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Evaluation of Greengram Genotypes for High Temperature Tolerance at Seedling Stage by Temperature Induction Response (TIR)

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Abstract High temperature stress is one of the acute environmental stress that affect the agricultural productivity by causing injurious to plant cells. Developing cultivars that can tolerate high temperature stress is required to improve the productivity. Therefore the thermotolerance capacity of greengram genotypes were screened by temperature induction response (TIR) technique to identify the greengram genotypes tolerant to high temperature stress. Five greengram varieties (Vamban 1, VBN (Gg) 2, VBN (Gg) 3 and CO 8) were screened to standardize the optimum induction temperature and lethal temperature, then it was standardized that the optimum induction temperature was 46—52°C and the lethal temperature was 56°C for greengram. 108 greengram genotypes were screened to identify the tolerant genotypes. Among 108 greengram genotypes COGG 1319, PUSA 9072, TARM 1, VBN (Gg) 3, VGG 15029, VGG 17003, VGG 17004, VGG 17006, VGG 17009 VGG 1719 and VGG 17045 were the thermotolerant genotypes with higher survival percentage along with this the tolerant genotypes also showed higher proline content. Therefore the tolerance capacity of the genotypes is based on the tolerance against the oxidative stress by antioxidant activity.

Keywords Greengram, High temperature, Proline, Seedling, Survival.

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Introduction

The agricultural productivity was highly affected by

biotic and abiotic stress condition. Among various abiotic high temperature stress is the major abiotic stress that limits the plant growth and development. It causes irreversible damage to plant cells at all stages of crop growth and leads to less of agricultural productivity. The average world temperature has been predicted that it will increase in the range of 2.23—6.63°C by the year 2100 (EPA 2011). Extreme temperature leads to cell injury and cause cellular death due to long term exposure of high temperature (Howarth 2005). The biochemical reaction involved in plant growth and development are highly sensitive to extreme temperature. Response of plant to high temperature vary based on the duration of high temperature and the crop type (Hasanuzzaman et al. 2013). Greengram is one of the important pulse crop with short duration having wide adaptability and low input requirements, it is cultivated more than 6 million ha in the warmer region of the world (Nair et al.2012). Among legumes greengram has intrinsic tolerance mechanism against stress condition, the distinct advantage is being a short duration crop (Hanumantha Rao et al. 2016). Germination is the first growth stage affected due to extreme temperature. The temperature range which affect the germination is vary based on the crop species (Johkan et al. 2011, Kumar et al. 2011). The major impacts due to high temperature are reduction in germination percentage, abnormal seedlings, reduction in plumule and radicle growth are recorded in various plant species (Piramila et al. 2017, Toh et al. 2008) this reduction in growth is due to less of water content in the cell. The mean temperature for growing greengram is 28—30°C, it is the optimum temperature required for the crop growth (Poehlman 1977) beyond this temperature the plant growth get affected. Some plants adapt several physiological and biochemical characters to withstand under high temperature condition (Kumar et al. 2007).

Materials and Methods

Temperature induction response (TIR) is one of the potential tool to identify the thermotolerance level of seedlings by its recovery and survival growth during seedling stage. This method involves exposing the germinated greengram seedlings to induced high temperature followed by exposure of seedlings to the challenging lethal temperature for specific time period. After the stress induction the seedlings were allowed to recovered from high temperature shock at normal temperature. At the end of the recovery period the survival percentage was measured, then select thermotolerance genotypes based on survival percent at the end of the recovery period (Kumar et al. 2003).

Four greengram varieties such as Vamban 1, VBN(Gg) 2, VBN (Gg) 3 and CO 8 were used to standardize the optimum induction temperature and lethal temperature for screening the genotypes.

The greengram seeds were surface sterilized with 0.1% mercuric chloride for 2—3 minutes and then washed thoroughly with sterile distilled water. Twenty seeds were sown in pertridishes containing blotting paper moistened with water. Three replications were maintained for each variety. The greengram seedling were exposed to severe temperature to determine the challenging temperature based on the percent survival of seedlings. The 3-day old seedlings of Vamban 1, VBN (Gg) 2, VBN (Gg) 3 and CO 8 were exposed to different challenging temperature 50, 52, 54, 56°C with 60% relative humidity in the temperature and relative humidity controlled growth chamber (Labtherm).

The optimum induction temperature was determined by exposing the seedlings to gradual increase in temperature at the rate of 2°C per h (44-50,46-52,48-54°C) then the seedlings were exposed to challenging temperature for 3 h after that the seedlings were allowed to recover at 30°C for 72 h with 60% relative humidity.

At the end of the recovery period the survival percentage of seedlings were measured by using the formula.

$$\text{Survival of seedlings (\%)} = \frac{\text{No. of seedlings survived}}{\text{Total No. of seedlings}} \times 100$$

108 greengram genotypes (Table 1) were exposed to optimum induction temperature of (46-52°C, @ 2°C per h) then the seedlings were exposed to challenging temperature 56°C for 3 h and it was allowed to recov-

Table 1. Details of greengram genotypes used in this study.

Sl. No.	Source	Sl. No.	Source	Sl. No.	Source	Sl. No.	Source
1	ADGG13009	28	VGG 15030	55	VGG 16065	82	VGG 17022
2	AGG35	29	VGG15031	56	VGG 16066	83	VGG 17023
3	CO6	30	VGG 15032	57	VGG 16067	84	VGG 17024
4	CO8	31	VGG 15035	58	VGG 16068	85	VGG 17025
5	COGG1319	32	VGG 15036	59	VGG 16069	86	VGG 17026
6	COGG1332	33	VGG 15038	60	VGG 16070	87	VGG 17027
7	COGG1339	34	VGG 15040	61	VGG 17001	88	VGG 17028
8	LGG607	35	VGG 16003	62	VGG 17002	89	VGG 17029
9	MGG385	36	VGG 16005	63	VGG 17003	90	VGG 1700
10	MGG387	37	VGG 16006	64	VGG 17004	91	VGG 17032
11	NBL722	38	VGG 16008	65	VGG 17005	92	VGG 17034
12	OBGG56	39	VGG 16016	66	VGG 17006	93	VGG 17035
13	OBGG57	40	VGG 16026	67	VGG 17007	94	VGG 17036
14	OBGG58	41	VGG 16027	68	VGG 17008	95	VGG 17037
15	PUSA9072	42	VGG 16028	69	VGG 17009	96	VGG 17038
16	Samrat	43	VGG 16029	70	VGG 17010	97	VGG 17039
17	TARMI	44	VGG 16035	71	VGG 17011	98	VGG 17040
18	TMGG11035	45	VGG 16036	72	VGG 17012	99	VGG 17041
19	Vamban l	46	VGG 16046	73	VGG 17013	100	VGG 17042
20	VBN (Gg)^{2}	47	VGG 16054	74	VGG 17014	101	VGG 17043
21	VBN (Gg)^{3}	48	VGG 16055	75	VGG 17015	102	VGG 17045
22	VGG 05009	49	VGG 16057	76	VGG 17016	103	VGG 17046
23	VGG 10008	50	VGG 16058	77	VGG17017	104	VGG 17047
24	VGG 15013	51	VGG 16061	78	VGG 17018	105	VGG 17048
25	VGG 15015	52	VGG 16062	79	VGG 17019	106	VGG 17049
26	VGG 15016	53	VGG 16063	80	VGG 17020	107	VGG 17050
27	VGG 15029	54	VGG 16064	81	VGG 17021	108	VMGG12005

er at 30°C for 72 h to identify the high temperature tolerant germplasm by TIR (Temperature induction response).

Measurement of proline content

For assessing the proline content the greengram seedlings were homogenized in 3% sulfosalicylic acid and centifuged at 11500 × g. The supernatant was mixed with acid ninhydrin, glacial acetic acid and phosphoric acid. Incubate the mixture at 100°C for 1 h then cool it and add toluene to separate the chromophore containing toluene and it was read spectrophotometrically at 520 nm (Bates et al. 1973).

Statistical analysis

The data were statistically analyzed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1. Principal component analysis (PCA) was performed using Clust Vis.

Results and Discussion

Standardization of optimum induction and lethal temperature

Based on the recovery growth of greengram seedlings the optimum induction temperature was identified 46-52°C @ 2°C per h (Table 2) nearly 50% of mortality was observed at this temperature. At 46-52°C the survival percent was higher (49.53%) inVBN (Gg) 2 and lower (14.26%) in CO8. The lethal temperature was standardize that 100% mortality was observed at 56°C in greengram (Table 2), in soybean it is recorded that 48°C is the lethal temperature (Ange et al. 2016). This induction temperature varies between the crop based on the ability of thermotolerance.

The 108 greengram genotypes were exposed to

Table 2. Survival percentage of greengram varieties under optimum induction temperature and lethal temperature. Significant differences are indicated *, p<0.05; ** , P<0.01; ***, p<0.001; G-Genotype; T-Treatment.

	Survival percentage (%) Standardization of optimum induction temperature			Survival percentage (%) Standardization of lethal temperature			
Temperature range	44-50°C	48-52°C	48–54°C	50°C	52°C	54°C	56°C
Vamban 1	27.12	7.78	2.56	57.72	63.89	26.19	0
VBN (Gg) 2	77.81	49.53	3.51	96.67	34.76	21.67	0
VBN (Gg) 3	88.33	43.84	3.52	98.33	69.30	48.33	0
CO8	18.98	14.26	3.42	98.33	60.88	17.11	0
Mean	53.06	28.85	3.25	87.76	57.20	28.32	0.00
	G	T	G × T	G	T	G × T	
SEm	3.63	4.19	7.26	4.33	4.33	8.66	
CD (p<0.05)	8.64***	7.48***	14.97***	8.14**	8.14***	16.29***	

optimum induction and lethal temperature based on the standardization to screen and identify the temperature tolerant genotypes. The greengram genotypes such as COGG 1319 (70.00%), PUSA 9072(83.67%), VGG 17004 (85.00%), VGG 17006 (50.00%), VGG 17009 (58.33%), VGG17019 (65.00%), VGG 17028 (61.67%) and VGG 17045 (62.00%) were showed higher survival percentage (>50%) among the screened genotype. Therefore the selected high temperature tolerant genotypes. Therefore the select-

Table 3. Survival percentage and proline content in greengram genotypes seedlings. Significant differences are indicated *, p<0.05; **, p<0.01; ***, p<0.001; G-Genotype; T-Treatment.

	Survival percentage (%)						Proline content (µM/g FW)				
Genotypes	Control	Induced	Genotypes	Control	Induced	Genotypes	Control	Induced	Genotypes	Control	Induced
ADGG13009	100.00	18.33	VGG 16065	100.00	8.33	ADGG13009	1.90	0.97	VGG16065	1.27	1.51
AGG35	100.00	35.00	VGG 16066	100.00	15.00	AGG35	1.52	2.18	VGG 16066	1.21	1.11
CO6	100.00	11.67	VGG 16067	100.00	36.67	CO6	2.04	0.81	VGG 16067	1.86	0.72
CO8	100.00	6.67	VGG 16068	100.00	11.67	CO8	1.69	0.56	VGG 16068	1.44	1.39
COGG1319	100.00	70.00	VGG 16069	100.00	38.33	COGG1319	0.99	2.77	VGG 16069	1.83	2.28
COGG1332	100.00	31.67	VGG 16070	100.00	5.00	COGG1332	1.97	1.67	VGG 16070	1.97	1.11
COGG1339	100.00	38.04	VGG 17001	100.00	32.78	COGG1339	1.35	1.55	VGG 17001	2.37	3.65
LGG607	100.00	25.00	VGG 17002	100.00	38.33	LGG607	1.44	3.21	VGG 17002	1.80	3.14
MGG385	100.00	23.33	VGG17003	100.00	48.33	MGG385	0.90	0.82	VGG17003	1.78	3.83
MGG387	100.00	39.67	VGG 17004	100.00	85.00	MGG387	1.42	1.92	VGG 17004	2.28	3.99
NBL 722	100.00	28.33	VGG 17005	100.00	18.33	NBL722	1.24	1.48	VGG 17005	1.56	1.46
OBGG56	100.00	36.67	VGG 17006	100.00	50.00	OBGG56	1.55	2.06	VGG 17006	2.42	3.47
OBGG57	100.00	16.67	VGG 17007	100.00	33.33	OBGG57	1.15	0.89	VGG 17007	1.55	2.28
OBGG58	100.00	20.00	VGG 17008	100.00	35.00	OBGG58	0.88	1.18	VGG 17008	1.97	1.99
PUSA9072	100.00	83.67	VGG 17009	100.00	58.33	PUSA9072	0.56	3.43	VGG 17009	2.07	3.90
Samrat	100.00	30.67	VGG 17010	100.00	43.33	Samrat	1.37	0.74	VGG 17010	1.72.	3.04
TARMI	100.00	48.33	VGG 17011	100.00	10.00	TARMI	1.41	2.23	VGG 17011	0.99	1.48
TMGG11035	100.00	25.00	VGG 17012	100.00	13.33	TMGG11035	0.73	2.49	VGG 17012	1.42	1.62
Vamban l	100.00	18.33	VGG 17013	100.00	37.67	Vamban l	0.65	0.56	VGG 17013	1.99	2.09
VBN (Gg)2	100.00	45.00	VGG 17014	100.00	33.33	VBN (Gg)2	1.42	2.00	VGG 17014	1.89	1.88
VBN (Gg)3	100.00	46.67	VGG 17015	100.00	23.33	VBN (Gg)3	0.41	1.88	VGG 17015	1.56	1.42
VGG 05009	100.00	6.67	VGG 17016	100.00	28.33	VGG 05009	1.42	1.56	VGG 17016	1.13	2.28
VGG 10008	100.00	25.00	VGG 17017	100.00	16.67	VGG 10008	1.88	1.02	VGG 17017	1.67	1.74
VGG 15013	100.00	23.33	VGG 17018	100.00	40.00	VGG15013	0.84	2.42	VGG 17018	1.77	2.35

Table 3. Continued.

	Survival percentages (%)					Proline content (M/g FW)				
Genotypes	Control	Induced	Genotypes	Control	Induced	Genotypes	Control	Induced	Genotypes	Control	Induced
VGG 15015	100.00	35.00	VGG 17019	100.00	65.00	VGG 15015	1.42	1.18	VGG 17019	1.92	3.70
VGG 15016	100.00	25.00	VGG 17020	100.00	23.33	VGG 15016	1.81	1.04	VGG 17020	1.79	1.06
VGG 15029	100.00	46.67	VGG 17021	100.00	5.00	VGG 15029	1.34	3.06	VGG 17021	1.35	2.11
VGG 15030	100.00	40.00	VGG 17022	100.00	8.33	VGG 15030	1.79	1.18	VGG 17022	1.93	2.20
VGG 15031	100.00	21.67	VGG 17023	100.00	16.67	VGG 15031	1.61	1.85	VGG 17023	2.04	2.20
VGG 15032	100.00	40.00	VGG 17024	100.00	5.00	VGG 15032	0.92	1.93	VGG 17024	1.72	1.81
VGG 15035	100.00	13.33	VGG 17025	100.00	23.33	VGG 15035	1.41	1.53	VGG 17025	1.66	2.02
VGG 15036	100.00	31.67	VGG 17026	100.00	38.33	VGG 15036	2.06	1.11	VGG 17026	1.79	1.04
VGG 15038	100.00	6.67	VGG 17027	100.00	20.00	VGG 15038	1.69	1.49	VGG 17027	1.23	2.20
VGG 15040	100.00	18.33	VGG 17028	100.00	61.67	VGG 15040	1.71	1.34	VGG 17028	1.25	3.47
VGG16003	100.00	35.00	VGG 17029	100.00	31.67	VGG 16003	1.25	1.37	VGG 17029	0.97	2.80
VGG 16005	100.00	31.67	VGG 17030	100.00	28.33	VGG 16005	1.71	1.56	VGG 17030	0.92	1.62
VGG 16006	100.00	16.67	VGG 17032	100.00	11.67	VGG 16006	2.27	1.65	VGG 17032	1.28	0.69
VGG 16008	100.00	26.67	VGG 17034	100.00	5.00	VGG 16008	0.88	1.86	VGG 17034	1.70	0.55
VGG 16016	100.00	30.00	VGG 17035	100.00	36.67	VGG 16016	1.74	1.27	VGG 17035	0.72	1.07
VGG 16026	100.00	29.00	VGG 17036	100.00	35.00	VGG 16026	1.64	1.81	VGG 17036	1.25	1.57
VGG 16027	100.00	15.00	VGG 17037	100.00	34.93	VGG 16027	1.72	0.48	VGG 17037	1.70	1.67
VGG 16028	100.00	21.67	VGG 17038	100.00	33.33	VGG 16028	1.13	0.41	VGG 17038	1.06	2.07
VGG 16029	100.00	16.67	VGG 17039	100.00	3.33	VGG 16029	1.28	1.16	VGG 17039	1.95	2.67
VGG 16035	100.00	8.33	VGG 17040	100.00	26.67	VGG 16035	0.81	0.88	VGG 17040	1.20	1.76
VGG 16036	100.00	18.33	VGG 17041	100.00	28.33	VGG 16036	1.64	1.97	VGG 17041	1.14	1.58
VGG 16046	100.00	43.33	VGG 17042	100.00	39.44	VGG 16046	1.42	0.99	VGG 17042	1.96	1.83
VGG 16054	100.00	23.33	VGG 17043	100.00	16.67	VGG 16054	1.93	0.79	VGG 17043	1.46	0.97
VGG 16055	100.00	25.00	VGG 17045	100.00	62.00	VGG 16055	1.51	1.18	VGG 17045	1.60	2.30
VGG 16057	100.00	26.67	VGG 17046	100.00	10.00	VGG 16057	2.11	1.51	VGG 17046	1.88	1.49
VGG 16058	100.00	23.33	VGG 17047	100.00	16.67	VGG 16058	1.53	1.46	VGG 17047	1.35	1.58
VGG16061	100.00	16.67	VGG 17048	100.00	11.67	VGG 16061	1.87	1.69	VGG 17048	1.49	1.51
VGG 16062	100.00	20.00	VGG 17049	100.00	20.00	VGG 16062	2.02	1.16	VGG 17049	1.84	1.04
VGG 16063	100.00	35.00	VGG 17050	100.00	23.33	VGG 16063	0.90	1.55	VGG 17050	1.05	0.88
VGG 16064	100.00	18.33	VMGG12005	100.00	43.33	VGG 16064	1.46	0.90	VMGG12005	1.49	3.99
Grand mean	Control	Induced					Control	Induced			
	100.00	28.31					1.51	1.77			
	G	T	G × T				G	T	G × T		
SE	5.18	0.71	7.33				0.43	0.05	0.62		
CD (p<0.05)	10.18***	1.38***	14.40***				0.86***	0.11***	1.22***		

ed high temperature tolerant genotypes were used to study the influence of high temperature stress on vegetative and reproductive phase of greengram.

Proline content was analyzed in all the seedlings exposed to optimum induction and lethal temperature. Proline the osmoprotectant may increase under stress condition and it increases the tolerant capacity of plants by osmotic adjustment (Gill and Tuteja 2010). Significant difference was observed among the genotypes for proline content. The tolerant genotypes recorded higher proline content COGG 1319 (2.77 µM/g FW), PUSA 9072 (3.43 µM/g FW), VGG 17004 (3.99 µM/g FW), VGG 17006 (3.47 µM/g FW), VGG 17009 (3.90 µM/g FW), VGG 17019 (3.70 µM/g FW), VGG 17028 (3.47 µM/g FW) and VGG 17045 (2.30 µM/g FW), some other genotypes such as VGG 17001 (3.65 µM/g FW), VGG 17003 (3.83µ M/g FW), LGG607 (3.21 µM/g FW) and VMGG 12005 (3.99 µM/g FW) also recorded higher proline content but the survival percentage was lower when compared to the tolerant genotypes.

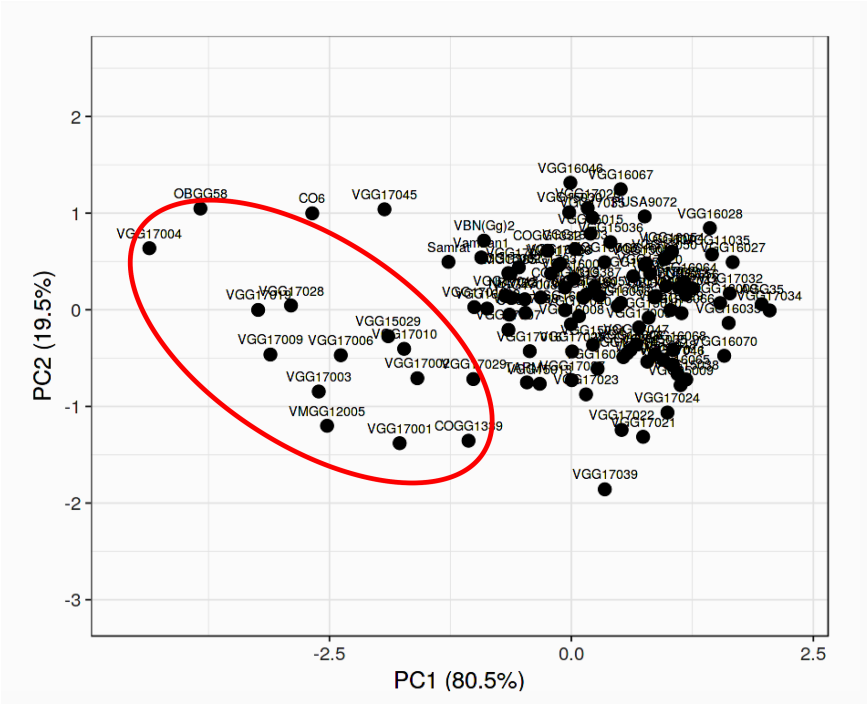

Fig. 1. Principal component analysis for survival percentage under high temperature stress and proline content.

Principal component analysis (PCA)

Principal component analysis (PCA; Fig. 1.) for traits such as survival percentage and proline was compared in all 108 greengram genotypes. In this study PCI showed higher variance than the other component, PCI describes 80.5% of the variance, PC2 describes 19.5% of the variance. Among 108 genotypes VGG 17019, VGG 17004, VGG 17028, VGG 17045, VGG 17009, VGG 17006, VGG 17003, VGG 17001, COGG 1339, OBGG58, VGG 17045, VGG 15029 and VMGG 12005 these are the tolerant genotypes which are spread diversely with higher survival percentage and proline content.

Conclusion

The greengram genotypes were screened for high temperature tolerance on the basis of survival percent and the proline content. The genotypes such as COGG 1319, PUSA 9072, VGG 17004, VGG 17006, VGG 17009, VGG 17019, VGG 17028 and VGG 17045 were showed higher survival percent and proline content to high temperature among the screened genotypes. Therefore these greengram genotypes will survive under high temperature stress during seedling stage.

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