.95	20.55	81.45	133.40	122.71
LGG 486 (V_3)	36.30	33.41	46.44	56.14	49.49	76.93	33.71	56.91	52.84	26.61	19.63	21.01	82.48	138.74	129.53
TM 96-2 (V_4)	37.94	37.29	59.97	62.41	60.51	83.95	32.22	39.06	35.19	25.19	22.89	25.21	82.20	162.51	154.76
LGG 407 (V_5)	35.22	30.98	44.04	55.08	46.58	67.09	54.96	70.79	59.17	26.01	17.74	19.18	82.77	105.56	101.15
CD (P=0.05)	NS	2.58	2.76	NS	2.28	5.24	NS	6.24	6.05	NS	2.37	1.66	NS	6.56	6.74
Interaction V × W	NS	NS	NS	NS	S	S	NS	S	NS	NS	S	NS	NS	S	S
CD(P=0.05)					3.96	9.08		24.68			4.10			11.36	11.67
W × V CD(P=0.05)					6.05	11.05		22.65			4.35			12.25	11.18

Leaf area duration denotes leafiness of crop till harvest. Significant differences were observed between genotypes and waterlogging treatments throughout the crop growth for LAD (Table 2). The LAD gradually increased from 25 DAS to 55 DAS and then declined in all the genotypes irrespective of treatments. Imposition of water logging for two days and four days were significantly reduced the LAD at all stages of plant growth. At 55 DAS, control plants showed highest LAD (18.10 cm^2 d^{-1}), where as four days water logging showed lowest LAD (14.45 cm^2 d^{-1}). Waterlogging for two days was found less detrimental to the LAD compared to waterlogging for four days. LAD was decreased in all the treatments compared to control which was mainly due to limited cell enlargement which is responsible for reduced LAI under waterlogged conditions makes the canopy inefficient in receiving the light energy (Trung *et al.,* 1987).

The genotypes tested for waterlogging tolerance were also significantly varied for LAD at all stages. Among the genotypes tested, TM96-2 recorded higher LAD followed by LGG 460 and the lowest LAD was recorded by LGG407 and remaining genotypes LGG450 and LGG 486 were on par with each other. Similar differences in genotypes were also observed in greengram by Islam *et al.,* (1994).

The RWC gradually increased from 15 to 35 DAS in control plants (Table 2). Imposition of waterlogging for two days and four days were significantly reduced the RWC. RWC decreased under waterlogged condition in all genotypes. The decline was great in susceptible genotypes. The tolerant genotypes of waterlogged showed higher RWC even four days of waterlogging.

The SPAD chlorophyll meter readings (SCMR) and CSI gradually increased from 15 to 35 DAS in the control plants (Table 3). Significant differences were observed between waterlogging treatments and genotypes during waterlogging and during the recovery period. Maximum chlorophyll content CSI was found at 35 DAS in control plants. Waterlogging for two days was found less detrimental to the chlorophyll content and CSI compared to waterlogging for four days. Four days waterlogging decreased the chlorophyll content and CSI by 35.09 and 22.28 percent and two days waterlogging decreased the chlorophyll content and CSI by 28.63 and 14.84 percent respectively. Reduction in SCMR values and CSI attributed to the reduction in chlorophyll content under waterlogging conditions (Kumutha *et al.,* 2008). Similar results were also reported in greengram (Kumar *et al.,* 2013). The genotypes tested for waterlogging tolerance were also significantly varied for chlorophyll content and CSI. Among the genotypes TM 96-2 recorded the highest chlorophyll content and CSI fallowed by LGG 460 and the lowest chlorophyll content and CSI was recorded by LGG 407 and the remaining genotypes LGG 450, LGG 486 were on par with each other. Higher chlorophyll content and CSI value in TM 96-2 might be due to higher leaf area, higher total drymatter, higher LAI, higher rate of photosynthesis, highest plant height and leaf growth and due to quick recovery of photosynthesis after waterlogging, higher photosynthetic rate as reflected through the total drymatter (Ahmed *et al.,* 2002). Similar differences in genotypes were also observed in greengram (Kumutha *et al.,* 2008; Sairam *et al.,* 2009a).

The membrane injury index gradually decreased from 15 to 35 DAS (Table 3). Imposition of waterlogging for two days and four days were significantly increased the membrane injury index. MII increased under waterlogging condition in all genotypes. The increase was more in susceptible genotype. The tolerant genotypes of waterlogging showed lower MII values even four days of waterlogging. Waterlogging for two days was found less detrimental to the MII compared to waterlogging for four days. Four days waterlogging increased the MII by 50.28 percent and two days waterlogging increased the MII by 41.70 percent over the control. Waterlogging results in disintegration of membrane and cells, this consequently acts as a hindrance to water uptake (McKersie, 1996). Increase in membrane injury and loss in water uptake, which was greater in susceptible genotype as compared to tolerant one in greengram (Kumutha *et al.,* 2008). Membrane disintegration is one of the consequences of oxygen deprivation (Rawyler *et al.,* 2002). Similar results for decrease in Membrane Stability Index (MSI) were also reported in greengram (Kumar *et al.,* 2013).

The total sugars gradually increased from 15 to 35 DAS in the control plants (Table 3). Imposition of waterlogging for two days and four days were significantly reduced the total sugars. Total sugars

decreased under waterlogging condition in all genotypes. The decline was great in susceptible genotype (LGG 407). The tolerant genotype (TM 96-2) of waterlogging showed higher chlorophyll content even four days of waterlogging. Waterlogging for two days was found less detrimental to the total sugars as compared to waterlogging for four days. Four days waterlogging decreased the total sugars by 49.05 percent and two days waterlogging decreased the total sugars by 36.61 percent over the control. Reduction in total sugars in waterlogging treatment was due to oxygen deficiency and anaerobic conditions and less root activity. Reduction in total sugars was mainly due to impairment of water absorbing ability of the plants or inhibition of synthesis and transport of photosynthetic assimilates (Wample and Thorton, 1984). Similar reduction of sugars due to waterlogging was also reported in greengram (Kumutha *et al.,* 2008) and in pegionpea (Kumutha *et al.,* 2009).

Significant differences were observed between waterlogging treatments and genotypes after waterlogging and during the recovery period for leaf proline content (Table 3). Imposition of waterlogging for two days and four days were significantly increased the proline content. The maximum proline content was found at 25 DAS in four days waterlogged plants. Four days waterlogging increased the proline content by 91.04 percent and two days waterlogging increased the proline content by 62.21percent over the control. Proline content increased under waterlogging condition in all genotypes. The increase was higher in tolerant genotypes (TM 97-2). The susceptible genotypes under waterlogging showed lower proline content values even four days of waterlogging. Waterlogging for two days was found less detrimental to the proline content as compared to waterlogging for four days. Proline is the common osmolyte, whose accumulation provides an osmoprotection to the plants. Proline content was accumulated in response to high concentration of inorganic ions and leaf proline is increased under waterlogged conditions. Similar results were also reported in maize by Yadav and Srivastava (2010), in cotton by Naidu and Thota (2012).

Significant differences were recorded between waterlogging treatments and genotypes with regards to seed yield (Table 3). Waterlogging decreased the seed yield significantly over the control. Imposition of waterlogging for two days and four days were significantly reduced the seed yield. Four days waterlogging decreased the seed yield by 70.51 percent and two days waterlogging decreased the seed yield by 24.81 percent over the control. Reduction in seed yield in waterlogging treatment was due to oxygen deficiency and anaerobic conditions and less mineral uptake and less root activity (Wample and Thorton, 1984). Similar results were also reported in wheat (Olgun *et al.,* 2008) and blackgram (Pallavi *et al.,* 2004). The genotypes tested for waterlogging tolerance were also significantly varied for seed yield. Among the genotypes tested TM 96-2 recorded highest seed yield followed by LGG 460 and the lowest seed yield was recorded by LGG 407 and the remaining genotypes LGG 450, LGG 486 were on par with each other. Similar differences in genotypes were also observed in greengram (Laosuwan *et al.,* 1994; Yadav and Saxena, 1998). Highest seed yield in TM 96-2 was recorded due to higher leaf area, higher total drymatter, higher LAI, higher rate of photosynthesis and leaf growth and due to quick recovery of photosynthesis after waterlogging, higher photosynthetic rate as reflected through the total drymatter (Ahmad *et al.,* 2002). From the above results it can be concluded that waterlogging decreased the Physiological parameters like LAI, CGR,RGR,NAR,LAD and biochemical parameters like Prolin ,total sugars and SCMR and TM96-2 and LGG460 are considered to possess submergence tolerance among the five genotypes studied.

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