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Evaluation of different pigeonpea varieties for desiccation tolerance at seedling level

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Abstract
The laboratory experiment was conducted during 2020-21 having 7 treatments with 3 replication planned with CRD design. Four varieties of pigeonpea namely GRG 811, TS-3R, GRG 152 and Maruti were exposed to desiccation and rehydration treatments in laboratory. For desiccation treatments seeds were exposed to different days of desiccation for 3, 5 and 7 days respectively. For rehydration treatments seeds were exposed to desiccation for 3, 5, 7 days and rehydrated for one day in petriplate respectively. Results revealed that treatment control *i.e.,* no desiccation treatment (T_1) showed significantly highest Seed germination at first count (34.77%), seed germination at final count (87.08%) shoot length (17.35 cm), seedling vigour index -I (3032), whereas (T_5) desiccation treatment for 3 days + rehydration treatment for one day in petriplate showed significantly highest root length (17.84 cm), seedling dry weight (12.22 mg) and speed of germination (18.98) in the variety TS-3R, followed by GRG 811. lowest was observed in T_4 (desiccation treatment for 7 days) pertaining to Maruti variety. Highest plant height (76.5 cm) was recorded in T_1 in GRG152 variety (69.25 cm) however, there was no plant growth in T_7 treatment of Maruti and first flowering initiation occurred in TS-3R variety.

Keywords: Desiccation, pigeonpea, rehydration, TS-3R

Introduction
Among the various food legumes, pigeonpea (*Cajanus cajan* L.) occupies an important place and has been rated as the best as far as its biological value is concerned. It has been recommended for a balanced diet with cereals, especially to fill in the nutritional gap for proteins amongst the poorer section in developing economies that cannot afford a non-vegetarian diet. At present, the protein availability in developing countries is about one-third of normal requirements and with ever growing population; various nutritional development programs are facing a tough challenge to meet the protein demand. Pigeonpea is mainly cultivated for its dry seeds and green vegetables in dry regions of the tropics and subtropics. The major pigeonpea producing countries are India, Eastern Africa, Central and South America, the Caribbean, Myanmar and West Indies (Vijayalakshmi *et al.*, 2013) [15]. Pigeonpea is the second most important pulses crop after chickpea in India and rank fifth in the world after soybean. In India, the crop is extensively grown in Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Gujarat. Globally, pigeonpea occupies an area of about 6.99 million hectares with an annual production of 5.96 million tonnes with a mean productivity of 852 kg/ha. India covers an area of 4.43 million hectares, production of 3.56 million tonnes and productivity of 806 kg per ha. Karnataka covers an area of 1.3 million hectares, production of 0.91 million tonnes and productivity of 860 kg per ha (Anon., 2021) [2].

Drought stress (DS) poses a constant challenge for agricultural crops and has been considered a severe constraint for global agricultural productivity; its intensity and severity are predicted to increase in near future. Legumes demonstrate high sensitivity to DS, especially at vegetative and reproductive stages. They are mostly grown in dry areas and are moderately drought tolerant, but severe DS leads to remarkable production losses. The most prominent effects of DS are reduced germination, stunted growth, serious damage to the photosynthetic apparatus, decrease in net photosynthesis, and reduction in nutrient uptake. To curb the catastrophic effect of DS in legumes, it is imperative to understand its effects, mechanisms, and the agronomic and genetic basis of drought for sustainable management. (Emefiene *et al.,* 2013) [8]. Seedling survival after desiccation, though an important agricultural trait, has not been studied extensively and reports on plant genetic resources displaying seedling survival after desiccation are relatively meagre.

Desiccation tolerance refers to the tolerance of further dehydration to below 0.1 g H_2O g $^{-1}$ dry weight, when the hydration shell of molecules is lost, without accumulation of lethal damage (Bewley 1979) [5]. Drought tolerance can be considered as the tolerance of moderate dehydration, down to a moisture content of - 0.3 g H_2O g $^{-1}$ dry weight, at which there is no bulk cytoplasmic water presence (Hoekstra *et al.* 2001). Tolerance to drought in pigeon pea, particularly of short-duration pigeonpea, can be ascribed to the crop's ability to maintain total dry matter, a small pod size, few seeds in the pod, high seed mass and low flowering synchronization. (Lopez *et al.* 1996) [11].

Material and Methods

The seeds of four pigeonpea variety *viz.*, GRG 811, TS-3R, GRG152, Maruti were obtained from seed unit, University of Agricultural Sciences Raichur. The research was carried out at two conditions *viz*, laboratory and field, laboratory experiments were conducted at Department of Seed Science and Technology, College of Agriculture Raichur during October 2020-21, and the data was analysed using two factorial completely randomised design. Seeds of pigeonpea varieties namely TS-3R, GRG 811, GRG 152 and Maruti (400 seeds) were sterilized by 1.5 percent chlorine and 70 percent ethanol followed by several washes with autoclaved double distilled water (DDW). Seeds were placed on filter paper in a petri plate containing murashige and skoog Media (MS) medium, incubated for four days in 4 ^{o}C (stratification) after which plates were transferred for germination at 20 ^{o}C. Treatment which were imposed to these seeds are T_1- no desiccation treatment, T_2- desiccation treatment for 3 days, T_3- desiccation treatment for 5 days, T_4- desiccation treatment for 7 days, T_5- desiccation treatment for 3 days+ rehydration treatment for one day in petriplate, T_6- desiccation treatment for 5 days+ rehydration treatment for one day in petriplate, T_7- desiccation treatment for 5 days+ rehydration treatment for one day in petriplate. The seeds which were survived in this laboratory condition after the exposure of seeds to the above treatments were then selected and transferred to pots as per the respective treatments to check either the seedlings which were subjected to desiccation could overcome the desiccation stress or not.

Desiccation Treatment

Germinated seeds on filter paper having short and long radicles were transferred to new empty, sterile petri plates for slow drying at 20 ^{o}C. For the drying purpose, plates were not sealed with parafilm but were simply covered to avoid fast desiccation. After the completion of desiccation treatment seedlings were sown in pots as per the respective treatments (T_2-T_4).

Rehydration treatment

Desiccated seeds were exposed to rehydration for one day in petriplate with distilled water then those rehydrated seeds were used for germination test. Those seedlings which were good in germination were then sown in pots as per the respective treatments (T_5-T_7). The seedlings which were sown in pots were monitored till flowering for each treatment in each replication.

After imposition of these treatments seed quality parameters like first count of seed germination, final count of seed germination, root length, shoot length, seedling vigour index I, seedling dry weight were recorded and results obtained were furnished below.

First and final count of seed germination (%)

The seed germination test was conducted in petriplates (25 seeds in each) to place 400 seeds for each variety, in filter paper containing murashige and skoog media incubated for four days in 4 ^{o}C (stratification) after which plates were transferred for germination at 20 ^{o}C in germination chamber. The number of normal seedlings from each replication were counted at the end of the 4th day (first count) and 10th day (final count) and the mean germination percentage was calculated (ISTA, 2013) [9].

$$\text{Germination (\%)} = \frac{\text{No. of normal seedlings}}{\text{Total no. of seeds}} \times 100$$

Root length (cm)

From the germination test, five normal seedlings were selected randomly from each treatment on 10th day. The root length was measured from the tip of primary root to base of hypocotyls and mean root length was expressed in centimeters.

Shoot length (cm)

From the germination test, the five random seedlings used for measuring shoot length**.** The shoot length was measured from the base of primary leaf to the base of hypocotyls and the mean shoot length was expressed in centimeter.

Seedling dry weight (mg)

From the germination test the same five seedlings used for measuring the root and shoot length along with another five seedlings were kept in a butter paper packed and dried in hot air oven maintained at 70 ^{0}C for 24 hours. Then the seedlings were cooled in a desiccator for 30 minutes and the weight of dried seedling were recorded using an electronic balance and was expressed in milligram per seedling.

Seedling vigour index I

The seedling vigour index I was computed using the formula as suggested by Abdul-Baki and Anderson (1973) [1] as follows

Vigour index-I = Germination (%) x Mean seedling length (cm)

Pot experiment

A load of fine red sandy soil was bought and it was mixed thoroughly with vermicompost and FYM. Then 84 (3 Replication X 7 Treatments X 4 Varieties) pots were arranged after filling the soil in these pots to the 3/4th of the pot, 21 pots were taken for each variety then these pots were arranged in 3 rows as one pot per each treatment in each replication.

The seeds which were tested for desiccation in laboratory with the above treatments were sown according to the treatments in three replications. In each pot 10 seeds were sown by simple dibbling method and watered to maintain sufficient soil moisture. Thinning was done after the emergence of seedlings to the height of 15 cm, and 3 good plants were maintained to test the seedling growth subjected to desiccation treatment to record the plant height and flower initiation.

Plant height (cm)
The length of main stem of tagged plants in each pot is randomly selected, and measured in centimeters from ground level to the tip of the plant and average was worked out and expressed in centimeter.

Flower initiation
This was done by seeing the visual initiation of flowers at 75 days of sowing, to check whether the flower initiation occurs in the treated seedlings or not.

Results and Discussion
Among the various treatments, significantly highest (34.77%) germination at first count was observed under the treatment T_1 (control) followed by T_5 (34.68%) (DT for 3 days + RT) and T_2 (33.93%) (DT for 3 days). Among the varieties V_2 TS-3R showed significantly highest (35.47%) germination followed by V_1 GRG 811 (33.07%), (Table 1) However, among the various treatments, significantly highest (87.08%) germination percentage at final count was observed under T_1, T_1 was on par with T_5 (83.92%) and among the varieties V_2 showed significantly highest (82.19%) germination at final count (Table 2). Here the seed was considered tolerant when it germinated and produced a normal seedling, or intolerant when it did not germinate or did not produce a normal seedling. (Corbineau and Come 2017) [7]. During desiccation tolerance seed germination progressively reduced as moisture content decreased. Similar findings were obtained in pigeonpea by Lisboa that the performance of drying to the less water content of below 10 percent certainly contributed for the decrease in germination due to low metabolism indexes of the reserves, especially in the protein fraction, whose centesimal composition in the seed is at 23 percent. Certainly, the elevated water contents of 14 percent and 16 percent contributed for the increased germination percentage due to acceleration in breathing rates of the reserves which occurred due to rehydration. (Lisboa *et al.*,2017) [10].
Significantly highest (17.84 cm) root length was observed under the treatment T_5, among the varieties highest (18.54 cm) root length was found in V_2 TS-3R, However, significantly highest (17.35 cm) shoot length was observed under the control treatment T_1 followed by the treatment T_5 (17.23 cm), highest (16.98 cm) shoot length was recorded in the variety TS-3R. Seedlings with larger root volumes have a better ability to take up water, avoid planting stress and become established after planting therefore, seedling initial root system size in relation to shoot size can have a direct effect on seedling water balance just after planting (Apostol *et al.*, 1990) [3]. (Polania *et al.*, 2017) [14] reported the importance of recording shoot and root mass under drought condition. The role shoot growth is more sensitive than root growth in drought screening (Pace *et al.*, 1999) [13] Under drought stress, plants allocate fewer resources to shoot because resources are mostly used for water uptake and reducing the evaporation rate (Chimungu *et al*., 2014) [6].
Among the various treatments, significantly highest (12.22 mg) seedling dry weight was observed under T_5 followed by T_1 (11.64 mg) and the highest seedling dry weight was recorded in V_2 (11.11 mg), (Table 3).This results were in contrast with the findings of Hay (1995) that the rate of decline in percent moisture content after mass maturity did not appear to increase significantly , however changes in the rate of decline in rate of seed fresh weight and, particular seed water content, indicate that there was active water loss from the seeds after mass maturity, that is the seeds have entered the post abscission phase of seed development despite the loss of water from the seeds resulting in the low seedling dry weight at very extreme desiccation and high seedling dry weight was achieved when the seeds were rehydrated to overcome desiccation tolerance.
Among the various treatments, significantly highest vigour (3,032) was observed under the treatment T_1 followed by the treatment T_5 (2,914) and in the varieties highest (2923) Seedling vigour index I was recorded in V_2 followed by V_1 (2,705), (Table 4). Similar findings were noticed in *Garcinia imberti* the fast desiccation method improved germination and seedling vigour in comparison to slow desiccation. Thus, fast drying was less adverse than slow drying, probably due to the embryo like structure being safe guarded from too much drying. It was reported that the mechanisms by which the fast drying of recalcitrant seeds confer greater tolerance to lower water content have yet to be explained (Berjak and Pammeter 2008) [4]. It has been suggested that the metabolic functions of cells are distorted in seeds with moisture content in between that of the fully hydrated state and the lower threshold of tolerance for a longer period as in the case of slow desiccation (Walters *et al.*, 2001) [16] The maximum seed vigour index under both fast and slow desiccation below the critical moisture content, exhibited less germination percentage and seed vigour index. The seedling length and seed vigour index were also reduced during continuous desiccation. It was reported that, the whole plant length was negatively affected by the rate of dehydration of the seeds that delayed seedling growth (Nunes *et al.*,2015) [12].
The results on plant height were obtained at 60, 75 and 100 days after planting the seeds in pot which were already imposed with desiccation and rehydration treatments in laboratory (Table 5). Significantly highest plant height of 57.41 cm, 65.5 cm, 76.5 cm was obtained in T_1 treatment at 60, 75 and 100 days respectively followed by T_5 treatment, highest plant height of 54.86 cm, 62.01 cm, 69.25 cm was obtained in V_3 variety at 60, 75 and 100 days respectively followed by V_1 variety. However, lowest mean plant height was obtained in Maruti variety as there was no plant growth in T_7 treatment as we found Maruti seeds were very much susceptible to desiccation treatments in laboratory.
The days taken for flower initiation was recorded in all the treatments and varieties. The flower initiation started at 75 days of sowing in TS-3R in T_1 (control) and T_5 (DT for 3 days + RT) followed by GRG 811 in the same treatment in other varieties such as GRG 152 and Maruti showed poor flower initiation. Among the treatments at 75 days of sowing flowering was seen in each treatment of TS-3 R and GRG 811 (excluding T_4) flowering in GRG 152 variety was seen only in T_1 and T_2 where as in Maruti flowering was seen in T_1 and T_4 only. Whereas at 100 days of sowing we can see initiation of flowering in rest of the treatments also.

Table 1: Effect of desiccation and rehydration treatments on the first count of seed germination percentage in pigeonpea

First count of seed germination percentage					
Treatments	**Varieties**				**Mean**
	V1	**V2**	**V3**	**V4**	
T1	35.60	37.07	33.83	32.57	34.77
T2	33.94	36.25	33.20	32.33	33.93
T3	32.36	35.92	31.00	28.98	32.06
T4	30.27	33.44	29.37	28.23	30.33
T5	34.86	37.20	33.93	32.72	34.68
T6	33.27	34.54	31.80	30.90	32.63
T7	31.22	33.86	31.50	29.50	31.52
Mean	33.07	35.47	32.09	30.75	32.84
	SE.m ±		CD @ 1%		
T	0.18		0.50		
V	0.13		0.38		
TxV	0.36		1.01		

Table 2: Effect of desiccation and rehydration treatments on the final count of seed germination percentage in pigeonpea

Final count of seed germination percentage					
Treatments	**Varieties**				**Mean**
	V1	**V2**	**V3**	**V4**	
T1	88.00	90.33	87.33	82.67	87.08
T2	85.00	86.00	82.00	79.67	83.17
T3	81.67	81.67	69.33	67.67	75.08
T4	74.67	75.00	63.00	62.67	68.83
T5	86.00	87.00	82.33	80.33	83.92
T6	81.33	80.00	71.33	68.67	75.33
T7	74.00	75.33	66.33	65.67	70.33
Mean	81.52	82.19	74.52	72.48	77.68
	SE.m ±		CD @ 1%		
T	0.72		2.06		
V	0.55		1.55		
TxV	1.45		4.11		

Table 3: Effect of desiccation and rehydration treatments on the Seedling dry weight (mg) in pigeonpea

Seedling dry weight (mg)					
Treatments	**Varieties**				**Mean**
	V1	**V2**	**V3**	**V4**	
T1	12.13	12.17	12.23	10.03	11.64
T2	9.63	9.33	9.47	10.27	9.68
T3	9.23	10.90	8.97	9.07	9.54
T4	10.13	9.00	10.07	8.83	9.51
T5	11.67	12.60	11.97	12.63	12.22
T6	10.90	11.70	12.40	11.27	11.57
T7	11.97	12.10	9.63	11.60	11.33
Mean	10.81	11.11	10.68	10.53	10.78
	SE.m ±		CD @ 1%		
T	0.19		0.54		
V	0.14		0.41		
TxV	0.38		1.09		

Table 4: Effect of desiccation and rehydration treatments on the seedling vigour values of the seedlings in pigeonpea

Seedling vigour index					
Treatments	**Varieties**				**Mean**
	V1	**V2**	**V3**	**V4**	
T1	3,133	3,348	2,955	2,692	3,032
T2	2,884	3,118	2,722	2,576	2,825
T3	2,645	2,936	2,150	1,962	2,423
T4	2,260	2,507	1,851	1,769	2,096
T5	2,998	3,237	2,794	2,629	2,914
T6	2,705	2,762	2,269	2,122	2,464
T7	2,310	2,551	2,089	1,937	2,222
Mean	2,705	2,923	2,404	2,241	2,568
	SE. m±		CD @ 1%		
T	30.47		86.55		
V	23.03		65.43		
TxV	60.94		173.10		

Table 5: Effect of desiccation treatments on the plant height (cm) at 100 days

Plant height (cm)					
Treatments	**Varieties**				**Mean**
	V1	**V2**	**V3**	**V4**	
T1	85.51	70.35	84.53	65.63	76.50
T2	67.20	69.30	69.30	57.38	65.79
T3	53.03	51.45	65.63	57.23	56.83
T4	55.65	73.38	49.35	71.40	62.44
T5	66.15	69.83	68.25	68.78	68.25
T6	67.20	37.80	80.00	67.20	63.05
T7	52.50	33.08	67.73	0.00	38.33
Mean	63.89	57.88	69.25	55.37	61.60
	SE.m ±		CD @ 5%		

T	0.35	1.00
V	0.26	0.75
TxV	*0.70*	2.00

T_1- no desiccation treatment, T_2- desiccation treatment for 3 days, T_3- desiccation treatment for 5 days, T_4- desiccation treatment for 7 days, T_5- desiccation treatment for 3 days+ rehydration treatment for one day in petriplate, T_6- desiccation treatment for 5 days+ rehydration treatment for one day in petriplate, T_7- desiccation treatment for 5 days+ rehydration treatment for one day in petriplate. V_1- GRG 811, V_2- TS-3R, V_3- GRG 152, V_4- Maruti.

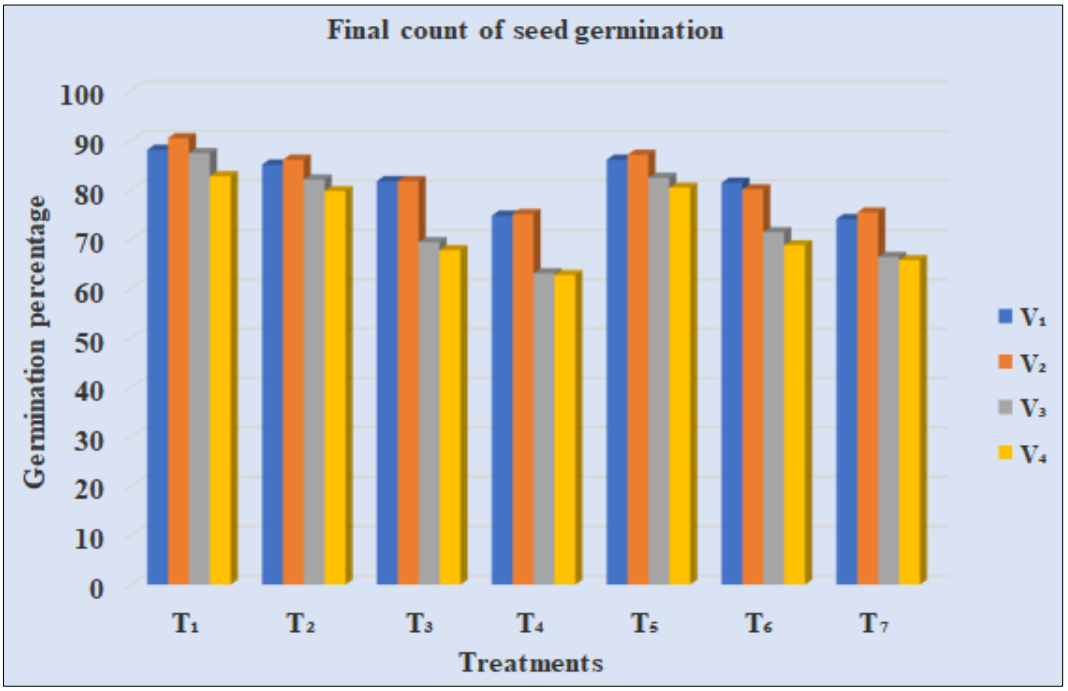

Fig 1: Effect of desiccation and rehydration treatments on the final count of seed germination percent of seedlings in pigeonpea

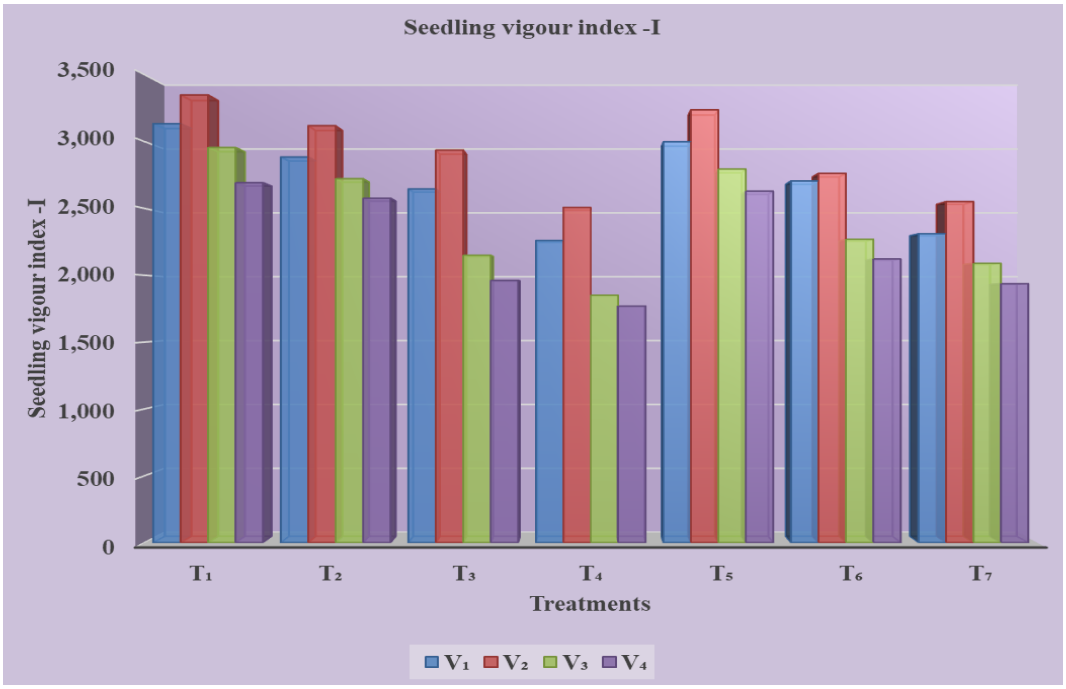

Fig 2: Effect of desiccation and rehydration treatments on the seedling vigour index -I of seedlings in pigeonpea

Conclusion

Among the four varieties used in the study TS-3R was found as a best variety to mitigate the drought stress and among the various treatments which were imposed in the lab to the seeds the treatment T_5 (DT for 3 days + RT) was found as best treatment this shows the ability of pigeonpea to regain its metabolic and physiological functions when rehydrated. Seedlings which were in pots have overcome desiccation and showed normal vegetative and flowering behaviour in T_5 treatment is an evidence to say that it was the best treatment.

But the variation in flowering duration is an evidence to say the intrinsic nature of depends on the nature of varieties to flower and also to tolerate drought stress period as early flowering in case of TS-3R was encountered.

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