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Physiological Responses of Cotton at Seedling Stage to Waterlogged Stress

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Abstract: In Jianghan plain as well as south China, cotton at seedling stage often encounter waterlogged stress, by which normal growth of cotton plants is affected, the purpose of the study is to analyze the responses to Waterlogging stress. Therefore flooding experiments of cotton in the seedling stage was made and a series of physiological indices were observed such as Chlorophyll Relative value (R_C), chlorophyll fluorescence (F_0, F_m), Malondialdehyde (MDA), nomadic Proline (Pro), Dissoluble Sugar (DS), Peroxidase (POD) and Superoxide Dismutase (SOD), Analytic results indicated that, after Waterlogging, chlorophyll hydrolysis rate was higher in the first 3 days, the peak value of chlorophyll fluorescence decrease occurred between the 6th to 9th day. It figured that stagnant water on the field should be drained off in 3 days after Waterlogging stress, to avoid the photosynthetic efficiency being strongly inhibited. The balance of normal physiological metabolic process in cotton leaf was broken after Waterlogging, some new negative changes occurred, as MDA content increasing and the activity of SOD decline. Some other positive changes were accompanied, as the increasing of DS and Pro content and the activity of POD, for protecting active tissues. These physiological indices appeared regularly changing characterized by fast-slow-fast, which can be simulated in unary cubic regression curve model.

Keywords: Cotton, leaf, physiological metabolism, waterlogged stress

INTRODUCTION

Jianghan plain was a main producing area for cotton in china. Generally, the beginning of raising seedling and transplanting stage were early April and early May. The Seedling stage after transplant lasted 40 to 50 days, in which normally was the beginning of plum rain season in the middle and lower reaches of Yangtze River. Nevertheless, seedling stage required the least of water requirement in cotton field, 55 to 65% of field moisture capacity was useful for seedling growth. When heavy rain happened, stagnant water on the field should be drained off in time, otherwise, Waterlogging stress should be occurred. The direct response to Waterlogging stress was root oxygen deficit, which caused a great quantity of reducible by-products which was bad for root gravitropic growth. The aboveground part damage of plant was mainly due to root oxygen deficit, including enzyme protection system activity weaken and membrane lipid peroxidation accelerating (Liu *et al*., 1995; Yan *et al*., 1996; Kumutha *et al*., 2009). In Waterlogging stress, Catalase (CAT) and Superoxide Dismutase (SOD) activity inside of leaf cell membrane significantly changed. The study on soybean (Glycine max) revealed that, the activity of these antioxidase changed in 4th-6th day of flooding process, which probably caused by NADPH oxidase activity decrease (Sairam *et al*., 2009; Zhang *et al*., 2009; Liu *et al*., 2011). Generally, the content of MDA and Pro were tending to increase after Waterlogging and regularity changed as time went on. Presently, the earlier researches on water stress of dry crops focused on drought stress. However, in Jianghan plain, Waterlogging disaster was more common and influenced more widely. In this research, pot experiment was carried to reveal cotton physiological metabolic processes after Waterlogging stress in seedling stage, which should be used for evaluating the losses from disaster and formulating post-disaster production program.

MATERIALS AND METHODS

Materials: Pot experiment was carried in the agro-ecological station of Agriculture College of Yangtze University, at 30°21′N and 112°09′E, where is located at the middle and lower reaches of Yangtze River, an agro-climatic region of monsoon in the east of northern subtropics, with 16.5°C of annual average temperature, 5094.9-5204.3°C·d of more than 10°C accumulative temperature, 1095 mm of annual average precipitation and 1718 h of annual average insulation duration. Cotton variety for the experiment was Eza No.15 and the seedlings were fostered by nutritive bowls in April 18, 2011, transplanted in May 5. Before transplanting, soil from farmland that was filled in the pots was

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Table 1: Comparison of chlorophyll relative value and chlorophyll fluorescence in cotton leaf

T_W (days)	R_C Waterlogging	R_C CK	F_0 Waterlogging	F_0 CK	F_V/F_m Waterlogging	F_V/F_m CK	F_V/F_0 Waterlogging	F_V/F_0 CK
0	41.5±0.42aA	41.3±0.64aAB	152±11.5cC	155±9.5aA	0.779±0.009aA	0.784±0.006aA	3.525±0.186aA	3.634±0.132aA
3	38.9±0.56bB	40.7±1.25abAB	159±1.5cBC	156±4.5aA	0.758±0.007bA	0.780±0.01aA	3.138±0.127bB	3.550±0.198aA
6	37.3±0.5cC	42.0±0.49aA	171±3.5bB	156±6.7aA	0.706±0.011cB	0.782±0.006aA	2.409±0.127cC	3.594±0.126aA
9	36.0±0.42dCD	41.5±0.89aAB	194±6.4aA	153±6.1aA	0.653±0.016dC	0.776±0.005aA	1.888±0.137dD	3.473±0.096aA
12	35.4±0.95dD	39.4±0.57bB	206±6.2aA	149±8.1aA	0.608±0.012eD	0.776±0.003aA	1.550±0.074eE	3.456±0.059aA

[a]: Within columns, values followed by the same letter do not differ significantly, small letter means p = 0.05, capital letter means p = 0.01; [b]: FW means observed value in conditions of fresh, the same below

completely mixed and added to 10 g of compound fertilizer (N:P:K = 18:12:10), the soil weight of each pot was approximate 21 kg. Waterlogging stress started on 11 June 2011, keeping the water depth at 1/2 plant height during the flooding process. Four conditions of Waterlogging time as 3, 6, 9, 12 days were set, 6 times each, leaving 20 pots as control group.

Analysis methods: Testing pots were moved out on schedule, a series of physiological indices in functional leaf (top fourth leaf), such as chlorophyll Relative Value (R_C), chlorophyll fluorescence (F_0, F_m) be observed immediately and the top second leaf was cut off to determine its Chlorophyll content (Chl), Malondialdehyde (MDA), nomadic Proline (Pro), Dissoluble Sugar (DS), Peroxidase (POD) and Superoxide Dismutase (SOD) in conditions of fresh in the lab. The measurements were: RC-SPAD-502 chlorophyll tester; chlorophyll fluorescence-OS-30P plant stress analyzer; Chl-ethanol colorimetric method; MDA, DS-TBA coloration method, Pro-ninhydrin coloration method, SOD-NBT illumination method, POD-guaiacol oxidation method (Li, 2000; Zou, 2000).

RESULTS AND DISCUSSION

Response of leaf chlorophyll content and chlorophyll fluorescence activity: In light of Table 1, it is obvious that the most immediate response was leaf yellowing, which was caused by chloroplast pigment constitute changing. R_C reflected chlorophyll content in cotton leaf, which was positively correlated with absolute concentration. Chlorophyll fluorescence parameters were the comprehensive reflection of chlorophyll activity that had close ties with stress level. Initial fluorescence, which also means 0-level fluorescence, was related to chlorophyll content. F_v/F_m was maximum photochemistry efficiency of PSⅡ, reflecting primary light energy conversion efficiency. F_v/F_0 represented PSⅡ light energy transmission capacity (Su *et al*., 2002). Table 2 was the observations of the parameters above. It indicated that R_C, F_V/F_m and F_V/F_0 were negatively correlated with T_W in each and the difference. The difference of neighboring treatment was significant ($p<0.05$), if interval time was 6 days or more, the difference could be extremely significant ($p<0.01$). F0 was positively correlated with T_W (a period of time that crop suffer from Waterlogging, in day).

Table 2: Change rate of chlorophyll relative value and chlorophyll fluorescence each 3 days

Period (days)	R_C	F_0	F_V/F_m	F_V/F_0
0→3	-6.340	4.160	-2.640	-10.97
3→6	-4.030	7.770	-6.880	-23.24
6→9	-3.480	13.65	-7.480	-21.62
9→12	-1.76 0	6.000	-6.990	-17.92
0→12	-15.61	31.58	-23.99	-73.75

The change rate (Rg) of neighboring observations was calculated according to formula 1 and Calculating result was showed in Table 2:

$$Rg(\%) = \frac{Vi - Vi-3}{Vi-3} \times 100 \quad (1)$$

In which, V_i represented the observations of day i, i = 3, 6, 9, 12.

It was indicated that, in 12 days after Waterlogging, R_C declined from fast to slow, the increasing/decreasing rate of F_0, F_V/F_0 and F_V/F_m reached a maximum in the 9th, 9th, 6th day after Waterlogging, respectively and then dropped down. In general, during the cotton seedling Waterlogging progress, R_C and chlorophyll fluorescence had different response sensitivities (the time of reaching the peak value), they were: $R_C>F_V/F_0>F_V/F_m$, F_0. The 12 days accumulating change amounts were: $F_V/F_0>F_0>F_V/F_m>R_C$.

Response of leaf metabolic byproduct: Under waterlogged stress, metabolic byproduct content in leaf cell membrane such as MDA, DS and Pro was maintaining a high level. Determining results in Table 3 showed that, MDA content was significantly increased compared with the control by 195% at maximum (the 12th day after Waterlogging); DS content continuously increased ($p<0.05$); Pro content was also sharply increased after Waterlogging, in the 3rd day, its content was 120% higher than the control and then carried on rising to 4 times greater than CK in the 12th day.

Response of leaf protective enzyme activity: Various kinds of enzyme as SOD, POD and CAT composed a

Table 3: Change rate of chlorophyll relative value and chlorophyll fluorescence each 3 days

T_W (days)	MDA (μmol•g^{-1} FW)		Pro (μg•g^{-1} FW)		DS (mmol•g^{-1} FW)	
	Waterlogging	CK	Waterlogging	CK	Waterlogging	CK
0	0.20±0.014dC	0.22±0.008bB	9.65±1.130dD	10.10±0.328aA	0.54±0.021cC	0.51±0.022bB
3	0.31±0.033cC	0.22±0.004bB	21.27±0.287cC	9.70±0.471aA	0.65±0.020cC	0.41±0.022cC
6	0.43±0.041bB	0.21±0.017bB	27.29±0.564bBC	11.45±0.666aA	0.92±0.010cB	0.54±0.055bAB
9	0.43±0.028bB	0.26±0.011aA	29.12±1.590bB	11.27±1.731aA	1.01±0.050bAB	0.61±0.007aA
12	0.59±0.032aA	0.25±0.011aA	54.97±5.907aA	10.70±1.597aA	1.17±0.043aA	0.55±0.009bAB

[a]: FW means observed value in conditions of fresh, the same below

Table 4: Comparison of the activity of protective enzyme in cotton leaf

T_W (days)	POD (⊿A•min^{-1}•g^{-1} FW)		SOD (U•g^{-1} FW)	
	Waterlogging	CK	Waterlogging	CK
0	26.9±4.72dC	25.7±0.47aA	150.9±5.56aA	153.9±3.23abAB
3	36.1±4.78cdBC	25.1±4.77aA	137.3±4.53bB	148.3±2.96bB
6	45.8±1.91bcB	26.3±0.95aA	96.9±4.12cC	150.4±1.60bAB
9	48.7±4.35bB	27.1±0.97aA	97.4±5.66cC	158.5±4.36aA
12	67.6±1.65aA	29.1±3.04aA	76.5±3.07dD	156.7±1.52aAB

Table 5: Regression effect between physiological indices and flooding duration (T_W, day)

Regression equation	R^2	F
$Y_{MDA} = 0.002T_W^3 - 0.038T_W^2 + 0.271T_W - 0.205$	0.927	33.886**
$Y_{Pro} = 0.174T_W^3 - 3.369T_W^2 + 21.354T_W - 17.171$	0.964	70.649**
$Y_{SOD} = -0.384T_W^3 + 9.183T_W - 71.898T_W + 280.73$	0.974	98.599**
$Y_{POD} = 0.142T_W^3 - 2.939T_W^2 + 20.75T_W - 3.533$	0.827	12.717**

[a]: $F_{0.01} = 7.59$; **: $p<0.01$; Y_i: Observed value of each parameter

protection system to active oxygen infection in active tissues. But the balanceable state would be destroyed in an adverse condition because the ability of elimination of active oxygen would weaken, some enzymes were over-expressed and other ones were inhibited. In Table 4, leaf POD activity was promoted, as well as SOD activity was continuously decreased. Multiple comparison showed that the difference of POD activity between 3, 6, 9 days flooding treatment and 12 days flooding treatment was extremely significant ($p<0.01$), but the difference among the 3, 6, 9 days flooding treatments are not significant. POD activity promotion was plant stress induced physiological response, which would be conductive to protecting active tissues. SOD activity was quickly decreased after Waterlogging, except for the 6 and 9 days flooding treatments, the differences were extremely significant ($p<0.01$).

Through deep analysis to the response regularity of MDA, Pro, SOD and POD change by Waterlogging period, it suggested that, their change rate had stable or dissension trend in medium term and then significantly changed again in the 12th day. That was to say that during the 12 days Waterlogging progress, the change rate of these 4 indexes had a fast-slow-fast law which could be simulated in unary cubic regression curve mode (Table 5).

SUMMARY

Conclusion: Seedling stage was the least water demanding stage of cotton, the demand of which accounted for 10% of total amounts. Too much or too little surface water was useless even harmful to crops. Waterlogging experiment indicated that, it was chlorophyll content drastically declined period in the first 3 days after Waterlogging in cotton leaf, then change rate slowed down, leaf etiolated; Chlorophyll fluorescence activity decrease appeared in the 6th-9th day, photosynthetic efficiency decreased. As a matter of fact, stagnant water on the field should be drained off in 3 days after Waterlogging stress, to avoid the Photosynthetic efficiency being strongly inhibited.

During the waterlogging stress progress, the balance of radical producing and eliminating in leaf cell membrane were destroyed, some positive and negative change occurred. Observing result showed that the negative change included MDA content increasing and SOD activity decline, some protective change included DS, Pro content increasing and POD activity promoting. The change rate of them had a fast-slow-fast law.

Discussion: Leaf PSⅡ maximum photochemical efficiency (F_v/F_m) could reflect its primary light conversion efficiency of chlorophyll. Normally, this parameter was very steady, maintaining at the amount of 0.75-0.85 (He *et al*., 2005). This research indicated that in the 3rd day of Waterlogging, F_V/F_m was 0.758, closing to normal level; by day 6, the value was lower, which meant the photosynthetic capacity of PSⅡ system was starting to fall. The same was F_0 and F_V/F_0. Obviously, at the earlier stage, chlorophyll hydrolyzed in quantity, but photosynthetic capacity was basically normal; if stress was continue, photosynthesis would be

grievous injured. In addition, F_V/F_0 activity had been extremely reduced in the 3rd day ($p<0.01$), it indicated that the negative effect to photosynthesis was firstly performed at photic-energy transferring.

The influence to leaf cell membrane of Waterlogging mainly was destroying the existing balance and rebuilding a new one. This research indicated that, except for DS content, which was increased progressively, MDA, Pro content and SOD, POD activity change had a common law with fast-low-fast. So it can be projected that, the significant change in the 3rd day was an irritability response of leaf, the relative steady in the 6th to 9th day was an adaptable response and the obvious change of day 12 was caused by plant tolerance decline. That was to say, the 3rd, 9th and 12th day of Waterlogging was the 3 crucial timings of cotton seedling physiological metabolism ability. In the field management, reduction of output could be completely avoided if surface water be drained off in the 3rd day of Waterlogging; if it was done in 6th or 9th day and effective remedies were coming up, output lost could be reduced; when flooding period went on for 12 days or more, recovery difficulty would be extremely hard.

By researching on dynamic responses of main physiological indices to Waterlogging stress, it was very conductive to providing available agronomic method of reducing the negative effect of Waterlogging disaster. What's more, it was extraordinary instructive for cotton thoroughbred breeding and seedling cultivation. Leaf MDA accumulation and the difference in active oxygen scavenge system mainly reflected the Waterlogging-enduring strength of cotton breeds. Some research showed that, after hypoxia preconditioning at seedling stage, hypoxic endurance of cotton increased significantly (Williams *et al*., 2000), which showed a decline of membrane lipid peroxide production and an enhancement of the ability to eliminate active oxygen in physiologically.

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