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Respiratory Activities are Affected by Water Stress in Cotton Seedling Roots

Saghir Ahmed Sheikh, Mohammad Ibrahim Keerio and Riasat Ali Kubar
Sindh Agriculture University, Tando Jam, 70060, Sindh, Pakistan

Abstract: The result suggested that the fresh weights of the root tips decrease with increasing germination time at 25°C. The respiration rate decreases with increasing germination time and the pattern was very similarly in both quartiles. Waterlogging treatment showed large reductions on root growth and their fresh and dry weights. Waterlogging treatment of 12 hours or more caused greater reductions in respiratory activities.

Keywords: Cotton, Respiration, Roots, Waterlogging,Fresh weight

Introduction

Respiration is the process of gaseous exchange associated with the oxidative catabolism of organic substrates. This produces carbon skeletons for biosynthetic purposes and energy for ion transport and assimilation, growth and cellular maintenance processes. Rumpho and Kennedy (1981) stated that higher plants have an absolute requirement for oxygen for the maintenance of metabolism and growth. Under normal conditions in well-drained soils, the aerobic respiration of plant roots and soil microorganisms requires typically 5 and 24g oxygen for each square metre of land surface per day during the growing season (Russel, 1973). During flooding, the soil pore space is totally water filled and gaseous exchange between soil and atmosphere is virtually eliminated. Due to the low diffusivity in water, the oxygenated zone at the soil surface may extend to a depth of only a few millimeters. Depending upon the soil temperature and tissue respiration rates, therefore, the dissolved oxygen in the soil water will be exhausted in only hours or days and the soil will become anaerobic. Waterlogging creates an anaerobic environment and this in turn brings changes in metabolism from aerobic to anaerobic pathways. This results in reduced energy yield, accumulation of toxic compounds and rapid depletion of organic substrates (Ueda and Tsuji, 1971; Gupta, 1977). The adverse effects of root anaerobiosis in mesophyte plants can be primarily attributed to the deficiency of molecular oxygen which prevents normal aerobic metabolism. Lee and Lee (1991) and Del-Rosario and Fajardo (1991) reported that waterlogging reduces the respiration rate of apple, soybean, mungbean, tomatoes, cotton, pumpkins and rice seedling roots.

Materials and Methods

The experiment was undertaken at the School of Biological Sciences, University of Wales, Bangor, U.K. The seeds of cultivar Qalandari supplied by the Cotton Research Institute, Sakrand, (Sind), Pakistan was used. **Seed treatment:** The seeds were placed in a 200 cm^3 beaker and approximately 4 cm^3 of concentrated sulphuric acid were added. The seeds and acids were stirred thoroughly with a glass-rod for 3 minutes and left for 5 minutes. They were then stirred for 2 minutes and transferred to a Buchner funnel where they were washed with running tap water for 3 minutes. The seeds were then placed in 200 cm^3 1% sodium bicarbonate solution for 10 minutes to neutralise remaining acid and washed thoroughly with distilled water. The delinted seeds were soaked for an hour in 1 litre of distilled water. After this time, the floaters were discarded and the sinkers were placed in a 1.5% sodium hypochlorite solution for 20 minutes to further sterilize them. Finally, the seeds were washed eight times with sterile distilled water to remove the hypochlorite. Germination and selection of quartiles: Fifty delinted and sterilized seeds were placed in 10 rows with 5 seeds in each row, on a sheet of Whatman No. 1 filter paper. The filter paper was moistened with 40 cm^3 sterile distilled water. The filter paper was then rolled up round a wooden stick and placed in a polythene bag. The top of the bag was folded over and tied lightly to maintain sterile conditions and allow air exchange. Finally, the bag and its contents were stood upright in a 500 cm^3 beaker and incubated in an incubator in the dark at 25°C. After 24 and 48 hours, the bag and its contents were rolled to re-moisten the seeds with water that had collected at the bottom of the bag. For each analysis, 100 seeds were germinated in this way in two filter paper rolls. After 48, 54, 60, 66 and 72 hour germination periods, the seeds were harvested and the best 75 seedlings were selected. The 75 seedlings were divided into three quartiles according to their root lengths as follows:

i. First quartile: the longest 25 roots
ii Second quartile: the medium 25 roots
iii Third quartile: the shortest 25 roots

Waterlogging Treatments: Cotton seeds were germinated in rolled filter papers for 48 hours as described above. The rolled filter papers along with their contents were taken out from the plastic bags and placed in glass jars filled with water up to 26 cm. This was enough to completely cover the rolled paper and its seedlings. All these treatments were carried out under controlled conditions at 25°C. The jar was covered with a glass lid and a piece of cling film was wrapped over it to prevent air exchange between the inside of the jar and the outside atmosphere. The jar and its contents were then incubated at 25°C for 6, 12 or 24 hours. The oxygen concentration of the water in the jar was monitored before and after each waterlogging treatments. After this waterlogging treatment, the paper roll containing the seedlings was drained and returned to the plastic bag and incubated at 25°C for a period of recovery. The recovery period was adjusted so that the total experimental period was 72, 96 or 120 hours for each waterlogging treatment. At the end of these times the roots were harvested and

the root length and fresh weight were made.The oxygen concentration of the water in the Waterlogging treatments was measured using an oxygen electrode (Model PHOX62). The instrument was calibrated with distilled water saturated with 100% oxygen at 25°C. The sensor of the oxygen meter was immersed in the water and rotated to ensure adequate water flow past the sensor membrane. The oxygen concentration was measured in units of mgl^{-1}. The respiration rate of the root tip segments was measured with a Clark-type oxygen electrode connected to a chart recorder (Kipp & Zonen, Model DB-111). The water jacket of the electrode was connected to a temperature controlled water bath to maintain it at 25°C. Five approximately 5mm long tips were used for each measurement. Two cm^3 buffer were placed in the sample compartment of the electrode and the tissue was added. The sample compartment was then closed and oxygen uptake was measured over a 20 minute period. The results are presented in units of nmoles min^{-1}.

Statistical Analysis: All means, standard deviation and standard error values were calculated using a pocket scientific calculator. The statistical analysis was done using a personal computer with the Systat/Sygraph software. A probability value of 0.05 or less was considered to be significant and any value above that was considered to be insignificant.

Results and Discussion

The results are shown in Table 1. In seeds germinated at 25°C (control), root tip fresh weight and dry weight decreased with increasing germination time. In each case, the curves followed a similar pattern. The respiratory activities of the tips also followed a similar decreasing course. Moreover, the general patterns were qualitatively and quantitatively very similar for both quartiles. For example, in the first quartile, the root tip fresh weight decreased from 24.6 mg at 24 hour to 8.7 mg at 120 hours, while in the third quartile the fresh weight decreased from 25.6 to 9.1 mg over a similar period. The changes were highly significant statistically. The rate of respiration in the first quartile also significantly decreased from 17.6 nmoles min^{-1} at 24 hours to 4.0 nmoles min^{-1} at 120 hours, while in the third quartile the rate decreased from 16.8 to 3.6 nmoles min^{-1}. Seedlings that had been waterlogged for 3 to 24 hours showed no significant changes in fresh weight in both quartiles during the waterlogging treatments. During recovery following 6 hour waterlogging treatment, the fresh weight was also not affected and even showed small but insignificant increases in both quartiles at 120 hour compared with the 120 hour controls. At the end of the recovery period following 12 hour treatment, the fresh weight was reduced from 8.7 to 6.9 mg in the first quartile and from 9.1 to 6.1 mg in the third quartile. These reductions were statistically insignificant however. During recovery following 24 hour waterlogging there were similar decreases. In this case, the fresh weight at 120 hour was reduced significantly in the third quartile compared with its 120 hour control. The dry weight patterns following waterlogging were very similar to those seen for fresh weights. In this case, seedlings waterlogged for 6 hours also showed small increases during the recovery period. Waterlogging for 12 hours had no significant effect on both quartiles. At the end of the recovery following 24 hour waterlogging treatment, the dry weight was significantly decreased from 0.60 to 0.37 mg in the first quartile and from 0.77 to 0.40 mg in the third quartile. Waterlogging for 3 hours sharply reduced the respiration rates in both quartiles. The rate at the end of the waterlogging treatment was reduced from 11.6 to 5.6 nmoles min^{-1} in the first quartile and from 11.4 to 5.6 nmoles min^{-1} in the third quartile. The 6 hour waterlogging treatment caused similar reductions; in this case, they were reduced to 5.0 nmoles min^{-1} in the first quartile and to 4.8 nmoles min^{-1} in the third quartile. All of these reductions were highly significant. During recovery following the 6 hour waterlogging, the root tips rapidly recovered their respiratory activities. At 120 hours, they showed similar respiration rates in both quartiles compared with their controls. Seedlings waterlogged for 12 hours also showed large reductions in root tips respiration rates in both quartiles. These reductions were greater than for the 6 hour treatment, down to 1.6 nmoles min^{-1} in the first quartile and 1.4 nmoles min^{-1} in the third quartile. During recovery, the tips rapidly recovered their respiratory activities within 12 hours. At 120 hour, the rates for both quartiles were up to the control levels and there were no significant differences between them. The 24 hour waterlogging treatment caused even greater reductions in the respiration. It decreased to 1.0 nmoles min^{-1} in the first quartile and to 0.8 nmoles min^{-1} in the third quartile. During the first 24 hours of recovery, respiration recovered to 3.6 and 3.4 nmoles min^{-1}, respectively, in the first and third quartiles. The respiration rate then fell off again, however. At 120 hours, it was reduced by 45 and 39%, respectively, in the first and third quartiles compared with the controls. The results suggests that the fresh and dry weights of the root tips decrease with increasing germination time at 25°C. The decreases in fresh and dry weights were mainly a consequence of the growth of the roots; as roots grow longer they become thinner. Immediately following waterlogging treatments, the root tips showed no apparent changes in fresh or dry weights. This suggests that waterlogging does not cause extensive physical damage to the tips. During recovery following 6 hour waterlogging, the fresh and dry weights were also not affected. Waterlogging treatments of 12 hour or more caused damage, however. These treatments even caused weight reductions during the recovery periods. That is, there was actually a loss of tissue material. Meek and Stolzy (1978) reported that the root tip is more sensitive than the other parts of the root to low oxygen levels. Anaya (1972) found that root elongation in wheat was 25, 32 and 36 centimetres for oxygen levels of 2, 6 and 10%, respectively, after 21 days. Guyot and Prioul (1985); Orchard *et al.* (1985) working on sorghum, sunflower and wheat reported that waterlogging reduces the dry weight accumulation. This was mainly due to damage to root tips and even cell death leading to a loss of turgor and shrivelling of the tissue. In the present study, the fresh and dry weights suggest that short term flooding did not affect the fresh and dry weights while 12 hours and more significantly damage the apical regions of roots. Meek and Stolzy (1978) reported that roots grown in poor aeration are shorter and thicker than those grown with sufficient aeration. In the case of respiration rate, the present results show that the rate of respiration decreases with increasing germination time and the pattern was very similar in the first and the third quartile. This presumably reflects the reducing tissue mass in the tip section. Hanson *et al.* (1959) have reported that, during germination of corn seeds (Zea mays), the respiratory activity of the scutellum increases for 3-4 days, then declines. Opik (1965) also stated that oxygen uptake by the intact cotyledons of *Phaselous vulgaris* rises to a peak between the third and fifth days of germination. A vegetative tissue

Table 1: Effects of Waterlogging on Fresh Weights and Respiration Rates.

Treatment (h)	Total Germ. Period (h)	Fr. Wt. (FQ)	Fr. Wt. (TQ)	Resp.Rate. (FQ)	Resp.Rate. (TQ)
		Mean ± se.	Mean ± se.	Mean ± se.	Mean ± se.
Control	24	24.6 ± 2.0	25.6 ± 0.3	17.6 ± 1.0	16.8 ± 0.1
	36	16.0 ± 0.2	20.2 ± 0.1	13.2 ± 0.6	13.2 ± 0.1
	48	12.7 ± 0.8	13.7 ± 0.8	11.6 ± 0.4	11.4 ± 0.6
	51	12.7 ± 0.7	14.0 ± 0.3	11.0 ± 0.4	10.6 ± 0.8
	54	11.9 ± 0.4	13.5 ± 0.6	10.8 ± 0.6	9.4 ± 0.4
	60	11.9 ± 0.3	11.9 ± 0.4	8.8 ± 0.8	7.4 ± 0.8
	72	10.6 ± 0.2	10.6 ± 0.1	7.6 ± 0.8	6.6 ± 0.8
	96	10.1 ± 0.6	10.2 ± 0.1	5.4 ± 04	5.2 ± 0.2
	120	8.7 ± 0.9	9.1 ± 0.4	4.0 ± 0.4	3.6 ± 0.1
3h	51	12.5 ± 0.4	13.3 ± 0.5	5.6 ± 0.4	5.6 ± 1.0
6h	54	12.1 ± 0.4	12.5 ± 0.5	5.0 ± 0.4	4.8 ± 0.2
	72	12.7 ± 0.5	12.1 ± 0.5	6.8 ± 1.0	5.8 ± 0.8
	96	8.9 ± 0.9	11.1 ± 0.1	4.8 ± 0.8	4.2 ± 0.6
	120	11.0 ±0.3	10.3 ± 0.7	4.6 ± 0.4	3.8 ± 0.2
12h	60	11.2 ± 0.4	12.3 ± 0.7	1.6 ± 0.1	1.4 ± 0.1
	72	12.0 ± 0.1	13.3 ± 0.5	4.6 ± 0.8	3.8 ± 0.4
	96	6.3 ± 0.8	7.5 ± 0.5	5.2 ± 0.8	4.6 ± 0.8
	120	6.9 ± 1.0	6.1 ± 0.9	4.8 ± 0.4	3.8 ± 0.4
24h	72	9.4 ± 0.6	10.8 ± 0.8	1.01± 0.1	0.8 ± 0.1
	96	9.2 ± 0.6	9.4 ± -0.1	3.6 ± 0.4	3.4 ± 0.6
	120	6.1 ± 0.5	3.9 ± 0.4	2.2 ± 0.4	2.2 ± 0.1

All values of fresh weight are mean (mg 5 tips) $^{-1}$) ±se form five experiments.
All values of respiration rate are mean (nmoles min $^{-1}$ (5 tips) $^{-1}$) ±se form five experiments.

NOTE:-
FQ = First Quartile
TQ = Third Quartile

such as root might have been expected to continue respiration at a high level. Waterlogging caused great reductions in the respiration rates in both cultivars. This was presumably due to the reduction in oxygen concentration during the waterlogging treatments. This in turn might have converted aerobic into anaerobic respiration. Crawford (1978) has pointed out that waterlogging causes a deficiency of oxygen that shifts respiratory metabolism from aerobic to anaerobic pathways. Crawford (1978) found that, in flood intolerant species, a reduction in soil aeration causes a rapid increase in glycolysis as well as a dramatic induction of alcohol dehydrogenase activity. It was also noted in the present experiment that, although waterlogging treatment for 12 hour or more caused visual damage to the root tips, they could still respire. This suggests that mitochondria of cotton roots show great tolerance to waterlogging. Interestingly, Vartapetian *et al.* (1978) reported that the mitochondria of cotton roots maintained their ultrastructure longer under anoxia than those of rice (very tolerant to flooding).

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