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Pre-germination anaerobic stress tolerance in tropical maize (*Zea mays* L.)

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Abstract

Pre-germination anaerobic stress caused by temporary soil water-logging at the planting and seedling establishment stage is an important constraint for maize (*Zea mays* L.) production in the tropics, especially in South and Southeast Asia. Whilst it is commonly accepted that the occurrence of excessive soil moisture following planting adversely affects the establishment and early growth of maize crops, the threshold limit and genotypic variability for pre-germination anaerobic stress tolerance in tropical maize germplasm have not been determined. We aimed to identify the threshold limit for pre-germination anaerobic stress tolerance and available genotypic variability in tropical maize for this stress. A set of 100 elite maize inbred lines selected from a wide genetic background were exposed to different durations of pre-germination anaerobic stress (12, 36, 72, 96, and 120 h). Two independent but key traits for crop establishment, germination percentage (> 80%) and delay in coleoptile emergence (<5 days), were compared under normal conditions and anaerobic stress conditions. These traits were used as the basic criteria for identifying the threshold limit of pre-germination anaerobic stress tolerance. By varying the duration of managed anaerobic stress screening, it was observed that 72 h of exposure to the stress resulted in the best identification of available genotypic variability. Any further increase in stress duration (96 and 120 h) masked the genotypic variation as almost all entries were susceptible. After identifying the threshold limit, we tested two different sets of test-cross progenies to identify the available genotypic variability in tropical maize for pre-germination anaerobic stress tolerance. This included 214 entries of test-cross progenies of recombinant inbred lines (developed by crossing vegetative stage water-logging tolerant and susceptible lines) and 296 entries of drought tolerant maize (DTM) association mapping panel, representing a wide genetic background of tropical maize. Our results clearly indicate significant genotypic variability in tropical maize for pre-germination anaerobic stress tolerance, which could be systematically selected for and further improved using a managed anaerobic stress screening technique. This study also showed that test weight (100 seed weight) is significantly ($P<0.01$) correlated with seedling vigor (shoot and root dry weight) under pre-germination anaerobic stress; however, there was no relationship with the test weight and seed germination or time to seedling emergence.

Keywords: anaerobic stress, excess moisture, germination, maize, water-logging.
Abbreviations: ASI - anthesis to silking interval, CIMMYT- International Maize and Wheat Improvement Center, CRD - completely randomized design, DTM-panel - drought tolerant maize association mapping panel, EM - excessive moisture, EPP - ears per plant, GCA - general combining ability, GY - grain yield, ICRISAT - International Crop Research Institute for Semi-Arid Tropics, IITA - International Institute for Tropical Agriculture, RILs-TC - recombinant inbred line test crosses, WL-RIL - water-logging recombinant inbred line.

Introduction

Rain-fed maize crops represent over 80% of the total maize area in the Asian tropics. They are grown during the summer-rainy season and therefore occasionally face extreme climatic conditions which limit crop establishment and yield potential. Among various abiotic stresses, excessive soil moisture is one of the most important constraints for maize production in Asia. The rain-fed maize crops often receive early monsoon rainfall during planting, resulting in temporarily water-logged soils and anaerobic conditions, even in well-drained fields (Joshi et al., 2005). These water-logged soils adversely affect seed germination, seedling establishment, early growth of maize seedlings, and eventually overall plant stand and final yield potential. In South and Southeast Asia alone, over 18% of the total maize growing area is frequently affected by water-logging (Zaidi et al., 2008). The loss in potential crop yield as a consequence of this environmental stress is not trivial. Moreover, the increasing demand for maize in Asia is rapidly transforming cropping systems in certain parts of the region from a rice monoculture to the more profitable rice–maize systems (Waddington et al., 2006), which currently occupy approximately 3.5 million ha in Asia (Timsina et al., 2010). However, maize production in this newly emerged rice–maize system frequently face the problem of early stage excessive soil moisture, as the soils of paddy fields are often saturated from late monsoon rains. Due to a shorter turn-around time, maize planting is regularly conducted in wet fields, which may cause germination failure or damage to young maize seedlings (Zaidi and Singh, 2006). In addition, soil water-logging in rice-based rotations is exacerbated by the land preparation practices used for rice, which result in the formation of a compacted hardpan that impedes drainage (Setter and Waters, 2003). Soil water-logging causes major changes in the physical and chemical properties of the rhizosphere (Zaidi et al., 2003), as gaseous diffusion rates in water-logged soil are approximately 100 times lower than air (Kennedy et al., 1992). Respiration of soil micro-flora and fauna leads to rapid exhaustion of soil oxygen, resulting in hypoxic (low oxygen) or anoxic (no oxygen) conditions,

Table 1. Mean, minimum and maximum values, and standard deviations (SD) from mean for germination and delay in germination after exposure of seeds to different durations of excessive soil moisture stress at planting.

	Normal moisture		12 h		36 h		72 h		96 h		120 h	
	Germ. (%)	Delay (d)	Germ. (%)	Delay (d)	Germ. (%)	Delay (d)	Germ. (%)	Delay (d)	Germ. (%)	Delay (d)	Germ. (%)	Delay (d)
Mean	95.6	–	80.2	1.2	70.3	2.4	43.9	4.7	19.9	6.4	9.2	14.9
Min.	92.3	–	20.0	0.0	5.0	0.5	0.0	1.5	0.0	4.0	0.0	10.0
Max.	100.0	–	100.0	4.0	100.0	7.5	95.0	25.0#	65.0	25.0#	45.0	25.0#
SD	3.6	–	17.9	0.9	21.6	1.1	20.9	1.4	18.8	3.0	12.0	2.5
P	G	T	G × T									
Germination	**	**	**									
Delay	**	**	**									

** Significant at $P<0.01$, # no germination until 25 days after planting (after the trial was concluded), G = genotype, T = treatment, SD = standard deviation.

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	*df*	Germination (%)	Total SS (%)	Delay in germination (d)	Total SS (%)
Treatment (T)	5	114070.97**	68.24	3865.63**	68.59
Error	6	194.50	0.14	2.56	0.05
Genotype (G)	99	3038.53**	17.81	56.09**	9.75
T × G	495	363.62**	10.66	20.57**	17.88
Error	594	89.40	3.14	3.56	3.72
CV (%)		23.95		26.09	
h^2		0.88		0.84	

** Significant at P<0.01, df = degrees of freedom, CV = coefficient of variance, SS = Sum of Squares, h^2 = *heritability*

which form the primary plant stresses in water-logged soils. Maize is highly susceptible to anaerobic soil conditions during germination and early growth stages (Mano et al., 2002; Zaidi et al., 2003). Good seed germination and seedling growth in anaerobic soils necessitates above-normal tolerance of the low-oxygen conditions usually experienced in water-logged soils. Seeds with carbohydrate reserves, including maize seeds, are generally more tolerant of hypoxia or even anoxia than seeds with fatty acid reserves (Al-Ani et al., 1985; Raymond et al., 1985). Therefore, maize seed can germinate under wet soil conditions in the presence of nominal amounts of oxygen (Van Toai et al., 1995). However, further growth is highly susceptible to excess soil moisture stress. Germinating maize seedlings retain a high tolerance to anoxia of the embryo, but this tolerance is lost within 2–3 d following germination. Previous studies have indicated considerable genetic variation for tolerance to anaerobic conditions in germinating maize seedlings (Lemke-Keyes and Sachs, 1989; Porto, 1997; Mano et al., 2002; Zaidi et al., 2003). Such responses inevitably raise the question as to whether there are any fundamental differences between tolerant and susceptible genotypes in their responses to imposed anaerobiosis. The available genotypic variation could be exploited in developing maize varieties that can tolerate pre-germination conditions of hypoxia or anoxia. This study aimed to identify the threshold of pre-germination anaerobic stress tolerance and the available genotypic variability in a wide genetic background of tropical maize, including germplasm originating from Asia, Sub-Saharan Africa and the Latin American tropics.

Results

Threshold of pre-germination anaerobic stress tolerance

Pre-germination anaerobic stress significantly (P<0.01) affected both duration to coleoptile emergence and

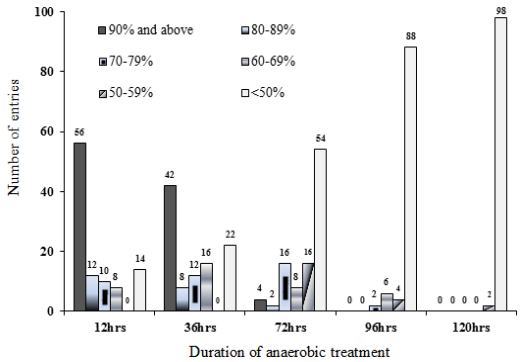

Fig 1. Frequency distribution for germination (%) after exposure of seeds to different durations of excessive soil moisture stress immediately after sowing.

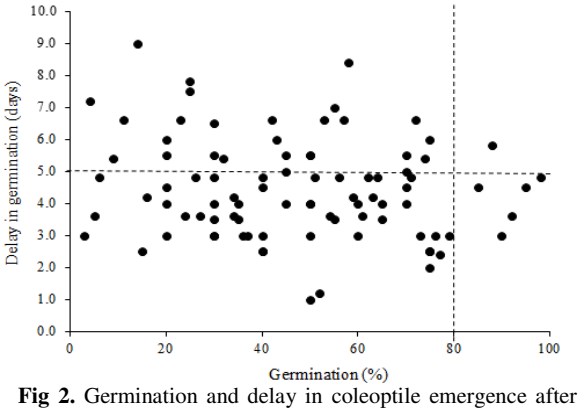

Fig 2. Germination and delay in coleoptile emergence after seeds were exposed to 120 h excessive soil moisture stress (72 h submergence and 48 h saturated soil conditions) immediately after planting.

Table 3. Mean, minimum and maximum values, and standard deviations from mean for seed germination and seedling biomass under optimal moisture and after exposure of maize genotypes to pre-germination anaerobic stress.

		Germination (%)		Days to maximum germination		Delay in germination (days)		Shoot dry weight (g/plant)		Root dry weight (g/plant)		Shoot:root ratio	
		NM	EM	NM	EM	NM	EM	NM	EM	NM	EM	NM	EM
RILs test-crosses													
Mean of the best entries		98.3	92.2	7.5	10.2	0.0	3.8	0.32	0.22	0.15	0.12	2.13	1.82
Population mean		93.2	47.3	7.8	14.3	0.0	6.1	0.30	0.14	0.14	0.09	2.11	1.57
Maximum		100.0	100.0	8.3	17.0	0.0	10.5	0.67	0.42	0.35	0.28	2.46	2.01
Minimum[#]		94.8	7.0	5.4	7.8	0.0	2.0	0.15	0.03	0.06	0.01	1.06	0.72
SD		14.9	21.8	1.7	1.4	0.0	1.7	0.11	0.07	0.05	0.05	0.44	0.87
P	Environment (E)	**		*		**		*		**		**	
	Genotype (G)	**		**		**		**		**		**	
	G x E	**		**		**		**		**		**	
CV (%)		13.24		19.76		21.23		17.98		23.99		20.36	
Heritability (h^2)		0.85		0.43		88.00		0.67		0.81		0.72	
DTM-panel													
Mean of the best entries		100.0	91.2	7.1	10.5	0.0	4.1	0.30	0.19	0.18	0.14	1.69	1.35
Population mean		92.0	44.8	6.8	15.2	0.0	8.2	0.34	0.12	0.19	0.12	1.76	1.04
Maximum		100.0	94.3	9.5	24.0	0.0	15.0	0.72	0.35	0.33	0.21	2.90	1.94
Minimum[#]		93.5	3.0	5.7	9.0	0.0	3.0	0.11	0.02	0.21	0.02	0.64	0.39
SD		12.4	23.2	1.3	1.5	0.0	2.2	0.10	0.08	0.08	0.07	0.37	0.31
CV (%)		16.25		15.64		33.21		24.19		36.10		28.23	
P	Environment (E)	**		**		**		**		**		**	
	Genotype (G)	**		**		**		**		**		**	
	GXE	**		**		**		**		**		**	
CV (%)		16.25		15.64		33.21		24.19		36.10		28.23	
Heritability (h^2)		0.74		0.51		0.63		0.75		0.60		0.66	

* and ** indicate statistical significance at P<0.05 and P<0.01, NM = normal moisture, EM = excessive moisture, [#] = except in case of germination, excluding those 12 and 15 entries with no germination in the case of RILs-TC and DTM-panel, respectively, CV = coefficient of variance, SD = standard deviation.

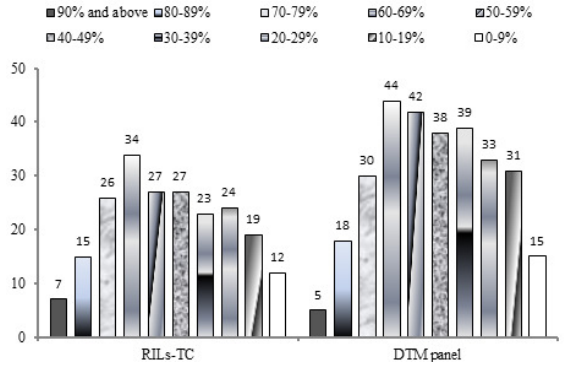

Fig 3. Frequency distribution for seed germination (%) of RILs-TC and DTM-panel after exposure to pre-germination anaerobic stress.

germination percentage (Table 1), but had a comparatively greater inhibitory effect on coleoptile emergence, and the effect increased exponentially with an increase in the duration of stress treatment. An anaerobic stress duration of 12 h resulted in a 4 d delay in coleoptile emergence in susceptible genotypes, which increased to 7.5 days with exposure to 36 h of stress, even though 56% and 42% of the entries, respectively, had 90% or more germination (Fig. 1).

The existing genotypic variability for pre-germination anaerobic stress tolerance was clearly evident with 72 h of anaerobic stress treatment, both in terms of germination and delay in coleoptile emergence (Fig. 2). Germination ranged from 0 (seven entries) to 98% (four entries with ≥ 90% germination). Similar variation was also observed in the delay of coleoptile emergence, from 1 d to the seven entries with no germination at all. The top ranking best entries in terms of both traits were the five inbred lines: POOL16BNSEQC3F6x38-1-1-2-1, POOL16BNSEQC3F22-x1-3-2-2-2, CA-14507, CML-193, and CA-14514, which had >80% germination and < 5 d delay in coleoptile emergence, in comparison to normal moisture conditions (Fig. 2). Further increases in stress duration masked the available genotypic variability (Fig. 1). At 96 h of stress treatment, 88% of the entries showed < 50% germination, coupled with significant delays in coleoptile emergence (mean of 7.1 days), and seven entries had no germination at all. Anaerobic stress for 120 h almost completely inhibited germination; just 2 of the 100 entries showed germination of 50–59%, with >10 d delay in final emergence. Analysis of variance indicated that anaerobic stress treatment (T), genotype (G) and T × G were highly significant (P<0.01) for both germination and delay in coleoptile emergence (Table 2), though the contribution of T was higher (70%) than for the other two factors for both variables. Genotype contributed more than the interaction effects for germination percentage, whilst the opposite was found for the delay in emergence. Coefficient of variation (CV) was high for both the traits, which may be due to the large variation among genotypes for the traits with a low mean for the trial. However, for both traits the broad-sense heritability (H^2) was also high, indicating high repeatability of the results.

Genotypic variability for pre-germination anaerobic stress tolerance

Pre-germination anaerobic stress severely affected seed germination, coleoptile emergence and seedling vigor in both DTM-panel and RILs-TC. However, significant genotypic variability was observed for all traits. An extremely wide range in germination rates was observed under the stress. Mean germination was < 50% for both RILs-TC and DTM-panel, which varied from 0 to 100% and 0 to 94.3%, respectively (Table 3). Of the 214 RILs-TC, only 22 entries achieved ≥ 80% germination, while 12 entries had no germination (Fig. 3). Similarly, 23 entries showed ≥ 80% germination of the 295 DTM-panel entries evaluated and 15 entries had no germination. Pre-germination anaerobic stress, in general, delayed coleoptile emergence in almost all the entries (Table 3), including those entries that had good germination (> 80%) under pre-germination anaerobic stress. The effect was comparatively more pronounced in DTM-panel where mean delay in coleoptile emergence was approximately 8 d in comparison to normal moisture, with a range of 3–15 d in those entries where at least one seed germinated. The top ranking tolerant entries with ≥ 80% germination and <5 d of delay in emergence were identified in both RILs-TC (13 entries) and DTM-panel (11 entries). Anaerobic stress also affected seedling vigor, decreasing the dry weights of both shoots and roots (Table 3). The mean decrease in shoot dry weight was 53.8% in RILs-TC and 64.2% in DTM-panel, while root weight loss was 35.7% and 39.2%, respectively. Shoot dry weight was more affected by anaerobic stress with a significant decrease in the shoot:root ratio in both RILs-TC and DTM-panel being observed. The reductions in shoot and root dry weights were comparatively less in cases of the top ranking entries of both RILs-TC and DTM-panel. All traits showed high levels of heritability (h), indicating high repeatability of the findings. Analysis of variance indicated that the T, G and T × G effects were highly significant (P<0.01) for all traits in both DTM-panel and RILs-TC, except the effect of T on days to maximum germination, and shoot and root dry weight of RILs-TC which was significant at P<0.05 (Table 4). For RILs-TC, the contribution of G was highest (40.57%) in days to maximum germination, T was highest for percentage germination (47.98%) and delay in coleoptile emergence (51.52%), while T × G interaction effects were highest for shoot dry weights (36.74%), root dry weights (42.97%) and shoot:root ratio (39.43%). For DTM-panel, T contributed >50% variation in all the traits, except in the case of root dry weight (36.8%) and shoot:root ratio (39.1%), where the contribution of G was the highest.

Relationship among the traits

Correlation analysis (Table 5) showed highly significant (P<0.01) phenotypic correlation of test weight with shoot dry weight under pre-germination anaerobic stress in both DTM-panel (r = 0.513**) and RILs-TC (r = 0.474**). The relationship was also significant (P<0.05) under normal conditions for RILs-TC (r = 0.402*), but it was non-significant at P<0.05 for DTM-panel (r = 0.312). The correlation between test weight and root dry weight was significant for both DTM-panel and RILs-TC under normal moisture conditions (P<0.05), and further increased under anaerobic stress conditions (P<0.01). The correlation of test weight with both germination percentage and time taken to emergence was positive but weak and non-significant at P<0.05 under both normal and stress conditions. Delay in germination due to pre-germination anaerobic stress was significantly and negatively correlated with shoot (r = –0.547***) and root dry weight (r = –0.412**) and also with shoot:root ratio (r = –0.292*) (Table 5). However, linear regression analysis indicated that only the shoot dry weight

Table 4. Variance components for seed germination and seedling growth traits across optimal moisture and after exposure of maize genotypes to pre-germination anaerobic stress.

Source	*df*	Germination (%)	Days taken in maximum germination	Delay in germination (days)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Shoot:root ratio
				RILs-TC			
Treatment (T)	1	357155.64**	1447.16*	5112.69**	2.60*	0.19*	4.21**
		(47.98)	(22.08)	(51.52)	(21.95)	(16.00)	(16.87)
Error	2	6436.13	216.52	0.38	0.01	0.23	0.007
		(1.73)	(6.61)	(0.01)	(1.00)	(0.31)	(0.06)
Genotype (G)	213	1006.02**	8.18**	10.61**	0.02**	0.01**	0.04**
		(28.79)	(40.57)	(22.79)	(33.16)	(36.51)	(35.06)
G x T	213	590.61**	7.31**	10.61**	0.02**	0.01**	0.046**
		(16.90)	(23.75)	(22.79)	(36.74)	(43.97)	(39.43)
Error	426	80.41	3.24	0.67	0.00	0.00	0.01
		(4.60)	(6.99)	(2.88)	(7.15)	(3.21)	(8.53)
	DTM-panel						
Treatment (T)	1	660271.62**	6025.54**	13983.75**	12.37**	1.84**	23.74**
		(57.12)	(57.25)	(65.68)	(50.32)	(18.51)	(30.23)
Error	2	690.88	22.00	1.16	0.00	0.00	0.00
		(0.12)	(0.42)	(0.01)	(0.01)	(0.09)	(0.00)
Genotype (G)	295	815.20**	6.15**	8.87**	0.02**	0.01**	0.062**
		(21.11)	(17.25)	(12.29)	(25.71)	(36.77)	(39.14)
G x T	295	557.04**	4.91**	8.87**	0.01**	0.01**	0.04**
		(14.43)	(13.76)	(12.29)	(16.31)	(26.32)	(19.58)
Error	590	123.08	2.02	3.50	0.00	0.0	0.01
		(6.38)	(11.31)	(9.71)	(7.65)	(18.30)	(11.05)

* and ** indicate statistical significance at P<0.05 and P<0.01, respectively. Values in parentheses are the percentage of total sum of square, df = degrees of freedom.

Table 5. Correlation coefficient between test weight and traits related to seed germination and seedling vigor when seeds were exposed to normal and excessive moisture stress at the time of germination.

	DTM-Panel		RILs Test crosses	
	NM	EM	NM	EM
Germination	0.045	0.035	0.047	0.092
Days taken to emergence	0.021	0.047	0.102	0.111
Shoot dry weight	0.349*	0.513**	0.402*	0.474**
Root dry weight	0.393*	0.405**	0.345*	0.412**

* = significant at P<0.05, ** = significant at P<0.01, N = normal moisture, EM = excessive moisture.

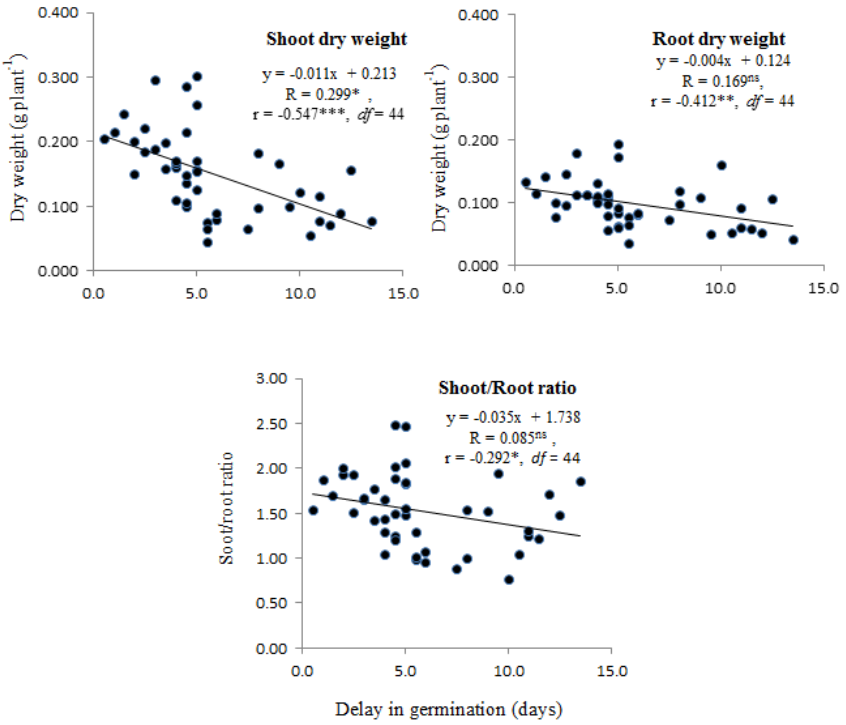

Fig 4. Relationship between delay in germination and seedling biomass in maize genotypes after exposure to pre-germination anaerobic stress. Regression equation coefficients (R) and correlation coefficients (r) were calculated using top ranking entries with at least 80% germination after exposure to pre-germination anaerobic stress in the RILs-TC and DTM-panel trials. *, ** and ** indicate statistical significance at P<0.05, 0.01 and 0.001, respectively. df = degrees of freedom, ns = not significant.

was significantly (R = 0.299*) dependent on test weight under pre-germination anaerobic stress.

Discussion

Previous studies on excessive moisture/water-logging stress tolerance in tropical maize largely focused on seedling or vegetative stages and found that the stress adversely affects maize at every growth stage, but susceptibility varied at different growth stages. These studies concluded that maize is highly susceptible to excess soil moisture stress before tassel emergence (Evans et al., 1990; Rathore et al., 1998; Zaidi et al., 2004). Our study examined the effect of pre-germination anaerobic stress due to excessive soil moisture and showed that pre-germination anaerobic conditions are highly detrimental for maize seed germination and emergence (Table 1). Previous studies of pre-germination/emergence stage flooding in maize have been conducted in temperate regions by soaking seeds in water (Martin et al., 1988, 1991), or in plastic pans (Fausey and McDonald, 1985), and have shown varied responses under different durations of pre-germination anaerobic stress. In our study, at 36 h of stress exposure, > 50% of the entries showed a significant decrease in germination, and at 72 h, with the exception of five tolerant entries, the germination of most of the entries was significantly reduced and emergence was delayed by more than 5 d (Fig. 1 and 2). Similar findings were reported in previous studies on temperate maize germplasm, where it was observed that 48–96 h of pre-emergence flooding at 25°C soil temperature (Fausey and McDonald, 1985) or seed soaking for 48 h at 35°C (Martin et al., 1991) resulted in a significant inhibition of germination in maize inbred lines. Pre-germination anaerobic stress (due to excessive moisture) may inhibit seed germination by restricting the seed respiratory metabolic processes essential for germination. Woodstock and Taylorson (1981) reported that soaking soybean seed at reduced water potential slowed water uptake and respiration process in seeds, but this could be reversed with the addition of polyethylene glycol (300 g kg^{-1} seed). Martin et al. (1991) found that with pre-emergence anaerobic stress, maize inbred lines excreted ethanol and acetaldehyde but that the amount excreted was not significantly correlated with stress tolerance. They concluded that soaking-induced inhibition of germination results from the accumulation of volatile metabolites(s) other than ethanol and acetaldehyde. Other than germination percentage, duration to emergence was comparatively more severely affected due to pre-germination anaerobic stress (Fig. 2). However, significant genotypic variability was observed for these two traits (Tables 1 and 2), which might be related to the variability in tolerance to low-oxygen in the enzyme-related breakdown of starch and the utilization of its products (Ismail et al., 2009). Van Toai et al. (1995) suggested that maize seed can germinate under wet soil conditions in the presence of nominal amounts of oxygen, but that further growth was highly susceptible to excess soil moisture stress, which concurs with our findings for delay in coleoptile emergence, including some of those entries with good final germination percentage (> 80%) under stress (Fig. 2). Means of all traits related to seed germination and seedling vigor were low in both RILs-TC and DTM-panel, but there was significant genotypic variability for germination (Tables 3 and 4; Fig. 3) and for other traits as well in both sets of entries (Tables 3 and 4). Significant genotypic variability for seed germination and early seedling growth under fully saturated soil conditions (hypoxia) was

observed in our previous study on Indian maize inbred lines (Zaidi et al., 2004), and also reported by others (Porto, 1997; Mano et al., 2002). Such variability might be related to the ability of different genotypes to utilize the stored assimilates in the endosperm through anaerobic metabolism for germination and radicle/coleoptile development (Xia and Saglio, 1992). RILs-TC progenies showed comparatively better performance than DTM-panel, which might be due to the fact that RILs were derived from a population with a female parent highly tolerant to vegetative stage water-logging stress. In our previous studies (Zaidi et al., 2003, 2004) we found a significant relationship between excessive moisture at the germination and vegetative stage (V_{7-8}) for water-logging tolerance and also found that hypoxia pre-treatment enhanced water-logging tolerance at the vegetative stage. However, Mano et al. (2002) suggested that pre-germination flooding tolerance was independent to seedling stage flooding tolerance. In addition to affecting germination and duration to coleoptile emergence, anaerobic stress strongly influenced further growth of seedlings, by reducing shoot and root dry weights, and the shoot:root ratio (Table 3). Reductions in shoot and root growth due to excessive moisture at the early seedling stage were also observed in our previous studies (Zaidi and Singh, 2001; Zaidi et al., 2003), and also reported by Porto (1997). Van Toai et al. (1995) suggested that germinating maize seedlings retain a high tolerance to anoxia in the embryo, but that this tolerance is lost within 2–3 d following germination. Following pre-germination anaerobic stress, the newly emerging leaves showed strong chlorotic symptoms, particularly in susceptible entries which had much delayed emergence (data not shown), indicating poor chlorophyll content. Reduced chlorophyll content may have resulted in low current photosynthetic activity, reducing production of photo-assimilates (Lizaso and Ritchie, 1997), and leading to poor seedling growth and development at the early stage (Zaidi et al., 2003). Similar findings were also reported by Loaiza and Ramirez (1993), who suggested that reduced growth of seedlings was related to reduced nitrate reductase activity in root tissues. Alternatively, genotypic variability in post-stress seedling growth and recovery might be related to variation in starch accumulation and the ability of genotypes to utilize it efficiently for shoot and root development (Ismail et al., 2009). Analysis of the relationship of test weight with various traits related to germination and seedling growth indicated that test weight had a positive relationship with both shoot and root dry weight under NM, which further strengthened under pre-germination anaerobic conditions (Table 5). However, the test weight was not related to germination and duration to emergence under both normal and stress conditions, as observed in the present study (Table 5) and also reported by Eagles and Hardacre (1979), under low temperature stress. In a study on the importance of kernel position in the ear on germination capacity and seedling vigor, Msuya and Stefano (2010) found that test weight was significantly correlated ($r = 0.56^*$) with seedling dry weight, and large kernels from the basal position in the ear produced significantly ($P<0.05$) more vigorous seedlings. However, other traits such as germination percentage, germination time, and shoot and root length had no relationship with test weight. Xia and Saglio (1992) suggested that genotypic variability for excessive moisture tolerance in maize at early growth stages might be related to stored assimilates in the endosperm and their utilization through anaerobic metabolism for germination and radicle/coleoptile development. Shoot and root dry weights and also shoot:root ratio showed a significant negative correlation in delay of coleoptile emergence under anaerobic stress (Fig. 4). However, regression analysis showed that only shoot weight was significantly dependent on a delay in emergence. Msuya and Stefano (2010) also reported a highly significant negative correlation ($r = –0.73^{**}$) between mean germination time and shoot length of maize seedlings. The relationship was also negative between root length and total dry weight of seedlings, but this was not statistically significant. Rapid emergence, in general, is one of the key traits for selection of seedling vigor, which is likely related to the fact that early emergence gives early autotrophy to the young seedlings for photosynthetic activity and synthesis of photo-assimilates and dry matter accumulation. Our results clearly demonstrate that pre-germination anaerobic stress at soil temperature > 25°C is detrimental for maize seeds in terms of germination, duration to coleoptile emergence, and seedling vigor. Significant genotypic variability exists in tropical maize, which was clearly expressed at 72 h of exposure to stress, and could be selected using managed stress screening for use in developing maize with an enhanced level of pre-germination anaerobic stress tolerance. Further increases in anaerobic stress duration masked the available genotypic variability, as most of the genotypes lost seed viability. Combined selection for per cent germination along with time taken in emergence under anaerobic conditions might help identifying potential maize germplasm suitable for breeding pre-germination anaerobic stress tolerant cultivars for early stage water-logging prone maize growing areas in tropics.

Materials and methods

Maize germplasm

To study the threshold level of pre-germination anaerobic stress tolerance, a set of 100 elite, advanced generation maize lines with good individual and cross performance in the Asian tropics which represent genetic diversity of tropical germplasm in the Asian region were used. These lines were derived from CIMMYT's lowland tropical and sub-tropical maize pools, populations, and elite × elite pedigree crosses; including Pool 16 BN Sequia, Pool 18, Pool 25, Pool 32, Population 31, 45, 66, 67, 68, 145, EY-DMR, AMATL, 444, 445, KTX3752 and KTX3753, and lines from the Indian maize program, including those derived from CM-118, 199, 501, Cargil 633, and Population H3191. A genotypic variability study was conducted on two different sets of test-cross progenies. The first set consisted of 180 advanced stage (S_6) water-logging recombinant inbred lines (WL-RILs) crossed with two tester lines – one each from CIMMYT's heterotic groups A and B. The WL-RILs were developed by crossing two elite advanced generation maize inbred lines with highly contrasting responses to vegetative stage water-logging stress, i.e. CML311-2-1-3 (highly tolerant) and WL-36-4-B (highly susceptible). The lines were selected on the basis of their consistent performance in the line evaluation trials which were conducted on tropical/subtropical lines from the Indian maize program and CIMMYT from 1998 to 2003. CML-311-2-1-3 was derived from selected highly tolerant segregants in the CIMMYT inbred line CML-311, which was derived from the synthetic variety S89500. WL-36-4-B was derived from CM-500, an open-pollinated variety from the Indian maize program. Further details on the genetic background of these lines and line evaluation trials for identifying water-logging tolerant maize inbred lines can be found in our earlier publication (Zaidi et al., 2007). A bi-parental population was developed by crossing CML-311-2-1-3 with WL-36-4-B during the dry season of 2005/2006 in

Hyderabad, India. The population was advanced to F_3 generation, and thereafter a single-seed descent method was followed for developing the RILs. In the dry season of 2009/2010 the 180 RILs (S_6 stage of inbreeding) were test-crossed with two CIMMYT testers, CL-02450 (HG-A) and CML-451, (HG-B), and the F_1 seeds of 214 test-crosses (RILs-TC) were harvested with enough seed for conducting pre-germination anaerobic stress tolerance studies. The second set consisted of 291 lines of the drought tolerant maize association mapping panel (DTM-panel) crossed with one tester line with high general combining ability (GCA). In creating the DTM-panel, a collection of maize germplasm was assembled, which represented the genetic diversity of CIMMYT and the International Institute for Tropical Agriculture (IITA) stress breeding programs. An initial set of 850 advanced breeding lines was selected and evaluated in the dry season of 2006/2007 under two different water regimes (well-watered and anthesis stage drought stress) at CIMMYT's experimental station in Tlaltizapán, Mexico (18°41'N, 99°07'W, 940 m.a.s.l). Grain yield (GY), ears per plant (EPP) and anthesis to silking interval (ASI) were measured. A total of 291 lines with superior grain yield under both water regimes were selected for further analysis. These lines were test-crossed with CML-312, and 291 crosses along with four commercial check entries were evaluated for pre-germination anaerobic stress tolerance.

Experimental site, cultural practices and stress treatment

The studies on pre-germination anaerobic stress tolerance were conducted in water-logging stress screening facilities (WL-pits) at the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, India. WL-pits are cemented structures constructed at ground level in open field conditions, where standing water can be maintained at a desired depth, and completely drained out after the completion of stress treatments. The size of each WL-pit was 400 cm × 360 cm × 25 cm, which allowed for accommodation of 500 pots of 15 cm × 15 cm with a volume of 2,157 cm^3. Each pot was filled with 2,000 cm^3 of sieved vertisol, alfisol soil and farm-yard manure (FYM), at a ratio of 2:2:1, respectively. All the trials were conducted during the summer-rainy season of 2010 in WL-pits at the ICRISAT campus, Patancheru, Hyderabad, India (17°53' N latitude, 78°27' E longitude and 545 m.a.s.l). In the trial to identify threshold levels for pre-germination anaerobic stress tolerance, six sets of 300 pots were prepared and placed in WL-pits. Each entry was planted in one pot, with 10 seeds per pot and three replications, using a completely randomized design (CRD-factorial). To avoid any variation in seedling emergence due to planting depth, seed depth (4 cm) was carefully managed at the time of planting by placing a circular piece of filter paper 4 cm deep in the pots and planting seeds on the paper before filling the pots with soil to the top of the pot. One set (normal moisture; NM) was placed in WL-pits to ensure similar conditions, but was maintained at optimal moisture levels throughout for proper seed germination, seedling emergence and growth. In the other five sets, different durations of pre-germination anaerobic stress treatment were administered by filling the WL-pits with water immediately after planting. The pots were completely submerged (water level 5 cm above pot surface) to insure anoxic conditions at seed depth. Each set was kept under submerged conditions for a period of anaerobic stress treatment (12, 36, 72, 96, or 120 h). In order to avoid sudden changes in moisture regimes and better simulate the field situation of pre-germination soil water-logging stress, the anaerobic treatment was followed by saturated soil conditions for the next 48 h by reducing and maintaining the water level in WL-pits at 5 cm (10 cm below the top of the pots). Soil temperature during the stress treatment was > 25°C. After this stage, the pits were completely drained, and the pots kept at optimal moisture conditions for observations on germination (coleoptile emergence) and days taken to maximum germination. The trial was concluded 25 d after planting, when there was no further possibility for new coleoptile emergence. For the trials on genotypic variability for pre-germination anaerobic stress tolerance, four sets of pots were prepared – two each for DTM-panel and RILs-TC – and placed in WL-pits. Each entry was planted in two pots with 10 seeds per pot and three replications using completely randomized experimental design. The first set (NM) was maintained at optimal moisture conditions for seed germination, seedling emergence and growth. For the second set (excessive moisture; EM), pre-germination anaerobic stress was applied for 72 h, followed by 48 h of saturated soil conditions, as with the first experiment. After the stress treatment, the WL-pits were completely drained, and moisture in the pots was subsequently maintained at an optimal level for seedling emergence and growth for 20 d.

Observations

Seed germination (%) was recorded in terms of the number of coleoptiles emerged on the soil surface as a percentage of total number of seeds sown. Observations on coleoptile emergence (visible tips) were noted on a daily basis starting from the fourth day after planting; the number of coleoptiles emerged was noted in each pot and this continued until maximum emergence was observed. The delay in germination percentage was calculated as the difference between duration to coleoptile emergence under excessive moisture conditions in comparison to the duration taken under optimal moisture for the same level of germination. At 25 d after planting, all seedlings in each pot were up-rooted and the roots carefully washed in water to avoid any root loss. Roots and shoots were separated and kept between layers of filter paper to soak excess water from the root or shoot surface. Roots and shoots were first dried in the shade for 24 h and then placed in an oven at 50°C for 1 d, and then raised to 70°C for the next 3 d. After complete drying of the samples, root and shoot dry weight was recorded and the total dry weight and shoot:root ratio was calculated. The test weight of each entry was noted by taking the weight of 100 kernels.

Experimental design and statistical analysis

Experiments were conducted using a completely randomized design (two-factors) with three replications. The normality of the original datasets collected in all the experiments was tested, and analysis of variance was computed using the MSTAT-C statistical software (MSTAT-C 1990). In order to avoid plant density bias in recording root and shoot dry weight, the entries with at least 80% germination after undergoing pre-germination anaerobic stress were selected from the two sets, including 23 entries from DTM-panel and 22 entries from RILs-TC, for computing the relationship of the delay in germination against dry weights of root and shoot under pre-germination anaerobic stress. Simple linear regression and Pearson phenotypic correlation between these traits was computed using MSTAT-C. Pearson phenotypic correlation was also computed between test weight and traits of germination, time taken in emergence, and dry weights of

root and shoot under normal and anaerobic stress for both DTM-panel and RILs-TC.

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