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Optimal Treatment Strategies of Drought and UV-B Stresses before Harvest for Promoting Antioxidants Accumulation in Kale Grown in Plant Factories

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ABSTRACT

Kale is one of the healthiest Brassica vegetables and its cultivation is increasing for either fresh consumption or as a source for functional foods and nutraceuticals. Among abiotic stresses, drought and UV-B radiation are effective in triggering extra secondary metabolites and can be easily applied to plant factories. The objective of this study was to determine the optimal treatment times of drought and UV-B stresses before harvest to achieve its maximum amount of antioxidants. Kales (*Brassica oleracea* L. cv 'Manchoo Collad') were grown at a temperature of 20°C and a photosynthetic photon flux density of 350 μmol·m^{-2}·s^{-1} (LEDs with red:blue:white = 8:1:1 and 16-8 h light/dark photoperiod) in a plant factory and harvested at 42 days after transplanting (DAT). Chlorophyll fluorescence (F_v/F_m), leaf water potential, dry weight, total flavonoid content, total phenolic content, and antioxidant capacity of the samples were determined. In the first experiment, for determining the optimal treatment time of drought stress to achieve its maximum amount of antioxidants, drought stress treatments lasting for 7, 6, 5, 4, 3, 2, and 1 days before harvest (T7, T6, T5, T4, T3, T2, and T1, respectively) were applied to kales. At T7, the F_V / F_m gradually decreased from 0.811 on DAT 36 to 0.563 on DAT 39, followed by a drastic decline to 0.286 on DAT 40. Leaf water potential decreased from -3.863 MPa at T2 to -6.27 at T3. However, it increased slightly to -6.04 MPa at T4 and then decreased again with longer drought stress. Both results indicated that the drought stress less than 4 days could ensure the function of leaf chlorophyll fluorescence and maintain normal leaf water potentials. As to the antioxidants such as total flavonoid content and total phenolic content, T2, T3, and T4 stresses significantly induced higher amount of antioxidants compared to the control and other

treatments. In the second experiment, a combined effect of drought and UV-B stresses on the accumulation of antioxidants was investigated at T4, T3, and T2 stresses from DAT 38 to 40. Compared to the control and single drought stress, leaf chlorophyll fluorescence and dry weight significantly decreased at combined stresses. The amounts of total antioxidants were higher at T3 and T4 drought stresses than any other treatments including the control, while the combined stresses showed no extra formation of antioxidants compared to those obtained at the single drought stress. Considering energy inputs, T3 drought stress could achieve the highest potential value of kale as a source of natural antioxidants.

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INTRODUCTION

Kale (*Brassica oleracea* L. var. acephala), which acts as an excellent source of dietary antioxidants, is commonly cultivated in many places around world and its cultivation is increasing for either fresh consumption or as a source for functional foods and nutraceuticals. The protective health effects of antioxidants are attributed to their radical scavenging and metal-chelating abilities (Heimler et al., 2007). However, many researches indicated that the production of those phytochemical compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Dixon 2001; Oksman-Caldentey and Inze, 2004). In kale, carotenoids, such as lutein and zeaxanthrin levels, are affected by growth factors such as maturity, climate, and farming practice (Walsh et al., 2015).

Abiotic factors such as high and low temperatures, drought, alkalinity, salinity and UV radiation are potentially harmful to plants and then induce secondary plant metabolites (Akula et al., 2011; María et al., 2013). Among abiotic factors, drought stress has been shown to induce a number of such phytochemicals, including α-tocopherol, β-carotene, and flavonoids, in a wide range of plant species (Keles and Öncel, 2002; Zobayed et al., 2007). UV-B radiation has been proved to be able to exert a considerable influence on increases in phenolic compounds (Mackerness 2000; Jansen et al., 2008; Inga et al., 2012).

Among various environmental factors, uses of water deficit as drought stress and UV-B radiation are relatively convenient to be applied in plant factories from practical aspects. In previous researches, the effects of drought stress and UV-B radiation on biomass production and metabolism in lettuce (Rajabbeigi et al., 2013) and on salicylic acid accumulation in barley roots and leaves (Bandurska et al., 2013) were studied. In addition, most of the previous related experiments were conducted in open fields or greenhouses and applied the stresses mostly to plant seedlings (Oh et al., 2010), young leaves (Sun et al., 2010), or plant organs (Schreiner et al., 2009), but rarely applied to whole mature plants which were

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produced for market. Furthermore, researches on the reactions of plants to drought and UV-B radiation in a plant factory level are insufficient and their quantifications for promoting antioxidants are unknown. Besides, optimal strategies of environmental stresses before harvest, which can contribute to maximum amount of antioxidants of plants, have not been established. The objectives of this study were to determine the optimal treatment time of drought stress before harvest and investigate the combined effect of drought and UV-B stresses to achieve the maximum contents of antioxidants in kale.

LITERATURE REVIEW

Antioxidants in kale and their functions

Consumption of vegetables and fruits that have plenty of antioxidants is reported to have health promoting effects like prevention of chronic disease (Finley et al., 2011; Wang et al., 2011). Brassica family especially is rich in phytochemicals which promote human health (Jahangir et al., 2009) and antioxidants activity (Podsedek, 2007). The contribution of Brassica vegetables to health improvement can be related to their antioxidant capacity. Phenolic compounds with vitamin C are the major antioxidants of Brassica vegetables, due to their high content and high antioxidant activity. On the contrary, lipid-soluble antioxidants (carotenoids and vitamin E) were responsible for up to 20% of the total antioxidant activity of Brassica vegetables (Podsędek et al., 2007). Consuming Brassica vegetables is believed to prevent cancer and cardiovascular diseases (Beecher, 1994; van Poppel et al., 1999; Cohen et al., 2000; Wang et al., 2004). Antioxidants activity of *Brassica* attributes its phenolic compounds and some flavonoids (Galati and O'Brien, 2004). Among Brassica vegetables, kale (*Brassia oleracea* L. var. acephala) especially has plentiful phenolic and flavonoid compounds (Hertog et al., 1992; Cao et al., 1996).

Effects of drought stress on accumulation of antioxidants in plants

Drought stress has been shown to induce a number of such phytochemicals, including α-tocopherol, β-carotene, and flavonoids, in a wide range of plant species (Keles and Oncel, 2002; Zobayed et al., 2007). The re-watering of plants following the stress period decreased their total phenolic concentration, suggesting that the levels of total phenolics (expressed on dry weight basis) are quite sensitive to the water status of plants (Oh et al., 2010). Drought stress just before harvest significantly increased total phenolic compounds and antioxidant capacity compared to the control (Oh et al., 2010). Drought stress

may also alter the synthesis of secondary plant compounds. For example, the content of phenolic compounds such as caffeic acid was found to be reduced in Ipomoea batatas roots (Mao et al., 2004), whereas total soluble phenols, quercetin and betulinic acid were increased in *Hypericum brasiliense* (shoots) (Abreu and Mazzafera, 2005) under drought stress conditions. Furthermore, selected amino acids are also involved in drought stress responses: Proline is widely distributed in plants and is accumulated in larger amounts than other amino acids in drought stressed plants (Irigoyen et al., 1992). For various crops, it was reported that the proline content significantly increased when water is limited, e.g. for maize and bean plants (shoots and roots) (Mohammad-Khani and Heidari, 2008), and for alfalfa plants (leaves) (Irigoyen et al., 1992). Thus, drought stress-mediated changes in primary and secondary plant compounds differ depending on the plant and morphological plant part used for consumption (Rajabbeigi et al., 2013).

Effects of UV-B radiation on accumulation of antioxidants in plants

During the last decade, it has become increasingly clear that UV-B (280-315 nm) radiation is an important regulator of plant secondary metabolism. Plants specifically detect and respond to UV-B radiation. This can be measured as characteristic changes in gene-expression (Ulm et al., 2004), physiology and/or morphology (Hectors et al., 2007; Lake et al., 2009), as well as altered accumulation of plant secondary metabolites (Schreiner and Huyskens-Keil, 2006; Huyskens-Keil et al., 2007; Jansen et al., 2008; Perez et al., 2009; Schreiner et al., 2009; Schmidt et al., 2011). Low, ecologically-relevant UV-B levels trigger distinct changes in the accumulation of, among others, phenolic compounds, carotenoids (Schreiner et al., 2012). A fundamental component of this plant-environment interaction is the formation of UV absorbing and photo-protective secondary metabolites such as phenolic compounds and carotenoids in response to UV-B radiation exposure (Treutter, 2005; Caldwell and Britz, 2006). The effect of UV-B radiation on plants includes accumulation of phenylpropanoids, such as phenolic acids and flavonoids (Liu et al., 1995; Kozlowska et al. 2007; Zhang and Bjorn, 2009).

Combined effect of drought stress and UV-B radiation on accumulation of antioxidants in plants

The promotion of phenol metabolism activity through water-deficit conditions was reported for pepper plants (Sung et al., 2005). Stress-mediated phenol metabolism synthesis induced by UV-B has been documented for lettuce (Caldwell and Britz, 2006) and white asparagus spears (Eichholz et al., 2012). Furthermore, plant responses to UV-B are known to interact with the water availability of plants, that is, water limitation is reported to lower the UV-B sensitivity of plants (Gwynn-Jones et al., 1999). However, the sensitivity of plants to UV-B or drought differs among species, populations and varieties, and depends upon physiological stage of the plant and duration of stress impact (Abreu and Mazzafera, 2005; Schreiner et al., 2009; Liu et al., 2011). Information on changes of the secondary metabolites as affected by drought stress in combination with UV-B radiation is contradictive to a certain extent and still limited (Hofmann et al., 2003).

MATERIALS & METHODS

Plant materials and growth conditions

Kale cultivar, 'Manchoo collard' (*Brassica olearacea* Acephala Group Manchoo collard, Asia Seed Company, Seoul, Korea) was cultivated in a plant factory module of Seoul National University. The seeds were sowed in deep flow systems under fluorescent lamps at a photosynthetic photo flux density (PPFD) of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After normal leafs appeared, nutrient solutions developed by National Institute of Horticultural and Herbal Science (Choi et al., 2005) for Brassica was applied with an electrical conductivity (EC) of 0.6 dS m^{-1}. Three weeks after germination, kale seedlings of uniform size were transplanted in the deep flow systems under light-emitting diodes (LED) (Red : Blue : White = 8 : 1 : 1, PPFD = 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the same nutrient solutions with an EC of 1.2 dS m^{-1} were applied. For 16-8 h light/dark photoperiod, temperature, relative humidity, and CO_2 concentration were maintained at 20 °C, 70 ± 5% and 500 ± 50 $\mu mol \, mol^{-1}$, respectively. Whole harvested samples were freeze dried at -80 °C. Chlorophyll fluorescence (F_v/F_m), leaf water potential, dry weight, total phenolic content (TPC), total phenolic content (TFC), and antioxidant capacity of the plants were measured and analyzed with three replications.

Drought and UV-B stress treatments

Drought stress was imposed by releasing all the nutrient solution from the root system in growing modules. The plants were irradiated with UV-B for 4 h a day from 10:00 to 14:00 using UV-B fluorescence lamp (Sankyo Ultraviolet Co. Ltd., Kanagawa, Japan) with intensity of 32.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Both stresses treatments were applied from designed days before harvest (42 days after transplanting, DAT) described as follows.

Two experiments (Exp 1 and Exp 2) were conducted as described in Table 1. In the first step of Exp 1 (Exp 1.1), seven drought stress treatments lasting for 7, 6, 5, 4, 3, 2, and 1 day before harvest (T7, T6, T5, T4, T3, T2, and T1, respectively) from DAT 35 to 41 were carried out. Based on the results of

Exp 1.1, drought stress treatments in the second step (Exp 1.2) were shortened to T4, T3, T2, and T1 to identify the optimal treatment time. For the control group, no stress was applied. Chlorophyll fluorescence (F_v / F_m), leaf water potential, dry weight, total phenolic content (TPC), total phenolic content (TFC), and antioxidant capacity of the plants were measured and analyzed with three replications. In Exp 2, plants were exposed to both drought and UV-B stresses at T4, T3, and T2 conditions from DAT 38 to 40. For the control groups, no stress and single drought stress were applied. Chlorophyll fluorescence (F_v / F_m), dry weight, total phenolic content (TPC), total phenolic content (TFC), and antioxidant capacity of the plants were measured and analyzed with three replications.

Measurements

Chlorophyll fluorescence. To evaluate the photosynthetic efficiency of kale, chlorophyll fluorescence was measured on the adaxial side of a fully expanded leaf with a chlorophyll fluorometer (PEA, Hansatech Instruments, Norfolk, UK) during the drought stress. The fully- expanded leaves from the middle of the canopy of kales were selected for the measurement. F_v / F_m ratio was measured during the day using the saturation pulse method after 30 min of dark adaptation. Three plants were used for each measurement.

Leaf water potential. Leaf water potential were measured at DAT 42 using a dewpoint potentiameter (WP4, Decagon Devices, Inc., Pullman, WA, USA). Prior to measuring the leaf water potential, one drop of distilled water was applied to the surface of recently-expanded leaf while the leaf was still attached to the whole plant, and the leaf was abraded gently and evenly about ten strokes with a 50×20 mm piece of 600 grit sandpaper to speed equilibration. After abrasion, the leaf surface was blot dried with Kimwipe® to remove any excess moisture. The leaf was excised about 4 cm diameter circular from the kale plant and then the leaf sample was placed into a WP4 sample cup. Following the immediate sealing the sample chamber, measurements of water potential were made. Equilibration for each measurement reached was typically within 30 min. All treatments for each measurement were replicated three times.

Antioxidants analysis. After kales were harvested, whole leaves were freeze-dried and ground to a fine powder with the aid of a mortar and pestle. The samples were assumed to be uniform. Each sample of 100 mg was extracted with 1 mL of 70% aqueous methanol (v / v, %).

Total phenolic compound was analyzed with Folin-ciocalteu procedure (Ainsworth and Gillespie, 2007). After incubated 48 h in dark condition with room temperature, the samples were centrifuged with 1.0×10^4 g for 10 min. The supernatant 50 μL was collected to 2 mL micro tube, and 10% Folin-cioalteu solution 750 μL and distilled water 135 μL were added. After vortexing, 600 μL 700 Mm Na_2CO_3 was added and incubated 2 h with room temperature. Absorbance in 765 nm was measured with a spectrophotometer (Photolab 6100vis, WTW, Germany). A standard curve was obtained with gallic acid and the results were expressed as mg gallic acid equivalent/g dry weight.

Total flavonoid compound was analyzed with Aluminum chloride colorimetric (Dewanto et al., 2002; Lee et al., 2012). After incubated 12 h   in dark condition with 4^oC, samples were centrifuged with 1.0×10^4 g for 10 min. The supernatant 150 μL was collected to 2 mL micro tube, and distilled water 135 μL and $NaNO_2$ 45 μL were added. After 5 min, 10% $AlCl_3$ 90 μL was added and incubated for 6 min. 1 M NaOH 300 μL and distilled water 165 μL were also added and after 5 min incubating, absorbance in 510 nm was measured with the spectrophotometer. A standard curve was obtained with catechin acid and the results were expressed as mg catechin equivalent/g dry weight.

Antioxidant capacity was analyzed with DPPH assay (Brand-Williams et al., 1995; Andarwulan et al., 2010). DPPH solution was prepared with methanol 100 mL and DPPH 24 mg. After incubated 48 h in dark condition with room temperature, samples were centrifuged with 1.0×10^4 g for 10 min. The supernatant 150 μL was collected to 2 mL micro tube, DPPH solution 1.35 mL was added. After 30 min incubating, absorbance in 517 nm was measured for samples and zero cell with the spectrophotometer. A standard curve was obtained with ascorbic acid and results were expressed as mg ascorbic acid equivalent antioxidant capacity/g dry weight.

Statistical analysis

Analysis of variance (ANOVA) was performed by SPSS (SPSS Statistics 23, IBM, USA). The experiment was conducted using a randomized complete block design with three replications. Means were compared using Duncan's multiple range tests.

RESULTS & DISCUSSION

Exp 1-1. Optimal treatment period of drought stress before harvest to maintain the normal function of the kale leaves

Seven drought stress treatments lasting for 7 to 1 days before harvest (DAT 35 to 41) were carried out. Leaf chlorophyll fluorescence F_V / F_m decreased gradually from 0.811 on DAT 36 to 0.563 on DAT 39, followed by a drastic decline to 0.286 on DAT 40 and to 0.077 on DAT 42 (Fig. 1). Leaf water potential decreased from -3.863 MPa at T2 to -6.27 at T3. However, it increased slightly to -6.04 MPa at T4 and then decreased again with longer drought stress (Fig. 2). In response to drought stress, a decreased activation leaf chlorophyll fluorescence was observed in kale. This response was consistent with the decline of leaf water potential. The similar trends were also observed in studies on blueberry that leaf water potential progressively decreased with increasing the water-withholding period (Kim et al., 2004). Water-stressed blueberry had more negative leaf water potentials than well-watered blueberry (Rho et al., 2012). (Oh et al., 2010） also reported the continuous decrease of leaf chlorophyll fluorescence and leaf water potential in water-stressed lettuce seedlings from 1 to 4 days. Both results indicated that when exposed to drought stress, the leaf chlorophyll fluorescence and leaf water potential of the plant declined with time and the drought stress longer than 4 days would obviously affect the function of the leaves.

Exp 1-2. Optimal treatment time of drought stress before harvest to promote antioxidants accumulation

Based on the results of Exp 1.1, drought stress treatments in the second step were shortened to 4 to 1 days before harvest (DAT 38 to 41) to identify the optimal treatment time. Under the drought stress, dry weight decreased with increase of treatment periodscompared to the control (no stress) (Fig. 3). The accumulation of dry weight from DAT 38 to harvest time showed significant differences between the control and treatments (Fig. 4). The amounts of TFC and total TPC were higher under T3 and T4 than

any other treatments (Figs 5 and 6). The same result was observed at antioxidant capacity (DPPH radical scavenging) analysis (Fig. 7).

Bray (2002) and Keles and O¨cnel (2002) reported antioxidants in a number of plant species by drought stress. In particularly drought stress just before harvest produced significant increases in total phenolic compounds and antioxidant capacity compared with the control (Munn´e-Bosch et al., 2001; Sofo et al., 2005). Also the response of lettuce seedlings to drought stress was very much dependent on plant age (Oh et al., 2010). In this study, the response of kale to drought stress was highly dependent on the exposed period to the stress. In response to the drought stress, the increase in TFC, TPC, and antioxidants capacity under T2, T3, and T4 was statistically significantly higher compared with those under T1 and the control. Thus, the results suggested that the drought stress lasting from two to four days could significantly contribute to the accumulation of antioxidants in kales.

Exp 2. The combined stress of drought and UV-B before on antioxidants accumulation

Combined stresses of drought and UV-B or single drought stress was applied for 4 to 2 days before harvest (DAT 38 to 40). Compared to the control and single drought stress, leaf chlorophyll fluorescence significantly decreased under the combined stresses (Fig. 8). Under the combined stresses of drought and UV-B or single drought stress, dry weight decreased with increase of treatment periods compared to the control (no   stress) (Fig. 9). The accumulation of dry weight from DAT 38 to harvest time showed significant differences between the control and treatment (Fig. 10). The amounts of TFC, TPC, and antioxidant capacity were higher under T3 and T4 than any other treatments (Figs 11, 12, and 13) However, there were no significant differences between T3 and T4. Besides, it is interesting to note that the combined stresses of drought and UV-B did not induce more antioxidants accumulation compared to those obtained at the single drought stress (Figs. 11, 12, and 13).

These results suggested that the exposure of plants to the combined stresses of drought and UV-Bs simultaneously did not have any extra accumulation compared with a single drought stress, which was

consistent with the studies on interaction of drought stress and UV-B radiation in lettuce (Rajabbeigi et al., 2013). However, Bandurska et al. (2012) reported that the positive effect of the combined stresses of drought and UV-B stress on accumulation of anthocyanin, proline content and phenolic acids was stronger than the individual drought in barley seedlings leaves and roots. Thus, from these results, we deduced that both stresses acted either synergistically or to some extent antagonistically in terms of inducing plant protective mechanisms (Rajabbeigi et al., 2013). It was surmised that the UV-B effect was perceived by water-stressed roots, which exhibited a reduction of lipid peroxidation and an accumulation of proline in water deficit-stressed plants exposed to UV-B. The drought stress as well as UV irradiation are the most adverse factors for plant growth and productivity (Reddy et al., 2004),   and the response of plants to UV-radiation or drought highly depends on the species, cultivar, plant organ, developmental stage, and furthermore is influenced by eco-physiological inter-actions (Rahbarian et al., 2011; Bandurska et al., 2012). Although UV-B radiation may enhance the resistance to water deficit and vice versa (Bandurska et al., 2013), it might be difficult to predict the effects of antioxidant accumulation under combined stresses of drought and UV-B or single stress.

CONCLUSION

The optimal treatment time of drought stress before harvest was determined to achieve its maximum content of antioxidants in kales and the combined effect of drought and UV-B stresses on the accumulation of antioxidants was investigated. Drought stresses less than 4 days before harvest could ensure the function of leaf chlorophyll fluorescence and maintain normal leaf water potential. In addition, drought stresses for 2, 3, and 4 days could significantly induce more antioxidants, while total antioxidant amounts were higher at 3 and 4 days than any other treatments including the control. However, combined stresses showed no extra formation of antioxidants compared to those obtained at single drought stress. Considering energy inputs, drought stress lasting for 3 days before harvest could achieve the highest potential value of kale as a source of natural antioxidants.

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Tables

Table 1. Detail schedules of the experiment 1 (Exp 1) and experiment 2 (Exp 2).

Experiment		Stress period before harvest
Exp 1	Exp 1.1	7, 6, 5, 4, 3, 2, 1 days, and no stress for drought stress (= T7, T6, T5, T4, T3, T2, T1, and control, respectively)
	Exp 1.2	4, 3, 2, 1 days, and no stress for drought stress (= T4, T3, T2, T1, and control, respectively)
Exp 1		4, 3, 2 days, and no stress for combined stresses of drought and UV-B (= T4, T3, T2, and control, respectively)

Figures
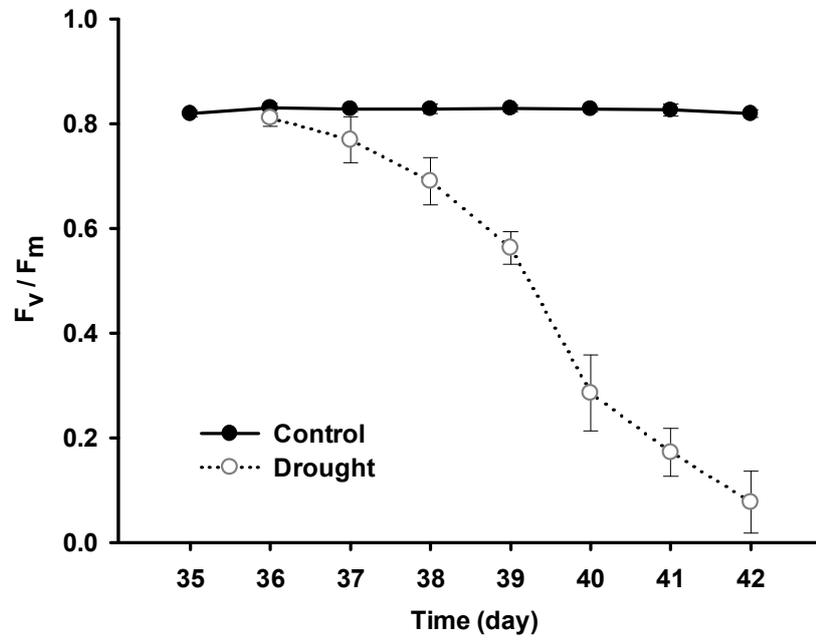
Fig. 1. Changes in leaf chlorophyll fluorescence (F_v / F_m) under different drought stress periods. The vertical bars represent the standard error of the mean (n = 3).
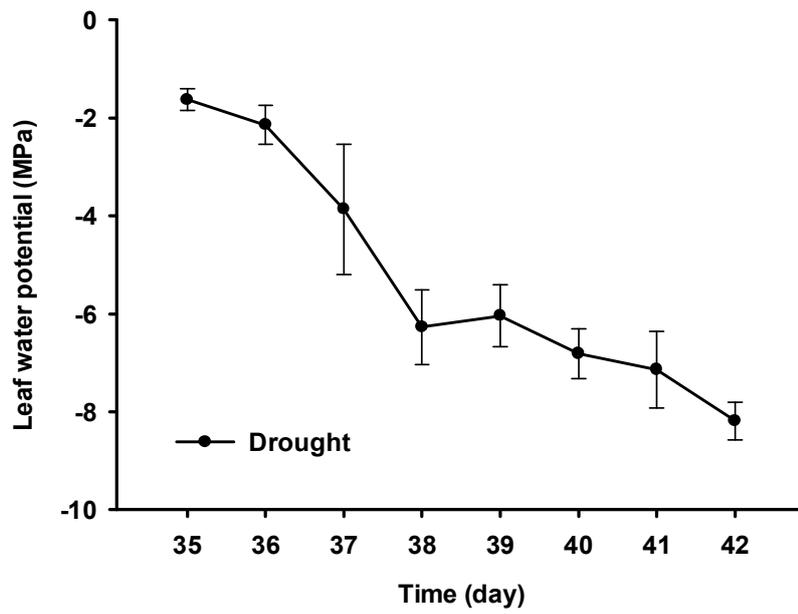
Fig. 2. Changes of leaf water potential under different drought stress periods. The vertical bars represent the standard error of the mean (n = 3).
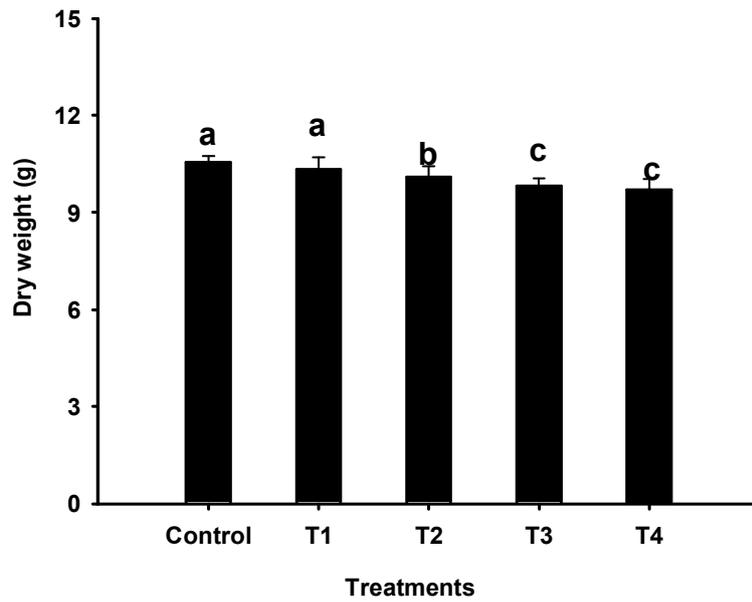
Fig. 3. Dry weight of kales under different drought treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.
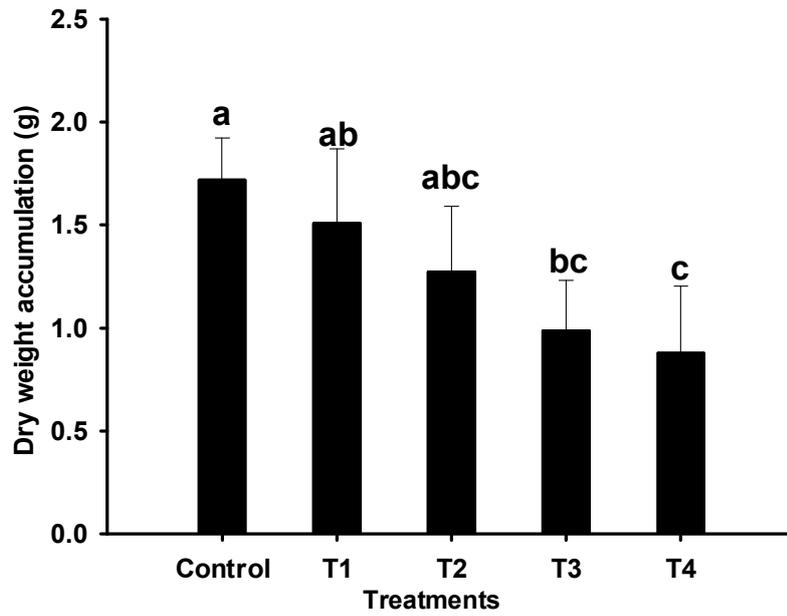
Fig. 4. Dry matter accumulation amounts of kale under different drought stress treatments (dry weight at 38 day after transplanting = 8.83g). The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test.   See Table 1 for the treatments.
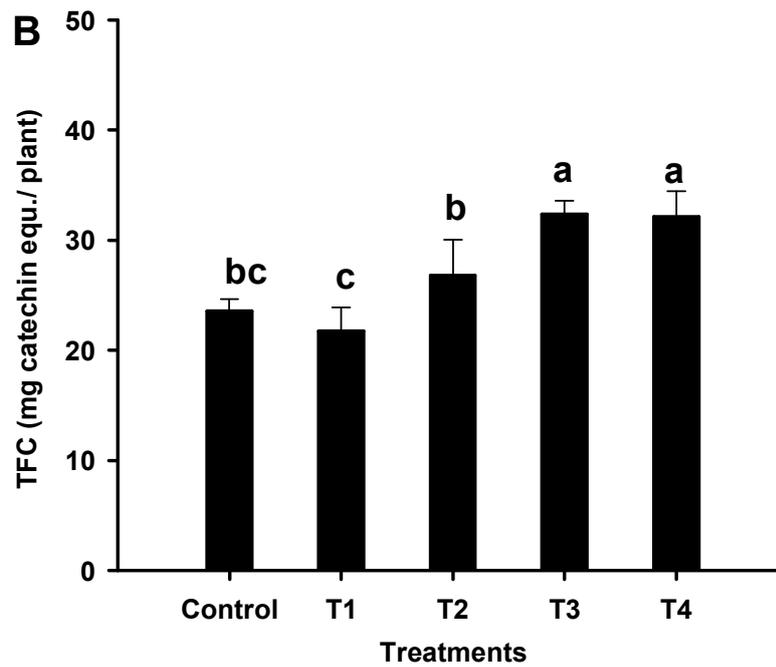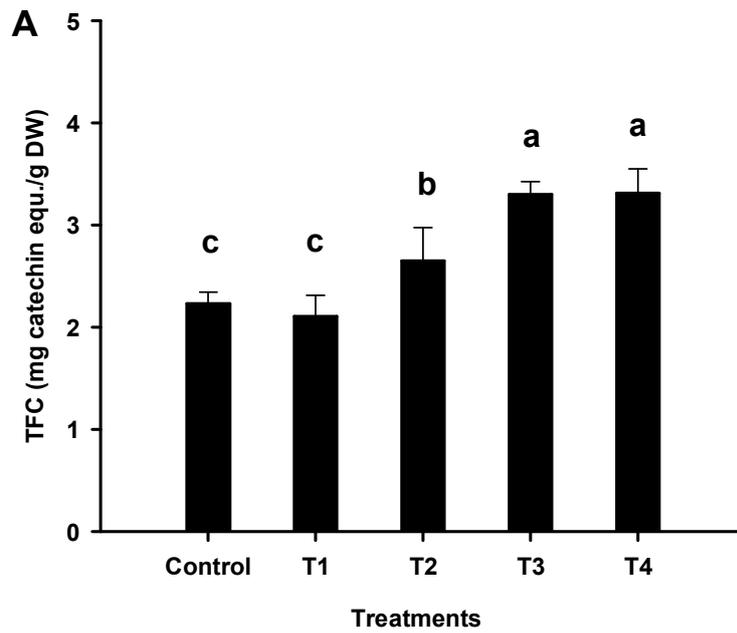
Fig. 5. Total flavonoid content (TFC) of kales under different drought stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p <$ 0.05) according to Duncan's multiple range test. See Table 1 for the treatments.
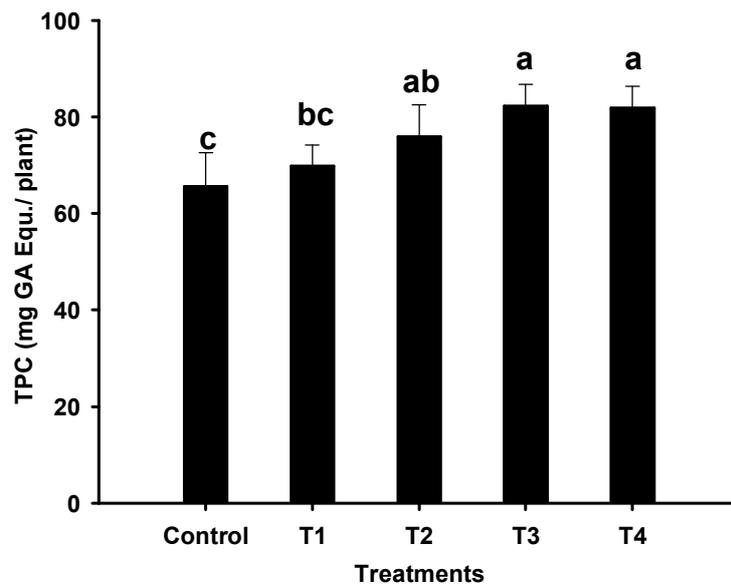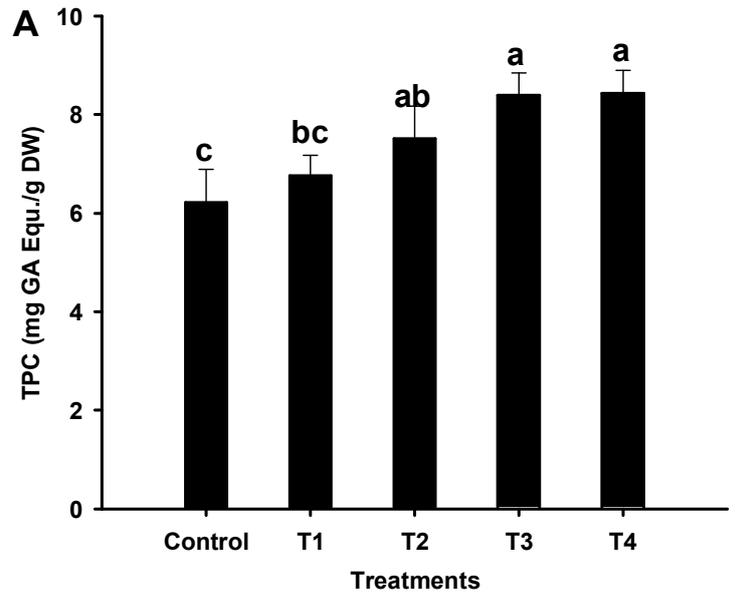
Fig. 6. Total phenolic content (TPC) of kales under different drought stress treatments. The vertical bars

represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p <$

0.05) according to Duncan's multiple range test. See Table 1 for the treatments.
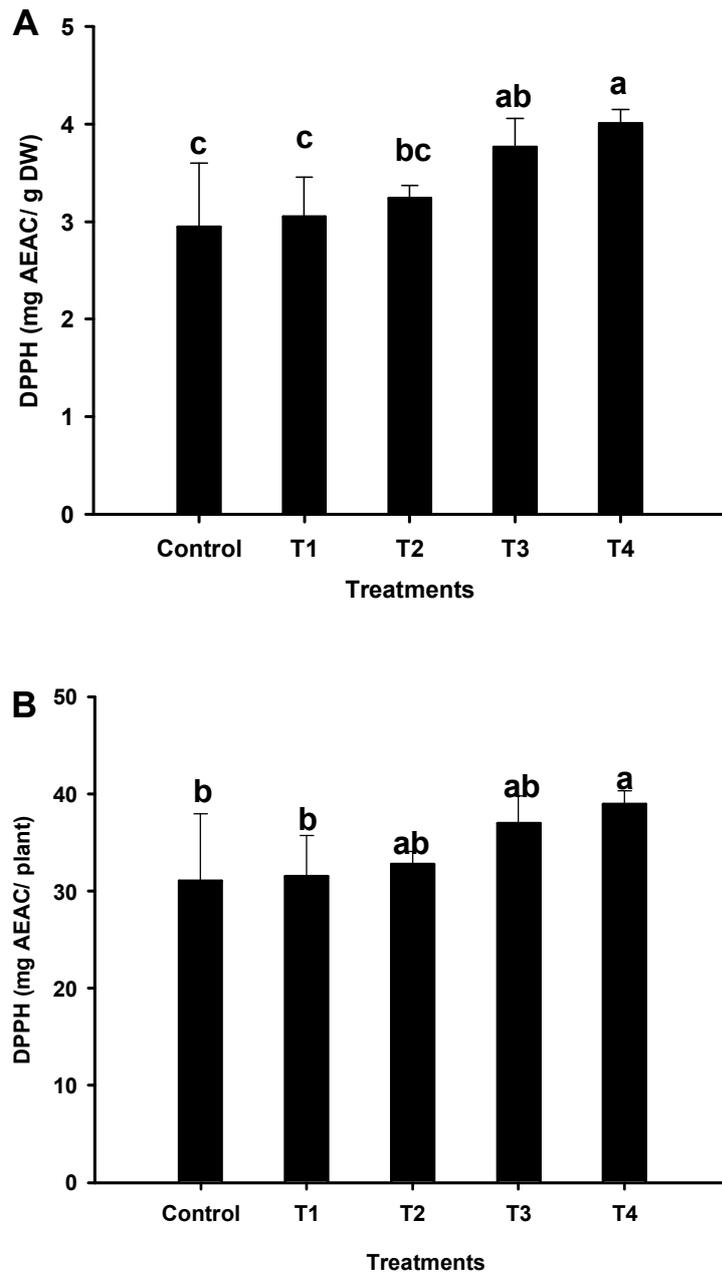
Fig. 7. Antioxidant capacity (DPPH radical scavenging) of kales under different drought stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.
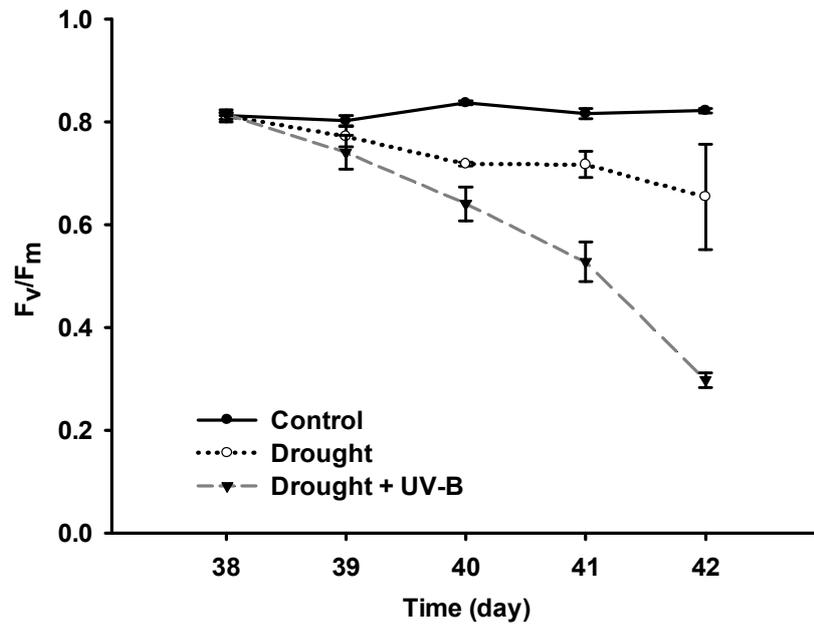
Fig. 8. Changes in leaf chlorophyll fluorescence (F_v / F_m) under different combined drought and UV-B

stress periods. The vertical bars represent the standard error of the mean (n = 3).
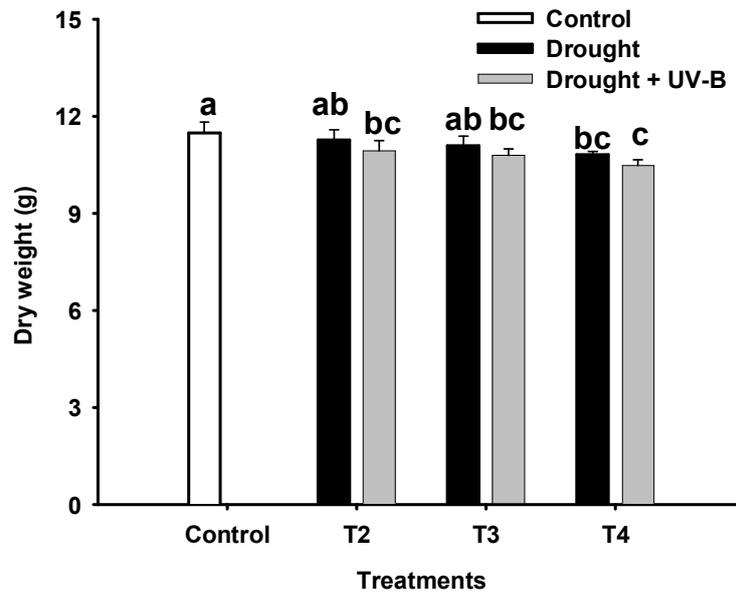
Fig. 9. Dry weight of kales under different stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.
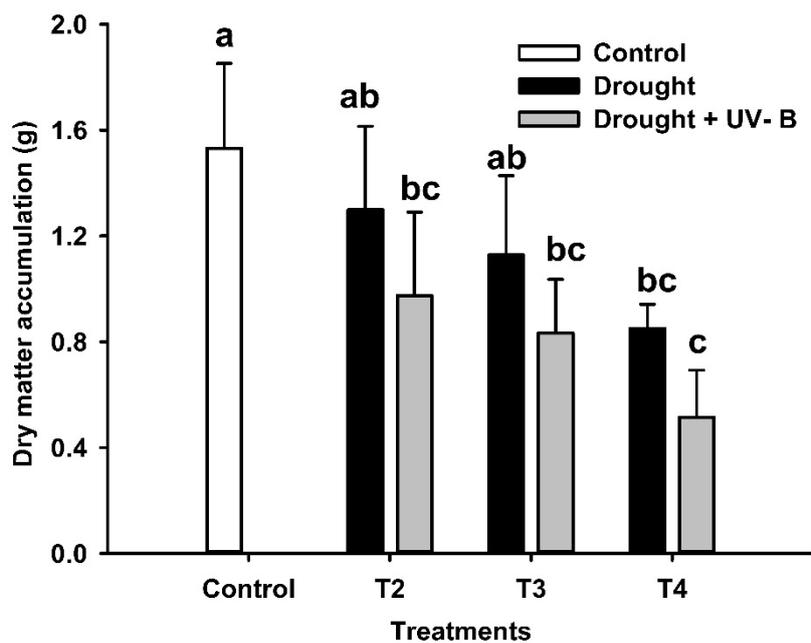
Fig. 10. Dry matter accumulation of kale under different stress treatments (dry weight at 38 day after transplanting = 9.96g). The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.
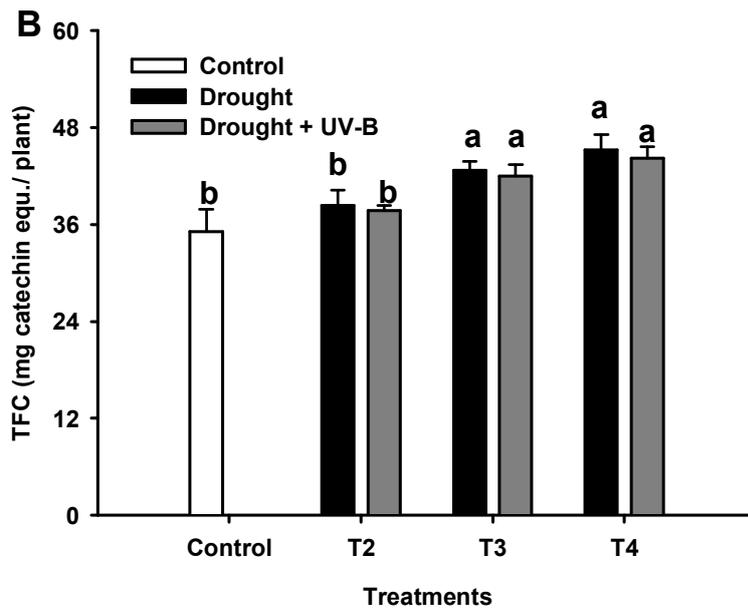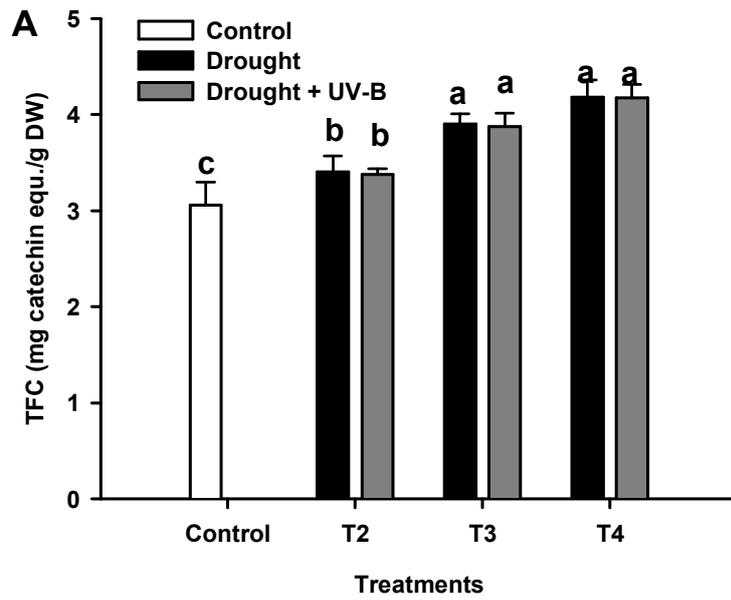
Fig. 11. Total flavonoid compounds (TFC) amounts of kales under different combined stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.
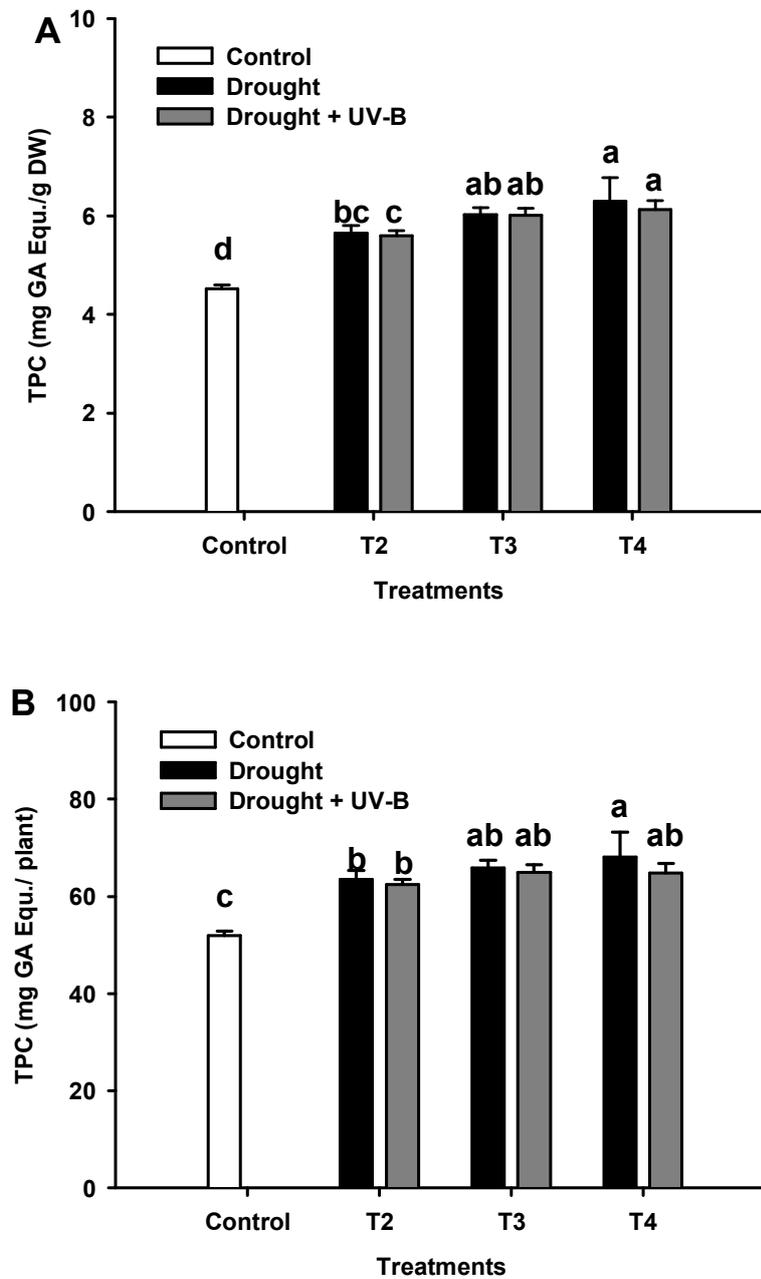
Fig. 12. Total phenolic compounds (TPC) amounts of kales under different combined stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.

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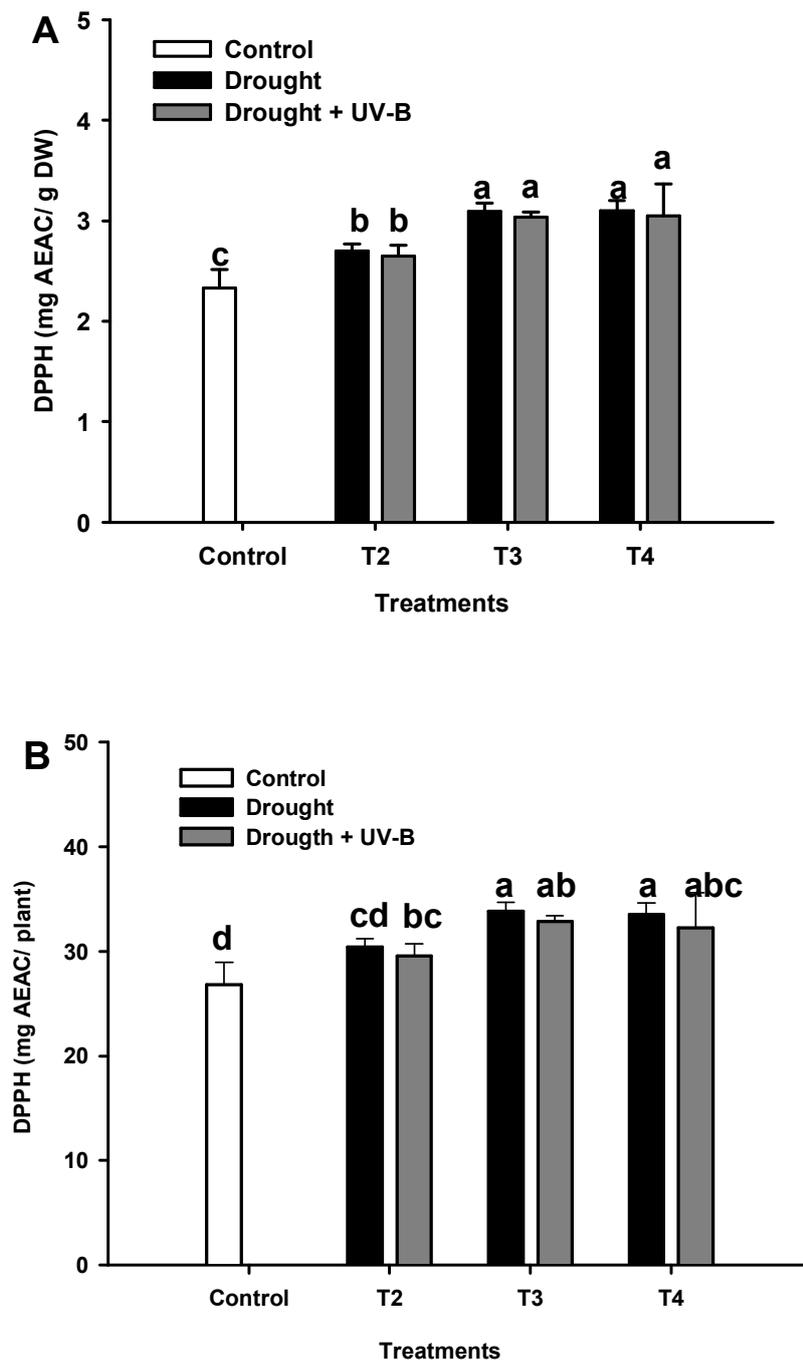
Fig. 13. Antioxidant capacity (DPPH radical scavenging) of kales under different combined stress treatments. The vertical bars represent the standard error of the mean (n = 3). Different letters indicate significant difference ($p < 0.05$) according to Duncan's multiple range test. See Table 1 for the treatments.

ABSTRACT IN KOREAN

케일은 신선 채소, 기능성및 영양 보조 식품의 공급원으로 재배가 증가하고 있다. 비생물적 스트레스 중 수분과 UV-B 는 2 차대사산물을 유발하는데 효과적이며 식물공장에서 편리하게 적용될 수 있다. 본 연구의 목적은 식물공장에서 수경재배 케일의 2 차대사산물 생산을 극대화하기 위하여 수확 전 수분스트레스와 UV-B 처리시기를 결정하는 것이다. 만추콜라드(*Brassica oleracea* L. ca 'Manchoo Collad')는 실내온도 20℃, 광양자속밀도 350μmol·m^{-2}·s^{-1}, 명기/암기 16/8 시간 조건에서 재배하여 정식후 42 일에 수확되었다. 광원은 적색:청색:백색=8:1:1 인 LED 를 사용하였다. 엽록소 형광 (Fv / Fm), 잎 수분포텐셜, 건조중량, 총플라보노이드 함량, 총페놀릭 함량, 항산화 능을 측정하였다. 첫 번째 실험에서 항산화물질을 최대로 하기 위한 처리 조건 결정을 위하여 수확 전 7, 6, 5, 4, 3, 2, 1 일 동안 지속되는 수분스트레스 (T7, T6, T5, T4, T3, T2, T1)를 적용하였다. T7 에서 FV / Fm 은 DAT 36 의 0.811 에서 DAT 39 의 0.563 으로 점진적으로 감소했으며 DAT 40 에서는 0.286 으로 급격히 감소했다. 잎수분포텐셜은 T2 에서 -3.863MPa 에서 T3 에서 -6.27MPa 로 감소했다. 이후 T4 에서 -6.04MPa 로 약간 증가한 이후 계속 감소하였다. 이러한 결과로부터 4 일 미만의 수분스트레스가 잎의 기능을 정상적으로 유지시킨 다는 것을 확인하였다. 다른 처리에 비하여 T2, T3 및 T4 에서 총 플라보노이드 함량, 총 페놀릭 함량 항산화능이 유의적으로 증가하였다. 두 번째 실험에서는 T4, T3, T2 과 대조군을 비고하였고, 대조 및 단일 수분스트레스와 비하여 복합처리에서 엽록소 형광 및 건조중량이 유의적으로 감소하였다. 제시된 총 항산화물질양은 T3 및 T4 에서 가장 높았으나 복합처리에서는 단일 수분스트레스 조건과 차이가 없었다. 따라서 에너지 투입량을 고려할 때, T3 조건이 가장 적합한 것으로 판단되었다.

주요어: 만추콜라드, 총페놀화합물농도, 총플라보노이드농도, 항산화능

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