INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0020/2018/20–1–71–76
DOI: 10.17957/IJAB/15.0363
http://www.fspublishers.org

Full Length Article

Comparison of Physiological Responses and Isoflavone Contents in Soybean *Glycine max* Cultivated under Different Irrigation Conditions

Eun-Hye Kim[1], Kyung-Cheol Lee[2], Seung-Hyun Kim[1] and Ill-Min Chung[1*]
[1]*Department of Applied Life Science, College of Life & Environmental Sciences, Konkuk University, Seoul 05029, Republic of Korea*
[2]*Department of Greenhouse Plants, Bereau of Biological Research & Management, Division of Botanic Research & Management, National Institute of Ecology, Chungcheongnam-do,33657, Republic of Korea*
[*]For correspondence: imcim@konkuk.ac.kr

Abstract

Two soybean cultivars, which had been grown under four different irrigation conditions, were analyzed for changes in isoflavone contents and physiological responses to water conditions. In this study, we examined leaf area (LA), leaf dry weight (LDW), specific leaf area (SLA), root dry weight (RDW), and shoot height (SH) under different irrigation conditions. The 50 mL/9 day irrigation period treatment produced the lowest LA, LDW, RDW, and SH values. In contrast, the drought treatments produced an increase in leaf water saturation deficits (WSD) in the soybean cultivars, Cheongjakong 3 and Taekwangkong. However, leaf water potential and stomatal conductance declined. Photochemical efficiency decreased in the 50 mL/1 day irrigation period treatment, and decrease in growth and development was noted during the 50 mL/9 day drought treatment. The 50 mL/1 day irrigation treatment produced the highest concentrations of total isoflavones in Cheongjakong 3 (2139.2±55.4 μg g^{-1}), whereas the 50 mL/9 day treatment led to the lowest isoflavone concentrations (870.3 ± 10.6 μg g^{-1}). The 50 mL/1 day treatment (2297.3±9.9 μg g^{-1}) and the 50 mL/3 day treatment (2312.4±123.2 μg g^{-1}) produced the highest total isoflavone contents in Taekwangkong, whereas the water deficit conditions led to the lowest contents of total isoflavones.

Keywords: *Glycine max*; Soybean; Irrigation periods; Physiological response; Isoflavones

Introduction

The water content in crop plants changes depending on the crop variety, cultivation period, and climatic conditions. Growth and development fruit quantity and enlargement are affected by soil water conditions (Eom *et al.,* 1983). According to Ryu *et al.* (1996) leaf area and photosynthesis are reduced by low soil moisture, and Griffin and Saxton (1988) reported that lower soil moisture caused reductions in soybean leaf area. They also showed that excess moisture during the soybean vegetative and reproductive growth periods caused the greatest reduction in crop growth rate (CGR) and in the dry weights of various plant tissues. In particular, the damage was greater during the vegetative growth stages than during the reproductive growth stages.

Soybean (*Glycine max* (L.) Merill) is a useful crop widely grown throughout the world. It is particularly popular in Korea, China, Japan, and other Asian countries. Many different parts of the soybean plant are used in various ways. For example, it is eaten as soybean sprouts, and used to make paste, soymilk, oil, and tofu (Kim *et al.,* 2014). Soybean contains various compounds that are beneficial to human health (Whent *et al.,* 2009; Yin *et al.,* 2015) and has been shown to help in the treatment of numerous diseases, such as cancer, cardiovascular disease, and osteoporosis. It contains various functional compounds, such as isoflavones, phenolics, saponins, and other compounds, and is particularly rich in isoflavones and the most common form of phytoestrogen (Kim *et al.,* 2014). Isoflavones are a group of polyphenol compounds which synthesized as antioxidants, mainly in the seed cotyledons and hypocotyl (Noguchi *et al.,* 2007) are an important human dietary constituent and needed for protection and nutrition. Our previous study investigated a number of functional compounds in soybeans, such as isoflavones, phenolic compounds, and anthocyanins, under different environmental conditions. It has been shown that the nutrient composition of crops is affected by environmental conditions, such as temperature and irrigation/precipitation (Riedle *et al.,* 2007).

This study investigated the effects of four different soil irrigation treatments (50 mL water/1 day, 50 mL/3 days, 50 mL/6 days and 50 mL/9 days) on isoflavone contents, photosynthesis, chlorophyll contents, leaf water potentials, and chlorophyll fluorescence reaction in two Korean soybean cultivars.

To cite this paper: Kim, E.H., K.C. Lee, S.H. Kim and I.M. Chung, 2018. Comparison of physiological responses and isoflavone contents in soybean (*Glycine max*) cultivated under different irrigation conditions. *Int. J. Agric. Biol.*, 20: 71–76

Materials and Methods

Plant Materials and Growth Conditions

Two Korean soybean cultivars, Cheongjakong 3 and Taekwangkong, were grown under four different irrigation treatments. The experiment was conducted in a laboratory and greenhouse run by the Department of Applied Life Science. The seeds were sown on July 24, 2012 in pots (30 cm in diameter) containing a silty-loam soil (60% silt, 20% sand, 20% clay). The experiment was a completely randomized design with 20 replicates. After germination, each soybean seed was treated with one of four different irrigation treatments which represented different lengths of time between irrigation events (50 mL water/1 day, 50 mL/3 days, 50 mL/6 days and 50 mL/9 days) from the August 1, 2012 to the October 31, 2012. A temperature and humidity-measuring device (HOBO H08-004-02, ONSET, USA) was installed in the greenhouse at 2-m height above the ground. The soil moisture content was measured using the gravimetric method on the day before irrigation treatment (from the October 2, 2012 to the October 5, 2012), using three replicate measurements.

Photosynthesis Measurement

Photosynthesis was measured the day before irrigation treatment from the September 22 to 30, by using a portable photosynthesis meter (Portable Photosynthesis system, Li-6400, Li-Cor Inc., NE, USA), which measured the fifth leaf from the apex. The net photosynthesis rate (Pn), stomatal conductance (g_s), stomatal transpiration rate (E), and intercellular CO_2 concentration (Ci) were measured in triplicate in a chamber (PPFD 1,000 μmol m^{-2} s^{-1}; CO_2 concentration, 400 ± 2 μmol CO_2 mol^{-1}; air, 500 μmol s^{-1}; temperature 25 ± 2°C) from 10:00 AM to 1:00 PM. Water use efficiency was defined as the ratio of photosynthesis to stomatal water conductance (μmol CO_2 mmol H_2O) (Ashraf *et al.,* 2002).

Chlorophyll Fluorescence and Chlorophyll Content

Chlorophyll fluorescence was measured using a chlorophyll fluorometer (OSI 30P, ADC, UK), which used the same leaf samples as the photosynthesis measurements. Before measurement, the leaf samples were covered using a sample clip for about 20 min (2,000 μmol·m^{-2}·s^{-1}) to prevent light reaching them. Then, the initial fluorescence reaction (Fo), maximum fluorescence reaction (Fm), and photochemical efficiency (Fv/Fm) were measured and analyzed.

Water Potential and Water Saturation Deficits

Water potential (WP) and water saturation deficits (WSD) were recorded and compared for each irrigation treatment. Water potential was measured using a pressure chamber (Model 3100 SAPS Console, Soil Moisture Corp. USA.), which measured the aerial parts of the samples at 6:00 AM. The water saturation deficits were calculated using:

$$WSD = (TW – DW)/(FW – DW)$$

The plant leaves were collected and then weighed to obtain a fresh weight (Fresh Weight, FW). The fully turgid weights (TW) of the samples were determined after soaked in deionized water at 20–25°C without light. Finally, the leaves were dehydrated at 80°C for 48 h to obtain the dry weight (DW) (Yoon, 2001).

Plant Growth Characteristics

We measured the leaf area, root dry weight, leaf dry weight, and stem length growth characteristics of the plants. Leaf area was measured using the LIA 3.2 program (version 0.377e, copy. Kazukiyo Yamato). For dry weight analysis, the plants under each treatment were oven dried at 80°C for 48 h until a constant weight. Specific leaf area was determined using the following equation:

$$\text{Specific leaf area (V)} = \text{leaf area} / \text{leaf dry weight}$$

Analysis of Isoflavones

Soybean seeds were harvested and dried in a freeze-dryer (Freezeone 4.5; Labconco, Kansas, Missouri, USA) under vacuum conditions, and then ground up. The extraction of isoflavones from soybeans was performed according to Wang and Murphy (1994), and the extractions were replicated twice per soybean sample. The extraction solvent consisted of 10 mL of acetonitrile and 2 mL of 0.1 N HCl per sample. The soybean powder (2 g) was extracted using the extraction solvent, and then the extract was stirred for 2 h at room temperature (Green-Sseriker; Vision Scientific Co. Ltd, Bucheon, Gyeonggi-Do, Korea), filtered through No. 42 Whatman filter paper (125 mm × 100 circles; Maidstone, UK), and concentrated using a vacuum evaporator (EYELA; Tokyo Rikakikai, Co. Ltd, Japan) at under 40°C. The residues were re-dissolved in 10 mL of 100% methanol (HPLC grade; J. T. Baker), filtered through a 0.2-μm nylon membrane syringe filter (17 mm, Titan; Sunsri, Rockwood, Tennessee, USA), and transferred into a 2-mL vial. The samples were then analyzed using HPLC.

Isoflavone analysis was performed according to Lee *et al.* (2003) with slight modification. A Younglin YL 9100 HPLC system, with a YL 9120 UV/VIS detector equipped with a YMC ODS AM-303; a 5 μm, 250 × 4.6 mm I.D. column; and a YL 9150 autosampler was used. The mobile phase consisted of solvents A and B. Solvent A was 0.1% glacial acetic acid in distilled water, and solvent B with 0.1% glacial acetic acid in ACN. The solvent flow rate was 1 mL·min^{-1}, the injection volume was 20 μL per sample, and the wavelength of the UV detector was 254 nm. The mobile

phase for solvent A was 0.1% glacial acetic acid in distilled water, and was 0.1% glacial acetic acid in acetonitrile for solvent B. The injection volume was 20 μL, and the gradient used in this experiment was as follows: 0 min, 85% A:15% B; 0–50 min, 65% A:35% B; and 50–60 min, 65% A:35% B. The run time was 60 min, and the flow rate was 1 $mL \cdot min^{-1}$.

The isoflavone standards were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The isoflavone standards were dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich, USA) at several concentrations (25, 50, 100, and 150 $\mu g \cdot mL^{-1}$), and a high linearity (r^2>0.996) was obtained for each compound. The 12 isoflavones were identified by their retention times, and their concentrations were calculated by comparing the peak areas of the samples.

Statistical Analyses

Statistical analyses were conducted using the general linear model (GLM) procedure in the SAS package (Version 9.3; SAS Institute Inc., Cary, NC, USA). All experiments in this study were independently repeated three times. The least significant difference (LSD) test was used with a 0.05 probability level.

Results

In this study, we investigated the air temperature during cultivation periods. The average temperature was 17.7°C during the soybean cultivation period from the August 1 to the October 31. The mean monthly temperature gradually decreased from 24.3°C in August to 18.5°C in September and 10.3°C in October (Fig. 1). The soil water contents (SWC) declined as the period between irrigations increased, particularly the SWC contents for the 50 mL/9 day treatment, which had the lowest SWCs at 29–31%. The water saturation deficit (WSD) and leaf water potential (WP) results gave an indication of the leaf moisture conditions. The Cheongjakong 3 WPs and the Taekwangkong WSDs for the 50 mL/1 day and 50 mL/3 day irrigation period treatments were not significantly different. However, there was a significant 3.2-fold increase between the 50 mL/6 day treatment and the 50 mL/1 day and 50 mL/3 day irrigation treatments for Cheongjakong 3, and about a 2-fold increase for Taekwangkong. The Cheongjakong 3 and Taekwangkong WSDs for the 50 mL/9 day irrigation treatments were 4.2 and 3.4-folds higher than the 50 mL/1 day and 50 mL/3 day irrigation treatments, respectively. The leaf water potentials gradually decreased from –0.1 MPa (50 mL/1 day) to –0.7 MPa (50 mL/9 day), but there were no differences between the two cultivars.

There were significant differences in leaf area (LA), leaf dry weight (LDW), root dry weight (RDW) and shoot height (SH) between the different irrigation periods. The 50 mL/9 day irrigation period produced the lowest LA, LDW, RDW, and SH values. Specific leaf area (SLA) was lowest in the 50 mL/6 day and 50 mL/9 day treatments for Taekwangkong (Table 1), but there were no significant differences in SLA for Cheongjakong 3.

Cheongjakong 3 and Taekwangkong showed decreases in leaf water potential and stomatal conductance when subjected to water deficits. Drought conditions also reduced the stomatal transpiration rate (E) and intercellular CO_2 concentration due the reductions in O_2 and CO_2 exchange. However, net photosynthesis rate was not significantly affected by any of the treatments.

The water use efficiency (WUE) increased when the stomatal transpiration rate declined. These responses were caused by stomatal closing to prevent moisture loss in the water stressed plants. The 50 mL/6 day treatment produced the highest levels of photochemical reaction efficiency, while the 50 mL/1 day treatment led to the lowest photochemical reaction efficiency levels.

The 50 mL/1 day irrigation treatment produced the highest total isoflavone concentrations in Cheongjakong 3 (2139.2 ± 55.4 $\mu g\ g^{-1}$), while the 50 mL/9 day treatment (870.3 ± 10.6 $\mu g\ g^{-1}$) led to the lowest concentrations. The 50 mL/3 day and 50 mL/1 day treatments produced the highest total isoflavone concentrations in Taekwangkong (2312.4 ± 123.2 $\mu g\ g^{-1}$ and 2297.3 ± 9.9 $\mu g\ g^{-1}$, respectively), whereas the 50 mL/9 day treatment led to the lowest isoflavone concentrations (513.8 ± 8.6 $\mu g\ g^{-1}$). Of the 12 isoflavones identified, malonyl-genistin concentrations were highest in Cheongjakong 3 under the 50 mL/1 day irrigation treatment (1314.2 ± 13.7 $\mu g\ g^{-1}$) and highest in Taekwangkong under the 50 mL/3 day irrigation treatment (1310.4 ± 84.7 $\mu g\ g^{-1}$) (Table 2).

Discussion

The results of present study showed that leaf area is affected by genetic and environmental factors, and soil water content is one of the most important factors (Nagasuga *et al.,* 2014; Ahmed *et al.,* 2017). An improvement in soil water conditions through rainfall or irrigation can increase both crop growth rate (CGR) and LAI (Nagasuga *et al.,* 2014). A previous study found that water stress at the flowering stage caused the largest reductions in leaf area, total chlorophyll contents, dry weights and photosynthetic rates in soybean (Kataria and Singh, 2014). Water stress accelerates leaf senescence of soybean and reduces yield by reducing the number of seeds (de Souza *et al.,* 1997).

The changes in water use efficiency correlated with plant growth and increases in water use efficiency under water deficit conditions (Wright *et al.,* 1993). Photochemical reaction efficiency (Fv/Fm) is an important physiological indicator of changes to photochemical energy efficiency in plants subjected to abiotic stress (Krause and Weiss, 1991; Rascher *et al.,* 2000).

Table 1: The soil water contents (SWC), water potentials (WP), water saturation deficits (WSD), and growth characteristics of soybean cultivated under different irrigation treatments

Cultivar	Treatment (days)	SWC (%)	WP (-MPa)	WSD (%)	LA (cm^2)	LDW(g)	SLA ($cm^2 \cdot g^{-1}$)	RDW (g)	SH (mm)
CJ-3	1	53.60d	0.10a	4.20a	50.80b	0.17b	425.60^{ns}	1.11b	1049.50 b
		(0.9)	(0.015)	(1.04)	(2.1)	(0.069)	(357.4)	(0.12)	(53.3)
	3	45.80c	0.19b	5.29a	47.40b	0.10 ab	481.20;	1.11b	1077.00 b
		(0.1)	(0.015)	(0.33)	(9.7)	(0.046)	(116.0)	(0.10)	(75.3)
	6	37.50b	0.49c	13.50b	41.80b	0.13ab	358.80	0.94b	1080.00 b
		(1.3)	(0.026)	(2.99)	(9.0)	(0.025)	(140.8)	(0.43)	(129.8)
	9	30.50a	0.73d	17.81c	22.20a	0.06a	441.00	0.52a	777.80 a
		(1.2)	(0.044)	(0.50)	(3.2)	(0.013)	(166.7)	(0.06)	(97.4)
TK	1	52.60 d	0.11 a	5.62 a	33.80 a	0.10ab	351.20 ab	1.04b	1061.00 b
		(0.9)	(0.010)	(0.77)	(5.2)	(0.014)	(30.6)	(0.12)	(66.1)
	3	44.00 c	0.19b	6.40 a	53.50 b	0.12ab	471.10 b	1.05b	1108.70 b
		(0.4)	(0.015)	(0.74)	(4.8)	(0.014)	(26.8)	(0.10)	(130.3)
	6	37.20 b	0.48c	11.07 b	40.00a	0.12b	307.60 a	1.07b	1075.00 b
		(0.3)	(0.021)	(0.39)	(12.7)	(0.037)	(15.4)	(0.15)	(83.3)
	9	29.40 a	0.72d	19.20 c	27.30 a	0.09a	335.40 a	0.55a	755.30 a
		(0.3)	(0.015)	(1.19)	(7.2)	(0.021)	(158.3)	(0.07)	(92.7)

Each value is expressed as the mean ± SD (n = 6). Mean separation within columns was calculated by Tukey's HSD at the 5% level.
CJ-3, Cheongjakong 3; TK, Taekwangkong; LA, leaf area; LDW, leaf dry weight; SLA, specific leaf area; RDW, root dry weight; SH, shoot height

Table 2: Comparison of 12 Isoflavones found in soybean plants cultivated under different water conditions

Isoflavones		Cheongjakong 3				CV	LSD(0.05)	Taekwangkong				CV	$LSD_{(0.05)}$
		1	3	6	9	(%)		1	3	6	9	(%)	
		----------μg·g-1----------											
Glycoside	Din	90.70± 8.40a	71.30± 2.10b	46.60± 4.30c	46.30± 0.60c	7.6	5.8	96.50± 8.40a	91.80± 3.70a	36.60± 2.60b	19.40± 0.30c	7.7	5.7
	Gly	30.40± 3.50b	33.50± 2.10a	19.40± 1.60c	14.60± 1.10d	9.2	2.7	28.60± 3.80a	25.40± 0.50b	16.80± 0.80c	12.60± 0.90d	9.5	2.4
	Gin	153.00± 10.60a	80.80± 2.30b	64.50± 4.30c	42.30± 1.20d	6.9	7.0	99.10± 8.60b	113.40± 5.50a	33.90± 3.20c	17.30± 0.70d	8.1	6.5
Malonyl-glycoside	Mdin	403.80± 13.20a	309.90± 17.60b	203.60± 3.10b	257.00± 1.70c	15.0	53.2	633.50± 12.10a	575.00± 35.10b	250.70± 0.80c	130.80± 0.50d	4.7	22.4
	Mgly	106.80± 7.10a	121.90± 93.00b	74.70± 2.00c	54.00± 1.10d	6.7	7.2	185.90± 12.30a	164.50± 9.20b	120.30± 1.8c	87.00± 7.60d	6.2	10.4
	Mgin	1314.20± 13.70a	852.70± 3.90b	619.90± 14.90c	468.30± 13.50d	1.5	14.8	1224.80± 22.70b	1310.40± 84.70a	489.70± 4.90c	231.90± 5.00d	5.4	52.9
Acetyl-glycoside	Acdin	3.00± 2.60a	3.50± 1.40a	3.60± 0.40a	2.70± 2.50a	61.1	2.3	4.80± 0.36a	4.70± 0.20a	2.20± 0.90b	2.80± 1.50b	25.0	1.1
	Acgly	15.50± 2.20a	10.20± 2.60b	10.40± 0.40b	6.30± 1.90c	18.5	2.4	9.50± 0.50a	9.70± 0.60a	6.30± 0.70b	5.60± 1.90b	13.9	1.3
	Acgin	6.90± 2.00c	10.30± 3.20b	14.30± 1.30a	5.80± 2.70c	25.8	2.9	1.50± 0.60b	5.60± 1.20a	6.60± 3.00a	2.90± 0.60b	39.7	1.9
Aglycone	Dein	8.40± 0.10a	6.30± 0.50b	3.40± 0.40c	4.60± 0.50d	7.2	0.5	7.80± 0.30;a	6.90± 0.80b	3.60± 0.70 c	2.00± 0.60d	12.8	0.7
	Glein	2.00± 0.80ab	1.40± 0.10b	2.70± 1.50a	2.10± 0.60ab	44.3	1.1	2.00± 0.30a	1.40± 0.10b	1.50± 0.20b	1.50± 0.50b	17.7	0.3
	Gein	4.60± 0.30a	2.80± 0.10b	1.90± 0.10c	1.50± 0.10d	6.8	0.2	3.10± 0.20b	3.60± 0.40a	1.30± 0.30c	0.10± 0.10d	13.6	0.3
Total		2139.20± 55.40a	1504.70± 21.00b	1065.10± 28.70c	870.30± 10.60d	3.8	64.8	2297.30± 9.90a	2312.40± 123.20a	969.60± 2.50b	513.80± 8.60c	4.1	74.6

Each value is expressed as the mean ± SD (n = 6). 1, 3, 6, and 9 refer to days between irrigation events; Din; Daidzin, Gly; Glycitin, Gin; Genistin, Mdin; Malonyldaidzin, Mgly; Malonylglycitin, Mgin; Malonylgenistin, Acdin; Acetyldaidzin, Acgly; Acetylglycitin, Acgin; Acetylgenistin, Dein; Daidzein, Glein; Glycitein, Gein; Genistein

From our results, excess moisture in soil is a stress factor that can affect plant growth. In contrast, drought conditions caused an increase in the leaf water saturation deficits (WSD). Consequently, Cheongjakong 3 and Taekwangkong WSDs increased in the water deficit treatments, whereas WP and stomatal conductance declined (Table 1, Fig. 2). This meant that there was a fall in the stomatal transpiration rate and CO_2 concentration in mesophyll cells as the water use efficiency increased. Photochemical efficiency decreased in the 50 mL/1 day irrigation treatment due to excess moisture, whereas drought conditions in the 50 mL/9 day treatment led to a decrease in plant growth and development.

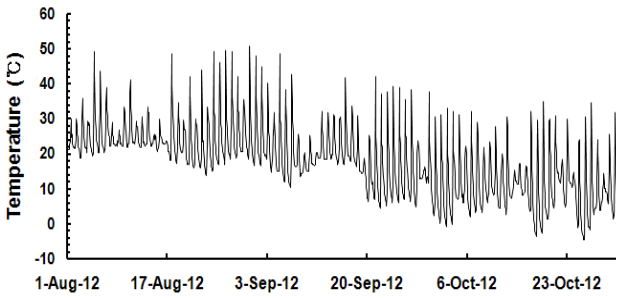

Fig. 1: Change in temperature during the soybean plant growth period

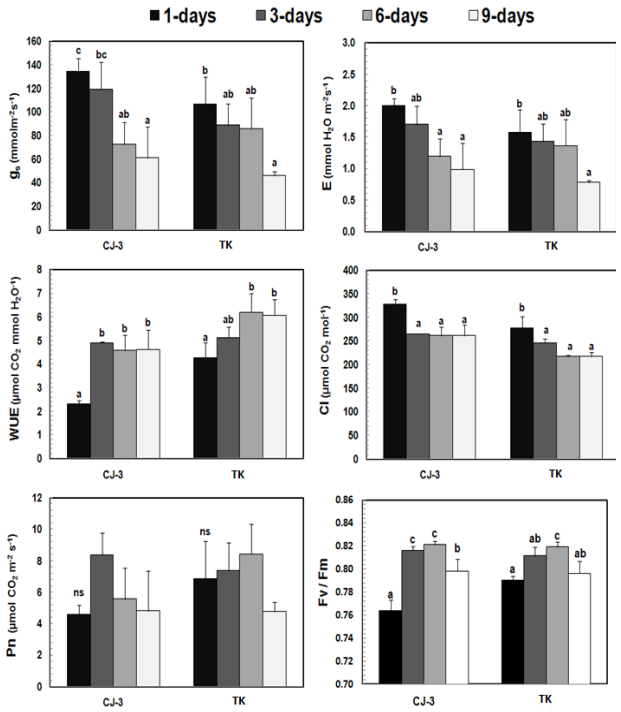

Fig. 2: Photosynthetic parameter changes in soybean grown under different irrigation treatments. CJ-3, Cheongjakong 3; TK, Taekwangkong; g_s, stomatal conductance; E, stomatal transpiration rate; WUE, water use efficiency; Ci, intercellular CO_2 concentration; Pn, net photosynthesis rate; Fv/Fm, photochemical efficiency. Significant differences between columns are marked with different letters ($P < 0.05$)

Isoflavone is major functional compounds of soybean crops. Isoflavone concentrations showed variation by many factors. Environmental factors, such as temperature and irrigation, also affect isoflavone composition (Riedle *et al.*, 2007). Seasonal rainfall deficits account for much annual variation in yield of crop. In addition there was a growing concern about environmental effects on soybean seed composition such as daily mean temperature, daily maximum and minimum temperature and daily mean solar radiation (Carrera *et al.*, 2009). The total concentration of isoflavone in soybean was influenced by genotype, location and cultivation year (Wang and Murphy, 1994). Tsukamoto *et al.* (1995) reported that reduction of isoflavone concentration was varied by high temperature during seed development of soybean. Another study indicated that content of isoflavone affected by ultra-violet radiation (Dixit *et al.*, 2010).

A previous study indicated that irrigated soybean cultivars showed increases in total isoflavone contents (Bennett *et al.*, 2004). The contents of seed oil and protein in soybean are influenced by water deficit (Carrera *et al.*, 2009). Therefore, the results of this experiment and previous studies showed that well-watered conditions improve isoflavone concentrations and could improve the growth of soybean cultivars (Table 2). A limited number of soybean varieties could be used for initial evaluation to identify isoflavones under different irrigation conditions. More extensive test would be desirable to select a best irrigation condition.

Conclusion

Application of 50 mL water in one and three days produced the highest total isoflavone contents while prolonged water deficit led to the lowest contents of total isoflavones.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2014R1A2A2A01002202).

References

Ahmed, Z., E.A. Waraich, R. Ahmad and M. Shahbaz, 2017. Morpho-physiological and biochemical responses of *camelina* (*Camelina sativa crantz*) genotypes under drought stress. *Int. J. Agric. Biol*., 19: 1–7

Ashraf, M., M. Arfan, M. Shahbaz, M. Ahma and A. Jamil, 2002. Gas exchange characteristics and water relations in some elite skra cultivars under water deficit. *Photosynthetica*, 40: 615–620

Bennett, J.O., O. Yu, L.G. Heatherly and H.B. Krishnan, 2004. Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. *J. Agric. Food Chem.*, 52: 7574–7579

Carrera, C., M.J. Martínez, J. Dardanelli and M. Balzarini, 2009. Water deficit on the relationship between temperature during seed fill period and soybean seed oil and protein concentrations. *Crop Sci.*, 49: 990–998

de Souza, P.I., D.B. Egli and W.P. Bruenung, 1997. Water stress during seed filling and leaf senescence in soybean, *Agron. J.*, 89: 807–812

Dixit, A.K., D. Bhatnagar, V. Kumar, A. Rani, J. Manjaya and D. Bhatnagar, 2010. Gamma irradiation induced enhancement in isflavones, total phenol, anthocyanin and antioxidant properties of varying seed coat colored soybean. *J. Agric. Food Chem.*, 58: 4298–4302

Eom, K.C., E.R. Son and S.H. Yoo, 1983. Fertilizer responses of Chinese cabbage to soil water potential. *J. Kor. Soc. Soil Sci. Fert.*, 16: 98–105

Griffin, J.L. and A.M. Saxton, 1988. Response of solid seeded soybean to flood irrigation. II. Flood duration. *Agron. J.*, 80: 885–888

Kataria, N. and N. Singh, 2014. Assessing the effects of applied potassium on selected *Vigna radiata* L. genotypes under water deficit. *Int. J. Adv. Res.*, 10: 369–385

Kim, E.H., O.K. Lee, J.K. Kim, S.L. Kim, J.H. Lee, S.H. Kim and I.M. Chung, 2014. Isoflavones and anthocyanins analysis in soybean (*Glycine max* (L.) Merill) from three different planting locations in Korea. *Field Crops Res.*, 156: 76–83

Krause, G.H. and E. Weiss, 1991. Chlorophyll fluorescence and photosynthesis: the basis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 42: 313–349

Lee, S.J., W.K. Yan, J.K. Ahn and I.M. Chung, 2003. Effect of year, site, genotype and their interactions on various soybean isoflavones. *Field Crop Res.*, 81: 181–192

Nagasuga, K., M. Kadowaki, S. Uchida, H. Kaji, A. Fukunaga and T. Umezaki, 2014. Effect of water condition on soybean (*Glycine max* L.) plant growth after flowering. *Environ. Control Biol.*, 52: 221–225

Noguchi, A., A. Satio, Y. Homma, M. Nakao, N. Sasaki, T. Nishino, S. Takahashi and T. Nakayama, 2007. A UDP-glucose: isoflavone 7-*O*-glucosyltransferase from the roots of soybean (*Glycine max*) seedlings: purification, gene cloning, phylogenetics and an implication for an alternative strategy of enzyme catalysis. *J. Biol. Chem.*, 282: 23581–23590

Rascher, U., M. Liebig and U. Lttge, 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant Cell Environ.*, 23: 1397–1405

Riedle, K.M., J.H. Lee, M. Renita, S.K. St Martin, S.J. Schwartz and Y. Vodovotz, 2007. Isoflavone profile, phenol content, and antioxidant activity of soybean seeds as influenced by cultivars and growing location in Ohio. *J. Sci. Food Agric.*, 87: 1197–1206

Ryu, Y.H., S.H. Lee, S.D. Kim and M. Kokuban, 1996. Effects of soil moisture content on leaf water potential and photosynthesis in soybean plants. *Kor. J. Crop Sci.*, 41: 168–172

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubum, K. Okubo and K. Kitamura, 1995. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.*, 43: 1184–1192

Wang, H.J. and P.A. Murphy, 1994. Isoflavone content in commercial soybean foods. *J. Agric. Food. Chem.*, 42: 1666–1673

Whent, M., J. Hao, M. Slavin, M. Zhou, J. Song, W. Kenworthy and L. Yu, 2009. Effect of genotype, environment, and their interaction on chemical composition and antioxidant properties of low-linolenic soybeans grown in Maryland. *J. Agric. Food Chem.*, 57: 10163–10174

Wright, G.C., K.T. Hubick, G.D. Farquhar and R.C. Nageswara Rao, 1993. Genetic and environmental variation transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut, *In: Stable Isotope and Plant Carbon-water Relations*, pp: 247–267. Ehleringer, J.R., A.E. Hall and G.D. Farquhar (eds.). Academic Press, New York

Yin, Y., R. Yang, Y. Han and Z. Gu, 2015. Comparative proteomic and physiological analyses reveal the protective effect of exogenous calcium on the germinating response to salt stress. *J. Proteomics*., 113: 110–126

Yoon, T.M., 2001. Water relations of 'fuji' apple trees in soil water controlled orchard. *Hortic. Environ. Biotech.*, 42: 549–552

(Received 07 January 2017; Accepted 26 April 2017)