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Screening of soybean genotypes for pre-germination anaerobic stress tolerance to waterlogging

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Abstract
Waterlogging is a common abiotic stress in *kharif* season (monsoon) dependent crops like soybean, the objective of this study was to evaluate the possibility of finding a morphological trait as selection criteria of soybean genotypes under waterlogging anaerobic stress. The experiment was carried out in the stage of germination. Percent germination was measured during water logging stress and stress recovery. Our results indicated the presence of genetic variability for germination. Percent germination associated with pre-germination under stress conditions can be used as selection criteria for waterlogging-tolerant soybean in breeding programs.

Keywords: soybean, genotypes, stress tolerance, waterlogging

Introduction
Soybean is an important source of oil and protein worldwide. In Indian soybean is a *kharif* crop grown in an area of 109.72 lakhha with a productivity of 114lakhMT. *Kharif* season is characterized by unprecedented rainfall that leads to water logging in fields. Waterlogging is a serious abiotic problem in Soybean causing a loss of nearly 16 percent in productivity worldwide. Transient water logging is seen to affect seedling growth and further crop yield in several crops like soybean (*Glycine max*), pea (*Pisumsativum*), wheat, maize, and rice (Magneschi and Perata, 2009; Al-Ani*et al*., 1985) [6]. Flooding affects early stages of soybean during crop season (Griffin and Saxton, 1988) [4]. Waterlogging affects early 30-45 days covering emergence, cotyledon and vegetative 1,2,3,4 trifoliate leave stages. Tolerance of genotype to pre-germination covering one to fifteen days (sowing/seed stage to vegetative cotyledonary stage) can help soybean crop tolerate flooded situation. Injuries due to water logging are mainly due to lack of oxygen (Dennis *et al*., 2000) [3]. However, breeding for tolerance to water logging in early stages of soybean is limited. Screening based on the key traits and its responses can assist selection while screening large number of genotypes. Reduction in percent germination would be the most vulnerable response of pre-germination water logging. Earlier studies support the reduction in germination percentage and duration of emergence of soybean seed (McDonald *et al*., 1988; Wuebker *et al*., 2001) [9]. Such responses can differentiate tolerant and susceptible genotypes (Zaidi *et al*., 2012) [10]. The available genotypic variation could be exploited in developing soybean varieties that can tolerate pre-germination conditions of low oxygen during waterlogging.Suitable screening techniques, morpho-physiological traits associated with tolerance and identification of promising genotypes are the requirements of selection and development of waterlogging tolerant soybean.This study aimed to identify tolerantand susceptible genotypes at seedgermination stage based on percent germination and delay in emergence from a wide range of genotypes.

Materials and Methods
This experiment was conducted to evaluate germination of 128 soybean genotypes that included germplasm and trial entries in a range of waterlogging durations. Based on literatures, the waterlogging duration above 2 days water logging was sufficient to inhibit germination. Five durations (3, 5, 7, 9 and 11 days waterlogging) were therefore evaluatedas treatments along with a well-drained (control) condition in this experiment. Three replications of 100 seeds of each genotype were used in a completely randomized design (CRD-factorial).Seeds were surface-sterilized and placed to germinate in perforated plastic cups with peat-vermiculite mixture (1: 1). These perforated cups were kept in traysfilled with water and maintained in germination chamber that provided a temperature of 24 ^{0}C. It is seen to maintain water level of 2cm above the soil level in each cup during the entire period of treatment. This arrangement is kept until treatment duration. After the water logging duration, the cups were

drained and retained for seven days as recovery period for noting the final readings. Stress reaction of the genotypes was measured in terms of percent germination and delay in coleoptile emergence. Seeds were considered germinated when radicle and shoot were longer than 15 mm. Percent germination (%) was recorded in terms of the number of seeds emerging from soil surface as a percentage of total number of seeds sown.The delay in germination percentage was calculated as the difference between duration to coleoptile emergence under excessive waterlogging condition in comparison to the duration takenin control for the same level of germination. Analysis of variance was calculated using the OPSTAT statistical software (Sheoran *et al*., 1998) [7].

Results and Discussion

Waterlogging stress severely affects soybean at every growth stage, but susceptibility varies with growth stages (Wuebker *et al*., 2001) [9]. The 128 genotypes evaluated in this experiment exhibited significant differences (P<0.01) in percent germination values and duration to coleoptile emergence (Table 1 and 2). At higher durations, the percent germination of genotypes decreased sharply (Figure 1). Also, high duration of waterlogging caused an evident suppression of early sprout (Figure 1). In reduced water potential,soybean seed slows down water uptake and respiration in seeds (Woodstock and Taylorson, 1981) [8]. Significant genotypic variability in percent germination and delay in emergence, might be related to variability in tolerance to low-oxygen in the enzyme-related breakdown of starch and the utilization of its products (Ismail *et al*., 2009) [5].

Waterlogging duration of3 days resulted in maximum 2 days delay in emergence of coleoptile in genotypes, which increased to 9.7 days on exposure to 11 days of waterlogging stress, with only two entries showing 70% or more germination. The prevailing genotypic variability for pre-germination anaerobic stress tolerance was evident with excessive duration of water logging treatment, both in terms of germination and delay in coleoptile emergence. The top ranking best entries in terms of both traits were the two genotypes: WT3 and WT8, which had >70% germination and < 5 d delay in coleoptile emergence, in comparison to control conditions (Figure 2).At 9 days stress treatment, seventy eight entries showed < 50% germination, coupled with significant delays in coleoptile emergence (mean of 5.7 days), and seventy three entries had no germination at all. Stress for 11 daysalmost completely inhibited germination; just 10 of the 128 entries showed germination of 50–59%, with delay infinal emergence. Analysis of variance indicated that stress treatment (T), genotype (G) and T × G were highly significant (P<0.01) for both germination and delay in coleoptile emergence (Table 2). Contribution of T was higher (70%) for the variable percent germination whereas interaction of TxG contributed more than other effects for delay in emergence. Coefficient of variation (CV) was high for delay in emergence, which may be due to the large variation among genotypes for delays in emergence with a low trial mean (Table 2).

According to Bacanamwo and Purcell (1999) [2] water-logging tolerance has been defined as minimal inhibition of germination and biomass under water-logging. Speedy emergence is one of the key traits for selection of seedling vigor as it provides early autotrophy to the young seedlings for photosynthetic activity and dry matter accumulation (Zaidi *et al*., 2012) [10]. Tolerant and susceptible genotypes could be established here. In the tolerant group, genotypes presented the highest percent germination values even after eleven days waterlogging. The genotypes with more than 70% germination in extreme stress condition were chosen as tolerant ones. Minimum germination percent set by Indian minimum seed certification standards in soybean is 70%. Two genotypes designated as WT3 and WT8 was less affected by increasing duration of waterlogging, presenting the lowest inhibition of germination even after eleven days of waterlogging (Figure 2). At this stress level, the germination performance of WT3 was excellent (GP84.4%) and good in the genotype WT8 (GP72.3%). In the susceptible group, the genotypes showed no germination at all. Highly susceptible genotypes (DS9712 and Pusa12-13) were further identified in this period by subjecting susceptible genotypes to less than 3 days (1 day) duration of water logging.

Conclusion

Germination percentage (>70%) and delay in coleoptile emergence (<5 days) can be basic criteria for choosing genotypes for pre-germination anaerobic stress tolerance. Traits can further assist screening large number of genotypes and in identifying the tolerant and susceptible genotypes. Genotypes designated as WT3, WT-8 were found tolerate pre-germination anaerobic stress due to water logging.

Table 1: Mean, minimum and maximum values and standard deviation from mean for germination and delay in germination after exposure of seeds to different days of water logging stress after sowing

	Well drained		3		5		7		9		11	
	G	D	G	D	G	D	G	D	G	D	G	D
Mean	93.3	-	83.7		62.3		46.7		23.9		13.7	2.1
Minimum	44.3	-	51.7	0*	0	0*	0	0*	0	0*	0	0*
Maximum	100	-	100	2	99	2	95.3	3.3	95	5.7	80.7	9.7
SD	14.48		11.69	0.25	27.22	0.37	22.58	0.44	29.76	1.17	20.07	2.89
**Significant at P<0.01, G=Genotype, D-Delay, T-Treatment, SD=standard deviation;*Due to no germination at all												

Table 2: Variance components for seed germination and delay in germination after exposure of seeds to different durations of excessive soil moisture stress at planting.

Source	Germination (%)	Total SS (%)	Delay in germination (d)	Total SS (%)
Treatment (T)	391624.63**	63.25	10.45**	0.6
Genotype (G)	785.91**	3.22	7.52**	19.4
T × G	1513.68**	31.04	10.06**	78.04
Error	49.94	2.48	0. 09	1.87
CV (%)	13.02		46.08	
h2				
** Significant at P<0.01, df = degrees of freedom, CV = coefficient of variance, SS = Sum of Squares, *h2 = heritability*				

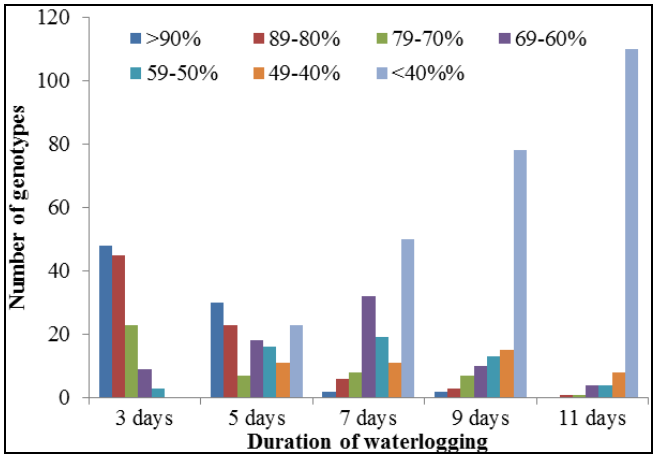

Fig 1: Frequency distribution for germination percent of 128 soybean genotypes as affected by increasing waterlogging duration. Readings are mean values of three replications in 3, 5, 7, 9, 11 days of water logging

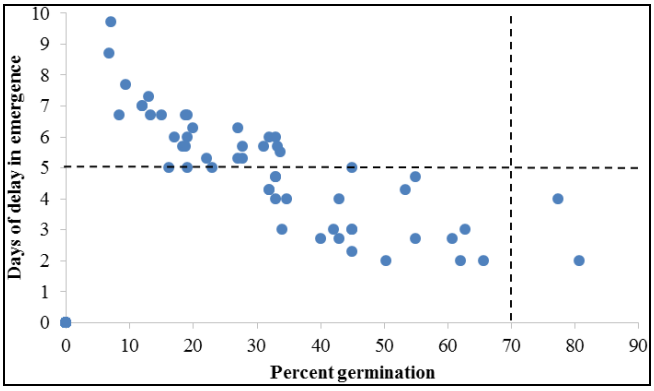

Fig 2: Germination and delay in emergence after seeds were exposed to 11 days excessive waterlogging stress (11 days waterlogging and 7 days recovery) after sowing

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