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FLOODING TOLERANCE IN COTTON (*GOSSYPIUM HIRSUTUM* L.) AT EARLY VEGETATIVE AND REPRODUCTIVE GROWTH STAGES

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Abstract

Periodic flooding at any growth stage greatly affects growth and yield of crops. In order to develop flooding tolerant cotton cultivar and to identify the most sensitive growth stage to periodic flooding, a field experiment was conducted in which 60-cultivars/accessions/lines were subjected to two week flooding at seedling/early vegetative, flower and boll formation growth stages. Pre- and post-flooding soil analysis was also carried out. Nitrate-N was greatly reduced due to flooding applied at all growth stages, whereas NH_4-N increased significantly. Similarly, Fe and Mn were also increased to many folds in flooded soils. Under hypoxic conditions, depletion of nitrates and toxic effects of accumulated NH_4, Fe and Mn caused severe damages to cotton plants and even death of plants. Of the three growth stages, early vegetative growth stage is most sensitive to two week flooding. Flooding imposed at the flowering and boll formation growth stages caused a substantial amount of yield penalty. On the basis of survival percentage, the 60-cultivars/accessions/lines were categorized into tolerant (≥61%), moderately tolerant (31≥60%) and sensitive (31% ≥) to short term flooding. At the seedling or early vegetative growth stage, genotypes DPL-SR-2 followed by 124-F and MNH-427 were most tolerant to flooding, while AET-5, N-KRISHMA, LRA-5166, CEDIX and H-142 were ranked as sensitive to flooding stress. At the flowering stage, the genotype NIAB-92 followed by S-14 and MNH-427 were highly tolerant to flooding. At the boll formation stage, genotypes DPL-70010-N followed by GH-11-9-75 and B-2918-2 were highly tolerant waterlogging. More than 50% of the genotypes maintained the degree of flooding tolerance at three growth stages. However, on the basis of survival percentage at three growth stages, genotypes MNH-564, FH-114, MNH-786 and CIM-573 were included in the tolerant group and the genotypes N-KRISHMA, LRA-5166, CEDIX and H-142 were included in the sensitive group. These genotypes/cultivars maintaining high degree of stress tolerance at different growth stages are of considerable importance for the development of tolerant cultivar.

Introduction

Cotton is a widely studied crop and is the main source of natural fiber worldwide. In Pakistan, it is main cash crop and lifeline of the textile industry. To meet increasing fiber demands the sufficient production of cotton for ever increasing world's population is now universally realized (www.cotton.org). In tropical and subtropical regions, severe crop losses are always caused by prolonged seasonal rainfall or periodic flooding. Periodic flooding increased in frequency and intensity over the past 50 years throughout the world and caused severe crop losses such as more than $3 billion in United States. In Pakistan, severe flooding caused damages to the wheat, cotton and rice crops up to $4.45 billion in 2010 (Arshad & Shafi, 2010). Similarly, flooding caused considerable crop losses in other parts of the world such as in Europe and Australia (Olesen *et al.*, 2011).

Periodic or seasonal flooding affects the plant growth by depletion of O_2 in the plant rhizosphere and thus caused hypoxic and anoxic conditions in soil (Kozlowski, 1997). Depletion of rhizospheric O_2 due to flooding resulted in a decrease in soil redox potential to levels about -250 mV (Ashraf & Rehman, 1999a; Ashraf & Rehman, 1999b). In most plant species, such hypoxic and anoxic conditions in soil reduces the capacity of roots to supply nutrients and water for plant growth and development (De Simone *et al.*, 2002; Abiko *et al.*, 2012) that resulted in reduction in growth (Ashraf & Rehman, 1999a; Ashraf & Rehman, 1999b; Smethurst & Shabala, 2003) and yield (Tan *et al.*, 2008; Tan *et al.*, 2010). The detrimental effects of water logging on various crops have been demonstrated in many species, such as wheat (Huang *et al.*, 1997; Wu *et al.*, 2014), rice (Ismail *et al.*, 2013), barley (Pang *et al.*, 2004), maize (Subbaiah & Sachs, 2003) and lentil (Ashraf & Chishti, 1993). However, some plant species thrive well on flooded soil, because they possess some adaptive mechanisms at whole plants and/or cellular level (Ashraf & Mehmood, 1990; Jackson & Colmer, 2005; Tan *et al.*, 2010; Abiko *et al.*, 2012). Similarly, intra-specific variation for flooding tolerance has also been found in a number of species, e.g. lentil (Ashraf & Chishti, 1993), wheat (Huang *et al.*, 1994a; Huang *et al.*, 1994b), and rice (Kato-Noguchi & Morokuma, 2007). In view of this information, it is suggested that tolerant cultivars of crop specie can be selected through screening and selection.

It has been noticed that the cotton crop experiences short-term flooding during the monsoon season (July-August) as a result of heavy rainfall. The crop growth is severely inhibited, which ultimately leads to low cotton yield and poor fibre quality. Under such conditions, genotypes or cultivars/lines of cotton that possess high tolerance to flooding would certainly be of considerable economic value. It is generally known that plant tolerance to any abiotic stress varies with the change in growth stage of most plant species, although this is not true in some other plant species (Ashraf, 1994). Similarly, tolerance to water logging also varies with change in growth stage in wheat (Li *et al.*, 2001; Sharma *et al.*, 2010) and rice (Adkins *et al.*, 1990). (Adkins *et al.*, 1990) found that rice plants are more sensitive to waterlogging at the early growth stages than at the later growth stages. Thus one of the major objectives to carry out the present study was to assess variation in flooding tolerance at different growth stages by screening available germplasm of cotton.

Materials and Methods

Screening of sixty cultivars of cotton at three growth stages were carried out in two independent experiments in the research area of the Department of Agriculture, Cotton Research Station, Multan, Pakistan; (30°11 N and 71°28 E). The description is as follows:

Screening at the seedling stage: Cotton seeds of 60 cultivars/genotypes/accessions were obtained from different cotton research organizations of Pakistan, e.g. Cotton Research Station, Multan, Cotton Research Institute, Faisalabad; Cotton Research Institute, Rahim Yar Khan; Cotton Research Station, Vehari; Nuclear Institute for Agriculture and Biology (NIAB) and Cotton Research Station, Bahawalpur. The list of countries of origin of all the cultivars/ genotypes/ accessions is presented in Table 1. The field was thoroughly prepared for planting the crop. Fertilizer, P_2O_5 (57.5) kg ha^{-1} was applied at the time of field preparation. The experiment was conducted in randomized complete block design with two treatments, hypoxia/flooding and non-flooding/control with triplicate replications. The main plot comprised of two subplots (control and flooding) and each sub-plot was further divided in 180 sub-plots. The size of each subplot was 7.50 x 1.75 meter. The inter-row and inter-plant distance was kept 75 and 30 cm respectively. Two seeds per point/hill were sown with hand and later thinned to one seedling per point after emergence when the seedlings attained 3-5 true leaves (25 d) after the sowing. The waterlogging/hypoxia treatment to subplots was subjected to flooding for 14 days. Water was applied up to the stage when there was no further leaching downward or horizontally. The source of irrigation was tube well water. The redox potential of the soil was also recorded three times in a day for two weeks, which changed from (460±2.5 to -38±1.35 mV). All other agronomic and cultural practices were kept constant. Pre-and-post analysis of soil used for the study was carried out following (Allen *et al.*, 1986). A soil sample was taken from 0-20 cm depth from each subplot from the determination of mineral nutrients and other soil characteristics. For the measurement of exchangeable, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} of soil, the samples were extracted in 1.0 *M* ammonium acetate solution. For waterlogged soil samples 1.0 *M* deoxygenated ammonium acetate solution (to deoxygenate N gas was passed through solution for 5 min) was used. Exchangeable cations in extracts were then analyzed on an atomic absorption spectrometer (Perkin Elmer, Analyst 100). Nitrate-and ammonium-N ($NaHCO_3$-P) were extracted and analyzed following (Allen *et al.*, 1986). There is a minute decrease of physicochemical characteristics of the soil before and after the flooding in electrical conductivity (ECe.), and pH of the soil saturated paste. Whereas, the value of N, P, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} are decreased in flooding soils as compared to the control. Fe^{2+} is decreased from 4.5 ± 0.4 to 3.8 ±0.4 (control to flooding). Two weeks after flooding, the total number of seedlings was recorded and the survival percentage was calculated. The 60 cultivars were categorized into three groups, i.e. tolerant, moderately tolerant and sensitive to hypoxia/flooding. Physio-chemical characteristics of the original soil before and after flooding are given in Table 2.

Table 1. List of cultivars/accessions/genotypes of cotton (*Gossypium hirsutum* L.) screened for waterlogging tolerance along with their countries of origin.

S. #	Cultivars/accessions/genotypes	Countries
1.	AET-5	USA
2.	LRA-5166	India
3.	H-2918-2	Pakistan
4.	ACALA 3080	USA
5.	ACALA-4-42	USA
6.	COKER	USA
7.	DEXI KING	Australia
8.	D.P.L.-SR-2	USA
9.	LUMAIN-1	USA
10.	ALBACALA 69/11	USA
11.	G.H.11-9-75	USA
12.	ACALA-1517/70	USA
13.	GREEG-25V	USA
14.	CEDIX	USA
15.	LAMBRIGHT	Australia
16.	E-288	USA
17.	D.P.L.61	USA
18.	DUNN	USA
19.	EARLY COT-31	Australia
20.	BRYCOT-396	Australia
21.	D.P.L-70010-N	USA
22.	CHINES L-1	China
23.	ALLEN-333-61	China
24.	D.P.L-90	USA
25.	DELTAPINE ORIGIN	USA
26.	ACALA-1821-88	USA
27.	ACALA-1517/BR	USA
28.	GENETIC COTTON	Pakistan
29.	H-142	Pakistan
30.	S-12	Pakistan
31.	MNH-147	Pakistan
32.	N-KRISHMA	Pakistan
33.	S-14	Pakistan
34.	S.L.S-1	Pakistan
35.	MNH-415	Pakistan
36.	AC-134	Pakistan
37.	MNH-93	Pakistan
38.	MNH-407	Pakistan
39.	CIM-573	Pakistan
40.	GR-156	Pakistan
41.	NIAB-78	Pakistan
42.	MNH-395	Pakistan
43.	BH-36	Pakistan
44.	LSS	Pakistan
45.	MS-84	Pakistan
46.	MNH-427	Pakistan
47.	CIM-109	Pakistan
48.	NIAB-92	Pakistan
49.	124-F	Pakistan
50.	MNH-786	Pakistan
51.	MNH-456	Pakistan
52.	CIM-240	Pakistan
53.	B-557	Pakistan
54.	SHAHEEN	Pakistan
55.	MNH-564	Pakistan
56.	FH-114	Pakistan
57.	FH-682	Pakistan
58.	CIM-70	Pakistan
59.	MNH-512	Pakistan
60.	VH-189	Pakistan

$$\text{Survival percentage} = \frac{\text{Number of survived seedlings under waterlogged condition}}{\text{Number of total seedlings survived in control}} \times (100)$$

Table 2. Physio-chemical characteristics of the normal and two week waterlogged soil.

Characteristics	Normal soil	Waterlogged soil
Electrical conductivity (ECe) of the soil saturated paste (mS/cm)	2.50 ± 0.5	2.9 ± 0.4
pH of soil saturated paste.	7.170 ± 0.45	7.7 ± 0.42
Textural class	Loam	Loam
Saturation percentage	31.0 ± 0.4	32.6 ± 0.7
Nitrate –N (mg kg^{-1} dry soil)	54.5 ± 1.32	1.68 ± 0.3
Ammonium –N (mg kg^{-1} dry soil)	9.2 ± 0.95	21.6 ± 3.4
Potassium (mg kg^{-1} dry soil)	95.0 ± 12.4	138.7 ± 11.4
Phosphorous (mg kg^{-1} dry soil) extracted with $NaHCO_3$	16.8 ± 1.24	26.4 ± 4.1
Calcium (mg kg^{-1} dry soil)	1249.5 ± 19.85	1130.5 ± 1.6
Magnesium (mg kg^{-1} dry soil)	57.0 ± 3.8	50.0 ± 1.2
Manganese (mg kg^{-1} dry soil)	26 ± 3.28	147.6 ± 21.52
Iron (mg kg^{-1} dry soil)	6.5 ± 1.4	384 ± 20.4

Screening at flowering and boll formation growth stages: This experiment was conducted in same way as mentioned above except flooding was applied at the flowering stage and boll formation growth stages. Flooding was applied for (7 d) at the flowering and boll formation stages when the crop attained maturity of 100 days (August-September). The soil remained saturation up to the next week; hence the flooding period was for a period of two week. Pre-and-post analysis of soil used for the study was carried out as mentioned in the screening experiment. Pesticides were applied accordingly, keeping in view the pest attack situation of the crop. Survival percentage at the seedling stage was recorded as number of plants survived out of total number of seedling emerged. While at the flowering and boll formation growth stages, number of plants wilted after two week flooding were considered as dead.

Statistical analysis of data: A completely randomized design (CRD) with four replicates was used for setting up the experiment. The COSTAT computer package (CoHort software, Berkeley, USA) was used for working out analyses of variance of all variables. The least significance difference test (Snedecor & Cochran, 1989) was used to compare the means.

Results

Two week flooding caused a significant reduction in soil redox potential (-96 to -109 mV), which reflects hypoxic conditions of soil. Flooding caused significant changes on availability of different mineral nutrients. However, there were non-significant differences in various mineral nutrients of flooded soil from all sub-plots in which different genotypes were growing. Similarly, effects of two week flooding on soil mineral nutrients in three different experiments were same. Thus, the data for soil analysis from three different experiments were pooled and presented once (Table 2). From these results, it is clear that NO_3-N in the soil was almost completely depleted due to two week flooding, whereas NH_4^+-N was increased to a great extent (Table 2). Concentrations of P and K also increased in flooded soils. However, concentrations of Ca and Mg in flooded soils remained almost unaffected (Table 2). Moreover, Fe and Mn contents were much higher in flooded soil than in non-flooded soils.

Analysis of variance of the data for survival percentage showed that all cotton genotypes significantly affected due to flooding at the three growth stages and genotypes also significantly differed under both normal and flooded conditions (Table 3). The mean performance of the 60 genotypes under normal and flooded conditions as survival percentage is given in Table 4, on the basis of which the 60-cultivars/ lines were categorized into tolerant, moderately tolerant and sensitive cultivars/lines/genotypes (Table 5). For tolerant (survival percentage 61≥) which contained 23 cultivars. Among flooding tolerant genotypes at the seedling stage, DPL-SR-2 followed by 124-F and MNH-427 had highest survival percentage. The moderately flooding tolerant group had survival percentage (31≥60) and comprised 32 cultivars. For moderately tolerant group, MNH-93 and ALBACALA 69/11 got the highest rank. The flooding sensitive group had 5 cultivars/lines (survival percentage 30≤) including H-142, CEDIX, NIAB KRISHMA, AET-5 and LRA-5166.

On the basis of the performance of survival percentage, screened at the flowering stage was categorized into two groups flooding tolerant (survival percentage 76≥; 52 cultivars) and sensitive group (survival percentage 75≤; 8 cultivars). Among flooding tolerant, NIAB-92, followed by S-14 and MNH427 had maximum survival percentage respectively (Table 6). However, in flooding sensitive category CEDIX, E-288, ACALA-3080, and N-KRISHMA were found to be the most flooding sensitive cultivars.

Table 3. Analysis of variance of data for survival percentage of the 60 cultivars/genotypes/accessions of cotton (*Gossipyumm hirsutum* L) at seedling, flowering and boll formation stages when subjected to waterlogging/hypoxia /flooding for two weeks.

Source of variance	df	Mean squares		
		Seedling stage	Flowering stage	Boll formation stage
Main block effects	2	57.63*	4.52 ^{NS}	18.53*
Cultivars/genotypes/accessions	59	309.5***	129.51***	79.69***
Flooding	1	82506.9***	10261.34***	7093.34***
Interaction (Cont X Hyp)	59	311.3***	58.13***	46.54***
Error	238	9.87	8.33	5.99
LSD (0.05)		0.566	0.519	0.441

NS = Non-significant, * = p≤0.05, ** = p≤0.01, *** = p≤0.001 Significant respectively

Table 5. Grouping of 60 cotton (*Gossypium hirsutum* L.) cultivars/accessions/genotypes on the basis of their performance at seedling stage when subjected to (14 d) flooding.

Tolerant / moderately tolerant / sensitive group	Number of genotypes	Cultivars/accessions/genotypes
Tolerant (Survival percentage ≥ 61) and above)	23	H-29-18-2, ACALA-3080, ACALA-4-42, DEXI KING, S-12, G.H.11-9-75, DPL-SR-2, COKER, FH-114, GREEG-25-V, AC-134, MNH-407, CIM-573, BH-36, MS-84, MNH-427,124-F,MNH-786,CIM-240, B-557, SHAHEEN, MNH-564, CIM-70
Moderately tolerant (Survival percentage 31 ≥ 60) Ranging from 31 to 60	32	LUMAIN-1, ALBACALA-69/11, ACALA-1517/70, VH-189, LAMBRIGHT, E-288, D.P. L-61, DUNN, EARLYCOT-31, BRYCOT-396, DPL-70010-N, CHINESE-L1, DPL-90, LSS, DELTAPINEORIGIN, ACALA-1821-88, MNH-147, ACALA-1517/BR, GENETICCOTTON, S-14, SLS-1, MNH-415, MNH-93, GR-156, CIM-109, NIAB-92, MNH-456, NIAB-78, MNH-395, FH-682 ALLEN-333-61, MNH-512
Sensitive (Survival percentage ≤30 and below)	5	AET-5, LRA-5166, CEDIX, H-142, N-KRISHMA,

Table 6. Grouping of 60 cotton (*Gossypium hirsutum* L.) cultivars/accessions/genotypes on the basis of their performance at flowering stage when subjected to (14 d) flooding.

Tolerant/ / sensitive group	Number of genotypes	Cultivars/accessions/genotypes
Tolerant (Survival percentage 76 and above)	52	AET-5, H-2918-2, ACALA-4-42, COKER, DEXI KING, D.P.L-SR-2,LUMAIN-1,MNH-512, CIM-70, ALBACALA 69/11, G.H.11-9-75, GREEG-25-V LAMBRIGHT, D.P.L-61, DUNN, EARLYCOT-31, BRYCOT-396, D.P. L-70010-N, ALLEN-333-61, D.P.L-90, DELTAPINE ORIGIN, MNH-147, AC-134, ACALA-1821-88, ACALA-1517/BR, S.L.S-1, GENETIC COTTON, S-12, S-14, MNH-415, B-557, MNH-93, MNH-407, CIM-573,FH-114, GR-156, NIAB-78, MNH-395, BH-36, MNH-427, CIM-109, NIAB-92, MNH-786, MNH-456, CIM-240, FH-682, SHAHEEN, 124-F, L.S.S, MS-84, VH-189, MNH-564
Sensitive (Survival percentage 75 and below)	8	LRA-5166, ACALA-3080, ACALA-1517/70, CEDIX, E-288, CHINESE L-1,H-142, N-KRISHMA

Table 4. Mean survival percentage of 60 cultivars/accessions/genotypes of cotton (*Gossypium hirsutum* L.) for screening at seedling, flowering and boll formation growth stages during 2009-10.

Sr.#	Cultivars/ lines / genotypes	Seedling stage			Flowering stage			Boll formation stage		
		Normal	Waterlogging	Waterlogging % of control	Normal	Waterlogging	Waterlogging % of control	Normal	Waterlogging	Waterlogging % of control
1.	AET-5	66.00±2.54	19.60±1.47	29.69	76.30±4.71	69.00±2.12	90.43	74.30±1.47	65.00±1.87	87.48
2.	LRA-5166	64.00±0.71	18.00±0.70	28.13	89.30±0.82	64.60±1.47	72.34	75.30±0.41	57.00±0.71	75.69
3.	H-2918-2	56.00±1.41	49.67±0.82	88.69	72.30±2.27	63.00±1.87	87.03	72.60±2.48	70.30±2.94	96.83
4.	ACALA 3080	61.00±2.12	54.66±0.82	67.48	86.60±4.08	65.00±1.23	75.05	78.30±0.82	72.00±1.25	91.95
5.	ACALA-4-42	72.30±1.08	48.33±1.08	66.84	73.00±1.22	64.67±1.63	88.58	74.70±3.27	68.30±2.04	91.43
6.	COKER	71.67±3.49	43.33±0.82	60.45	78.60±1.63	61.00±0.71	77.60	74.67±0.41	69.00±1.87	92.40
7.	DEXI KING	72.00±2.55	52.00±0.72	72.22	76.00±2.83	66.67±0.41	87.72	74.30±3.49	67.00±0.71	90.17
8.	D.P.L.-SR-2	71.67±1.78	64.67±1.63	90.23	82.00±1.41	70.67±1.08	86.18	76.33±2.27	63.67±1.08	83.41
9.	LUMAIN-1	66.33±1.78	42.33±0.41	63.81	77.00±0.82	70.33±0.41	91.33	77.67±2.86	64.67±1.08	83.26
10.	ALBACALA 69/11	73.67±1.08	43.67±1.47	59.27	81.33±1.66	68.67±1.08	84.43	75.00±2.12	65.67±0.41	87.56
11.	G.H.11-9-75	82.00±1.41	41.67±1.78	50.81	76.67±0.83	69.33±0.41	90.42	76.30±1.08	72.67±0.41	95.24
12.	ACALA-1517/70	88.00±2.45	36.00±1.41	40.90	69.00±0.72	50.00±0.71	72.46	71.67±0.41	54.00±1.25	75.34
13.	GREEG-25-V	73.33±1.47	55.67±1.08	75.91	77.67±1.78	59.30±1.08	76.34	76.30±1.47	66.33±1.08	86.93
14.	CEDIX	73.33±1.63	21.07±0.82	28.73	76.67±2.27	57.00±1.23	74.34	76.67±0.82	55.33±1.08	72.16
15.	LAMBRIGHT	75.67±3.19	40.33±1.08	53.29	89.33±0.82	68.30±2.86	76.45	76.67±2.04	65.67±1.34	85.65
16.	E-288	82.00±2.45	28.67±1.47	34.96	82.00±1.10	63.33±1.47	77.23	76.67±2.04	67.33±0.82	87.81
17.	D.P.L.61	88.67±0.82	32.67±1.19	36.84	86.00±1.41	69.67±1.08	81.01	78.67±2.27	71.33±1.63	90.66
18.	DUNN	71.67±3.16	40.33±1.08	56.27	67.30±0.82	62.67±0.82	86.68	73.33±1.08	65.67±1.47	89.55
19.	EARLY COT-31	61.33±1.08	33.67±2.16	54.89	70.00±1.23	58.30±1.16	83.28	69.00±0.71	57.00±1.25	82.60
20.	BRYCOT-396	88.33±2.04	35.67±1.60	40.38	73.30±1.08	62.00±1.87	84.58	69.30±0.82	63.30±1.63	91.34
21.	D.P.L-70010-N	81.00±6.04	30.33±1.63	37.44	69.00±2.12	63.20±0.41	91.59	87.67±1.78	75.30±1.78	85.89
22.	CHINES L-1	79.67±6.42	37.00±2.23	46.44	87.30±3.27	57.30±1.87	65.63	73.30±1.08	50.07±1.08	68.30
23.	ALLEN-333-61	64.00±2.12	29.00±1.23	45.31	75.00±2.55	61.67±0.82	82.22	73.30±1.41	68.00±2.00	92.76
24.	D.P.L-90	67.67±2.27	29.00±1.23	42.85	77.00±1.87	71.30±0.82	92.59	70.67±0.42	66.69±1.08	94.36
25.	DELTAPINE ORIGIN	64.67±1.08	36.33±1.78	56.17	70.67±1.08	65.67±1.08	92.92	78.33±2.04	73.30±1.47	93.57
26.	ACALA-1821-88	75.00±1.26	33.00±1.08	44.00	87.30±1.63	76.67±0.41	87.82	85.67±0.42	73.30±1.08	85.56
27.	ACALA-1517/BR	76.00±0.71	29.5±1.23	38.80	76.00±2.29	65.00±1.23	85.53	89.00±1.25	67.67±1.47	76.03
28.	GENETIC COTTON	80.67±0.82	32.33±0.82	40.07	79.67±1.90	69.00±0.71	86.60	75.33±2.86	70.30±1.78	93.33
29.	H-142	77.00±1.87	21.67±0.82	28.14	80.00±1.41	59.00±0.71	73.75	79.30±4.08	56.67±2.04	71.46
30.	S-12	72.33±0.42	58.67±2.04	81.11	78.00±1.41	70.67±1.08	90.60	79.30±1.47	66.67±0.82	84.07

Table 4. (Cont'd.).

Sr.#	Cultivars/ lines / genotypes	Seedling stage			Flowering stage			Boll formation stage		
		Normal	Waterlogging	Waterlogging % of control	Normal	Waterlogging	Waterlogging % of control	Normal	Waterlogging	Waterlogging % of control
31.	MNH-147	88.00±2.45	36.00±1.87	40.90	75.67±1.78	65.00±0.71	85.89	71.67±2.04	68.00±1.41	94.87
32.	N-KRISHMA	74.00±1.23	20.33±0.78	27.47	80.67±1.16	69.67±0.42	86.36	94.67±2.90	66.30±0.41	70.03
33.	S-14	68.33±2.86	37.00±0.71	54.14	73.00±1.87	61.00±0.71	83.56	78.30±2.48	70.33±0.82	89.82
34.	S.L.S-1	67.00±1.87	29.67±1.16	44.28	69.67±2.27	60.30±1.08	86.55	74.67±2.27	71.07±1.08	95.17
35.	MNH-415	73.33±1.78	30.33±0.82	41.36	74.00±3.95	60.00±1.23	81.08	88.00±2.45	80.0±1.414	90.90
36.	AC-134	81.00±0.71	51.00±1.83	62.69	89.00±0.71	76.30±2.48	85.73	87.19±1.08	73.30±1.47	84.06
37.	MNH-93	74.33±1.47	45.33±1.78	60.98	87.30±1.08	82.00±1.41	93.92	67.67±1.43	62.30±1.08	92.06
38.	MNH407	61.00±1.22	51.67±1.08	84.70	84.00±0.74	70.30±0.41	83.69	74.00±1.41	64.00±1.25	86.48
39.	CIM-573	79.33±0.82	61.00±1.41	76.89	87.30±0.27	77.67±0.82	88.96	82.00±2.45	73.30±1.08	89.39
40.	GR-156	74.33±2.27	30.00±1.68	40.36	80.47±0.82	73.00±1.87	90.71	75.67±2.67	73.00±1.25	96.47
41.	NIAB-78	68.67±1.63	28.67±1.94	41.76	85.33±1.56	78.30±0.41	91.76	86.67±1.47	79.3±0.817	91.49
42.	MNH-395	64.00±0.71	40.67±1.27	63.54	75.00±1.08	69.67±0.82	92.89	79.30±0.41	72.67±1.08	91.63
43.	BH-36	69.00±1.87	44.00±1.74	63.76	78.00±1.41	72.00±0.71	92.30	76.00±2.55	72.3±1.633	95.13
44.	LSS	72.33±0.41	37.00±0.41	51.15	85.33±1.56	78.67±1.90	92.19	74.00±3.08	70.30±1.08	95.00
45.	MS-84	75.67±2.67	55.67±1.08	73.56	82.67±2.16	74.00±1.41	89.51	78.30±1.08	71.67±2.27	91.53
46.	MNH-427	72.33±0.41	63.33±1.78	87.55	76.30±2.48	73.00±1.63	95.67	74.67±1.78	68.00±1.16	91.06
47.	CIM-109	66.67±2.27	31.67±1.63	47.50	76.00±1.25	71.00±0.71	93.42	74.00±0.71	67.67±2.04	91.44
48.	NIAB-92	76.33±0.41	34.67±1.47	45.42	78.00±1.41	77.00±1.87	98.72	80.67±0.82	76.00±1.41	94.21
49.	124-F	72.34±0.41	64.67±1.78	88.98	88.67±1.63	71.67±1.78	80.82	86.00±2.45	73.00±1.23	84.88
50.	MNH-786	76.67±2.16	64.33±1.08	83.90	89.33±0.82	79.67±0.82	89.18	80.67±2.16	71.30±1.08	92.10
51.	MNH-456	64.67±1.08	38.33±1.47	59.27	87.33±1.16	73.00±3.24	83.59	78.00±1.41	66.30±0.41	85.00
52.	CIM240	62.67±0.41	51.33±1.82	81.90	86.67±1.16	73.67±1.47	85.00	75.30±1.47	66.30±0.41	88.04
53.	B-557	67.00±0.71	53.00±1.41	79.10	83.30±1.97	69.00±0.71	82.83	76.30±0.41	66.67±1.08	87.37
54.	SHAHEEN	66.33±0.41	39.67±2.16	59.80	87.67±1.47	82.67±1.08	94.29	76.00±2.45	68.83±1.86	90.56
55.	MNH564	88.33±0.41	70.67±1.16	80.00	89.30±0.41	82.00±1.23	91.82	88.00±1.45	83.67±0.71	95.07
56.	FH-114	62.33±1.08	51.67±1.90	58.49	89.30±0.82	74.00±0.71	82.86	77.30±1.78	67.67±1.23	87.84
57.	FH-682	84.00±1.41	69.33±1.08	82.53	86.67±2.16	77.30±1.63	89.18	85.67±1.08	76.67±1.47	89.49
58.	CIM-70	71.00±1.87	33.00±1.41	46.48	89.30±0.82	81.30±0.82	91.04	76.67±2.04	73.00±1.87	95.21
59.	MNH-512	71.67±0.41	56.33±1.47	78.59	75.30±0.42	63.78±2.94	84.70	82.33±2.86	68.30±2.27	82.95
60.	VH-189	65.67±2.04	35.00±1.45	53.29	80.67±0.82	71.33±1.47	88.42	72.00±2.12	64.67±1.63	89.81

Table 7. Grouping of 60 cotton (*Gossypium hirsutum* L.) cultivars/accessions/genotypes on the basis of their performance at boll formation when subjected to (14 d) flooding.

Tolerant/ sensitive group	Number of genotypes	Cultivars/accessions/genotypes
Tolerant (Survival percentage ≥76 and above)	54	AET-5, H-2918-2, ACAL-3080, ACALA-4-42, COKER, DEXIKING, D.P.L-SR-2, LUMAIN-1, ALBACALA 69/11, G.H. 11-9-75, GREEG-25-V, LAMBRIGHT, E-288, D.P. L-61, DUNN, MNH-415, EARLYCOT-31, RYCOT-396, VH-189, D.P. L-70010-N, ALLEN-333-61, D.P.L-90, ELTAPINE ORIGIN, ACALA-1821-88, ACALA-1517/BR, GENETIC COTTON, S-12, MNH-147, S-14, S.L.S-1, AC-134, MNH-93, MNH-407, CIM-573, GR-156, NIAB-78, MNH-395, BH-36, L.S.S, MS-84, MNH-427, CIM-109, NIAB-92, 124-F, MNH-786, MNH-456, MNH-395 CIM-240, B-557, SHAHEEN, FH-114, FH-682, CIM-70, MNH-564
Sensitive (Survival percentage 75 and below)	6	LRA-5166, ACALA-1517/70, CEDIX, CHINESE L-1, H-142, N-KRISHMA

Grouping of the 60 cultivars/lines were screened at the stage of boll formation and categorized into tolerant (the survival percentage 76≥; 54 cultivars) and the sensitive group (the survival percentage 75≤; 6 cultivars) (Table 7). On the basis of performance of the survival percentage, DPL-70010-N, GH-11-9-75 and B-2918-2 had the highest survival percentage. Whereas ACALA-1517/70 followed by LRA-5166 and H-142 were found to be most flooding sensitive cultivars.

In view of yield potential and survival percentage of the 60 cultivars/lines at seedling, flowering and boll formation growth stages, most flood tolerant and the most flood sensitive cultivars selected for further study in physiological and quantitative parameters of cotton (*Gossypium hirsutum* L.). They were classified as under:

Tolerant group: MNH-564, FH-114, MNH-786, CIM-573.
Sensitive group: NIAB KRISHMA, LRA-5166, CEDIX, H-142.

Discussions

Periodic flooding increased throughout the world due to climate change and caused severe crop losses (Ashraf, 2003; Olesen *et al.*, 2011). Development of flooding tolerant cultivar is the way to overcome this problem. Various strategies are being used to improve flooding tolerance in different crops including cotton (Pang *et al.*, 2004; Parelle *et al.*, 2010). In natural ecosystem, periodic flooding act as selection pressure and can develop genetic differences for flooding tolerance within populations of a species. Since mechanism of flooding tolerance is still not completely understood, genetic variability for waterlogging tolerance can be assessed indirectly as survival percentage, damage indices, negative impact on growth and yield or directly evaluating traits contributing in tolerance to flooding stress (Parelle *et al.*, 2010). In the present study, plant responses to flooding stress were recorded in terms of survival percentage i.e., extreme response, thus survival rate under hypoxic conditions is an important mean to assess the degree of flooding tolerance (Xu & Mackill, 1995; Nandi *et al.*, 1997; Cornelious *et al.*, 2005; Martin *et al.*, 2006). The advantage of measuring survival rate is that variability in this trait is directly related with genetic variability for flooding tolerance (Parelle *et al.*, 2010). From the present study, it is clear that two week flooding stress caused inhibition in growth of all cotton genotypes/cultivars or even death at all three growth stages and even. From pre and post-experiment soil analysis, it was revealed that soil redox potential become lowered to ~-110 mV. At such lowered redox potential, growth medium become hypoxic with accumulation of Fe^{2+} and Mn^{2+} to a toxic level as has been observed earlier (Ashraf & Rehman, 1999a; Ashraf & Rehman, 1999b). Under such conditions, nitrate-N is used by soil microorganism as an alternative electron acceptor. Manganese oxides are next electron acceptor, followed by iron (Fe) and sulphate (Shabala, 2011). In waterlogged soils, the main form of plant-available nitrogen (N) is NH_4 (Kirk, 2004), and plant adaptations to NH_4 vs NO_3 nutrition may be important under waterlogging (Kirk & Kronzucker, 2005). Similarly, under NH_4 nutrition, plants become unable to exclude Fe^{2+}, therefore and plant traits for internal tolerance or detoxification of Fe^{2+} will be important (Dufey *et al.*, 2012). In the present study, nitrate-N was greatly reduced due to flooding applied at all growth stages, whereas NH_4-N increased significantly. Thus, flooding caused the depletion of nitrates and over-accumulation of NH_4, Fe and Mn thereby resulting death of cotton plants. Moreover, genotypes had greater survival percentages might have better adaptive feature for NH_4 vs NO_3 nutrition and detoxification of Fe^{2+}.

From the screening of 60 cultivars/lines/accessions of cotton (*Gossypium hirsutum* L.) at three different growth stages, seedling stage was found to be the most sensitive growth stage than the other two flowering and boll formation growth stages. A considerable amount of genetic variation exists for flooding tolerance in 60 cultivars/genotypes of cotton. The intra-specific variation for flooding tolerance in cotton is parallel to that has earlier been observed in different crops, e.g., lentil (Ashraf & Chishti, 1993), wheat (Huang *et al.*, 1997; Li *et* *al.*, 2001), and rice (Kato-Noguchi & Morokuma, 2007). In addition, sensitivity to flooding stress was higher at the seedling stage. These results can be explained in view of some earlier findings in which it was found that at the seedling stage flooding caused damage to root growth and establishment in maize, rice and wheat (Huang *et al.*, 1994a; Subbaiah & Sachs, 2003; Visser *et al.*, 2003). Greater sensitivity of seedling stage in cotton germplasm to flooding stress could be due to non-development of any adaptive metabolic pathway or aerenchyma (Parelle *et al.*, 2010). Furthermore, seeds with carbohydrate reserves such as rice and wheat are generally more tolerant of hypoxia (low O_2) or even anoxia (absence of O_2) than seeds with fatty acid reserves such as sunflower and cotton (Al-Ani *et al.*, 1985; Raymond *et al.*, 1985). From these results and some earlier reports it is suggested that selection at the seedling stage can enhance tolerance to flooding stress (Yamauchi *et al.*, 1993; Redoña & Mackill, 1996; Biswas & Yamauchi, 1997). A few years back, (Ling *et al.*, 2004) found that such success is limited because knowledge of the physiological basis of tolerance was inadequate.

It is well evidenced that flooding tolerance in plants is highly dependent on intensity and duration of flooding, so selected cultivars become intolerant in different flooding environment or at different developmental growth stages (Setter *et al.*, 2009). This suggests that factors other than the aerobic root respiration such as oxidative stress, hormonal balance and photosynthetic capacity play an important role in overall waterlogging tolerance. It is, therefore, cotton germplasm was screened at flowering and boll formation growth stages. Most of the strains maintained their degree of flooding tolerance at the three growth stages. For example, N-KRISHMA, LRA-5166, H-142 and CEDIX were ranked as flooding-sensitive at all three growth stages. Similarly NIAB-78, S-12, BH-36, CIM-240, B-557, SHAHEEN, FH-682 and many others were flooding tolerant at all three different growth stages. In contrast there are some strains which showed different degree of flooding tolerance at different developmental phases, e.g., AET-5 was sensitive at the seedling stage, but it was tolerant at the latter two growth stages. Since most of the strains screened, maintained their flooding tolerance consistently at three different growth stages, this aspect has considerable practical value. It has been emphasized that a species or cultivar maintaining its degree of stress tolerance, consistently at all developmental growth stages of life cycle would be of considerable importance, since selection made at any particular stage produced individuals which will maintain their tolerance throughout the plant life cycle.

Conclusion

From the results of the present study, it is concluded that depletion of NO_3-N and K along-with accumulation of toxic concentration of ammonium, Mn and Fe caused severe damages and death in cotton genotypes. A wide range of genetic diversity for flooding tolerance in cotton exists, which can be used for further improvement in flooding tolerance in cotton. The waterlogging-tolerant plants could be selected at any stage, and the selections would be more effective at the seed germination stage.

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