Responses of three soybean cultivars exposed to UV-B radiation

Ning Gao[1], Liyan Yang[2*]

[1,2*]College of Life Science, Shanxi Normal University, Linfen, Shanxi 041004, China

***Abstract*—** *To understand the responses of various soybean (Glycine max) cultivars to ultraviolet-B, Dongnong 42, Zhonghuang 57 and Jin 36 were cultured either under UV-B radiation (10.08 kJ / m-2 UV-B) or without UV-B for 25 days. Under UV-B irradiation, significant difference were observed in plant height, nodes length, contents of soluble sugar, proline, protein, flavionoids, GSH, ASA, relative content of anthocyanins, also the emzymes activities of APX ,SOD and GR. The lowest plant height was presented in Jin 36, followed Zhonghuang 57 and Dongnong 42. The node length of plant decreased from the first node to the fourth one, and the maximal reduction was observed in Jin 36. Contents of protein, flavionoids, GSH, ASA and anthocyanins as well as emzyme activities of APX, SOD and GR significantly increased under UV-B irradiation where content of protein and activities of emzyme APX, SOD and GR were higher in Zhonghuang 57 than those in Dongnong 42 and Jin 36; higher GSH, ASA, flavonoids, as well as the anthocyanins were showed in Donghong 42. Increased proline content was observed in the three cultivars upon UV-B (p<0.05) where Zhonghuang 57 >Dongnong 42 > Jin36; for the soluble sugar content, the order was Zhongnong 57>Jin 36>Dongnong 42 (p<0.05). Various soybean cultivars showed varied physiological responses upon UV-B.*

***Keywords*— *UV-B, soybean, cultivars, physiological characters, response*.**

I. INTRODUCTION

Ultraviolet-B (wavelengths from 280 to 320 nm) radiance resulting from depletion in the stratospheric ozone layer affects plant growth and metabolism with morphological, physiological, and biochemical changes is well documented (1). UV-B is recognized as one of the major environmental regulators that control gene expression, celluar and metabolic activities (2).

Plants have adopted the specific ways to deal with UV-B radiation. Leaf thickening is one mechanism which may mitigate the detrimental effects of UV-B by greater tissue absorption (3). Epidermal flavonoid accumulation is known as another adaptive mechanism which can alleviate harm on mesophyll that related to photosynthesis (4, 5). Enzymatic and non-enzymatic antioxidants that defense reactive oxygen species (ROS) induced by enhanced UV-B radiation (6, 7) has been illustrated too (8). UVR8, plant photoreceptor of UV-B, was a newly discovered mechanism which involved in the UV-B induced pathway (9-11).

Different species showed various responses, so is the cultivars of the same species (12, 13). It has been well established that dicotyledonous plants are more sensitive to UV-B than monocotyledons. At the present study, three soybean cultivars (Glycine max) were exposed to UV-B radiation, the growth and physiological traits were investigated.

II. MATERIAL AND METHOD

2.1 Plant material and treatment

Soybean (Glycine max) seeds were provided by the Wheat Research Institute, Shanxi Academy of Agricultural Sciences (SAAS), China. They are selected for uniform size and sterilized for 10 min with 0.1% HgCl2 and washed for 20 min with running water. Five seeds were sow in pots (15×20cm), and there were 6 treatments, with 3 replications each. When seeds were germinating, irradiation treatment was applied according to the light / dark period as given (Table 1).

UV-B radiation was generated by a filtered lamp (30 W, 297 nm, Qin brand, Baoji Lamp Factory, Baoji City, China.). The lamps were hung above the plants 40cm, the irradiation was obtained by adjusting the distance between the lamps and the pots. Daily dose were 0 and 10.08 kJ m-2 UV-B, respectively.

TABLE 1
LIGHT / DARK TREATMENT

Treatment	UV-B irritation (daily dose)		Dark
	0	10.08 kJ·m-2	
CK1	+	-	16 hr·d-1
B1	-	+	16 hr·d-1
CK2	+	-	16 hr·d-1
B2	-	+	16 hr·d-1
CK3	+	-	16 hr·d-1
B3	-	+	16 hr·d-1

CK1-3 soybean cultivars without UV-B radiation; B1-3: soybean cultivars with UV-B radiation

2.2 Measurement of plant height

Plant height and internode length were measured twice weekly, and three seedlings per replication were randomly selected from each treatment for the downstream measurements.

2.3 Soluble sugars analysis

Soluble carbohydrates were determined according to the anthrone method (14). One ml of leaf extraction was added to 3 ml (final volume) solution containing 1.08 M H2SO4, 1.09 mM thiourea and 2.1 mM anthrone. The mixture was heated at 100°C for 10 min and the absorbance at 620 nm was recorded. A calibration curve with glucose was a standard.

2.4 Assay of free prolines

Proline content was assayed followed the method described by Bates (15). Briefly, extraction from leaf was mixed with acid-ninhydrin and glacial acetic acid, and then heated at 100°C in a water bath for 10 min, the reaction was terminated on ice followed by toluene added in. The proline-ninhydrin chromophore in the upper phase was used to measure absorbance based on dry weight.

2.5 Determination of total protein content

Total protein content was quantified according to Bradford (16) where BSA (bovine serum albumin) was used as the standard.

2.6 Contents of flavonoids and relative anthocyanins assay

Leaves kept at 55° were frozen with liquid N2 and ground to fine powder then suspended with 60% aqueous alcohol. The supernatant was used to detect flavonoids. Briefly, 5% NaNO2 was added then the mixture was incubated, followed by adding Al(NO3)3 and NaOH. Absorbance was recorded and flavonoids were calculated on dry weight base. The relative anthocyanins content was assayed according to Zhang (17).

2.7 Analysis of SOD, APX, GR activity and ascorbic acid,GSH level

Superoxide Dismutase (SOD) activity was determined based on the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD activity is defined as the amount of enzyme that inhibite the NBT reduction by 50%. Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (18).Glutathione reductase (GR) activity and Glutathione (GSH) content were measured according to Ban Wang et al. (19). Ascorbic acid (ASA) content was determined by Mukherjee and Choudhouri (20).

2.8 Statistical analysis

Statistical significance is estimated at $P < 0.05$ followed Duncan's multiple range test. All data were given means ± SD.

III. RESULTS

3.1 Effects of UV-B irradiance on seedling height and node length of soybean

TABLE 2
RESPONSES OF PLANT HEIGHT AND NODE LENGTH UNDER UV-B RADIATION

Varieties	Length of nodes (cm)				Plant height cm
	1st	2nd	3rd	4th	
CK1	10.7±0.41a	8.6±0.52 a	11.2±0.67a	6.6±0.56a	44.5±0.53a
B1	9.5±0.63b	6.7±0.46d	7.9±0.35c	4.5±0.23c	34.3±0.48b
CK2	10.1±0.78a	7.8±0.93c	8.5±0.82b	5.3±0.23b	36.8±0.84b
B2	9.3±0.81b	6.9±0.73d	6.4±0.58d	3.5±0.20d	29.5±0.70c
CK3	9.5±1.12b	8±0.85b	7.7±0.95c	4.0±0.26c	33.8±0.97b
B3	8.5±0.63c	7±0.54d	4.9±0.69d	0.8±0.05e	28.8±0.62c

Different letters indicate significant differences between treatments at 0.05 level according to Duncan's multiple range test (n=4), the same below. CK_1: *Dongnong 42* without UV-B, CK_2: *Zhonghuang 57* without UV-B, CK_3: *Jin 36* without UV-B, B_1: *Dongnong 42* with UV-B; B_2: *Zhonghuang 57* with UV-B, B_3: *Jin 36* with UV-B.

UV-B treatment significantly suppressed plant height where they were 22.92%, 19.84% and 14.79% lower in *Dongnong 42*, *Zhonghuang 57* and *Jin 36* than the control *($p<0.05$)*. The lowest and the highest seedling height were observed in *Jin 36* and *Dongnong 42*, respectively.

The dwarf induced by UV-B radiation presented from the first node for each variety. For the first node the reduction level was *Dongnong 42>Jin 36>Zhonghuang 57*. The second node was much dwarfed than the first one where they were decreased by 22.09% in *Dongnong 42* group, 24.71% in *Zhonghuang 57* and12.50% in *Jin 36*. Considerable decreasing was found in the third node where the shortest node of 2.5cm showed in *Jin 36*. In the fourth node, maximum inhibition showed where it was 0.8 cm in *Jin 36* group *($p<0.05$)*（**Table 2**）.

3.2 Effects of UV-B irradiance on contents of flavonoids, anthocyanins and protein

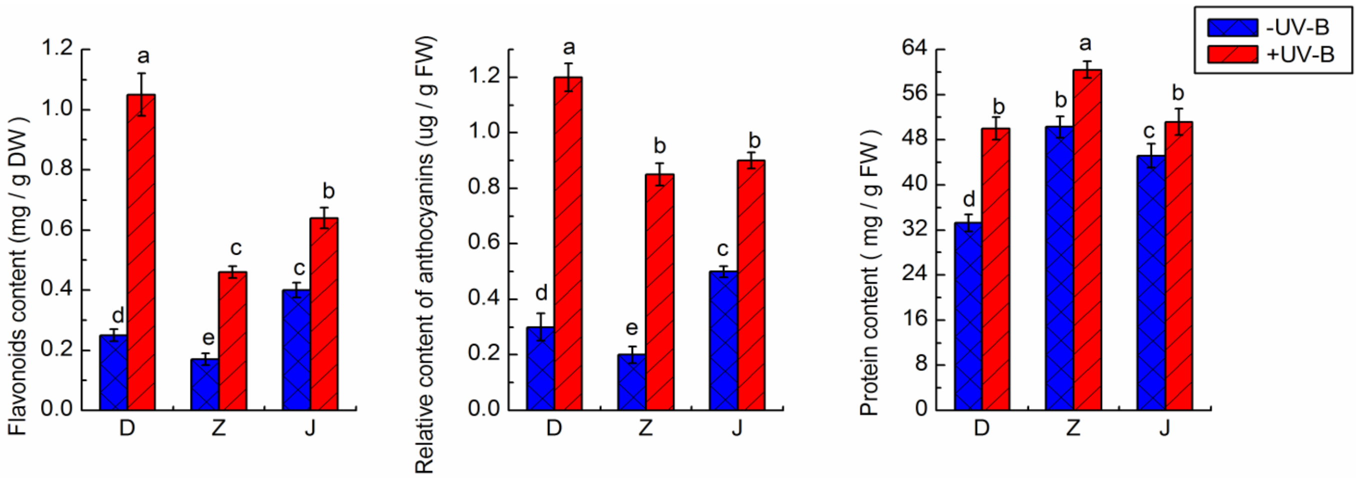

FIG.1 FLAVONOIDS, ANTHOCYANINS AND PROTEIN CONTENT UNDER UV-B RADIATION
Different letters indicate significant differences between treatments at 0.05 level according to Duncan's multiple range test (n=4). D: *Dongnong 42*; Z: *Zhonghuang 57;* J: *Jin 36*

The content of flavonoids in different soybean cultivars varied where *Jin 36 >Dongnong 42 > Zhonghuang 57*, but it increased in all groups when exposed to UV-B. The maximum content was observed in *Dongnong 42 (1.05mg/g)*, followed by *Jin 36 (0.64mg/g)* and *Zhonghuang 57 (0.46mg/g)*. It raised by 325%, 165% and 61% in *Dongnong42*, *Zhonghuang 57* and *Jin 36* groups, respectively *($p<0.05$)*.

The relative content of anthocyanins was significantly increased when exposed to UV-B radiation. It improved 400%, 325% and 80% in *Dongnong 42*, *Zhonghuang 57* and *Jin 36*, *($p<0.05$)* respectively. The highest content was showed in *Dongnong 42*, and it was the lowest in *Zhonghuang 57*.

After 25 day (from germination) of UV-B irradiation, protein content was significant increased *($p<0.05$)* where they increased 50.36%, 20.27% and 13.29% in *Dongnong 42*, *Zhonghuang 57* and *Jin 36*, respectively *($p<0.05$)*. Maximum protein content was found in *Zhonghuang 57* whenever with or without UV-B radiation (**Fig.1**).

3.3 Effects of UV-B irradiation on contents of proline and soluble sugar in various soybean cultivars

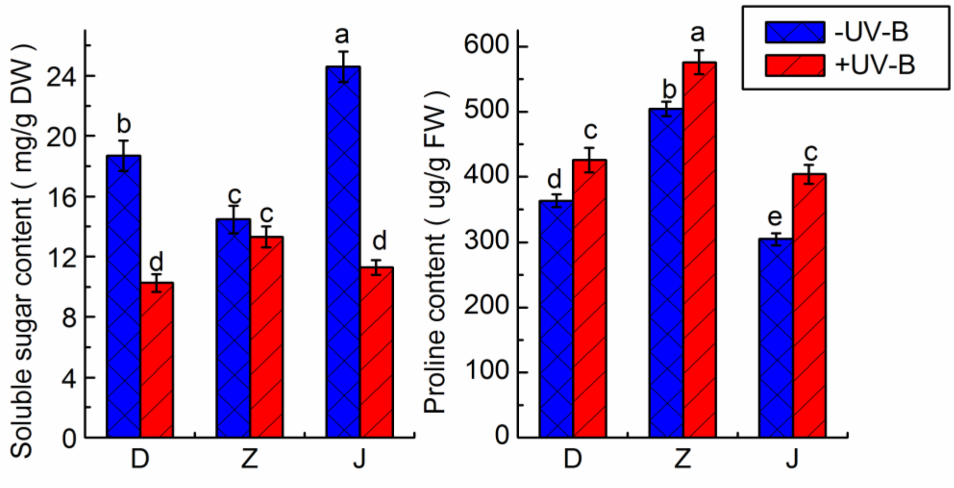

FIG. 2 UV-B AFFECTS CONTENTS OF PROLINE AND SOLUBLE SUGAR
Different letters indicate significant differences between treatments at 0.05 level according to Duncan's multiple range test (n=4) D: *Dongnong 42;* Z: *Zhonghuang 57*; J: *Jin 36*

Soluble sugar decreased under UV-B radiation and it was not the case for proline. For *Dongnong 42*, soluble sugar decreased by 45.09% and proline increased by 17.18% *($p<0.05$)*, they were 7.87% lower *($p>0.05$)* and 14.25% higher *($p<0.05$)* in *Zhonghuang 57*; 25.14% lower and 32.85% higher in *Jin 36* (*$p<0.05$*), respectively. The highest proline level and sugar both appeared in *Zhonghuang 57*(**Fig.2**).

3.4 Effects of UV-B irradiation on antioxidative enzyme activities of SOD、GR and APX

After 25 days of UV-B radiation, activities of antioxidative enzyme as SOD, GR and APX in the three cultivars considerably increased where they increased 45.22%, 62.49% and 96.91% in *Dongnong 42 ($p<0.05$)*; 39.10%, 65.82%, 55.44% in *Zhonghuang 57 ($p<0.05$)*, 32.39%, 66.02%,48.54% in *Jin 36($p<0.05$)*. The highest SOD, GR and APX activity turn up in *Zhonghuang 57* group, and the lowest SOD GR and APX activities occured in *Dongnong 42*(**Fig.3**).

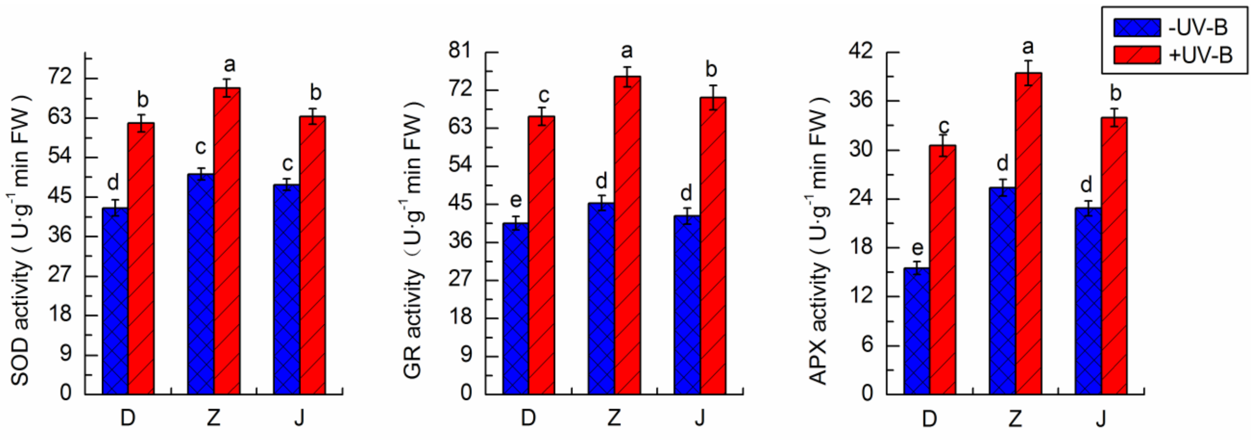

FIG.3 ANTIOXIDATIVE ENZYME ACTIVITIES OF SOD、GR AND APX EXPOSED TO UV-B.
Different letters indicate significant differences between treatments at 0.05 level according to Duncan's multiple range test (n=4) D: *Dongnong 42;* Z: *Zhonghuang 57*; J: *Jin 36*

3.5 Effects of UV-B irradiation on GSH and ASA

Significantly increased GSH and ASA were observed in three soybean cultivars under UV-B irradiation where they increased 252% and 83% in *Dongnong 42*; 145% and 78% in *Zhonghuang 57;* 68% and 31% in *Jin 36*, respectively, *($p<0.05$)*. The highest GSH and ASA presented in *Dongnong 42*, followed by *Zhonghuang 57*and *Jin 36 ($p<0.05$)* (**Fig.4**).

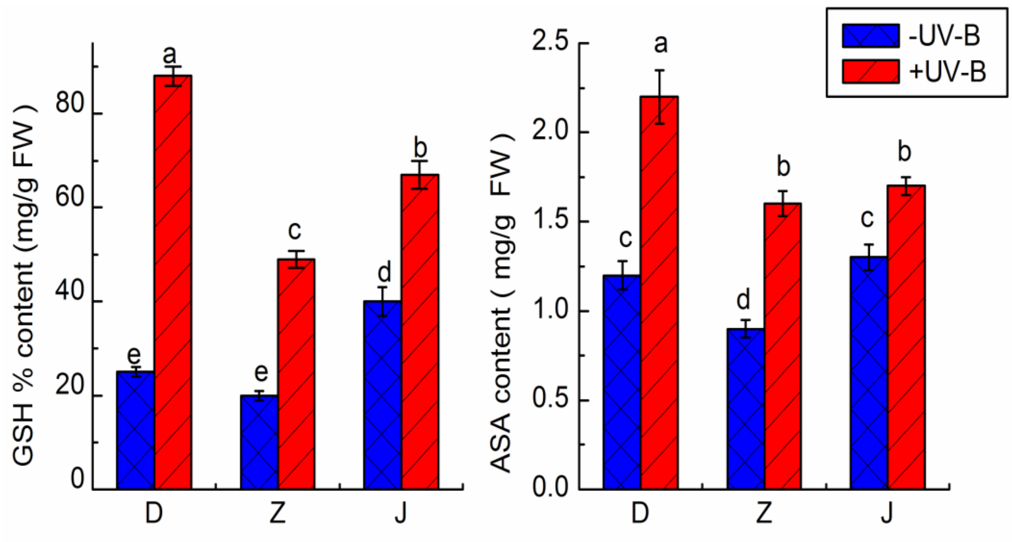

FIG.4 CONTENTS OF GSH AND ASA EXPOSED TO UV-B

Different letters indicate significant differences between treatments at 0.05 level according to Duncan's multiple range test (n=4). D: *Dongnong 42;* Z: *Zhonghuang 57*; J: *Jin 36*

IV. DISCUSSION

Plant height is an important agronomic trait. Reduction in plant height upon to enhanced UV-B has been reported in several crops (21). Tevini et al. (22) found that indoleacetic acid, a growth hormone, was absorbed in the UV-B range and believed that UV-B dependent photooxidation might be the reason for the dwarfed shoot. The present study showed UV-B significantly inhibited the plant height of the three soybean cultivars, especially *Dongnong42*. Given that nodes were fundamental part of plant height, the inhibitory occurred in nodes was easy to understand. UV-B seriously inhibited the fourth node of *Jin 36*. The first three nodes were more dwarfed in *Dongnong 42* than the other cultivars. Besides, the stems exposed to UV-B irradiation became thin and weak, especially in *Zhonghong 57* which might due to the varied substances in stems and the altered allocation of photosynthate (23).

Plant leaves are the target of solar radiation. Pal et al. indicated leaf orientation might be an important factor accounts for the sensitivity under UV-B (24). He illustrated that maize was less sensitive to UV-B might due to near-vertical leave than mung bean whose leaves are horizontal in orientation. Decreased leaf area under supplemental UV-B has been reported for a number of dicotyledonous species including cucumber, radish, soybean and garden bean (25-27). Murali et al. (3) manifested that leaf thickness was related to UV-B sensitivity, and he believed thicker leaves could mitigate UV-B radiation through additional tissue absorbance, thereby prevent a greater proportion of UV from reaching sensitive organelles in mesophyll tissues. We observed the leaves of three varieties soybean became thicker and smaller after UV-B radiation, which agreed with the above reports.

Besides leaf, UV-B sensitivity was also correlated with the change of certain biochemical substances in leaves. Most plants produce more flavonoids upon UV-B radiation (27), though the absolute amounts of constitutive and UV-B-Induced flavonoids varied greatly from species to species. Flavonoids accumulation occurs under both low and high UV-B radiation.

Couple of reports argued that the function of phenylpropanoids and flavonoids that accumulated via UV-B-mediated pathway is to increase ROS scavenging activity (5, 28). It was reported the UV-Induced improved quercetin: kaempferolrol ratio represented an increase in ROS scavenging activity, rather than an increase in UV absorbance (29) One of flavonoids, the anthocyanins (ANT), which is highly water soluble pigment and accumulates in vacuole (30), was considered beneficial to plants for alleviating damage to photosynthetic systems via UV-B absorption (31). It was believed the significantly ANT accumulation upon heat stress might account for the increased osmotic potential of leaves, so as to increase the water absorption and reduce transpirational losses (32). Based on this, we speculated the flavonoids accumulated upon UV-B radiation served for the absorption of UV-B, ROS scavenge, also the osmotic control. Lately study indicated that changes in ROS and antioxidant metabolism were intrinsic response to UV-B radiation. It was reported high level of UV-B might elevate ROS level or membrane-localized NADPH-oxidase, and genes expression which may be controlled through UVR8, COP1 and HY5 (2) Besides, UVR8 improved the expression of chloroplastic proteins such as SIG5 and ELIP, which might protect photosynthesis by regulating the secondary metabolites and photomorphogenesis (33).

At the present study, significantly improved contents of flavonoids, anthocyanins, GSH, ASA as well as increased APX, NR, SOD activities were observed in the three soybean cultivars when exposed to UV-B. *S*lightly higher SOD, GR and APX activities in *Zhonghuang 57* was detected compared with those in *Jin36* which might be related to higher protein content. However, higher levels GSH, ASA, flavonoids and anthocyanins in *Dongnong 42* were observed than other cultivars after UV-B radiation.

Chen and Murata (36) presented that proline, as a molecular chaperone can modify allosteric enzyme in adversity and protected the electron transfer of mitochondrial complex II. Park et al. (37) indicated proline can effectively protect and repair PSII damage in adversity. Smirnoff and Cumbes (38) suggested proline could act as active oxygen scavenger. We speculated the increased proline in three soybean cultivars might be the acclimation to UV-B radiation. Photosynthesis converts light energy into the chemical of sugar and organic compounds, therefore the levels of sugar in plant was closely related to photosynthesis. Approximately 80% of the CO_2 assimilated during photosynthesis was soluble sugar, which is the major transport form of organic carbon exported from the source to sink (39). The change of soluble sugar induced by stress can lead to loss in agricultural production (40). Thereby we deem the reduction of soluble sugar in three soybean cultivars under the UV-B indicated a greater movement of assimilates from the source to sink which is beneficial to yield formation. The results are consistent with Ana et al (41).

V. CONCLUSION

Various soybean cultivars showed varied physiological responses upon to UV-B, but the trend is consistent. The result further showed that enzymatic and non-enzymatic antioxidants SOD, APX, GR, ASA, GSH, increased in leaf of the three cultivars which developed a strong network defense system through UV-B radiation. Both flavonoids and protein contents significantly increased which may be related to the increased secondary metabolism of plants. The improvement of osmotic regulation substances as proline and anthocyanins may be a adjustment of plant to UV-B. The soluble sugar decreased among the three cultivars which suggested UV-B affect the photosynthesis, not negatively.

ACKNOWLEDGEMENTS

This research project is supported by the Fund Program for the Scientific Activities of Selected Returned Overseas Professionals in Shanxi Province.

REFERENCES

[1] K. Marek, S. Jerzy, K. Heli, et al, "Influence of solar UV radiation on the nitrogen metabolism in needles of Scots pine (Pinus sylvestris L.),"Environ. Pollut. Finland, vol.156, pp.1105–1111, April 2008.

[2] G.I. Jenkins, "Signal transduction in responses to UV-B radiation," Annu. Rev. Plant. Biol. United Kingdom, vol.60, pp.407-431, June 2009.

[3] N.S. Murali, A.H. Teramura, S.K. Randall, "Response differences between two soybean cultivars with contrating," Photochem. Photobio. USA, Vol.48, pp. 653-657, November 1988.

[4] C.W. Warner, M.M. Caldwell, "Influence of photon flux density in the 400-700nm waveband on inhibition of photo-synthesis by UV-B 280-320 nm irradiation in soybean leaves: separation of indirect and immediate effects," Photochem. Photobio. USA, vol.38, pp.341-346, September 1983.

[5] G. Agati, M. Tattini, "Multiple functional roles of flavonoids in photoprotection," New. Phytol. Italy, vol.186, pp.786-793, June 2010.

[6] S.B. Agrawal, S. Mishra, "Effects of supplemental ultraviolet-B and cadmium on growth, antioxidants and yield of Pisum sativum L," Ecotox.Environ.Saf. India, vol.72, pp.610–618, February 2009.

[7] Kumari R, Singh S, Agrawal SB. "Effects of supplemental ultraviolet-B radiation growth and physiology of Acorus calamus L, (sweet flag)," Acta Biol Cracov Bot. Lidia, vol. 51, pp.19–27. May 2009.

[8] C.J. Beggs , U. Schneider-Ziebert, E. Wellmann, "UV-B radiation and adaptive mechanisms in plants, In Strcrtospheric Ozone Reducrion, Solar Ultraviolet Radiation and Plant Life," R.C.Worrest and M.M.Caldwell, Eds. Springer-Verlag, Berlin, Germany,1986, pp. 235-250.

[9] C. Kami, S. P. lorrain, and C. fankhauser,"Light-regulated plant growth and development," Curr. Top. Dev. Biol. Switzerland, vol. 9, pp. 29-66, August 2010.

[10] B.A. Brown, G..I. Jenkins, "UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature Arabidopsis leaf tissue by requirement for UVR8, HY5 and HYH," Plant .Physiol. United Kingdom, vol. 146, pp. 276-588, February 2008.

[11] S. Singh, S.B. Agrawal, M. Agrawal, "UVR8 mediated plant protective responses under low UV-B radiation leading to photosynthetic acclimation," Phothochem. Photobiol. Iidia, vol.137, pp.67-76, April 2014.

[12] S.B. Agrawal, S. Singh, M. Agrawal, 'Ultraviolet-B induced changes in gene expression and antioxidants in plants," Adv. Bot. Res. India, vol.52, pp. 47–86, November 2006.

[13] K.K.Choudhary, S.B.Agrawal,"Ultraviolet-B induced changes in morphological, physiological and biochemical parameters of two cultivars of pea (Pisum sativum L.) Ecotox. Environ. Safe. India, vol.100, pp.178-187, February 2014.

[14] R.G. Spiro, "Analysis of sugars found in glycoprotein," In:Neufeld, E.S., Ginsburg, V. (Eds.), Methods in Enzymology, vol. VIII, Complex Carbohydrates, Academic Press, New York, 1966, pp. 3–26.

[15] L.S.Bates, R.P. Waldren, I.D.Teare, "Rapid determination of free proline for water-stress studies," Plant. Soil. USA, vol.39, pp. 205-207, August 1973.

[16] M.M. Bradford, "A rapid and sensitive method for quantitation of microgram quantites of protein utilizing the principle of protein-dye binding," Anal. Biochem. Georgia vol.72, pp. 248- 254, January 1976.

[17] Z.L. Zhang, W.j. Zhuo, X.F. Li, "Plant physiology experiment instruction," the 4th edition, higher education press, Beijing, China, 2009, pp.135-140.

[18] Y Nakano, K Asada, "Hdgrogen peroxide is scavenged by asscorbate-specific peroxidase in spinach chloroplasts," Plant and Cell Physiol. Japan,vol. 22, pp.867-880, June 1981.

[19] Wang B, Ma Y, Kong X, et al, "NAD(+) administration decreases doxorubicin-induced liver damage of mice by enhancing antioxidation capacity and decreasing DNA damage," Chem. Biol. Interact. China, vol. 212, pp.65-71, April 2014.

[20] S.P. Mukherjee, M. A.Choudhouri, "Implication of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in vigna seedings," Physiol. Plantarum. India, vol.58, pp.166-170, June 1983.

[21] J.E. Ambler, D.T. Krizek, P. Semeniuk, "Influence of UV-B radiation on early seeding growth and translocation of 65Zn from cotton," Plant Physiol, USA, vol.34, pp.177-181, July 1975.

[22] Tevini M, Braun J, Grusemann P, Ros J, "UV-Wirkungen auf Nutzpflanzen,''In Akad Natursch, Landschaftspl (Laufen/Salzach,Germany), pp.38-52, 1989.

[23] M. A. Jansen, A. M. Coffey, E. Prinsen, "UV-B induced morphogenesis:four players or a quartet ?" Plant. Signal. Behav,Ireland, vol.7, pp.1185-1187,September 2012.

[24] M. Pal, A. Sharma, Y.P. Abrol, and U.K. Sengupta , "Exclusion of UV-B radiation from nomal solar spectrum on the growth of mung bean and maize," Agr. Ecosyst. Environ. India, vol. 61, pp. 29-34, June 1997.

[25] N.S, Murali, A.H. Teramura , "Effects of supplemental ultraviolet-B radiation on the gowth and physiology of field-grown soybean,"Environ.Exp.Bot. USA, vol. 26, pp. 233-242,October 1986.

[26] Y.P. Cen, J.F. Bornman, "The response of bean plants to UV-B radiation under different irradiances of background visible light," Exp. Bot.Sweden, vol. 41, pp.1489-1495, April 1990.

[27] M. Tevini,A.H.Teramura,"UV-B effects on terrestrial plants,," Photochem. Photobiol.Germany, vol.50, pp.479-487, Ootober 1989.

[28] Fini A, Brunetti C, Tattini M, et al, "Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants," Plant. Singnal. Behav. Italy, vol.6, pp.709-711, May 2011

[29] K. G. Ryan, K. R. Markham, S. J. Bloor, et al, "UV-B radiation induced increase in quercetin:kaempferol ratio in wild–type and transgenic lines of Petunia," Photochem. Photobiol. New Zealand, vol. 68, pp.323-330, Sepember 1998.

[30] Chalker Scott L,"Environmental significance of anthocyanins in plant stress responses," Photochem. Photobiol. USA, vol.70, pp.166-72, July1999.

[31] J. Burger, G.E. Edwards, "Photosynthetic efficiency and photodamage by UV and visible radiation in red versus green leaf coleus varieties," Plant. Cell. Physiol. USA, vol.37, pp. 399-395, February 1996.

[32] Chalker Scott L, "Do anthocyanins function as osmoregulators in leaf tissues?" Adv Bot Res, USA,vol. 37, pp.103-106, December 2003.

[33] K. Sunita, J. Anjana, K.N. Guruprasad, "Impact of increasing Ultraviolet-B radiation on photosynthetic processes," Photochem Photobiol, India, vol. 137, pp.55-56, August 2014.

[34] E. Hideg, M.A. Jansen, A. Strid, "UV-B exposure ROS and stress: inseparable companions or loosely linked associates?" Trends.Plant. Sci, Hungary, vol.18, pp.107-115. February 2013.

[35] M. Tevini, W. Iwanzik, A. H. Teramura, "Effects of UV-B radiation on plant during mild water stress. II. Effects on growth, protein and flavonnoid content," Zeitschrift Fur Pflanzenphysiol, USA,vol.110, pp.459-467, June 1983

[36] T.H. Chen, N. E. Murata, "Enhancement of tolerance of abiotic atress by metabolic engineering of betaines and other compatible solutes," Curr. Opin. Biotechnol. USA, vol.5, pp. 250-257,Jun 2002.

[37] E.J. Park, Z. Jeknic, A. Sakamoto, et al, "Genetic engineering of glycinebetaine synthesis in tomato protects seed, plants, and flowers from chilling damage," Plant. J. USA, vol.40, pp.474-487, November 2004.

[38] N. Smirnoff, Q. J. Cumbes, "Hyroxyl radical scavenging activity of compatible solutes," Phytochemistry, U.K, vol. 28, pp.1057-1060, July 1989.

[39] K. Koch, "Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development," Curr.Opin. Plant. Biol. USA, vol.7, pp. 235-246, Jun 2004.

[40] L. Rizhsky, H.J. Liang, J. Shuman, et al, "When defense pathways collide.The response of Arabidopsis to a combination of drought and heat stress," Plant. Physiol. USA, vol.134, pp.1683-1696, March 2004.

[41] S.C. Ana, P.A. Francisco, C. Manuel, A. Manuel, "Polyamines as short- term salt tolerance in tomato," Plant .Sci. Spain, vol.138. pp. 9-16, November 1998.