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Physiological characterisation of green gram (*Vigna radiata* L.) genotypes for drought tolerance

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Abstract
Drought is the major constrains faced in rainfed areas so Screening of genotypes for drought tolerance is the need of the day, by using physiological traits as a parameter The present investigation was designed out in in-vitro and in-vivo conditions. In-vitro PEG6000 is used as a drought inducer to screen 17 genotypes based on seed vigour 7 best genotypes were selected for in-vivo. Field trail with 7 genotypes Based on field capacity different water treatments are taken to impose drought stress on green gram genotypes such as 100%, 80%, 50% and 30% irrigated conditions Among the genotypes protein content % KM-1423 and IPM 02-3, carotenoids KM-1423 and KM-1409, relative water content KM-1423 and IPM 02-14, chlorophyll a KM-1423 and KM-1415 and chlorophyll b, KM-1423 and IPM 02-14, Total chlorophyll KM-1423 and IPM 02-14 and proline content were found as KM-1423 and IPM 02-14. Over the genotypes KM-1423 found to be highly suitable for under both irrigated as well as rain fed conditions.

Keywords: PEG (*Polyethylene glycol*), green gram (*Vigna radiata* L), drought stress and screening

1. Introduction
Pulse crops are highly valuable grain legumes that are widely used as food, fodder and feed. Pulses are important constituent of the Indian diet and supply a major part of the protein requirement. Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought stress tolerance is seen in almost all plants but its extent, varies from species to species and even within species. It is a complex phenomenon and always coupled with moisture and high temperature stresses. Plants respond to drought by initiating a number of developmental, physiological, biochemical and molecular changes. Plants have developed a number of strategies to cope with the physiological and traits. The productivity and yield of green gram (*Vigna radiata* L) is significantly influenced by selection of suitable varieties, soil and environmental conditions as well as the management factors. Most of the green gram (*Vigna radiata* L) growing areas of the world experience environmental stresses like drought (water stress), Polyethylene glycol (PEG) could be used for evaluation of germination potential under variable water conditions since it stops the intake of water molecules and provides a controlled way to impose a physiological drought. Polyethylene glycol (PEG) compounds used to induce osmotic stress in Petri dish (in vitro) for plants to maintain uniform water potential during the experimental period. In vitro screening for drought tolerance has been proven to be a suitable method to effectively screen large sets of germplasm with good accuracy (Kulkarni and Deshpande 2007) [12]. In current study, PEG was used for drought stress induction in green gram (*Vigna radiata* L) seedling stage and tolerant genotypes are selected. (Pouresmael *et al.*, 2013) [16] Reported that using physiological traits to identify drought-tolerant genotypes is best method, but in almost all of them these indices have been used as a unique tool for screening or scoring drought tolerance using these indices in combination with agronomical indices has not yet been studied. Agronomical traits and evaluations of genotypes for either high yield potential or stable performance under different drought stress treatments are the starting points in selection for drought tolerance (Ahmad *et al.*, 2003) [2]. Therefore, based on yield loss under drought conditions in comparison to optimal conditions, different drought indices were defined that have been used for screening drought-tolerant genotypes, and also by Using conventional breeding methods to screen best genotypes are time consuming so taking physiological parameters into account is easy and convenient

method the main objective of the study is to find the best drought tolerant genotype by using physiological traits.

2. Materials and Methods

The present investigation was carried out at the field experimentation centre, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, U.P. during *zaid*-2018. Seeds were obtained from Indian institute of pulses Research (IIPR), Kanpur.These 17 genotypes of green gram (*Vigna radiata* L) such asKM-1401, KM-1404, KM-1405, KM-1406, KM-1408, KM-1409, KM-1410, KM-1413, KM-1414, KM-1415, KM-1422, KM-1423, KM-2195, KM-2241, T-44, IPM 02-3, and IPM 02-14. Out of 17 genotypes these genotypes found to be resistant to drought they are KM-1401, KM-1404, KM-1405, KM-1406, KM-1408, KM-1409, KM-1410, KM-1413, KM-1414, KM-1415, KM-1422, KM-1423 and KM-2195.

Field experiment was laid out in factorial randomized block design, replicated thrice with three main treatments and different genotypes of green gram (*Vigna radiata* L) The field level treatments was designed as T_0 100% irrigation, T_1 80% irrigation, T_2 50% irrigation and T_3 30% irrigation. The physiological parameters such as seed protein content, chlorophyll a, chlorophyll b, total chlorophyll content, Carotenoids, proline content, relative water content were taken for drought tolerance screening.

2.5 Seed vigour index I (Abdul baki and Anderson, 1973) [1]

Germination% x seedling length

2.1 Proline content (mg/f.wt.)

The frozen plant material is homogenized in 3% aqueous sulphosalicylic acid (0.01g/ 0.5 ml) and the residue is removed by centrifugation at 12,000 g for 10 min 1ml of the homogenized tissue reacts with 1 ml acid-ninhdrin and 1 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction is terminated in an ice bath. The reaction mixture is extracted with 2 ml toluene, mixed vigorously and left at room temperature for 30 min until separation of the two phases. The chromophore-containing toluene (1 ml, upper phase) is warmed to room temperature and its optical density is measured at 520 nm using toluene for ablankthe proline concentration is determined from a standard curve using D-Proline.

2.2 Carotenoid content (mg/f.wt.)

Carotenoid was determined according to (Wellborn 1983) [19]. 0.5 gm and homogenizedin 10 ml of acetone (80% acetone). Next to the centrifuged at 3000 rpm at 10 min. The absorbance was recorded at 470 nm.

It is calculated by the formula –

Total carotenoids = [1000A470- (3.27 Chl-a+104 Chlb)]/22.

2.3 Relative water content (%)

The relative water content was estimated by the method of Barrs and Weatherly (1962) [5]. Ten leaf discs were collected randomly in each treatment and weighed accurately up to third decimal an a single pan analytical balance. This was considered as fresh weight. The weighed leaf discs could float on distilled water in a Petri dish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. After drying in hot air oven at 720 ºC for 48 hours, the dry weight was recorded and RWC was calculated by using the following formulae.

$$RWC\ (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

2.4 Chlorophyll content (mg/f.wt.)

Chlorophyll was determined according to Wellborn (1983) [19]. 1gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 ppm for 5 minutes. The supernatant was read under 663, 645 nanometres. The readings were fed in the following formula and results were determined under spectrophotometer.

Chlorophyll content was calculated by using the following formula and expressed in mg/g fresh weight-1:

$$\text{Chlorophyll 'a'} = 12.7 \times (A663) - 2.69 \times (A645) \times \frac{V}{1000 \times w \times a} \quad (\text{Mg g-1 fr. wt.})$$

$$\text{Chlorophyll 'b'} = 22.9 \times (A645) - 4.68 \times (A663) \times \frac{V}{1000 \times w \times a} \quad (\text{mg g-1 fr. wt.})$$

$$\text{Total chlorophyll} = 20.2 \times (A645) + 8.02 \times (A663) \times \frac{v}{1000 \times w \times a} \quad (\text{Mg g-1 fr. wt.})$$

2.6 Statistical analysis

The analysis of variance was worked out to test the significance of F tests. It was carried out according to the procedure of factorial randomized complete block design for each character as per methodology advocated by (Fisher 1936). ANOVA helps in partitioning the total variance into three components viz. replication, treatments and error.

3. Results and Discussion

PEG treatment

Percent of seed germination, were severely damaged with increased level of PEG 6000 stress at 10% level of stress, the percent of decrease in germination % is a useful parameter to assess stress tolerance of genotypes presented in Table 1.

It was observed that with an increase in water stress (0 – 10%), there was a gradual depletion in rate of water uptake by green gram (*Vigna radiata* L) seeds of all genotypes. This reduction might be due to the fact that water moves from high potential to low potential due to differences in the free energy content. The gradient of water potential between dry seeds and pure water decrease rapidly with the addition of any soluble substances such as polyethylene glycol in water. The decrease in water potential gradient between seed and media will prevent the seeds to absorb the desired amount of water (Achakzai, 2009) [3]. Similar results were also reported in case of mungbean (Akhter, 1985) [4], and maize (Achakzai, 2009) [3]. Drought stress decreased the root length it may be due to declining vacuolar K+ because its accumulation in newly formed vacuoles drives cell expansion (Walker *et al*., 1998) [21]. It is a fact that the drought tolerant accessions had greater shoot and root lengths and biomass production than the sensitive accessions. Same results found in in alfalfa (Safarnezad, 2008) [18]. KM-1409, KM-1415, KM-1423, KM-1422, KM-2195, IPM 02-3 and IPM 02-14. These genotypes found to be more tolerant to the drought stress on the basis of seed vigour.

3.1 Protein content (%)

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 2, KM-1423 (20.133) and IPM 02-3 (19.037) recorded highest content of protein compared to other genotypes, IPM 02-14 (18.4), KM-1415 (16.5) and KM-2195 (16.267) recorded moderate protein content, whereas KM-1422 (15.533) and KM-1409 (16.01) recorded significantly low content of protein.

The effects of water stress on chickpea grains. The stressed seed showed reduction in protein in large size Kabuli chickpeas and small size Desi chickpea as compared to non-stressed chickpea grains was observed by (Nayyar *et. al.*,2006) [20].

3.2 Carotenoids

Significant differences were noticed between moisture stress treatments, genotypes and their interactions, presented in table 2. Carotenoids were significantly reduced due to imposition of stress 30% when compared to other treatments. Among the genotypes, irrespective of the irrigation and moisture stress treatments, KM-1423 (0.190) and KM-1409 (0.190) recorded highest carotenoid compared to other genotypes, KM-2195 (0.187), IPM 02-14 (0.183) and IPM 02-3 (0.177) recorded moderate carotenoid, whereas KM-1422(0.169) and KM-1415 (0.170) recorded significantly low carotenoid. Due to Water stress, reduction in the concentrations of carotenoids **(**Havaux, 1998; Kiani *et al.,* 2008) [10, 11], Oxidative damage generated by drought stress in the plant tissue is alleviated by the production of ROS in the thylakoids (Niyogi, 1999; Reddy *et al*., 2004) [15]. This proves the carotenoid pigments are sensitive to 30% than other treatments.

3.3 Relative water content

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 2, KM-1423 (65.36) and IPM 02-14 (61.76) recorded highest relative water content compared to other genotypes, IPM 02-3(60.50), KM-1422 (60.36), and KM-1409 (59.467) recorded moderate relative water content, whereas KM-1415 (56.96) and KM-2195 (57.400) recorded significantly low relative water content. Significant differences were noticed between moisture stress treatments, genotypes and their interactions. Relative water content were significantly reduced due to imposition of stress 30% when compared to other treatments.

Under drought stress conditions should be of high-content RWC. Under water stress decrease in RWC in plants under drought stress may depend on plant vigour reduction and have been observed in many plants (Liu *et al.,* 2002) [14]. Under water deficit, cell membrane subjects to changes such as penetrability and decrease in sustainability (Blokina *et al.,* 2003) [7].

3.4 Chlorophyll-a

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 3, KM-1423 (1.723) and KM-1415 (1.687) recorded significantly highest chlorophyll a content compared to other genotypes, KM-1422 (1.647), KM-2195 (1.640) and IPM 02-3 (1.620) recorded moderate chlorophyll a content, whereas IPM 02-14 (1.607) and KM-1409 (1.607) recorded significantly low chlorophyll a content.

Significant differences were noticed between moisture stress treatments, genotypes and their interactions. Chlorophyll content were significantly reduced due to imposition of stress 30% when compared to other treatments. Chlorophyll-a content of green gram (*Vigna radiata* L) plants showed a decreasing trend with the increasing duration of drought which proved that these photosynthetic pigments are sensitive to water deficit condition. The reduction in photosynthesis under water deficit stress can also be attributed to a decrease in chlorophyll content, such a decrease in total chlorophyll content due to drought stress was observed in Green gram (*Vigna radiata* L) (Lalinia *et al*., 2012) [13]. Drought stress at early flowering stage reduced the chlorophyll content in chickpea (Gupta *et al*., 2010) [9].

3.5 Chlorophyll-b

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 3, KM-1423 (0.22) and IPM 02-14 (0.20) recorded highest chlorophyll b content compared to other genotypes, IPM 02-3 (0.19), KM-2195 (0.187) and KM-1415 (0.180) recorded moderate chlorophyll b content, whereas KM-1409 (0.16) and KM-1422 (0.17) recorded significantly low chlorophyll b.

Chlorophyll b content of leaves declined significantly with increasing concentration of drought stress Chlorophyll decreased in different concentrations of water stress. The reduction in photosynthesis under water deficit stress can also be attributed such a decrease in chlorophyll b content due to drought stress was observed in Green gram (Lalinia *et al*., 2012) [13]. However, the most stress effected at 30% irrigated condition when compared to 50%, 80% and 100% irrigated conditions.

3.6 Total Chlorophyll

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 3, KM-1423 (1.987) and IPM 02-14 (1.970) recorded highest total chlorophyll content compared to other genotypes, IPM 02-3 (1.947), KM-2195 (1.910) and KM-1415 (1.873) recorded moderate total chlorophyll content, whereas KM-1409 (1.830) and KM-1422 (1.850) recorded significantly low total chlorophyll content. Due to imposition of drought stress the photosynthetic rate was declined due to low irrigated conditions low photosynthetic rate that leads to decline in total chlorophyll content similar results were obtained by Rao (2012) [17].

3.7 Proline content

Among the genotypes, irrespective of the irrigation and moisture stress treatments presented in table 2, KM-1423 (8.033) and IPM 02-14 (7.866) recorded highest proline compared to other genotypes, IPM 02-3 (7.800), KM-2195 (7.500) and KM-1422 (7.366) recorded moderate proline, whereas KM-1409 (7.066) and KM-1415 (7.333) recorded significantly low proline. Higher accumulation of proline betters osmotic balance in plant cells suffering from water deficit and accounted for their higher drought tolerance capacity keeping the slope of water potential at the threshold of drought stress (Baroowa *et al.,* 2015.) [6].

4. Conclusion

Green gram (*Vigna radiata* L) genotypes showed susceptibility in terms of physiological under different levels of drought stress. However, the effect was more pronounced when genotypes imposed to moisture stress at 30% irrigation.KM-1423 and IPM 02-3 are highly suitable to the severe drought zones under both irrigated as well as rain fed conditions. However, for drought prone areas KM 1423 is recommended due to its drought tolerance character.

Table 1: Evaluation of green gram (*Vigna radiata* L) genotypes with PEG6000 at 10

S.N0	Genotypes	Germination (%)		Seed Vigour	
		Control	Peg Treated	Control	Peg Treated
G_1	KM-1401	80	50	1200	600
G_2	KM-1404	60	30	720	240
G_3	KM-1405	80	20	1040	100
G_4	KM-1406	70	30	910	300
G_5	KM-1408	50	10	600	40
G_6	KM-1409	50	60	750	720
G_7	KM-1410	70	40	980	440
G_8	KM-1413	70	30	840	210
G_9	KM-1414	70	20	910	160
G_{10}	KM-1415	60	70	960	840
G_{11}	KM-1422	80	60	1200	660
G_{12}	KM-1423	90	50	1530	600
G_{13}	KM-2195	80	60	1120	600
G_{14}	KM-2241	90	30	1170	300
G_{15}	T-44	90	70	1080	560
G_{16}	IPM 02-3	80	40	1840	840
G_{17}	IPM 02-14	70	60	1260	720
		Standard Deviation 2.213		Standard Deviation 255.396	
		Variance 2.315		Variance 65227.941	
		T - Table (0.05) 3.230		T - Table (0.05) 2.037	

Table 2: mean comparison of physiological traits of green gram (*Vigna radiata* L) genotypes subjected to drought treatme

Genotypes	Protein Content				Proline Content				Carotenoids				Relative Water Content			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
KM-1409	22.3	21.3	20.6	16.1	2.3	3.20	4.63	7.06	0.247	0.177	0.210	0. 190	70.900	62.333	60.467	59.467
KM-1415	22.3	20.23	19.43	16.5	2.53	3.26	5.06	7.3	0.227	0.210	0.19	0.170	71.800	57.833	60.167	56.96
KM-1422	22.3	21.1	19.93	15.53	2.400	3.43	4.66	7.36	0.207	0.217	0.193	0.163	72.433	57.200	59.533	60.36
KM-1423	23.86	22.8	21.667	20.13	2.4	3.76	5.16	8.03	0.213	0.207	0.197	0.190	73.400	69.46	65.63	65.36
KM-2195	22.76	21.33	20.63	16.26	2.46	3.33	4.90	7.5	0.207	0.203	0.200	0.187	69.500	57.200	64.467	57.400
IPM 02-3	23.1	22.3	22.3	19.06	2.63	3.86	5.30	7.80	0.200	0.197	0.190	0.177	65.467	60.733	58.667	60.50
IPM 02-14	22.33	22.3	22.1	18.4	2.83	4.03	5.76	7.86	0.220	0.193	0.190	0.183	72.23	62.26	60.300	61.76
C.D (0.05%)	0.139 T	0.184 G	0.369 TXG		0.006 T	0.008 G	0.015 TXG		0.155 T	0.191 G	0.392 TXG		0.16 T	0.22 G	0.44 TXG	
S.E(m)	0.049 T	0.065 G	0.130 TXG		0.002 T	0.003 G	0.005 TXG		0.141 T	0.086 G	0.533 TXG		0.05 T	0.07 G	0.15 TXG	

T_0: 100% IRRIGATION T_1: 80% IRRIGATION T_2: 50% IRRIGATION, T_3: 30% IRRIGATION

Table 3: mean comparison of physiological traits of green gram (*Vigna radiata* L) genotypes subjected to drought treatments

Genotypes	Chlorophyll a				Chlorophyll b				Total chlorophyll content			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
KM-1409	1.863	1.720	1.667	1.607	0.270	0.220	0.193	0.16	2.133	1.973	1.943	1.830
KM-1415	1.767	1.733	1.727	1.687	0.247	0.237	0.233	0.180	2.000	1.933	1.907	1.873
KM-1422	1.827	1.777	1.713	1.647	0.240	0.220	0.207	0.17	2.033	1.997	1.887	1.850
KM-1423	1.920	1.780	1.773	1.723	0.263	0.243	0.233	0.22	2.193	2.080	2.077	1.987
KM-2195	1.787	1.720	1.703	1.640	0.273	0.233	0.200	0.187	1.903	1.827	1.907	1.910
IPM 02-3	1.860	1.840	1.700	1.620	0.260	0.220	0.21	0.19	2.030	1.973	1.880	1.947
IPM 02-14	1.813	1.780	1.74	1.607	0.257	0.240	0.207	0.20	2.100	1.987	1.933	1.970
C.D (0.05%)	0.023 T	0.030 G	0.060 TXG		0.008 T	0.011 G	0.022 TXG		0.023 T	0.030 G	0.061 TXG	
S.E(m)	0.008 T	0.011 G	0.021 TXG		0.003 T	0.004 G	0.008 TXG		0.008 T	0.011 G	0.021 TXG	

T_0: 100% IRRIGATION, T_1: 80% IRRIGATION, T_2: 50% IRRIGATION, T_3: 30% IRRIGATION

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