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Physiological Response, Fatty Acid Composition and Yield Component of *Brassica napus* L. under Short-term Waterlogging

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Abstract. The effects of short-term waterlogging on physiological responses, fatty acid compositions and yield components of rapeseed at both the vegetative growth and the reproductive growth stages were assessed in this study. Waterlogged plants were treated for a period of 10 days at the vegetative growth stage and 4 days at the reproductive growth stage. The results show that photosynthesis and stomatal conductance at both the vegetative growth and the reproductive growth stage were significantly reduced during the waterlogging period and after the recovery period. When the plants were removed from water, photosynthesis and stomatal conductance progressively restored similar values to those of control plants within 2~3 days. Fatty acid compositions were unaffected by waterlogging treatment. However, yield components (pod number and pod length) of the waterlogged treated plants at the reproductive growth stage were significantly reduced. These results suggest that short-term waterlogging may thus influence oilseed yield component.

Key words : oleic acid, photosynthesis, soil water content, waterlogging period

Introduction

Brassica napus L. (Brassicaceae), is one of major oilseed crops commonly cultivated cereal-growing areas after the rice harvest in Asia countries, has recently focused much attention as its seed is the primary source for biodiesels. In rapeseed growing regions of South Korea, typical double-cropping regions are constantly or periodically received with poorly drained soil condition. In recent, global warming has resulted in greater than normal rainfall during vegetative growth and reproductive growth stages and also causes waterlogging for several days.

The responses of plants to waterlogging are widely well known (Bange et al., 2004; Else et al., 2001; Graaini et al., 2007; Orchard et al., 1985). First of all, waterlogging has occurred the rapid condition of anoxia and hypoxia in the soil, with effects of O_2 supply, water and nutrient absorption (Araki, 2006). In addition, waterlogging damage has been attributed to reduction of plant growth (Issarakraisila et al., 2007; Orchard and Jessop, 1984; Zhou and Lin, 1995), reduction of stomatal conductance, photosynthesis and transpiration (Bange et al., 2004; Ortuno et al., 2005; 2007), and decrease of seed weight and seed oil content of rapeseed (Gutierrez Boem et al., 1996).

Under natural conditions, waterlogging induces the growth and development of the rapeseed plant in the field for several days, but when the plants are removed from the water, they recover from the waterlogging stress. Ortuno et al. (2007) previously reported that the leaf water potential of the lemon plant's response to flooding evidenced a progressive reduction, but when the plants were removed from the water, the leaf water potential increased, reaching values similar to those of the control plants 10 days later. In South Korea, winter oilseed rape has encountered the period from floral initiation to flow-

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ering to be susceptible stages to short-term waterlogging. These periods from floral initiation to flowering are important stage influencing seed production as well oil compositions. Therefore, responses of plants to short term waterlogging during vegetative stage and reproductive stage need and thereafter evidence the normal ability of plants to recover from root system waterlogging. The principal objective of this research was to assess the effects of short-term waterlogging on physiological responses, fatty acid compositions and yield components of rapeseed at both the vegetative growth and reproductive growth stages in the rapeseed plant.

Materials and Methods

Rapeseed seeds (*Brassica napus* L. cv. 'Young-San') were germinated and grown in the glass greenhouse. Two principal experiments were conducted in a glass greenhouse at the Chonnam National University, South Korea; one studied the effect of short term waterlogging on physiological responses at vegetable growth stage, the other studied the effects of short term waterlogging on physiological responses, fatty acid composition and yield components at reproductive growth stage.

In the first experiment, three-month-old seedlings were grown in plastic pots (21cm diameter×22cm high) and in the second experiment, five-month-old seedlings were grown in plastic pots (27cm diameter×26cm high) filled with a 2:1:1 v/v/v mixture of bed soil (Heung-Nong Bio Soil, South Korea), vermiculite, and perlite for 2 weeks in the glass greenhouse, respectively. Six plants in the first experiment and four plants in the second experiment were subjected to two different treatments: control plants irrigated daily during the experimental period, and waterlogged plants that were treated for a period of 10 days in the first experiment and 4 days in the second experiment. When symptom of waterlogging damage was appeared, both waterlogging periods were set. The pots in which the waterlogging-treated plants were enclosed and flooded by placing their pots inside larger pots filled with a 2-cm layer of water over the soil surface (Ortuno et al., 2007). The waterlogging-treated plants were removed from the water and separated from larger pots, then drained and treated as control plants.

Leaf stomatal conductance (mmol $m^{-2}s^{-1}$) was measured using Leaf Porometer (Steady State Diffusion Porometer, Decagon Devices, Inc, USA). Photosynthetic rate (μmol m^{-2} s^{-1}) and the transpiration rate (mmol m^{-2} s^{-1}) were employed for these measurements using LCA-4 (ADC Bio Scientific Ltd., UK). In the leaf chamber ($6.25cm^2$), we attached the third or fourth leaves from the shoot apex of each plant between 12:00 and 14:00 hours. Photosynthetic active radiation (PAR) at the leaf surface evidenced a maximum of up to 1076μmol m^{-2} s^{-1}, leaf surface temperature 27±3°C, ambient CO_2 concentration 350±5μmol mol^{-1}, and the boundary layer resistance (r_a) was 0.8s cm^{-1}. The volumetric soil water content was measured TDR (Field Scout TDR-300).

Analysis of the fatty acid composition of harvested seeds was determined the fatty acid methyl esters (FAMEs) method as previously described (Kim et al., 2008). In briefly, 0.02mg of dry rape seeds were weighed and added in 1ml of 5% (v/v) H_2SO_4 in methanol. Each sample was transmethylated at 90~95°C for one and half hours. Appropriate amount of Heptadecanoic acid (C17:0) was added as internal standard. After transmethylation, 1.5ml of aqueous 0.9% NaCl was added and fatty acids were extracted using FAMEs method with 2ml of hexane three times, and analyzed via gas chromatography. The oven temperature was ramped from 160°C to 220°C at 2.5°C min^{-1} (Shimadzu, Japan). Fatty acids were identified by comparing their sample retention and mass spectra with those of standards.

At harvest, the plant height from the soil surface to the shoot tip was measured using a flexible ruler. Stem diameter of rapeseed plants was measured at 2.0cm above the ground using digital calipers (Mitutoyo, Digmatic). Panicle length and pod length were measured using flexible ruler. Branching number and pod number were counted. The experiment was designed via a completely randomized design with 6 replicates for the first experiment and 4 replicates for the second experiment. The data were analyzed using the GLM procedure in the SAS package, version 9.1 (SAS Institute, Cary, North Carolina, USA).

Results

At the vegetative growth stage, the exposure of rapeseed plants to waterlogging for 10 days was shown in Fig. 1. From the outset of the waterlogged stress period,

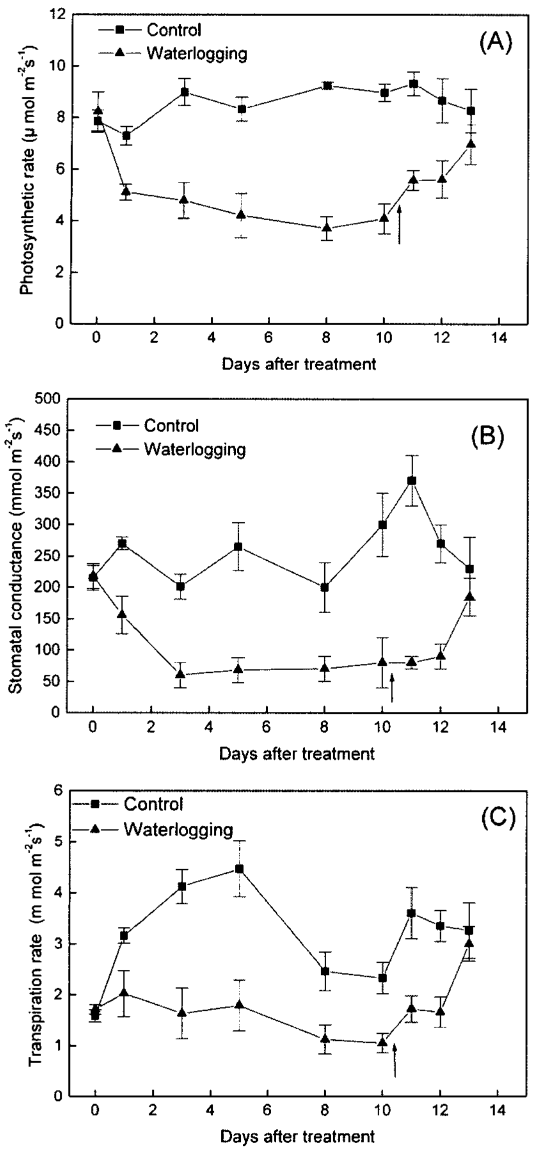

Fig. 1. Effects of waterlogging treatment at the vegetative growth stage on photosynthetic rate (A), stomatal conductance (B), and transpiration rate (C). Vertical arrows indicate the end of the waterlogging period and the start of the recovery period. Vertical bars represent SE (n = 6).

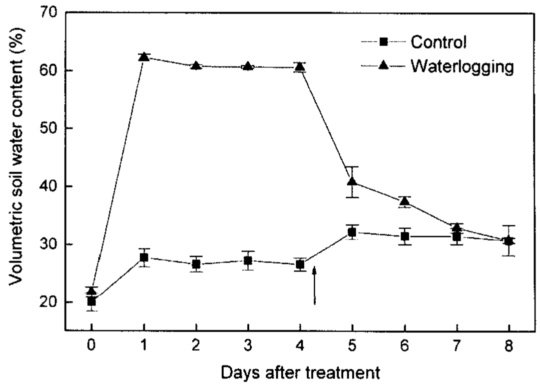

Fig. 2. Effects of waterlogging treatment at the reproductive growth stage on volumetric soil water content. Vertical arrow indicates the end of the waterlogging period and the start of the recovery period. Vertical bars represent SE (n = 4).

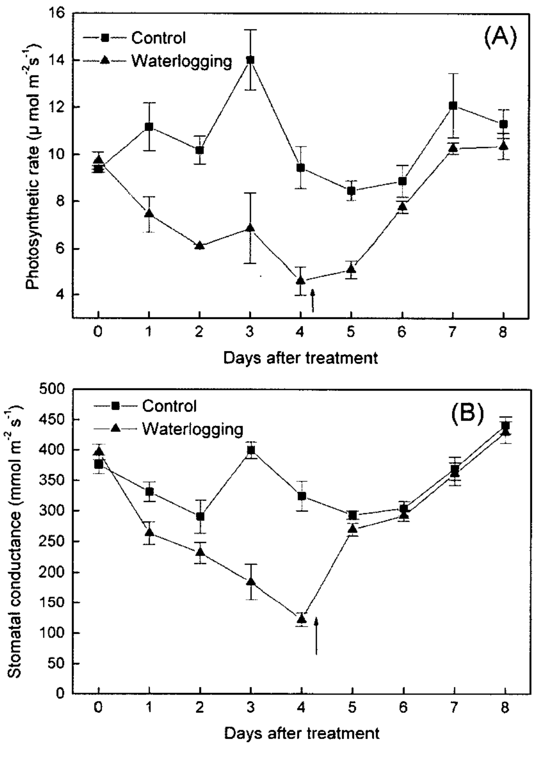

Fig. 3. Effect of waterlogging treatment at the reproductive growth stage on photosynthetic rate (A) and stomatal conductance (B). Vertical arrows indicate the end of the waterlogging period and the start of the recovery period. Vertical bars represent SE (n = 4).

photosynthetic rate, stomatal conductance and transpiration rate values were significantly decreased in waterlogged treated plants. Up to 10 days after the application of waterlogging treatment, photosynthetic rates, stomatal conductance and transpiration rate of waterlogging-treated plants were significantly less than those of the control plants (Figs. 1A, B and C). During the recovery period, the photosynthetic rates, stomatal conductance, and transpiration rates tended to decrease until 12 days

Table 1. Fatty acid compositions of waterlogged treated plants of *Brassica napus* L at reproductive growth stage.

Treatment	Saturated fatty acid			Monounsaturated fatty acid			Polyunsaturated fatty acid	
	C16:0	C18:0	C20:0	C18:1	C20:1	C22:1	C18:2	C18:3
Control	6.5 ± 0.5	1.9 ± 0.1	0.7 ± 0.0	69.9 ± 3.0	1.1 ± 0.0	0.4 ± 0.0	15.3 ± 2.1	4.2 ± 0.5
Waterlogging	7.1 ± 0.3	2.0 ± 0.2	0.7 ± 0.0	66.2 ± 1.8	1.0 ± 0.1	0.4 ± 0.0	17.9 ± 1.1	4.7 ± 0.4

±indicates the standard error of the mean (n=12).

Table 2. Effect of waterlogged treated plants on yield components of *Brassica napus* L at reproductive growth stage.

Treatment	Height (cm)	Stem dia (mm)	Panicle length (cm)	Branching (no.)	Pod (no.)	Pod length (cm)
Control	172.5 ± 5.1	11.7 ± 0.3	90.0 ± 5.0	16.5 ± 1.1	27.3 ± 0.9	4.1 ± 0.1
Waterlogging	133.5 ± 5.6	11.0 ± 0.5	59.0 ± 0.8	11.0 ± 3.7	9.3 ± 2.5	3.7 ± 0.2

±indicates the standard error of the mean (n = 4).

after waterlogging treatment. On day 13, these parameters did not differ statistically between the treatments, and partial recovery was observed.

At the reproductive growth stage, volumetric soil water content of the waterlogged treated plants was 2 times higher than those of non-treated plants (Fig. 2). During the experimental period, the volumetric soil water content reached 60% in waterlogged and 30% in control plants, suggesting that it may be indicated the stress of waterlogging. Like vegetative growth stage, photosynthetic rate and stomatal conductance were significantly reduced in the waterlogged treated plants (Fig. 3). When the rapeseed plants were released from water contained bigger pots, photosynthetic rate and stomatal conductance progressively increased, becoming similar values to those of the control plants on 3 days and 2 days, respectively. Fatty acid compositions were unaffected by waterlogging treatment (Table 1). However, plant height, panicle length and branching number of the waterlogged treated plants were significantly decreased compared to control plants. Also yield components related with pod number and pod length were reduced, these reductions becoming significant rape seed yield (Table 2).

Discussion

These experiments clearly demonstrated that photosynthetic rate and stomatal conductance of the waterlogging of rapeseed plants declined sharply as the result of 4- and 10-days of waterlogging both vegetative growth stage and reproductive growth stage (Figs. 1 and 3). Pod number per plant and pod length was reduced by short-term waterlogging (Table 2). These results agree with Zhou and Lin (1995) that the inhibition of physiological parameters of rape plants by short-term waterlogging leads to decrease plant photosynthesis and stomatal conductance, and consequently, to smaller seed yield components. When the plants were removed from the water, the photosynthetic rate and stomatal conductance were increased, recovering to a level similar to that of the controls by 2~3 days after the recovery period. The photosynthetic rate, stomatal conductance, and transpiration rate after the recovery period achieved values similar to those of the control plants, which were important for the survival of rapeseed plants grown in waterlogged regions. Waterlogged rapeseed plants recovered from reduced photosynthetic activity may possibly become the formation of new adventitious roots (Orchard et al., 1985; Iwanaga and Yamamoto, 2007) and enable the root system to grow, extend, and survive in waterlogged regions. Also, a significant reduction in the photosynthesis of waterlogged plants is a consequence of stomatal closure, whereas the rate of photosynthesis increased progressively after the recovery period (Yordanova et al., 2005). The present experiment indicated that the photosynthetic rate and stomatal conductance did evidence a significant relationship during the experimental period. Thus, it appears possible that stomatal factors may be responsible for an increase in the photosynthetic rate.

Our visual observation of rapeseed plants revealed no damage occurring during the waterlogging period at the vegetative growth stage (data not shown). However,

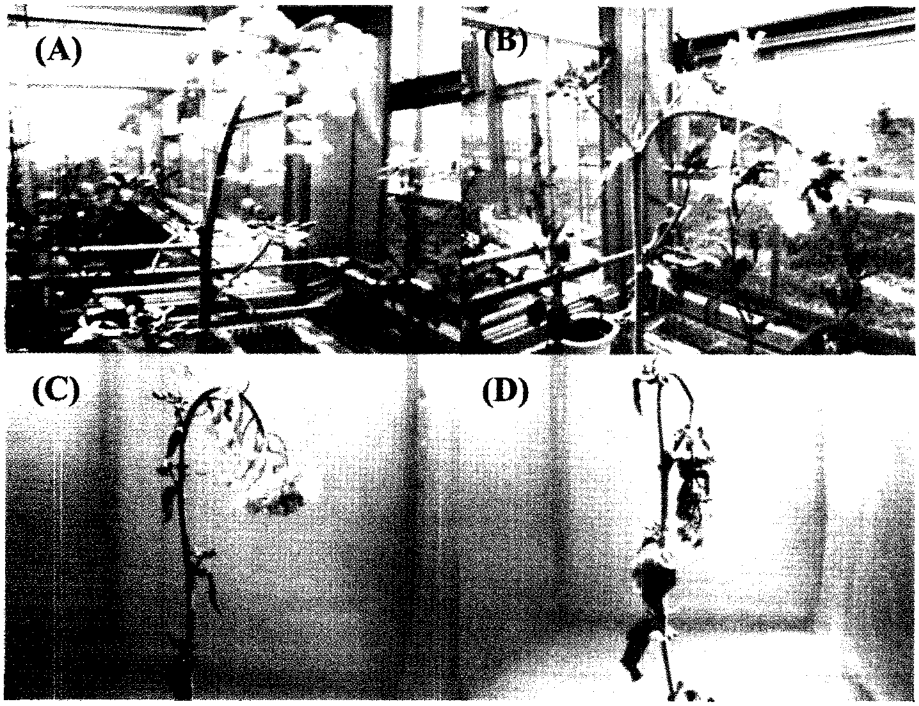

Fig. 4. Developing process of waterlogging stress. The more waterlogging progresses, the more damage occurs. Rape plants grow normally (A) and evidence a 90 degree curvature (B). They were severely damaged at the region approximately 12 cm region from the apical tip (C) and were nearly dead with two weeks after waterlogging (D).

plant height, panicle length and branching number of waterlogged plants at the reproductive growth stage were significantly reduced affected by waterlogging treatment even though stem diameter was similar (Table 2). The developing process of waterlogging damage is clearly described in Fig. 4. It is observed that waterlogged plants appear to evidence more damaged shoot growth which are severely damaged in a region of approximately 12 cm from the shoot apical tip, and the plants usually die completely within 2 weeks. During the reproductive growth stage, waterlogged rapeseed plants evidenced severely damaged shoot growth, whereas rapeseed plants evidenced no visual symptoms during the vegetative growth stage within ten days, although physiological responses including photosynthetic rate and stomatal conductance were reduced during the two growth stages. Thus, the short-term period of waterlogging of approximately four days during the reproductive growth stage severely damaged the growth and development of rapeseed plants.

Short-term waterlogging at reproductive growth stage had no effect on fatty acid compositions (Table 1). High oleic acid (C18:1) of the waterlogged treated plants slightly was lower, but had no significant difference between treatments. There was also no effect on fatty acid compositions at vegetative spear growth stage. However, yield components related with pod number and pod length may be an important factor with regard to final seed yield characteristics. Waterlogged treated plants at the reproductive growth stage were significantly reduced pod number and pod length, subsequently total production of rape seed can be significantly reduced. These results demonstrate that waterlogging at the reproductive growth stage exerted a relatively significant effect on plant growth and yield components as compared to those observed during the vegetative growth stage.

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Literature Cited

1. Araki, H. 2006. Water uptake of soybean (*Glycine max* L. Merr.) during exposure to O_2 deficiency and field level CO_2 concentration in the root zone. Field Crops Res. 96:98-105.
2. Bange, M.P., S.P. Milroy, and P. Thongbai. 2004. Growth and yield of cotton in response to waterlogging. Field Crops Res. 88:129-142.
3. Else, M.A., D. Coupland, L. Dutton, and M.B. Jackson. 2001. Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap. Physiologia Plant. 111:46-54.
4. Graaini, P., G.V. Indaco, M.L. Pereira, A.J. Hall, and N. Trápani. 2007. Responses to short-term waterlogging during grain filling in sunflower. Field Crops Res. 101:352-363.
5. Gutierrez Boem, F.H., R.S. Lavado, and C.A. Porcelli. 1996. Note on the effects of winter and spring waterlogging on growth, chemical composition and yield of rapeseed. Field Crops Res. 47:175-179.
6. Issarakraisila, M., Q. Ma, and D.W. Turner. 2007. Photosynthetic and growth responses of juvenile chinese kale (*Brassica oleracea* var. *alboglabra*) and caisin (*Brassica rapa* subsp. *parachinensis*) to waterlogging and water deficit. Sci. Hort. 111:107-113.
7. Iwanaga, F. and F. Yamamoto. 2007. Growth, morphology and photosynthetic activity in flooded *Alnus japonica* seedlings. J. For. Res. 12:243-246.
8. Kim, M.J., Y.S. Go, S.J. Ahn, C.H. Chung, and M.C. Suh. 2008. Functional complementation of a perilla ω-3 fatty acid desaturase under the seed-specific *SeFAD2* promoter. J. Plant Biol. 51:174-179.
9. Orchard, P.W. and R.S. Jessop. 1984. The response of sorghum and sunflower to short-term waterlogging. I. Effects of stage of development and duration of waterlogging on growth and yield. Plant Soil. 81:119-132.
10. Orchard, P.W., H.B. So, and R.S. Jessop. 1985. The response of sorghum and sunflower to short-term waterlogging. III. Root growth effects. Plant Soil. 88:421-430.
11. Ortuño, M.F., J.J. Alarcón, E. Nicolás, and A. Torrecillas. 2007. Water status indicators of lemon trees in response to flooding and recovery. Biologia Plant. 51:292-296.
12. Ortuño, M.F., J.J. Alarcón, E. Nicolás, and A. Torrecillas. 2005. Sap flow and trunk diameter fluctuations of young lemon trees under water stress and rewatering. Environ. Exp. Bot. 54:155-162.
13. Yordanova, R.Y., A.N. Uzunova, and L.P. Popova. 2005. Effects of short-term soil flooding on stomata behaviour and leaf gas exchange in barley plants. Biologia Plant. 49: 317-319.
14. Zhou, W. and X. Lin. 1995. Effects of waterlogging at different growth stages on physiological characteristics and seed yield of winter rape (*Brassica napus* L.). Field Crops Res. 44:103-110.

단기간 침수처리 하에서 유채의 생리적 반응, 지방산 조성과 수확량

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적 요. 단기간 침수처리에 의한 유채의 영양생장과 생식생장기의 생리적 반응, 지방산 조성과 수확량에 관하여 조사하였다. 영양생장 단계에서 10일과 생식생장 단계에서 4일 침수한 결과를 영양생장과 생식생장기 모두 광합성, 기공전도도와 증산량은 침수처리 기간과 회복시기에 현저하게 감소하였다. 침수처리가 끝난 처리구에서 광합성과 기공전도도는 2~3일 내에 대조구와 비슷한 경향을 보였다. 침수처리된 작물의 지방산 조성은 대조구에 비해 통계적 유의성의 차이를 보이지 않았지만 수확량과 관련된 요인들에서 현저하게 감소함으로 생식생장 단계에서의 단기간 침수처리가 수확량 요인에 큰 영향을 줌으로서 종자 수확량에 영향을 미칠 것으로 사료된다.

주제어 : 광합성, 올레인산, 침수기간, 토양수분 함량