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Effects of waterlogging on seed germination, electric conductivity of seed leakage and developments of hypocotyl and radicle in sudangrass

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Abstract. Sudangrass (*Sorghum sudanense* Stapf) is a short-period forage species adapted to growth in Taiwan. The objectives of the experiment were to determine the effects of waterlogging on seed germination, electric conductivity of seed leakage and developments of hypocotyl and radicle in sudangrass and provide information for selecting the lines with tolerance to waterlogging stress. Sudangrass, CV. Taishi No. 1, was used for the experiment. In the germination test, the seeds were waterlogged for 3 days at 0, 1, 2 and 3 days after imbibing. The seeds waterlogged at 0 day after imbibing had the lowest germination percentage. The potassium contents of the seed leakages were increased and contents of both total sugar and reducing sugar in the seed leakages were decreased with an increase in imbibing period. The relationship between germination percentage and electric conductivity was negatively correlated ($p<0.05$), indicating that the electric conductivity could be used to evaluate the germination ability of sudangrass. For the emergence test, the sudangrass seeds were waterlogged for 0, 1, 2, 3, 4, 5, 6 and 7 days after sowing. The emergence percentages of sudangrass were not significantly different among treatments, while the emergence rate indices and the corrected emergence rate indices decreased with the increase in waterlogging period. This indicated that the emergence ability of sudangrass was reduced by waterlogging after sowing. The hypocotyl or the radicle cortex of sudangrass with a low germination or emergence percentage was damaged during waterlogging. The results demonstrated that: (1) the germination percentage of sudangrass was seriously affected when seeds were waterlogged before imbibing, (2) the electric conductivity of the seed leakage could be used to evaluate the germination ability of sudangrass.

Keywords: Electric conductivity; Hypocotyl; Radicle; Seed germination; *Sorghum sudanense*; Waterlogging.

Introduction

Sudangrass (*Sorghum sudanense* Stapf) is a short period forage species with high forage yield, drought tolerance, tillering ability, and fast regrowth. It has high amounts of crude protein and low amounts of crude fiber and hydrocyanic acid. It is adapted to growth in tropic and subtropic areas. *Sorghum* spp. has been used for forage in countries with a developed animal industry for a long time (McDonald et al., 1968; Sotomayor-Rios and Torres-Cardona, 1984).

The germination in this species is very sensitive to waterlogging. Decreases in germination ability have been attributed to a shortage of oxygen due to waterlogging (Orchard and Jessop, 1984). Respiration and electron transport are inhibited and ATP formation is decreased during germination when oxygen is deficient (Al-Ani et al., 1985; Johnson et al., 1989; Tsai et al., 1997). When the formation of ATP is reduced, the oxidation-reduction state between cell membranes becomes unbalanced and membrane permeability is increased. Thus, the solute leakage is increased (Johnson et al., 1989), and electric conductivity is increased (Givelberg et al., 1984; Lott et al., 1991). The cell membrane deteriorates and the contents of carbohydrates, amino acids, organic acids and ions are increased in the leakage leading to an increase in electric conductivity when the seeds are waterlogged (Loomis and Smith, 1980; Mckersie and Stinson, 1980; Mukhear and Laidman, 1982; Simon and Raja-Harum, 1972). The seed vigor can be evaluated by the electric conductivity of the leakage (Agrawal, 1977; Yaklich and Abdul-Baki, 1975).

The root injury caused by waterlogging is due to oxygen stress in saturated soils (Drew, 1983). Drew et al. (1985) reported that many adventitious roots were formed when corn was under oxygen stress. In addition, air space development has been reported in the cortex of the root in several mesophytic species (Pezeshki, 1994). The objectives of this experiment were to determine the effects of waterlogging on seed germination, electric conductivity of seed leakage, and developments of hypocotyl and radicle in sudangrass. In addition, the results provide information necessary to develop screening tools for selecting the lines with tolerance to waterlogging stress, thus, improving field emergence and survival percentage of sudangrass during waterlogging.

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Materials and Methods

The seeds of sudangrass, CV. Taishi No. 1, were used for the germination test. Petri dishes with 9-cm diameter were used. A piece of sponge with the same diameter as the dish was used and saturated with distilled water. One filter paper was put on the top of the sponge. Then 50 seeds of sudangrass for each treatment were uniformly distributed on the filter paper. The seeds were waterlogged for 3 days at 0 (S_0), 1 (S_1), 2 (S_2) and 3 days (S_3) after imbibing, in the incubator at 25°C with an 8 h light period, respectively. Each Petri dish received 50 ml distilled water for waterlogging treatment. Another piece of sponge was put on top of the seeds to keep them in the water during the waterlogging period. A factorial design with 4 replications was used. The Petri dishes were put in the incubator at 25°C with an 8 h light period. The seeds were considered germinated when the radicle reached 2 mm. Germinated seeds were counted and germination percentages were calculated. Final germination percentages were arcsin-transformed prior to statistical analysis (Snedecor and Cochran, 1980).

Determination of Total Sugar in Seed Leakage

A 2 ml sample from the above-prepared solution was taken. To it were added 2 ml 4% H_2SO_4, and it was boiled for 15 min. Then, 4% NaOH was used to neutralize it, and 4 ml copper reagent was added and boiled for 10 min. To the cooled solution was added 4 ml ammonium paramolybdate reagent (prepared with 50 g ammonium paramolybdate, 42 ml 96% sulfuric acid, 6 g sodium hydrogen arsenate and distilled water to make 1000 ml) and distilled water to reach 100 ml. The final solution was used for determination of sugar content using a spectrophotometer at 560 nm.

Determination of Reducing Sugar in Seed Leakage

Two ml of above-prepared original solution was mixed with 2 ml copper reagent and boiled for 10 min. The cooled solution was added to 2 ml ammonium paramolybdate reagent, and distilled water was added to reach 100 ml. The final solution was used to determine reducing sugar content with a spectrophotometer at 560 nm.

Determination of Potassium Content in Seed Leakage

Potassium contents of the seed leakages from the above-mentioned original solution were determined by atomic absorption spectrophotometer (Hitachi E-6100) according to Thomas (1985).

Determination of Electric Conductivity of Seed Leakage

Another experiment was designed to determine the electric conductivity of seed leakage. Sudangrass seeds were imbibed for 0, 1, 2, 3, 4, 5 and 6 days before waterlogging for 6, 5, 4, 3, 2, 1 and 0 days, respectively. The seeds were incubated at 25°C with an 8 h light period. A completely randomized design (CRD) with 4 replications was used. The leakages were taken to determine electric conductivity with an electric conductive meter (Suntex, Model SC 170). The germination percentages for each treatment were calculated.

Pot Culture with Waterlogging Treatment

Plastic pots 100 cm wide, 70 cm long, and 70 cm high were filled with sandy loam soil to sow sudangrass. Fifty seeds of sudangrass were uniformly sown in two rows in the pot. The pots were waterlogged for 0 (CK), 1 (W_1), 2 (W_2), 3 (W_3), 4 (W_4), 5 (W_5), 6 (W_6) and 7 days (W_7) after sowing, respectively. Each treatment had four pots. The pot with no waterlogging treatment was used as CK. The emerged seeds were counted and marked by toothpicks once a day after sowing until emergence was terminated. The emergence rate index (ERI), as proposed by Hsu and Nelson (1986), was calculated as the summation of emergence percentage for each day divided by the total number of days after sowing. A corrected emergence rate index (CERI), as proposed by Hsu and Nelson (1986), was obtained by dividing ERI by the final emergence percentage and multiplying by 100. Emergence percentages, ERI and CERI, were used to evaluate emergence ability (Hsu and Nelson, 1986).

Paraffin-Cut Section and Anatomy Observation

The hypocotyls and the radicles of the above-mentioned treatments were taken and prepared to do a paraffin-cut section for anatomy observation following the procedures reported by Lin and Yeh (1996).

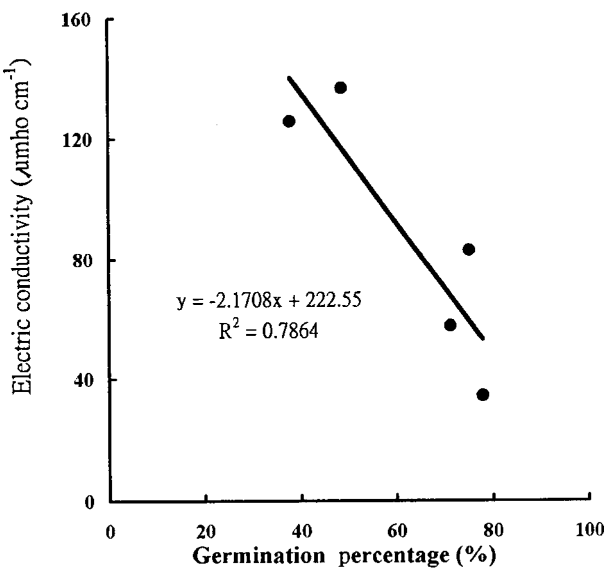

Figure 1. The relationship between germination percentage and electric conductivity of the leakage from sudangrass seeds.

Table 1. Effects of waterlogging at different imbibing stages of sudangrass seeds on germination percentage and leakage content.

Treatment	Germination percentage (%)	Leakage content (μg g^{-1})		
		Total sugar	Reducing sugar	Potassium
S_0[a]	12.7[b*]	985.6[a]	796.6[a]	10.4[b]
S_1	60.7[a]	859.4[ab]	595.1[ab]	24.7[b]
S_2	63.3[a]	692.9[b]	576.4[b]	39.4[b]
S_3	59.3[a]	658.0[b]	396.0[b]	75.2[a]

[a] S_0, S_1, S_2 and S_3 indicate the seeds waterlogged for 3 days at 0, 1, 2 and 3 days after imbibition, respectively.
* Means in each column followed by the same letter are not significantly different at 5% probability level.

Results and Discussion

The germination percentages of sudangrass were 12.7, 60.7, 63.3 and 59.3% for waterlogging 3 days at 0, 1, 2 and 3 days after imbibing, respectively (Table l). The seeds waterlogged before imbibing had the lowest germination percentage (So). The germination percentages of S_1, S_2 and S_3 were not significantly different. The results indicated that the germination ability was drastically reduced when the seeds were waterlogged before imbibing.

The contents of total sugar, reducing sugar and potassium were determined in the leakage as shown in Table l. The contents of total and reducing sugars were decreased and those of potassium were increased with the increase in imbibing period before waterlogging. Some reports also indicated that potassium and soluble carbohydrates were observed in the leakage (Givelberg et al., 1984; Lott et al., 1991).

The relationship between germination percentage and electric conductivity is shown in Figure 1. There was a negative correlation ($r = -0.88$, $p<0.05$). The seeds had the lowest germination percentage and the highest electric conductivity with waterlogging for the longest period.

Some reports also indicated that a negative correlation was observed between germination percentage and electric conductivity in soybean (Yaklich and Abdul-Baki, 1975), rice (Agrawal, 1977), and pea (Bradnock and Mattews, 1970; Perry, 1987). However, no such correlation was observed in melon (Pesis and Ng, 1983) or barley (Abdul-Baki and Anderson, 1970).

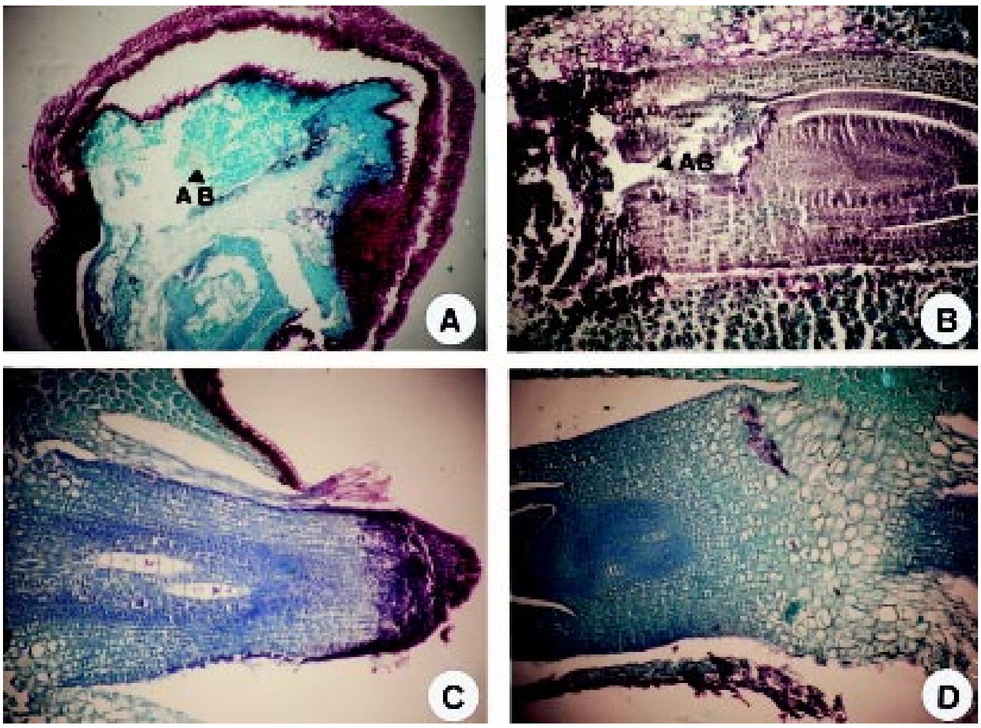

Figure 2. Development of sudangrass seedlings waterlogged for 3 days at 0-1 day after imbibing (×50). A, Abnormal seed waterlogged at 0 day after imbibing (S_0); B, Abnormal radicle waterlogged at 0 day after imbibing (S_0); C, Normal radicle waterlogged at 1 day after imbibing (S_1); D, Normal hypocotyl waterlogged at 1 day after imbibing (S_1). AB, Abortion.

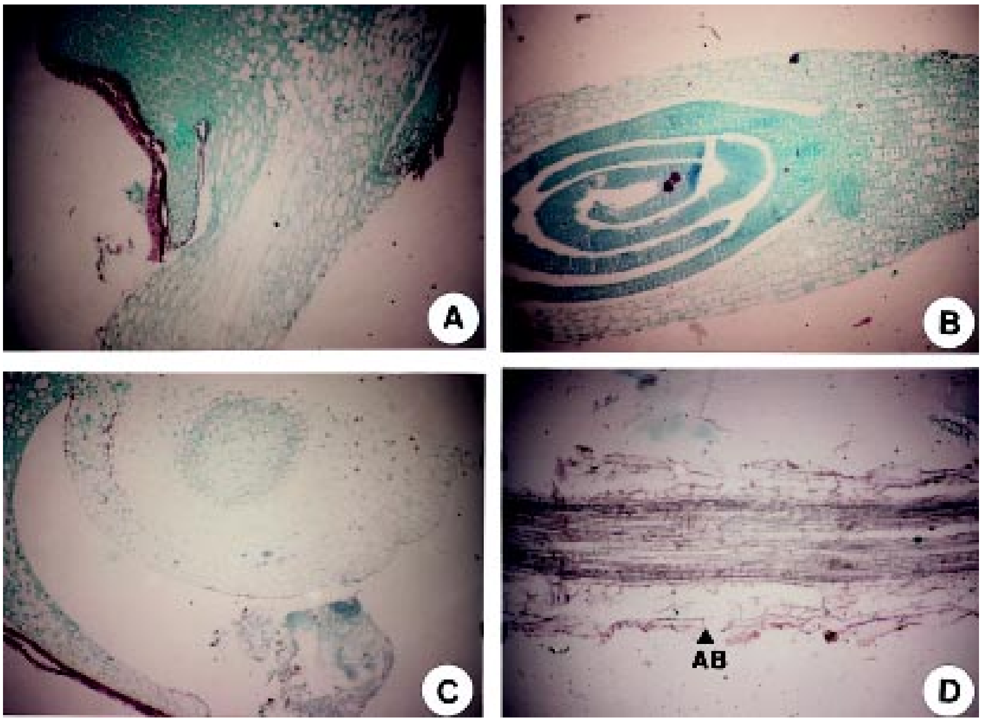

Figure 3. Development of sudangrass seedlings waterlogged for 3 days after imbibing. A, Normal radicle waterlogged at 2 days after imbibing (S_2) (×100); B, Normal plumule waterlogged at 2 days after imbibing (S_2) (×50); C, Normal root waterlogged at 3 days after imbibing (S_3) (×50); D, Abnormal root waterlogged at 3 days after imbibing (S_3) (×50). AB, Abortion.

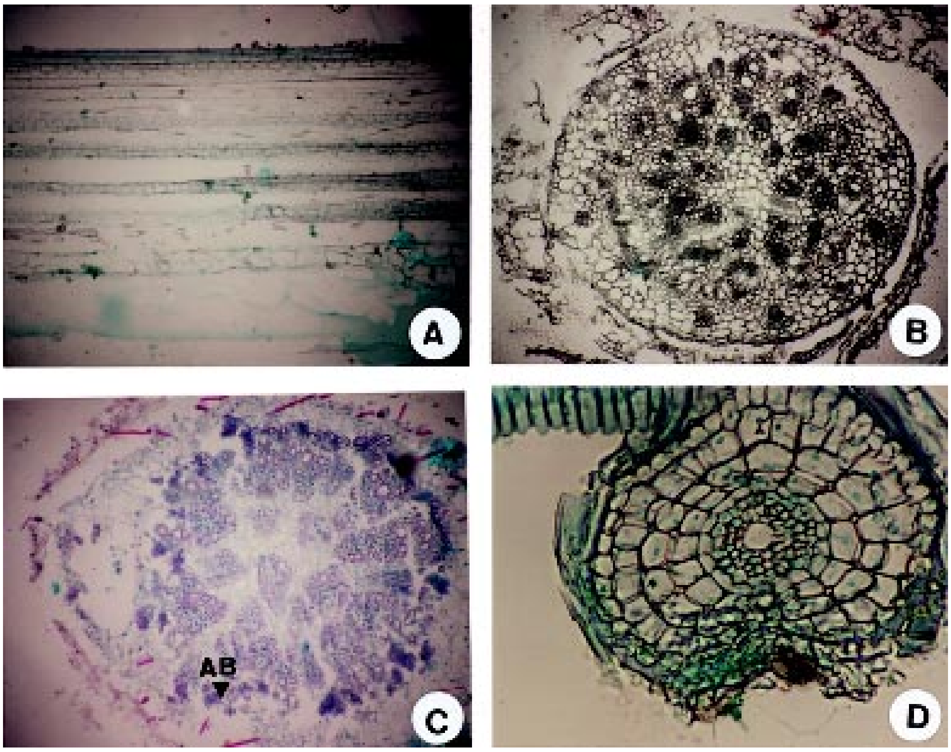

Figure 4. Development of sudangrass seedlings waterlogged at 0-2 days after sowing. A, Normal root waterlogged at 0 day after sowing (CK) (×100); B, Normal root waterlogged at 0 day after sowing (CK) (×50); C, Abnormal root waterlogged at 1 day after sowing (W_1) (×50); D, Normal root waterlogged at 2 days after sowing (W_2) (×50). AB, Abortion.

Table 2. Effects of different waterlogging periods after sowing on emergence ability of sudangrass.

Treatment	Emergence percentage (%)	Emergence rate index (% day^{-1})	Corrected emergence rate index (day^{-1})
CK[a]	66^{a*}	2.97^{a}	4.51^{a}
W_1	57^{a}	1.92^{bc}	3.37^{b}
W_2	80^{a}	2.47^{ab}	3.09^{c}
W_3	75^{a}	2.15^{bc}	2.84^{d}
W_4	78^{a}	1.98^{bc}	2.54^{e}
W_5	77^{a}	1.95^{bc}	2.53^{e}
W_6	67^{a}	1.54^{cd}	2.29^{f}
W_7	58^{a}	0.96^{d}	1.65^{g}

[a] CK, W_1, W_2, W_3, W_4, W_5, W_6 and W_7 indicate the seeds waterlogged for 0, 1, 2, 3, 4, 5, 6 and 7 days after sowing, respectively.
* Means in each column followed by the same letter are not significantly different at 5% probability level.

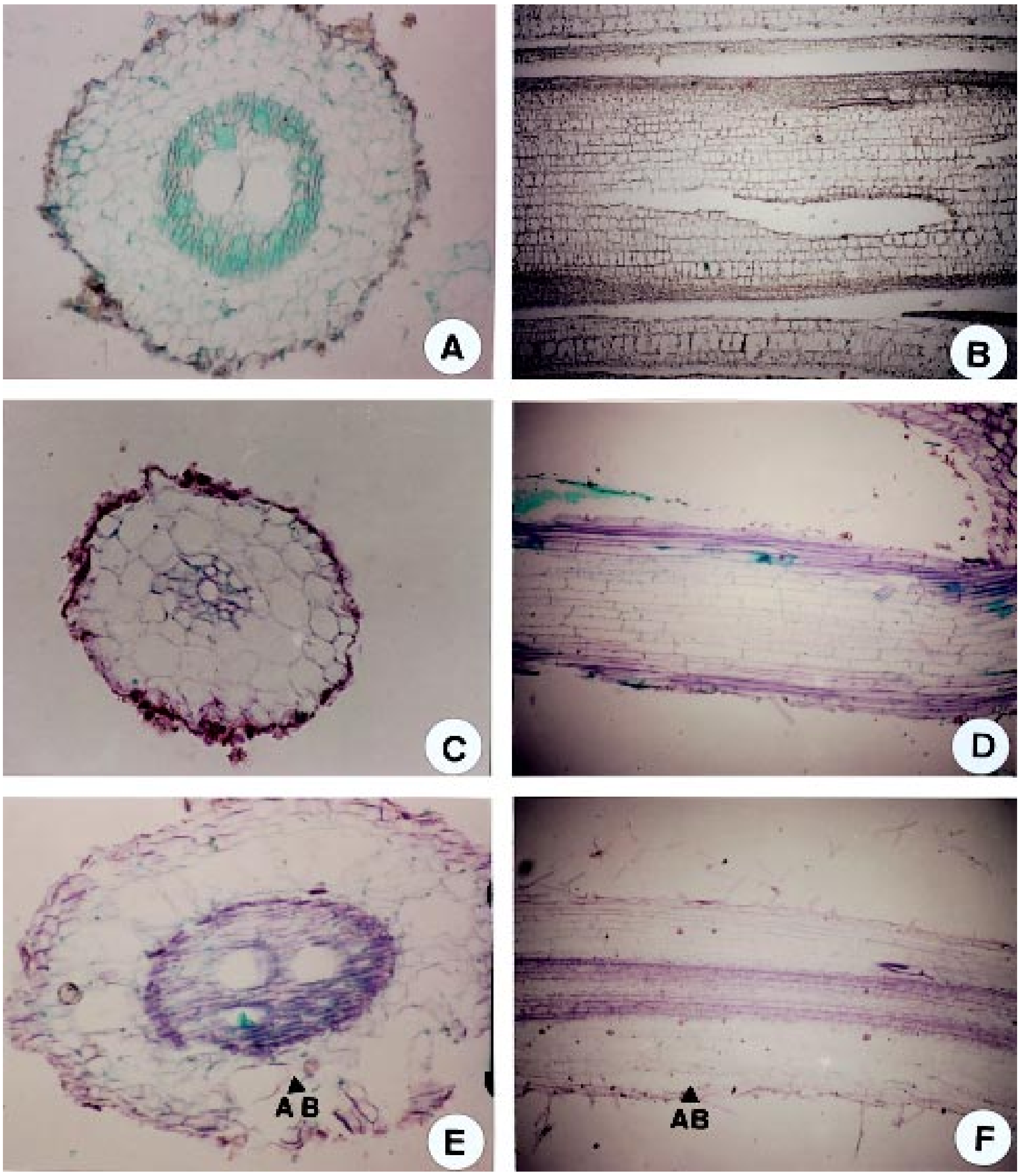

Figure 5. Development of sudangrass seedlings waterlogged at 3-7 days after sowing. A, Normal root waterlogged at 3 days after sowing (W_3) (×100); B, Normal root waterlogged at 4 days after sowing (W_4) (×100); C, Normal root waterlogged at 5 days after sowing (W_5) (×50); D, Normal root waterlogged at 5 days after sowing (W_5) (×50); E, Abnormal root waterlogged at 6 days after sowing (W_6) (×100); F, Abnormal root waterlogged at 7 days after sowing (W_7) (×100). AB, Abortion.

The emergence percentages of sudangrass seeds were not significantly different among waterlogging periods (Table 2). However, ERI and CERI decreased as waterlogging period increased. This showed that emergence ability and speed of emergence were decreased by waterlogging treatment.

The seeds waterlogged for 3 days before imbibing had the lowest germination percentage (Table 1). The anatomies of the seeds and the radicles showed abortion (Figures 2A, B). The radicles and the hypocotyls of the seedlings with waterlogging at 1 day after imbibing were normally developed (Figures 2C, D). The hypocotyl enlargement and differentiation were observed in the seeds with waterlogging at 2 days after imbibing (Figures 3A, B). Normal hypocotyl development was also observed in the seeds with waterlogging at 3 days after imbibing (Figure 3C). However, the epidermis of the seedling waterlogged at 3 days after imbibing was aborted (Figure 3D). This was similar to the result reported by Pezeshki (1994) and showed that the root epidermis was aborted by waterlogging.

Both longitudinal and transverse sections of the normal developing roots are shown in Figures 4A and B. The epidermis of the hypocotyl in the transverse section was abnormal with waterlogging for 1 day after sowing (Figure 4C). It was normal with waterlogging for 2, 3, 4 and 5 days after sowing (Figure 4D; Figures 5A, B, C, D), respectively. However, abortion was observed in the developing roots of the seedling waterlogged for 6 and 7 days after sowing (Figures 5E, F), respectively. The results explained the reason that the emergence of sudangrass seeds was more inhibited in the treatments with longer waterlogging periods.

Based on our results, the electric conductivity of the seed leakage could be used to evaluate germination ability of sudangrass. The germination ability of sudangrass was remarkably decreased when the seeds were waterlogged before imbibition. Further, the emergence ability of sudangrass was significantly inhibited by waterlogging. A more inhibitory effect on emergence was observed in the treatment with a longer waterlogging period. The anatomical studies indicated that the hypocotyl and the radicle were seriously damaged by waterlogging. That led to a decrease in the emergence ability of sudangrass during waterlogging.

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淹水對蘇丹草種子發芽、電導度及胚軸與胚根發育之影響

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蘇丹草（*Sorghum sudanense* Stapf）係一種短期芻料作物，適合台灣種植生產。為探討淹水對蘇丹草種子發芽、電導度及幼苗根組織之影響，並藉試驗結果尋求蘇丹草耐淹水品系選拔之依據。本試驗以蘇丹草台畜草一號為材料，進行不同淹水處理。在培養皿試驗，當種子浸潤 0、1、2 及 3 天後分別再淹水 3 天，結果顯示蘇丹草種子浸潤 0 天後直接進行淹水處理的發芽率最低；浸潤後 1、2 及 3 天後再淹水處理 3 天之發芽率、全糖及還原糖含量差異均不顯著；發芽率與種子浸出液電導度呈顯著負相關（$P<0.05$），顯示蘇丹草種子浸出液電導度可作為評估其種子發芽力之指標。在盆栽試驗中，種子播種後分別淹水 0、1、2、3、4、5、6 及 7 天，其萌芽率在各處理間沒有顯著性差異，但萌芽速率指數及校正萌芽速率指數均隨著淹水日數的增加而減少，顯示淹水對蘇丹草種子之萌芽力有影響。組織切片顯示，發芽及萌芽率低之處理的胚軸及胚根表皮均受損傷。綜合上述結果顯示，蘇丹草種子浸潤前受到淹水，其發芽率顯著地受影響，且種子浸出液電導度可作為評估蘇丹草種子發芽力之大小。

關鍵詞：電導度；胚軸；胚根；種子發芽；蘇丹草；淹水。