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A STUDY ON THE EVALUATION OF WATERLOGGING TOLERANCE OF DIFFERENT *DACTYLIS GLOMERATA* L. GERMPLASM RESOURCES AND THE DIFFERENCE IN ROOT MICROSTRUCTURE UNDER WATERLOGGING STRESS

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Abstract

In order to characterize the physiological mechanisms of waterlogging tolerance of *Dactylis glomerata* L. and to help rationally select waterlogging-tolerant species to alleviate the waterlogging damage to agricultural production, effects of waterlogging on the growth, morphological indexes, physiological changes and root microstructure of *Dactylis glomerata* L. were studied. A total of 20 varieties (lines) of *Dactylis glomerata* L. from different areas were flooded treatment for 0 day and 28 days, respectively. Morphological indexes and physiological indexes of 20 *Dactylis glomerata* L. resources were evaluated under waterlogging stress comprehensively. In addition, paraffin section technology was used for observing the microstructure changes on root of 6 types of *Dactylis glomerata* L. under waterlogging stress for 0 day, 14 days and 28 days respectively. Our results demonstrated that the orders of 20 *Dactylis glomerata* L. resources by ability of waterlogging tolerance were 'Dianbei' > 'Cambria' > 'Aldebaran' > 'Athos' > 'Donata' > 'Sparta'> 'PI 231482' >'Endurance' > 'PI 598418' > '02-114' > 'PI 311388' > '01472' > 'PG28' > 'Glorus Sweden' = 'PI593995'> 'Smithii PI 441032' = 'PI594994' > 'Geneal Belgnaio' > 'PG49' > 'Amba'. What's more, with the extension of waterlogging stress time, the number/size of vessel and the diameter of vascular cylinder were significantly (P<0.05) reduced. Cells ruptured and structure collapsed were found in the root microstructure of 'PI 594994', 'Geneal Belgnaio', 'PG 49' while structurally-complete of root in 'Dianbei', 'Cambria', 'Aldebaran' for under waterlogging stress 28 days. Therefore, they could be used as the key selection accessions and studied on their genes of waterlogging tolerance for further research.

Key words: *Dactylis glomerata* L., Waterlogging tolerance, Morphological changes, Physiological changes, Microstructure.

Introduction

Orchardgrass (*Dactylis glomerata* L.) is an important member of the family Gramineous genus *Dactylis* which are widely distributed in Europe, North America , and Japan for more than 100 years (Casler *et al*., 2000; Cristiane *et al*., 2020). There are abundant native orchardgrass germplasms with great potential of utilization in temperate zones, which contains great economical and ecological values. In China, the *Dactylis glomerata* L. germplasm resources are primarily distributed in the northwest and southwest areas, which mainly grow on shrubs, forest edges, and sub alpine meadows at altitudes from 1000 to 3600 m (Peng *et al*., 2008). The *Dactylis glomerata* L. has been studied extensively in China over 20 years since that it is considered as the vital forage and hay grass in many aspects (Shuai *et al*., 1997).

Waterlogging is one of the most challenging abiotic stresses and severe problem which affects the growth and yield of *Dactylis glomerata* L, particularly inirrigated areas and high rainfall environments (Zhu *et al*., 2015). Although in waterlogged soils carbon dioxide, ethylene, and trace elements may accumulate in concentrations potentially toxic to plants，oxygen deficiency is the most important cause of waterlogging injury (Zeng *et al*., 2013). The oxygen deficiency in rhizosphere restricts mineral nutrient and water uptake and alters plant metabolism, affecting the growth and development of plants. Meanwhile, waterlogging tolerance is an important breeding objective for *Dactylis glomerata* L.; however, it is a complex quantitative trait. From now on, because of the changes of the environment, it is difficult to screen a large number of breeding lines in the field unless under the control conditions. It has been reported that the important traits for waterlogging tolerance in plants are the architecture of root system and the formation of aerenchyma. Aerenchyma is a specialized plant tissue containing enlarged gas spaces and functions in aeration in plants (Abiko *et al*., 2012; Takahashi *et al*., 2014). Furthermore, the waterlogging tolerance which measured as grain yield is correlated with increased aerenchyma formation (Setter & Waters, 2003). These results indicated that the maintenance and use of ventilatory or functional root system respiration is important for root development under wet conditions, which helps to increase yield under waterlogging conditions (Mano & Omori, 2007; Kumawat *et al*., 2020). Based on this, the direct indexes and traits of aerenchyma development in roots was measured for waterlogging tolerance. Waterlogging also induces degradation of chlorophylls, an increase in membrane permeability, and an insufficient oxygen supply for the root system. Waterlogging tolerant plants neutralize the stress by forming hypertrophied lenticels and aerenchymas and developing adventitious roots to adapt to the waterlogging (Malavasi *et al*., 2016). However, only a few studies have been reported on the growth and the physiological function of root development in waterlogging tolerance.

The objective of the present study was performed on 20 orchardgrass accessions for 28 days after waterlogging stress. The growth indexes (plant height, leaf length, leaf width, stem diameter, root length, dry weight of root and the number of adventitious root) and leaf physiological indexes (chlorophyll content, MDA content, catalase activity, peroxidase and the activity of ascorbic acid peroxidase activity) were measured. Combined with the plant technology of paraffin tissue sections to evaluate the different tolerability of *Dactylis glomerata* L. root tissue. In order to providing a theoretical basis for the reasonable development and application of waterlogging tolerance of *Dactylis glomerata* L. resources.

Table 1. The names and origins of *Dactylis glomerata* L. in this research.

No.	Names	Origins	Types
1.	Amba	China	Cultivar
2.	Aldebaran	China	Cultivar
3.	Donata	China	Cultivar
4.	01472	China	Breeding line
5.	Athos	China	Cultivar
6.	Cambria	China	Cultivar
7.	Dianbei	China	Cultivar
8.	Endurance	China	Cultivar
9.	Sparta	China	Cultivar
10.	Geneal Belgnaio	New Zealand	Cultivar
11.	PG49	New Zealand	Cultivar
12.	PG28	New Zealand	Cultivar
13.	Glorus Sweden	New Zealand	Cultivar
14.	02-114	China	Breeding line
15.	Smithii PI 441032	New Zealand	Cultivar
16.	PI 311388	America	Cultivar
17.	PI 593995	America	Cultivar
18.	PI 594994	America	Cultivar
19.	PI 598418	America	Cultivar
20.	PI 231482	Algeria	Cultivar

Materials and Methods

Materials sampling: A total of 20 varieties *Dactylis glomerata* L. were studied in the experiment, which including all the cultivated varieties in China (Table 1). The porcelain flowerpots with 30 cm caliber and 30 cm depth without drainage holes and the same specifications of porcelain flowerpots with drainage holes were selected for the experiment. Each flowerpot was filled with the same amount of soil, and the height was lower than the basin edge of 8 cm. Different *Dactylis glomerata* L. materials in a petri dish was uniformed and identical to the pot seedling transplanting after germination, growth. Each pot fixed 6 strains, which placed in the light incubator, to ensure a constant growth environment of *Dactylis glomerata* L. resources. The setting program of light incubator was divided into two sections: light intensity 3000 Lx, temperature 22℃, 14 h; light intensity 0 Lx, temperature 12℃, 10 h. The early growth of the seedlings was managed according to the normal water and fertilizer.

Experimental design: After all the *Dactylis glomerata* L. materials were reached the jointing stag and grew to the 4-5 pieces of true leafs, the plant began to be flooded. According to the actual growth situation, rainwater was used for experiment in the flowerpot. There were 2 groups: Control group: under waterlogging stress 0 d (transplanting to a porcelain flowerpot with drainage holes); Waterlogging stress group (transplanting to a pottery flowerpot without drainage holes, and keeping the water surface 3 cm above the soil surface). 3 replicates were set up for each treatment.

Growth and physiological indexes measurements: The growth and physiological indexes were measured at 0 d and 28 d, respectively. The growth indexes including plant height, leaf length, leaf width, stem diameter, root length, dry weight of root and the number of adventitious root. The physiological indexes including chlorophyll content (Chl), malondialdehyde content (MDA), catalase (CAT) activity, peroxidase (POD) activity and ascorbic acid peroxidase (APX) activity. If the phenomenon of death occurred when *Dactylis glomerata* L. were 28 d under waterlogging stress, the index was recorded as 0.

Paraffin section for the roots of *Dactylis glomerata* L.: The newborn branch roots of *Dactylis glomerata* L. were acquired as the research object. The fresh tissues of *Dactylis glomerata* L. root were collected by series of steps including fixed, dehydration, paraffin embedding, slicing, wax dipping, dewaxing, rehydration, dyeing, mounting. Structure and morphology of cells were observed by a microscope and photographed at 200× magnification.

Statistical analysis

Statistical analysis were performed and carried out using SPSS 19.0 software and the following formulas. The statistical significance of the differences between groups was determined by ANOVA and all the data were statistically analyzed by mean ± S.D. p<0.05 (5% significant level) was considered statistically significant, and p<0.01 (1% significant level).

$$\text{Coefficient of waterlogging resistance } (\alpha) = \frac{\text{Measured value of waterlogging stress at 28 d}}{\text{Measured value of waterlogging stress at 0 d}} \times 100\% \quad (1)$$

$$U_x = (X_i - X_{\min}) / (X_{\max} - X_{\min}) \quad i=1, 2, 3\ldots, n \quad (2)$$

In formula (1) and formula (2), U_x represents the value of the membership function, X_i represents the measured value of the index. $X_{\max}$ and $X_{\min}$ represent the maximum and minimum of all the tested materials respectively.

$$wi = Pi / (P_1 + P_2 + P_3 + \ldots + P_n) \quad i=1, 2, 3,\ldots, n \quad (3)$$

In the formula (3), *wi* represents the main degree of the *i* common factor in all the common factors, and *Pi* was the contribution rate of the *i* factor of each material. The principal component analysis was carried out by SPSS 19.0, the coefficient and contribution rate of each comprehensive index were obtained, and the weight of each comprehensive index was calculated.

$$D=U_1\times w_1 + U_2\times w_2+ U_3\times w_3+\ldots U_n\times w_n \quad i=1, 2, 3,\ldots, n \qquad (4)$$

In formula (4), the value of *D* was the comprehensive evaluation value of the waterlogging resistance performance evaluated by the comprehensive indexes under the stress of waterlogging. The greater the *D* value, the stronger the waterlogging resistance. On the contrary, the more sensitive to waterlogging stress, the weaker the ability to resist waterlogging.

Results and Analysis

Evaluation of waterlogging resistance: Coefficients of waterlogging-resistance 20 *Dactylis glomerata* L. materials were obtained by the formula (1). It indicated that when the waterlogging tolerance coefficient <100%, it was considered as the index which was decreased under waterlogging stress. Compared with the control group, most of morphological indexes were decreased; stem diameter, the number adventitious root were increased after waterlogging stress 28 d. In physiological indicators, the mean value of Chl content, MDA content, CAT activity, POD activity and APX activity were consistent with <100%. Total Chl (82.20%) > root dry weight (54.58%) > leaf length (49.16%) > CAT (39.46%) > plant height (32.78%) > number of adventitious roots (31.86%) > POD activity (29.15%) > root length (16.51%) > leaf width (15.25%) > stem diameter (12.58%) > MDA (6.04%) > APX (0.58%). The results showed that total chlorophyll was most sensitive to waterlogging stress, followed by dry weight of root, leaf length and activity of CAT Tables 2 and 3).

Comprehensive evaluation: The waterlogging tolerance of pasture is a complex trait with many factors. It is difficult to fully reflect the real waterlogging tolerance of plants by using a single index as a direct evaluation criterion for waterlogging tolerance index. Therefore, the waterlogging tolerance of *Dactylis glomerata* L. were evaluated by 7 indicators and 5 physiological indexes. The 12 individual indicators of 20 *Dactylis glomerata* L. materials in waterlogging coefficient correlation were analyzed with SPSS 19.0, and there were a correction among the 12 single indicators (Table 4). The results of principal component analysis showed that the contribution rates of the first 4 comprehensive indexes were 45.672%, 21.705%, 8.938% and 6.820% respectively, and the cumulative contribution rate was 83.135% (Table 5). The values of 4 comprehensive indexes *C(x)* were obtained by scores of waterlogging tolerance coefficient of each single index and comprehensive indexes, and the values of U_x were obtained by formula (2) (Table 6). Then according to the contribution rate of the 4 comprehensive indexes, the weight of the 4 comprehensive indexes (0.549, 0.261, 0.108, 0. 082) were obtained by formula (3). Finally, the comprehensive evaluation of the 20 *Dactylis glomerata* L. resources under waterlogging stress value of material *D* were obtained by formula (4). Waterlogging stress had different level of inhibition effects on the growth of all *Dactylis glomerata* L. Based on the comprehensive performance on morphology and physiology of 20 *Dactylis glomerata* L. resources under waterlogging stress, the order by ability of waterlogging tolerance were 'Dianbei' > 'Cambria' > 'Aldebaran' > 'Athos' > 'Donata' > 'Sparta'> 'PI 231482' >'Endurance' > 'PI 598418' > '02-114' > 'PI 311388' > '01472' > 'PG28' > 'Glorus Sweden' = 'PI593995'> 'Smithii PI 441032' = 'PI594994' > 'Geneal Belgnaio' > 'PG49' > 'Amba'. What's more, 'Amba' had all died after waterlogging stress for 28 days.

Effect of waterlogging stress on the microstructure of root: The microstructure of *Dactylis glomerata* L. root consists of epidermis (marked with number 1, Fig. 1A), cortex (marked with number 2, Fig. 1A), endodermis (marked with number 3, Fig. 1A), pith (marked with number 4, Fig. 1A), epigenetic xylem (marked with number 5, Fig. 1A). Under waterlogging stress 0 d, the cortical cells were closely connected, no or less space was found, and the ventilatory tissues weren't formed in inner and outer cortex. When the *Dactylis* L. resources 'PI 594994', 'Geneal Belgnaio' and 'PG 49' under waterlogging stress, it can be seen that the cytoderm of the outer cortex cells were lignification and thrombus, and its endothelial cells were "U shaped" (marked with arrow, Figs. 2D, 2E, and 2F), while the "PG 49" root exfoliate cells were keratinized, showing "herring-bone form" (marked with arrow, Figs. 2G, 2H, and 2I). When under waterlogging stress, the formation time and quantity of ventilatory tissues were ordered as follow: 'Dianbei', 'Cambria' and 'Aldebaran' >'PI 594994', 'Geneal Belgnaio' and 'PG 49'. Compared with 'Dianbei', 'Cambria' and 'Aldebaran' at the prolongation of the time of waterlogging stress, the greater the degree of damage to the root cells of *Dactylis glomerata* L., the worse the integrity of the structure of tissue. Under waterlogging stress for 14 d, a slender gap in the root cortex was formed by separation among cells. Compared with the waterlogging tolerance *Dactylis glomerata* L. 'Dianbei', 'Cambria' and 'Aldebaran', the waterlogging sensitive *Dactylis glomerata* L. resources 'PI 594994','Geneal Belgnaio'and'PG 49' were no obvious intamescentia phenomenon among cells (marked with arrow, Figs. 1B, 1E, and 1H), cyto-architectures weren't broken, and the number of slender gaps was less visible (marked with arrow, Figs. 2B, 2E, and 2H). Under waterlogging stress for 28 d, 'Dianbei', 'Cambria' and 'Aldebaran' were seen in the shape of "wheel", many air chambers formed in the roots cells, radiation, and the cell walls of the adjacent air cavity were stacked together to form a double cytoderm, the root structure remains intact (marked with arrow, Figs. 1C, 1F, and 1I). 'PI 594994', Geneal Belgnaio'and 'PG 49' have several large air chambers in the cortex, but some of the cell walls were broken. There was a separation between the cortex and the vascular cylinder, some cells in the middle pillar ruptured and collapsed (marked with arrow, Figs. 2C, 2F, and 2I).

Table 2. Waterlogging-resistance coefficients of morphology indexes of 20 *Dactylis glomerata* L. materials after waterlogging stress for 28 days (%).

Names	Height	Leaf length	Leaf Width	Stem diameter	Root length	Dry weight of root	Number of adventitious root
Amba	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dianbei	58.71	32.08	61.70	90.48	31.13	40.00	140.50
Donata	64.53	34.50	96.88	153.85	89.67	25.00	174.25
Endurance	71.09	50.50	75.00	135.29	82.58	25.00	102.60
Aldebaran	69.58	27.49	71.11	80.00	88.54	25.00	184.14
Cambria	47.79	38.18	85.00	135.29	91.03	20.00	203.29
Sparta	50.62	26.20	110.00	115.00	93.58	75.00	162.21
Athos	51.02	50.79	97.44	119.05	94.19	50.00	102.54
‘01472’	76.04	49.73	100.00	100.00	98.36	66.67	208.04
‘Geneal Belgnaio’	67.42	50.76	100.00	106.67	84.92	75.00	133.30
‘PG49’	76.45	52.08	94.12	140.00	93.69	60.00	103.81
‘PG28’	71.65	83.31	97.56	126.67	97.74	40.00	155.56
‘Glorus Sweden’	86.31	83.97	77.50	135.29	89.68	60.00	189.39
‘02-114’	88.78	65.00	88.89	113.33	96.03	50.00	100.00
‘Smithii PI 441032’	82.01	70.07	93.94	150.00	93.16	50.00	129.48
‘PI 311388’	70.59	55.68	80.43	90.00	84.78	80.00	104.37
‘PI 593995’	79.95	60.39	90.32	114.29	91.38	50.00	110.44
‘PI 594994’	68.36	61.13	93.75	118.75	89.95	50.00	106.67
‘PI 598418’	86.53	72.87	92.50	106.25	94.75	33.33	107.18
‘PI 231482’	76.92	52.04	88.89	121.43	84.72	33.33	119.36
Average	67.22	50.84	84.75	112.58	83.49	45.42	131.86
Mean range	32.78	49.16	15.25	12.58	16.51	54.58	31.86

Table 3. Waterlogging-resistance coefficients of physiological indexes of 20 *Dactylis glomerata* L. materials after waterlogging stress for 28 days Unit: %

Name	Total Chl content	MDA content	CAT activity	POD activity	APX activity
Amba	0.00	0.00	0.00	0.00	0.00
Dianbei	24.02	124.92	67.21	92.22	280.63
Donata	30.57	86.90	57.30	109.32	205.55
Endurance	33.98	100.00	52.01	59.28	58.18
Aldebaran	27.88	117.50	85.14	65.67	166.81
Cambria	21.54	182.99	86.11	122.55	91.41
Sparta	35.94	104.29	73.90	139.77	163.86
Athos	21.46	147.84	104.94	91.45	69.03
‘01472’	46.97	89.02	61.47	74.76	57.39
‘Geneal Belgnaio’	31.29	92.50	55.24	53.33	114.88
‘PG49’	3.59	65.68	88.61	54.26	53.89
‘PG28’	4.46	97.81	38.57	63.16	118.71
‘Glorus Sweden’	26.36	92.96	46.85	83.00	131.68
‘02-114’	3.01	152.47	44.10	65.56	55.68
‘Smithii PI 441032’	8.23	52.23	71.94	82.14	153.76
‘PI 311388’	4.40	64.50	31.58	52.83	91.08
‘PI 593995’	7.84	60.91	55.86	56.70	64.49
‘PI 594994’	13.89	84.24	79.06	43.56	36.21
‘PI 598418’	7.22	46.96	25.29	42.17	28.14
‘PI 231482’	3.43	115.38	85.52	65.22	47.03
Average	17.80	93.96	60.54	70.85	99.42
Mean range	82.20	6.04	39.46	29.15	0.58

Table 4. Correlative coefficient matrix of 12 indexes of 20 *Dactylis glomerata* L. germplasm resources after waterlogging stress for 28 days.

Indexes	Height	Leaf length	Leaf width	Stem diameter	Root length	Dry weight of root	Number of adventious root	Total chl content	MDA content	CAT activity	POD activity	APX activity
Height	1											
Leaf length	0.805**	1										
Leaf width	0.672**	0.554*	1									
Stem diameter	0.661**	0.600**	0.792**	1								
Root length	0.741**	0.648**	0.901**	0.768**	1							
Dry weight of root	0.452*	0.402	0.614**	0.285	0.481*	1						
Number of adventious roots	0.398	0.208	0.556*	0.524*	0.548*	0.244	1					
Total chl content	0.003	-0.223	0.28	0.183	0.182	0.202	0.644**	1				
MDA content	0.161	0.056	0.384	0.396	0.360	0.031	0.529*	0.323	1			
CAT activity	0.185	-0.011	0.520*	0.518*	0.439	0.175	0.425	0.29	0.606**	1		
POD activity	0.103	-0.044	0.537*	0.569**	0.392	0.217	0.713**	0.534*	0.641**	0.556*	1	
APX activity	0.069	-0.138	0.143	0.236	-0.066	0.106	0.526*	0.404	0.277	0.235	0.616**	1

Table 5. Coefficients of comprehensive indexes and their contribution rate of 20 *Dactylis glomerata* L. germplasm resources after waterlogging stress.

Coefficients	Comprehensive indexes			
	1	2	3	4
Height	0.688	-0.566	0.113	0.231
Leaf length	0.522	-0.742	0.040	0.268
Leaf width	0.906	-0.229	0.010	-0.196
Stem diameter	0.854	-0.176	-0.174	0.211
Root length	0.853	-0.376	-0.109	-0.138
Dry weight of root	0.521	-0.289	0.493	-0.476
Number of adventious root	0.780	0.359	0.197	0.155
Total chl content	0.437	0.587	0.379	-0.252
MDA content	0.590	0.416	-0.466	0.026
CAT activity	0.625	0.317	-0.473	-0.267
POD activity	0.716	0.572	-0.018	0.057
APX activity	0.367	0.592	0.385	0.449
Contribution rate/%	45.672	21.705	8.938	6.820

Table 6. The values of comprehensive indexes C(*x*), subordinative function *U*(*x*) and comprehensive evaluation *D* of 20 *Dactylis glomerata* L. germplasm resources after waterlogging stress for 28 days.

Names	C (1)	C (2)	C (3)	C (4)	U (1)	U (2)	U (3)	U (4)	D
Amba	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dianbei	6.425	2.463	0.623	1.381	1.000	0.317	0.000	0.131	0.643
Donata	7.611	1.667	0.409	1.181	1.000	0.175	0.000	0.107	0.603
Endurance	5.924	0.262	-0.262	0.461	1.000	0.085	0.000	0.117	0.581
Aldebaran	6.701	1.640	0.133	0.827	1.000	0.229	0.000	0.106	0.618
Cambria	7.845	1.746	-0.606	0.670	1.000	0.278	0.000	0.151	0.634
Sparta	7.754	1.727	0.372	0.534	1.000	0.184	0.000	0.022	0.599
Athos	6.909	0.777	-0.628	0.171	1.000	0.186	0.000	0.106	0.606
‘01472’	7.212	0.588	0.253	0.234	1.000	0.051	0.003	0.000	0.563
‘Geneal Belgnaio’	6.532	0.496	0.320	0.397	1.000	0.028	0.000	0.012	0.557
‘PG49’	6.311	-0.230	-0.243	0.217	1.000	0.002	0.000	0.070	0.555
‘PG28’	6.874	0.225	0.125	0.861	1.000	0.015	0.000	0.109	0.562
‘Glorus Sweden’	7.479	0.586	0.412	0.913	1.000	0.025	0.000	0.071	0.561
‘02-114’	6.445	-0.038	-0.428	0.430	1.000	0.057	0.000	0.125	0.574
‘Smithii PI 441032’	7.073	0.381	0.294	0.882	1.000	0.013	0.000	0.087	0.559
‘PI 311388’	5.537	-0.095	0.369	0.369	1.000	0.000	0.082	0.082	0.565
‘PI 593995’	5.946	-0.218	0.009	0.387	1.000	0.000	0.037	0.098	0.561
‘PI 594994’	6.009	-0.218	-0.319	0.155	1.000	0.016	0.000	0.075	0.559
‘PI 598418’	5.411	-0.771	0.013	0.392	1.000	0.000	0.127	0.188	0.578
‘PI 231482’	6.340	0.198	-0.547	0.350	1.000	0.108	0.000	0.130	0.588
Weights					0.549	0.261	0.108	0.082	

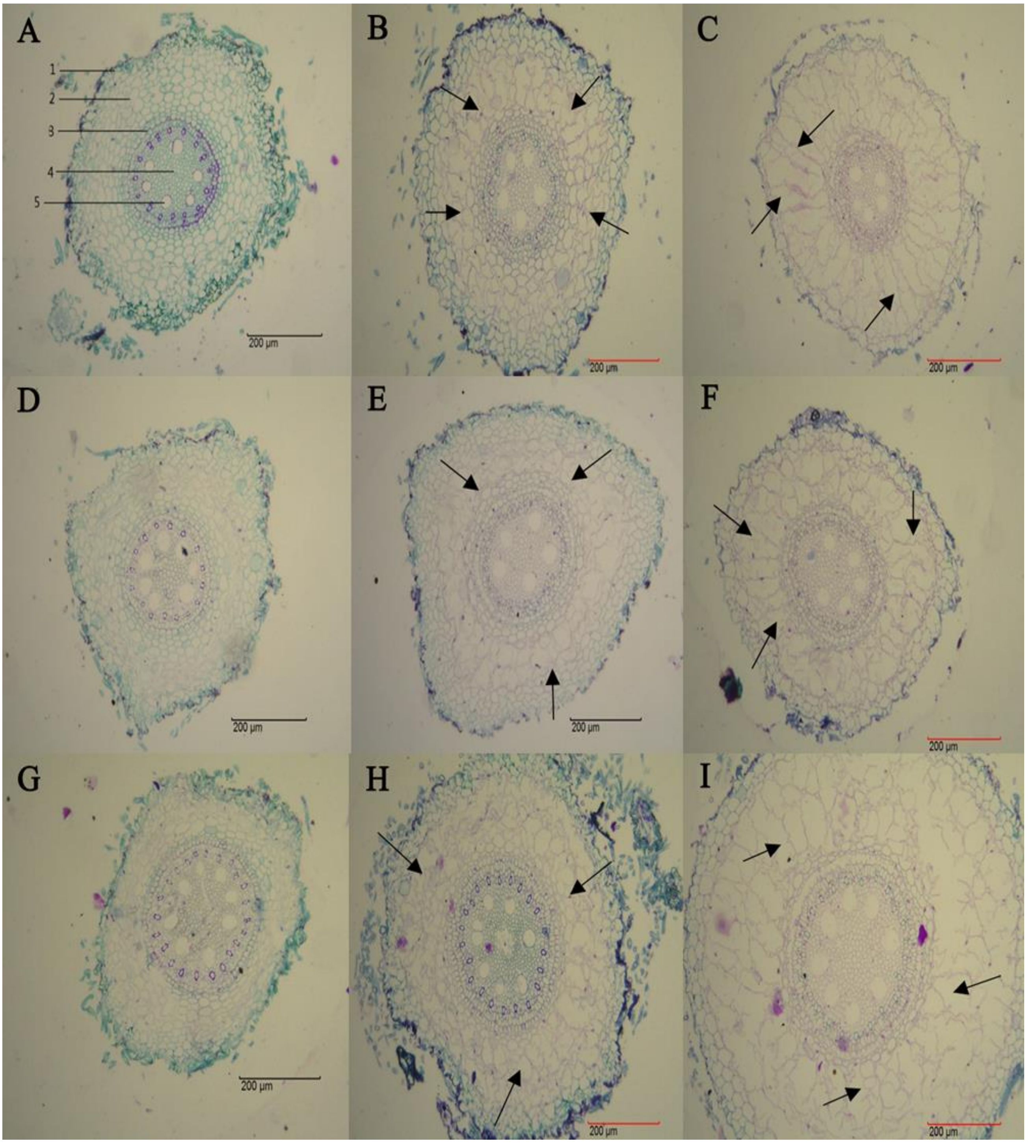

Fig. 1. The development of aerenchyma on 'Dianbei', 'Cambria' and 'Aldebaran' under waterlogging stress.

Effect of waterlogging stress on the number of vessels: Vessel is a tubular structure and an important part of plant dredging tissue that mainly transport water and inorganic salts in plant roots (Siddiqi *et al.*, 2008). In present experiment, with the increase of waterlogging stress time (0, 14, 28 d respectively), the number of vessels significantly decreased (p<0.05), and the degree of reduction was different due to the difference of waterlogging resistance (Table 7). After waterlogging stress, the number of vessels of *Dactylis glomerata* L. 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced with an average of 3.67 while 'PI 594994', 'Geneal Belgnaio' and 'PG 49' reduced with an average of 3. Compared with under waterlogging stress for 0 d, the number of vessels of *Dactylis glomerata* L. resources 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced when under waterlogging stress for 28 d (p<0.01) and 14 d (p<0.05). Compared with under waterlogging stress for 0 d, *Dactylis glomerata* L. resources 'PI 594994', 'Geneal Belgnaio' and 'PG 49' significantly reduced when under waterlogging stress for 28 d (p<0.01) and 14 d (p<0.01).

Effect of waterlogging stress on diameter of vascular cylinder: In the course of long-term evolution, the root structure of plant was responded adaptively to changes in the soil environment. The vascular cylinder is the middle axis of the endoderm and important part of the microstructure of the root of the plant, which is composed of the pericycle and the vascular tissue. With the increase of waterlogging stress time (0, 14, 28 d respectively), the diameter of vascular cylinder was significantly decreased ($p<0.05$) (Table 8). After waterlogging stress, the diameter of vascular cylinder of *Dactylis glomerata* L. resources 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced with an average of 133.24 μm while 'PI 594994','Geneal Belgnaio' and 'PG 49' reduced with an average of 51.23 μm. Compared with under waterlogging stress for 0 d, the diameter of vascular cylinder of *Dactylis glomerata* L. resources 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced when under waterlogging stress for 28 d ($p<0.01$) and 14 d ($p<0.01$). Compared with under waterlogging stress for 0 d, 'PI 594994', 'Geneal Belgnaio' and 'PG 49' reduced slowly with the increase of waterlogging stress time when under waterlogging stress for 28 d ($p>0.05$) and 14 d ($p>0.05$).

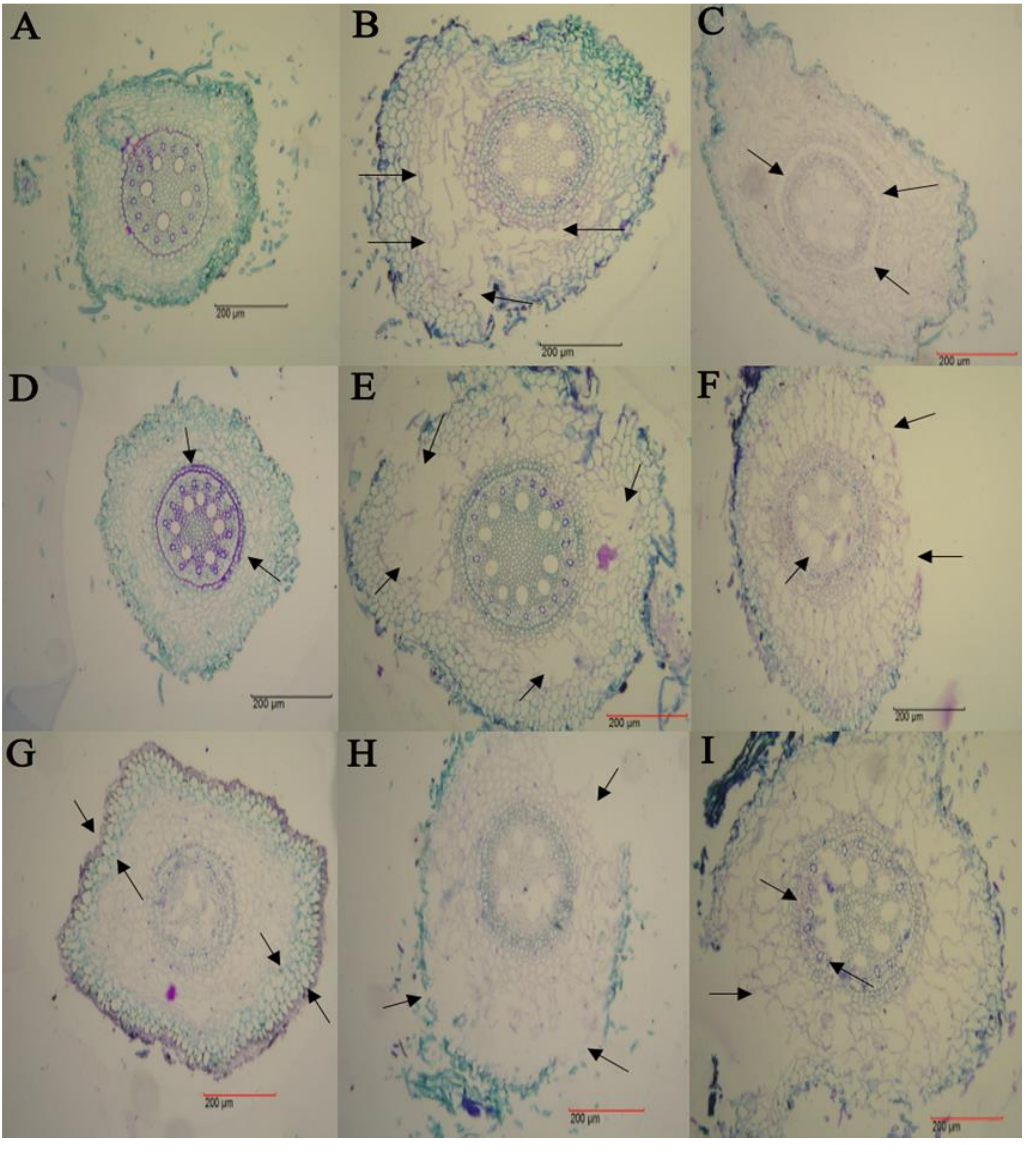

Fig. 2. The development of aerenchyma on 'PI 594994', 'Geneal Belgnaio' and 'PG 49' under waterlogging stress.

Table 7. The comparison on number of vessel under waterlogging stress (pcs).

Type of germplasm resources	Waterlogging stress for 0 day	Waterlogging stress for 14 days	Waterlogging stress for 28 day
Relative waterlogging enduring	9.00 ± 0.00^{Aa}	6.00 ± 1.73^{ABb}	5.33 ± 0.58^{Bb}
Relative waterlogging sensitive	8.67 ± 1.53^{Aa}	6.33 ± 0.58^{Ab}	5.67 ± 0.58^{Ab}

Table 8. The comparison on diameter of vascular cylinder under waterlogging stress (μm).

Type of germplasm resources	Waterlogging stress for 0 day	Waterlogging stress for 14 days	Waterlogging stress for 28 day
relative waterlogging enduring	349.77 ± 13.16^{Aa}	237.93 ± 27.37^{Bb}	216.53 ± 36.19^{Bb}
relative waterlogging sensitive	280.53 ± 58.61^{Aa}	260.17 ± 38.72^{Aa}	229.30 ± 22.12^{Aa}

Table 9. The comparison on size of vessel under waterlogging stress (μm).

Type of germplasm resources	Waterlogging stress for 0 day	Waterlogging stress for 14 days	Waterlogging stress for 28 day
relative waterlogging enduring	30.68 ± 4.85^{Aa}	26.12 ± 1.88^{AaB}	17.47 ± 1.12^{Bb}
relative waterlogging sensitive	32.82 ± 1.77^{Aa}	25.01 ± 1.09^{Bb}	23.87 ± 3.46^{Bb}

Effect of waterlogging stress on the size of vessel: As shown in Table 9, with the increase of waterlogging stress time (0, 14, 28 d respectively), the size of vessel was significantly increased ($p<0.05$). After waterlogging stress, the size of vessel of *Dactylis glomerata* L. resources 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced with an average of 13.21 μm while 'PI 594994','Geneal Belgnaio' and 'PG 49' reduced with an average of 8.95 μm. Compared with under waterlogging stress for 0 d, the size of vessel of *Dactylis glomerata* L. resources 'Dianbei', 'Cambria' and 'Aldebaran' significantly reduced when under waterlogging stress for 28 d ($p<0.01$) while the difference was not significant for 14 d ($p>0.05$). Compared with under waterlogging stress for 0 d, 'PI 594994', 'Geneal Belgnaio' and 'PG 49' significantly reduced when under waterlogging stress for 28 d ($p<0.01$) and 14 d ($p<0.01$).

Discussion

Under normal habitat conditions, all metabolic processes within the plant are in a relatively stable and coordinated state. When the plant is subjected to waterlogging stress, the normal growth and development were affected metabolic disorder, even induces programmed cell to death (Kuai *et al*., 2014). In present study, the results showed that the growth rate of aboveground parts of the 20 *Dactylis glomerata* germplasm resources were slowed, leaves were yellow, wilting and falling out, internode and petiole elongation morphologically after 28 days of waterlogging stress (Zengin *et al*., 2020). It is consistent with the research of Ito (Ito *et al*., 1999). In addition, underground parts were stem hypertrophy, formation of adventitious roots and aerobic growth of roots, which is consistent with the study of other scholar's research (Kozlowski *et al*., 1997). It was also revealed that 20 *Dactylis glomerata* germplasm resources were various degrees of yield reduction, even the roots of the 'Anba' plant were greatly reduced, and extensive rot occurred, and they were easily attacked by pests and diseases, eventually causing all plant deaths. It is consistent with Justin and Armstrong's study on the respiratory metabolism of plant roots under anaerobic conditions (Justin & Armstrong, 2010). When plant tissues are damaged by adversity, the cell membrane is damaged firstly, and the changes in the plasma membrane affects other physiological activities of the plant under waterlogging resistance (Pan & Xue, 2012). The content of Chl was decreased, the MDA was accumulated, SOD, CAT, POD, and APX were activated to alleviating the damage to the plant and contributed to the survival of the 20 *Dactylis glomerata* germplasm resources. In present study, the contents of Chl were decreased, leaf chlorosis and yellow can attenuated photosynthesis of plants. With the extension of flooding time, enzyme activities which related to photosynthesis were decreased, the content of Chl were decreased rapidly, the leaves declined prematurely, the area of green leaves decreased, and the leaves fell off. This is consistent with the results of Shah's research (Shah & Nahakpam, 2012). It was reported that waterlogging resistance can cause membrane lipid peroxidation, and also accumulation of MDA, and CAT and APX activity increase significantly. When the concentration is high enough, the plant might avoid damage under waterlogging stress (Wang *et al*., 2016). At the same time, in order to reduce the damage to *Dactylis glomerata* L. under waterlogging resistance, the activity of antioxidant enzymes such as POD and APX were significantly increased (Khezrian *et al*., 2020). With the duration of waterlogging stress, MDA was accumulated while the membrane lipid peroxidation was aggravated. On the contrary, accumulation of MDA may aggravated lipid peroxidation which forming a vicious cycle that caused premature failure of leaves (Ashraf *et al*., 2010). Due to the limited capacity which *Dactylis glomerata* L. resources resist against waterlogging stress, the antioxidant enzymes activity is not enhanced with the prolonging of stress time or infinite strength. Thus, the change of MDA content and activity of antioxidant enzymes is not linearly related to the time or intensity of waterlogging stress.

The waterlogging resistance of the *Dactylis glomerata* L. germplasm was evaluated by the membership function method. It was scientifically and comprehensively reflected by the variation of many indicators of the plant after 28 days of waterlogging stress. In previous studies, this method was widely used to evaluate the stress resistance of forage grass, and there are certain correlations among these indexes (Liu *et al*., 2015). The principal components analysis was performed on 12 individual indicators of 20 *Dactylis glomerata* L. materials after 28 days under waterlogging stress. Under the premise of retaining or reducing the loss of original information, a number of single indicators with certain relevance were converted into 4 comprehensive indicators. By combining the membership function method and the principal component analysis method, the one-sided of performance evaluated by each single index was avoided. Multiple indicators were fully integrated to improve the response to waterlogging stress between different materials or different growth stages of the *Dactylis glomerata* L.

There was a significant difference in adaptive response to waterlogging stress in *Dactylis glomerata* L. germplasm with different levels of waterlogging tolerance. Whether plants can better withstand waterlogging stress is often closely related to the degree of development of aerenchyma (Yamauchi *et al*., 2013). From the observation of the aerenchyma in the roots of three *Dactylis glomerata* L. germplasm resources at different levels, the aerated tissues were formed in all the test materials after waterlogging stress. It was reported that the formation of aeration tissue was a universal mechanism which *Dactylis glomerata* L. germplasm for the adaptive response of waterlogging resistance (Sairam *et al*., 2008). The most important physiological function of the roots is to absorb water and inorganic salts from the soil for the use of above-ground parts of the plants, whereas organic compounds from above-ground parts are transported to the roots through the drainage organization to ensure the normal growth and development of the plants (Takehisa *et al*., 2012). Under the waterlogging stress, the number of catheters contained in the roots of *Dactylis glomerata* L. germplasm, the lumen size, and the diameter of the vascular cylinder all showed a decreasing trend. It showed that in order to adapt to irrigated areas and high rainfall environments, the roots through narrowing the morphology of the transmitting tissue (catheter in the quantity, vascular cylinder size and the size of the catheter lumen, etc.), thus the ability of roots to transport water is also reduced, while the ability to absorb and utilize oxygen of soil or was dissolved in water is enhanced (Goyal *et al*., 2020).

For the relative waterlogging-enduring *Dactylis glomerata* L. germplasm resources (‘DianBei’, ‘Cambria’ and ‘Aldebaran’), the weakening of the drainage organization was evidently after 14 days of waterlogging stress while the weakening range was greater after 28 days of waterlogging. Whereas the relative waterlogging-sensitive *Dactylis glomerata* L. germplasm resources ('PI 594994', 'Geneal Belgnaio' and 'PG 49') only showed a clear weakening of the drainage tissue after 28 days of waterlogging stress, it may be due to poor integrity of the root structure after waterlogging stress for 14 days, and it has been subjected to waterlogging damage (Adebiyi *et al*., 2018). Therefore, it can no longer effectively resist waterlogging stress environment. In present study, the drainage organization of ‘DianBei’, ‘Cambria’ and ‘Aldebaran’ were obviously weaken under waterlogging stress for 14 d while the drainage organization of‘'PI 594994', 'Geneal Belgnaio', 'PG 49'’under waterlogging stress were for 28 d.

Conclusions

Waterlogging resistance of 20 *Dactylis* L. germplasm resources from strong to weak order: 01472 > PI 593995 > Smithii PI 441032 > 02-114 > PI 594994 > Glorus Sweden > PG49 > Cambria > PI 231482 > PI 311388 > Aldebaran > Sparta > Dianbei > Geneal Belgnaio > Donata > Athos > PG28 > Endurance > PI 598418 > Amba. Waterlogging resistant Dactylis L. germplasm resources ‘Dianbei’, ‘Cambria’ and ‘Aldebaran’ were tolerated for 28 d waterlogging stress environment. On the microstructure of root, the integrity of tissue structure, the number and the size of vessel, and the diameter of vascular cylinder all showed that the effective resistance and adaptation to the waterlogging stress environment. The waterlogging tolerant *Dactylis glomerata* L. cultivars found in this study will be available to elucidate the mechanism of the development of roots under waterlogging tolerance. In addition, to achieve a better understanding of the waterlogging tolerance of *Dactylis glomerata* L. germplasm resources, the photo-physiological recovery mechanism of *Dactylis glomerata* L. after waterlogging also remains to be investigated.

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References

Abiko, T., M. Obara, F. Abe, K. Kawaguchi, A. Oyanagi and T. Yamauchi. 2012. Screening of candidate genes associated with constitutive aerenchyma formation in adventitious roots of the teosinte zea nicaraguensis. *Plant Root.,* 6: 19-27.

Adebiyi, J.A., A.O. Obadina, O.A. Adebo and E. Kayitesi. 2018. Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. *Crit. Rev. Food Sci. Nutr.,* 58: 463-474.

Ashraf, M. and Q. Ali. 2010. Response of two genetically diverse wheat cultivars to salt stress at different growth stages:leaf lipid peroxidation and phenolic contents. *Pak. J. Bot*., 42.1: 559-565.

Casler, M.D., S.L. Fales, D.J. Undersander and A.R. Mcelroy. 2001. Genetic progress from 40 years of orchardgrass breeding in North America measured under management-intensive rotational grazing. *Can. J. Plant Sci.,* 81(4): 713-721.

Cristiane, J.D. and D.A. Luciano. 2020. Short-term nitrate supply decreases fermentation and oxidative stress caused by waterlogging in soybean plants. *Environ. Exp. Bot.,* 176: 104078.

Goyal, K., K. Kaur and G. Kaur. 2020. Foliar treatment of potassium nitrate modulates the fermentative and sucrose metabolizing pathways in contrasting maize genotypes under water logging stress. *Physiol. Mol. Biol. Plants.,* 26(5): 899-906.

Ito, O., E. Ella and N. Kawano. 1999. Physiological basis of submergence tolerance in rainfed lowland rice ecosystem. *Field Crop Res.,* 64(1-2): 75-90.

Justin, S.H.F.W. and W. Armstrong. 2010. Evidence for the involvement of ethane in aerenchyma formation in adventitious roots of rice (*Oryza sativa* L.). *New Phytol.,* 118(1): 49-62.

Khezrian, S., A.P. Salati, N. Agh and H. Pasha-Zanoosi. 2020. Effect of replacement of fish oil with different plant oils in Oncorhynchus mykiss broodstocks diets on egg and larval antioxidant defense development. *Vet. Res. Forum.,* 11(1): 83-88.

Kozlowski, T.T. 1997. Responses of woody plants to flooding and salinity. *Flood. Plant Growth.,* 1(7): 129-163.

Kumawat, G., J.P. Shahi and M. Kumar. 2020. Assessment of genetic diversity of *maize* (*Zea mays* L.) hybrids under water logging condition. *Elect. J. Plant Breed*., 11(1): 252-258.

Liu, C., Z. Yang and Y.G. Hu. 2015. Drought resistance of wheat alien chromosome addition lines evaluated by membership function value based on multiple traits and drought resistance index of grain yield. *Field Crop Res.,* 179: 103-112.

Malavasi, U.C., A.S. Davis and M.D.M. Malavasi. 2016. Estimation water in living woody stems - a review. *Cerne,* 22(4): 415-422.

Mano, Y., F. Omori, T. Takamizo, B. Kindiger, R.M. Bird and C.H. Loaisiga. 2006. Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. *Plant Soil,* 281(1-2): 269-279.

Pan, L. and L. Xue. 2012. Plant physiological mechanisms in adapting to waterlogging stress: a review. *Chin. J. Ecol.,* 31(10): 2662-2672.

Peng, Y., X. Zhang, Y. Deng and X. Ma. 2008. Evaluation of genetic diversity in wild orchardgrass (*Dactylis glomerata* L.) based on AFLP markers. *Hereditas,* 145(4): 174-181.

Sairam, R.K., D. Kumutha, K. Ezhilmathi, P.S. Deshmukh and G.C. Srivastava. 2008. Physiology and biochemistry of waterlogging tolerance in plants. *Biol. Plant.,* 52(3): 401-412.

Setter, T.L. and I. Waters. 2003. Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant Soil,* 253(1): 1-34.

Shah, K. and S. Nahakpam. 2012. Heat exposure alters the expression of sod, pod, apx and cat isozymes and mitigates low cadmium toxicity in seedlings of sensitive and tolerant rice cultivars. *Plant Physiol. Biochem.,* 57: 106-113.

Shuai, S., X. Zhang and D.U. Yi. 1997. Study on genetic characters in diploid and tetraploid of *Dactylis glomerata*. *Acta Agrestia Sin.,* 5: 261-267.

Siddiqi, E. and M. Ashraf. 2008. Can leaf water relation parameters be used as selection criteria for salt tolerance in safflower (*Carthamus tinctorius* L.). *Pak. J. Bot*., 40(1), 221-228.

Takahashi, H., T. Yamauchi, T.D. Colmer and M. Nakazono. 2014. Aerenchyma formation in plants. *Plant Cell Monogr.*, 21: 247-265.

Takehisa, H., Y. Sato, M. Igarashi, T. Abik, B.A. Antonio and K. Kamatsuki. 2015. Genome-wide transcriptome dissection of the rice root system: implications for developmental and physiological functions. *Plant J*., 69(1): 126-140.

Wang, X., M. Huang, Q. Zhou, J. Cai, T. Dai and W. Cao. 2016. Physiological and proteomic mechanisms of waterlogging priming improves tolerance to waterlogging stress in wheat. *Environ. Exp. Bot.*, 132: 175-182.

Yamauchi, T., S. Shimamura, M. Nakazono and T. Mochizuki. 2013. Aerenchyma formation in crop species: a review. *Field Crop Res.*, 152: 8-16.

Zeng, F., L. Shabala, M. Zhou, G. Zhang and S. Shabala. 2013. Barley responses to combined waterlogging and salinity stress: separating effects of oxygen deprivation and elemental toxicity. *Front. Plant Sci*., 4: 313-315.

Zengin, G., S. Ibrahime, G. Ak, F. Mahomoodally, M. Paksoy, C. Picot, J. Glamoclija, M. Soković, J. Jekő, Z. Cziáky, Rodrigues, J. Maria, C. Guerreiro Pereira and L. Custódio. 2020. Chemical profile, antioxidant, antimicrobial, enzyme inhibitory, and cytotoxicity of seven Apiaceae species from Turkey: A comparative study. *Ind. Crops Prod.*, 153.

Zhu, M., M. Wang, F.H. Li and Z.S. Shi. 2015. Physiological and photosynthesis response of popcorn inbred seedlings to waterlogging stress. *Pak. J. Bot*., 47(6): 2069-2075.

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