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Aging Effect to Accumulation of Lettucenin A in Lettuce after Elicitation with Various Abiotic Elicitors

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Abstract

A series of experiments were conducted to study the effect of age to accumulation of lettucenin A in lettuce (*Lactuca sativa*) after various elicitations. Lettuces were challenged with four different types of elicitation method; spray with silver nitrate ($AgNO_3$) or copper sulfate ($CuSO_4$), ultraviolet irradiation and freeze-thawed. Lettucenin A, a type of phytoalexin in lettuce accumulated with a higher concentration after challenged with chemical elicitors, silver nitrate ($AgNO_3$) and copper sulfate ($CuSO_4$). Physical elicitation with irradiation of short wavelength (254nm) ultraviolet radiation and freeze-thawed were also effective in eliciting the production of lettucenin A. However, the amounts were considerably lower compared to those elicited by chemical elicitors. Ultraviolet radiation and freeze-thawed stimulated lettuce to produce lettucenin A at a concentration of 1.52 μg/ml and 0.49 μg/ml respectively while $AgNO_3$ and $CuSO_4$ contributing to accumulation of 4.27 μg/ml and 4.26 μg/ml respectively. The elicitations of lettucenin A were both dependent on types of elicitor and the age of the plant. Treatments with $AgNO_3$ and ultraviolet radiation both showed the same pattern production of lettucenin A. Lettucenin A accumulation increased significantly from week nine to the maximum at week twelve before it decreased to minimum at week eighteen. There is a week relation (R^2= 0.69) between the accumulation of lettucenin A with the age of the lettuce with elicitation of silver nitrate. However, there is no relation with induction with ultraviolet radiation. Our results suggest lettuce produces the highest amount of lettucenin A after elicitation with chemical elicitors at twelve weeks old throughout the series of trial.

Keywords: Lettucenin A, Silver nitrate, Cooper sulphate, UV

1. Introduction

Lettuce (*Lactuca sativa*) is one of the most popular vegetable after tomato, citrus and potato in the world (Ryder, 1979). It contributes a lot for diet purpose as it provides little protein, fat, starch or sugar resulting in low energy value and the presence of the β-carotene make them more important among the salad (Siemonsma and Piluek 1994). The major problem in growing lettuce is the occurrence of disease such as downy mildew, powdery mildew, anthracnose, corky root rot, lettuce mosaic virus and many others (Peirce, 1987). The diseases are not only reducing the quality appearance of the lettuce but also affecting the growth and the yield when it involved large scale of vegetable production (Peirce 1987; Ryder 1979). Great economy loses encouraged researches to be conducted in understanding lettuce's defense mechanisms. Plants are constantly live under uncertainty environment with the mercy of wind, rain, UV radiation, herbivores and pathogens attack. To impede to this different uncertainties they need special defence mechanisms. Some plants may produce some antimicrobial compounds after pathogen attack such as secondary metabolites being known as phytoalexins. These compounds have demonstrated a striking activity *in vitro* against potential pathogens (Chong et. al. 2006). Lettucenin A, the phytoalexin in lettuce was first found and isolated by Takasugi et. al. in 1985. The finding of lettucenin A is considered new at that time compared to others phytoalexins that found in soya bean, pea and many other plants. Although there were research to study the response of lettuce in production of lettucenin A, but most of them are concentrated on the effect after biotic elicitation. In an experiment conducted by Bestwick et. al. 1995. using lettuce seedlings with red spot disorder, the accumulation of lettucenin A was reported vary in different time (days) after sowing. It is apparent that changes take place in the aging of plant tissues that render them to become more resistant to attack and conversely more susceptible to attack. In this study we studied the accumulation of lettucenin A in respond to different types of abiotic elicitor and the relation of lettucenin A production to different time of elicitation (weeks).

2. Research methods

2.1 Plants

Lettuce seeds were sown using top soil with mixture of compost to maintain the moisture of the soil. Seeds were grown in room temperature with 25°C. The lettuce seeds were first grown in trays before transferred to pots after two weeks old. Organic fertilizer was added at week three to enhance their growth.

2.2 Silver nitrate ($AgNO_3$) elicitation

Solution of $AgNO_3$ was prepared at the concentration of 10 mM (1%). The lettuces were sprayed with solution of $AgNO_3$ when they reached to their respective week before maintained for another three days (Mert-Turk et. al. 2003). For control, plants were placed in 25˚C under normal growing condition for three days before undergo extraction.

2.3 Copper sulfate ($CuSO_4$) elicitation

Solution of 50 mM (5%) of $CuSO_4$ was prepared and the whole plant was sprayed with the solution at the respective weeks (Al-Barwani and Eltayed 2004). Control plants were not sprayed with $CuSO_4$. All plants were then incubated for three days under the normal growing condition.

2.4 Ultraviolet radiation elicitation

Lettuces were irradiated under the short wavelength 254nm. Lettuces were placed 10 cm from the UV source for 30 minutes (Al-Barwani and Eltayed 2004), removed and incubated in dark at room temperature for one and a half days followed by another one and a half day in the normal growing condition. Plants for control were not subjected to irradiation but only incubated under the same condition as those irradiated.

2.5 Freeze- thawed treatment

Plants are kept under the temperature of -65ºC for five minutes. These partially freezed plants were later thawed by incubating them at 25ºC in dark (Hargreaves and Bailey 1978). Control plants were incubated in dark but not subjected to freeze-thaw treatment. A total of three replications were done for each of the elicitations mentioned above section 2.2-2.5).

2.6 Extraction of Lettucenin A

Treated leaf samples were first homogenized using mortar and pestle before addition of 60% methanol with a ratio of 10 ml of solvent per gram of tissue and left overnight in dark at room temperature. The homogenate was then filtered through Whatman No.1 filter paper and the residue was again re-extracted as described before. The extracts were pooled and evaporated at 45ºC using a rotary evaporator to about 30% from the total volume. The concentrate was then extracted three times with chloroform. The extract was filtered again through Whatman No.1 filter paper before concentrated to a volume of 2ml (Bennett et. al. 1994).

2.7 Detection of Lettucenin A

Detection of lettucenin A was done using High Performance Thin Layer Chromatography (HPTLC) plates. Methanolic extracts of treated tissue were applied onto 1 cm origin on pre-coated HPTLC plates (Merck Kiesel $60F_{254}$) and developed in chloroform:methanol (98:2,v/v) (Bennett et. al. 1994) the plates were later taken out and air-dried. The chromatograms were examined under UV with wavelength of 365 nm where a greenish yellow fluorescent spot (Takasugi et. al. 1985) was observed. Chromatograms were sprayed with 2,4-dinitrophenylhydrazine reagent (DNP) which reacts with lettucenin A to give a pinkish red coloration (Bennett et. al. 1994)

2.8 Quantification of Lettucenin A

A Varian Cary 50 Win UV was used to estimate the concentration of lettucenin A. The spectrophotometer was adjusted to 446 nm, the maximum absorption wavelength of the lettucenin A (Takasugi 1985) Absorbance of the methanolic lettucenin A were recorded. The final concentrations were adjusted to µg/ml.

3. Results and discussion

3.1 Accumulation of lettucenin A after different abiotic elicitation

Accumulation of lettucenin A was significantly different after elicited by the four different methods of elicitation

(Figure 1). Silver nitrate elicited the highest accumulation of lettucenin A (4.27 µg/ml) but not significantly different with copper sulfate which induced the production of 4.26 µg/ml (Figure 1). This followed by ultraviolet radiation contributed to 1.52 µg/ml of lettucenin A and freeze-thawed method induced the lowest production of lettucenin A with only 0.49 µg/ml (Figure 1). Results showed that accumulation of lettucenin A was higher in elicitation using chemical elicitors compared to non-chemical elicitor. The higher accumulation of lettucenin A elicited with chemical elicitors may due to release of plant's constitutive elicitors from the cell wall surrounding the damage cells caused by the elicitation or the increased activities of the enzymes that could be intermediates in the accumulation of phytoalexin in surrounding cells (Darvill and Albersheim 1984). In addition, Loschke et al. 1983 also reported the induction of PAL activities and other specific proteins by chitosan when tissues were challenged with biotic and abiotic inducer. The minimum productions of lettucenin A were obtained when lettuce is subjected to freezing injury compared to other three abiotic elicitors. This may due to the delay of *de novo* synthesis of phytoalexin. The *de novo* synthesis is required for the occurrence of the irreversible membrane damage (IMD) and for the onset of hypersensitive (HR) reaction (Gottlieb 1970; Woods et. al. 1988). Woods et al. 1989, in their experiments to investigate the effect of heat-shock on IMD occurring during the HR reaction of *Lactuca sativa* L. to infection of *Bremia lactucae* Regel demonstrated the application of heat shock would affect the protein synthesis by inhibiting the translation of mRNA other than those newly synthesis. The temporary suppression of the protein synthesis results in delay both the occurrence of IMD and also the onset of HR reaction. Temperature stress on lettuce might have causes the reduced in protein synthesis that might be essential to the biosynthesis of lettucenin A (Hess et. al. 1988).

3.2 Accumulation of Lettucenin A at different age (weeks)

Among the four different weeks tested, twelve weeks-old lettuce accumulated the highest amount of lettucenin A in both silver nitrate elicitation and UV radiation. The accumulation of lettucenin A significantly increased at week nine to week twelve and significantly decreased to the minimum at week eighteen. Plants elicited with silver nitrate accumulated lettucenin A with 2.30 µg/ml at week nine and drastically increased to 4.28 µg/ml at week twelve. This was followed with a decrease to the lowest concentration of lettucenin A at the age of eighteen weeks with only 1.57 µg/ml. Elicitation using non-chemical abiotic elicitor, such as UV radiation increased the production of lettucenin A from week nine; 0.55 µg/ml to the highest at week twelve (1.52 µg/ml) and decreased drastically to 0.66 µg/ml at week fifteen. The lowest accumulation of letttucenin A was at week eighteen with 0.54 µg/ml. Throughout the experiment all controls showed no production of lettucenin A. The accumulation of lettucenin A at different weeks of elicitation is shown in Figure 2. There is a week relation (R^2= 0.69) between the accumulation of lettucenin A with the age of the plant after elicitation with silver nitrate. However, for non-chemical elicitor, there is no relation with R^2 below 0.5. Different age of plant produces different amount of phytoalexins (Bestwick et. al. 1995; Daniel and Purkayartha, 1995; Paxton and Chamberlain 1969). The present study showed an increase in the accumulation of lettucenin A until it reaches maturity stage (10-12 weeks) (Siemonsma, and Piluek 1994; Splittstoesser 1979; Tindall 1983) and started to decrease later to the late stage of the plant growth. The increase in the production of lettucenin A in the early stage may due to increase in resistance of lettuce (Daniel and Purkayartha 1995) while the decrease in the late growing stage may due to the resistance of other defense mechanisms such as morphological barriers and production of other antimicrobial compounds that took over the role of lettucenin A in defending lettuce against whatever invasion (Chong et. al. 2007). The results in this research also suggesting the older the tissues, the ability for them to produce phytoalexins decreases (Lazarovits 1981). The finding suggesting, the production of lettucenin A in lettuce start to decrease gradually after week twelve and the increase in accumulation of lettucenin A only at the first half of the growing period. The outcome from this support the postulation by Paxton and Chamberlain 1969 which they proposed to the resistance of soya beans that may contributed by the production of phytoalexin which plays a role at the early stage of the plant growth and the other resistance occurs in older plant tissue which the production of phytoalexin are greatly reduced.

4. Conclusion

In conclusion, this study demonstrated a positive relation between the ages of plant (weeks) in the production of lettucenin A with the use of chemical abiotic elicitor but there is no significant different with non-chemical elicitors. Our results suggest lettuce produces the highest amount of Lettucenin A after elicitation with chemical elicitors at twelve weeks old throughout the series of trial.

References

Al-Barwani, F.M., & Eltayed, E.A. (2004). Antifungal compound from induced *Conium maculatum* L. plants. *Biochemical Systematics and Ecology*, 32, 1097-1108.

Bailey, J.A., & Deverall, B.J. (1983). *The Dynamics of Host Defence*; Academic Press Inc, Australia.

Bennett, M.H., Gallagher, M.D.S., Bestwick, C.S., & Rossiter, J.T. (1994). The phytoalexin response of

lettuce to challenge by *Botrytis cinerea*, *Bremia lactucae* and *Pseudomonas syringae* pv. *Phaseolicola*.

Physiological and Molecular Plant Pathology, 44, 321-333.

Bestwick, L., Bennett, M.H., Mansfield, J.W., & Rossiter, J.T., (1995). Accumulation of phytoalexins lettucenin A and changes in 3-hydroxy-3-methyglutaryl coenzyme a reductase activity in lettuce seedlings with the red spot disorder. *Phytochemistry*, 39, 775-777.

Chong, K. P., Kee, K. C., & Desyi. A. N. (2007). Comparison of Aging Effect on Accumulation of Sakuranetin in Paddy and Camalexin in Arabidopsis thaliana after Elicited by Silver Nitrate. *Asian Journal of Microbiology Biotechnology and Environmental Sciences*, 9, 3, 463-467.

Chong, K. P., Mansfield, J. W., Bennett, M. H., & Rossiter, J. F. (2006). Detection of antifungal compounds in Arabidopsis thaliana and Brassica oleracea by Thin Layer Chromatography. *ASEAN Journal on Science and Technology for Development*, 23, 1&2, 123-131.

Daniel, M., & Purkayartha, R.P. (1995). *Handbook of Phytoalexin Metabolism and Action.* Marcel Dekker, Inc, America.

Darvill, A.G., & Albersheim, P. (1984). Phytoalexins and their elicitors. *Annual Review Plant Physiology*, 35, 243-275.

Gottlieb, D., & Shaw, P.D. (1970). Mechanisms of action of antifungal antibiotics. *Annual Review of Phytopathology*, 8, 371-402.

Hammerschmidt, R., Lamport, D.T.A., & Muldon, E.P. (1984). Cell wall hydroxypoline enhancement and lignin deposition as an early event in the resistance of cucumber to *Cladosporium cucumerum.*

Physiological Plant Pathology, 20, 43-47.

Hargreaves, J.A., & Bailey, J.A. (1978). Phytoalexin production by hypocotyls of *Phaseolus vulgaris* in response to constitutive metabolites released by damaged bean cells. *Physiological Plant* Pathology, 13, 89-100.

Hess, W.M., Singh, R.S., Singh, U.S., & Weber, D.J. (1988). *Experimental and Conceptual. Plant Pathology*, Volume 3**.** Oxford and IBH Publishing Co. Pvt. Ltd, India.

Lazarovits, G., Stossel, R., & Ward, E.W.B. (1981). Age-related changes in specificity and glyceollin production in the hypocotyl reaction of soyabeans to *Phytophthora megasperma* var.*sojae*. *Phytopahology*, 71, 94-97.

Loschke, D.C., Hadwiger, L.A., & Wagoner, W. (1983). Comparison of mRNA population coding for phenylalanine ammonia lyase and other peptides from pea tissue treated with biotic and abiotic phytoalexin inducers. *Physiological Plant Pathology Physiological Plant Pathology*, 23, 163-173.

Maclean, D.J., & Tommerup, I.C. (1979). Histology and physiology of compatibility and incompatibility between lettuce and the downy mildew fungus, *Bremia lactucae* Regel. *Physiological Plant Pathology*, 14, 291-312.

Mert-Turk, F., Bennett, M.H., Mansfield, J.W., & Holub, E.B. (2003). Camalexin accumulation in *Arabidopsis thaliana* following abiotic elicitation or inoculation with virulent or avirulent

Hyaloperonospora parasitica. *Physiological and Molecular Plant Pathology*, 62, 137-145.

Paxton, J.D., & Chamberlain D.W. (1969). Phytoalexins production and disease resistance in soyabeans

as affected by age. *Phytopathology*, 59, 775-778.

Peirce, L.C. (1987). *Vegetables: Characteristic, Production and Marketing*; Library of Congress Cataloging in Publication Data, America.

Ryder, E.J. (1979). *Leafy Salad Vegetables*. Avi Publishing Company Inc, America.

Siemonsma, J.S., & Piluek, K. (1994). *Plant Resources of South-East-Asia*. No.8. Vegetables Prosea Foundation, Indonesia.

Splittstoesser, W.E. (1979) *Vegatables growing* handbook. AVI publishing company Inc, United States of America.

Takasugi, M., Okinaka, S., Katsui, N., Masamune, T., Shirata, A., & Ohuchi, M. (1985). Isolation and structure of lettucenin A, a novel guaianolide phytolaexin from *Lactuca sativa* var. *capitata* (Compositae). *Journal of The Chemical* Society, 10, 621-622.

Tindall H.D. (1983). *Vegetables in the tropics*. Macmillian Education Ltd, Hong Kong.

Woods, A.M., Fagg, J., & Mansfield, J.W. (1988). Fungal development and irreversible membrane damage in cells of *Lactuca sativa* undergoing the hypersensitive reaction to the downy mildew fungus

Bremia lactucae. *Physiological and Molecular Plant Pathology*, 32, 483-497.

Woods, A.M., Fagg, J., & Mansfield, J.W. (1989). Effect of heat-shock and inhibitors of protein synthesis on irreversible membrane damage occurring during the hypersensitive reaction of *Lactuca sativa* L. to *Bremia lactucae* Regel. *Physiological and Molecular Plant Pathology*, 34, 531-544.

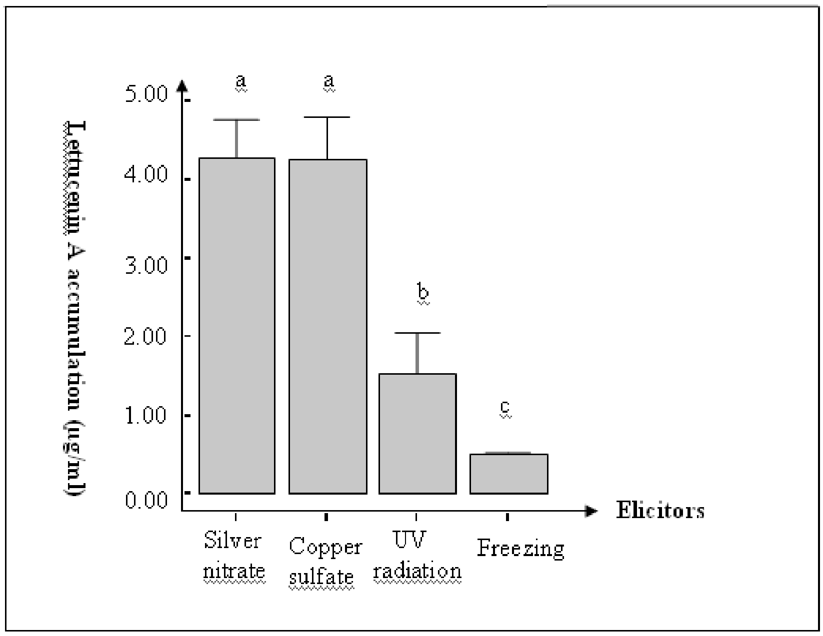

Figure 1. The concentration of lettucenin A accumulated in respond to different type of elicitors. Bar represented means of three replicates with 15g of lettuce leaves per replicate.

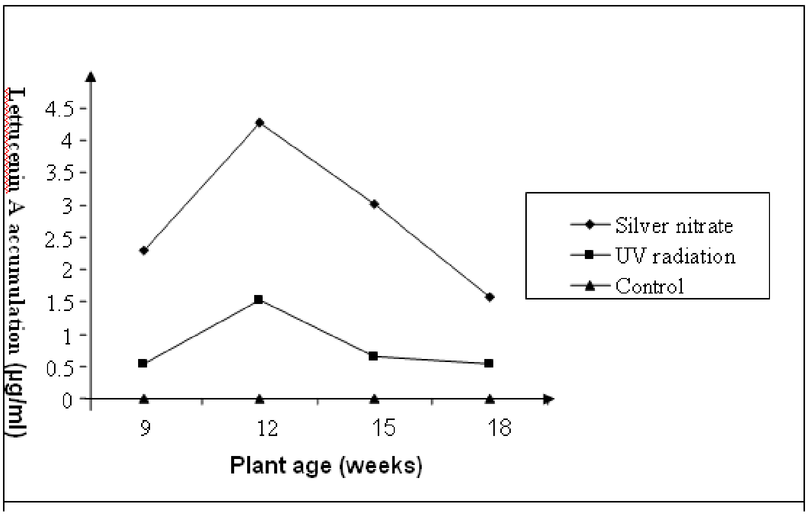

Figure 2. Accumulation of lettucenin A in lettuce at different age (weeks) after elicited by silver nitrate ($AgNO_3$) and ultraviolet radiation at 254nm. Data represent means of three independent experiments with 15g of lettuce leaves per treatment.