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Shading Application on Controlling the Activity of Polyphenol Oxidase and Leaf Browning of 'Grand Rapids' Lettuce

Benjawan Chutichudet and P. Chutichudet
Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand

Corresponding Author: Benjawan Chutichudet, Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand

ABSTRACT

The effects of preharvest different shading (0, 50, 60, 70 and 80%) on the contents of phenolic and quinone, leaf color, browning appearance and activity of Polyphenol Oxidase (PPO) were evaluated on lettuce cv. Grand Rapids under field conditions. A Completely Randomized Design was arranged with four replications and composed of five treatments: no shading (Control) compared with shading sunlight to 50, 60, 70 or 80% when the plants aged 15 Days After Planting (DAP). The results showed that plants grown under shading of 60% sunlight had the lowest contents of both phenolic and quinone substances. In addition, leaf color from plants treated with 60% shading showed the lowest a* value. Furthermore, at harvest, both the least browning damage and the minimal activity of PPO were achieved with the treatment of 60% reduction of sunlight.

Key words: Shading, polyphenol oxidase, lettuce, browning incidence, leaf color

INTRODUCTION

Lettuce (*Lactuca sataiva*) is a popular vegetable and considered as one of the most important all year round crops in Thailand (Chutichudet *et al*., 2009). In 2007, the total area for growing lettuce in Thailand was 2,119.2 ha with an estimated production of 15,499.87 tones/year. Generally, fresh lettuce is used for fresh consumption in fast food and prepared salads. In addition, it contains significant amounts of biologically active components that can impart health benefits, including dietary antioxidants which are known to have a protective effect against various forms of cancer and cerebrovascular diseases (Nicolle *et al*., 2004; Llorach *et al*., 2008). Therefore, it is not surprising to consider the lettuce as "healthier" foods (Ahvenainen, 1996; Dupont *et al*., 2000). By nature, lettuce shows a great sensitivity to enzymatic browning which leads to color change appearing on their leaf surfaces. This discoloration has long been considered as a physiological disorder that becomes the main quality problem for growers (Chutichudet *et al*., 2010). Some researchers presumed that this disorder is mainly associated with the enzymatic browning caused from the oxidation of phenolic compounds by the enzyme Polyphenol Oxidase (PPO) to produce quinones that polymerizes and form brown pigments in fresh lettuces (Jiang *et al*., 2004). Thus, leaf browning is a major factor that causes a decline in quality, a shortened storage life and economic loss. The appearance of these physiological disorders can be observed visually on leaf surfaces during the preharvest period (Chutichudet *et al*., 2009). Martyn *et al*. (2007) reported that browning in several vegetable crops often increases with high light intensity. Altunkaya and Gokmen (2008) also reported that lettuce is highly susceptible to enzymatic browning, especially in planting areas in tropical regions. The harvested lettuces in these zones usually face browning due to the hot weather (Misaghi *et al.*, 1992). However, even if high temperatures promote the severity of browning incidence in field-grown lettuce during the hot summer months (Borkowski and Szwonek, 1994) in these regions, many agricultural areas are still planting lettuce for commercial production throughout the year because of the high demand for fresh consumption. Shading has been reported to play an important role in controlling the browning process of several plants (Nixon, 1998). For example, the significantly reduced bract browning in waratahs is produced in the shade condition at 50% (Martyn *et al.*, 2007). Therefore, since the shading practice decreases this disorder during plant growth in these zones, it should be studied to increase the quality of harvested lettuce. At present, very little is known about a practical method to control browning disorder in lettuce planted in the field by using shading. Thus, the purpose of this experiment was to investigate the effect of shading treatment in order to cut off the sunlight to control the browning disorder of 'Grand Rapids' lettuce grown under field conditions.

MATERIALS AND METHODS

The experiment was carried out from May to July, 2009 at the experimental field, Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the northeast of Thailand. Seeds of 'Grand Rapids' lettuce were sown and transplanted at 15 DAP in 2-L pot filled with a sandy loam soil: rice husk: manure ratio 1: 1: 1. A Completely Randomized Design was arranged and composed of five treatments: seedlings were held in condition of full sunlight (control) compared with different shading at 50, 60, 70 and 80%. Since 15 DAP, seedling plants were exposed to various shadings by using commercial shading saran cutting off the sunlight to 50, 60, 70 and 80%. Individual shade shelters were constructed from a galvanised steel tube frame which covered the plants 120 cm above the ground. The individual shade netting saran (50, 60, 70 and 80%) covered all sides of these frames. Plants subjected to full sunlight were chosen as Control treatment. Each treatment was carried out in four replicates, ten plants per replication. The following determinations were evaluated at harvest date (60 DAP) for assessments of (1) Phenolic content was performed as described by Ribeiro *et al.* (2008). Content was expressed as absorption at 765 nm/100 g fresh weight of leaf. (2) Quinone content was extracted as described by Pirie and Mullins (1976). Quinone content was expressed as absorbance at 437 nm per g fresh weight. (3) Leaf color was measured on the leaf surface with a Hunter Lab Model No. 45/0-L, Serial No. 7092, USA. CIE color values L^* (black = -100 and white = +100), a^* (greeness) (- = green and + = red) and b^* (yellowness) (- = blue and + = yellow) were measured to describe the color of lettuce's leaf. (4) The level of browning incidence occurring on a lettuce leaf was expressed as a percentage for evaluating leaf browning incidence. (5) Activity of Polyphenol Oxidase (PPO) was analyzed from lettuce leaf and carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS, from China. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per 60 sec. The collected data were statistically analyzed using the SPSS Computer Programme, Version 6 (SPSS, 1999).

RESULTS AND DISCUSSION

After plants were supplied with different shadings, the recorded data received from analyzing the contents of phenolic compounds and quinone, leaf color, degree of leaf browning and PPO activity demonstrated the following results:

Table 1: Contents of phenolic and quinone substances in lettuce after different shading at harvest

Treatment	Phenolic substances (g/100 g FW)	Quinone content (g FW)
Outdoor	0.76[a]	3.52[a]
Shading 50%	0.79[a]	2.46[b]
Shading 60%	0.35[c]	1.42[c]
Shading 70%	0.62[b]	3.40[a]
Shading 80%	0.42[c]	1.98[bc]
F-test	**	**
C.V. (%)	19.86	21.77
LSD	0.063	0.4177

Same letters within columns indicate Least Significant Differences (LSD) at p** = 0.01

Table 2: Leaf color and browning percentage in lettuce after different shading at harvest

Treatment	L*	a*	b*	Leaf browning (%)
Outdoor	50.63	-7.74[a]	29.48	35.12[a]
Shading 50%	49.34	-8.29[ab]	29.84	15.37[c]
Shading 60%	50.00	-9.30[b]	29.89	14.37[c]
Shading 70%	54.99	-6.88[a]	25.88	21.62[b]
Shading 80%	55.07	-7.59[a]	25.49	21.12[b]
F-test	ns	*	ns	**
C.V. (%)	9.55	12.06	11.73	7.69
LSD	4.0386	0.7807	2.6821	1.08

Same letters within columns indicate Least Significant Differences (LSD) at p** = 0.01, p* = 0.05, ns: Non significant

Phenolic content: The analyzed phenolic contents taken from plants grown under different shadings are shown in Table 1. At harvest, in a comparison of the phenolic contents of lettuce's leaves after various shadings during plant growth, the data indicated that plants receiving 60 and 80% shading had a highly significant effect on lowering the phenolic compounds (0.35 and 0.42 g/100 g FW, respectively) while the maximal level of total phenolics in the leaf from the control plants and shaded plants at 50% were observed.

Quinone content: The results from Table 1 showed that control plants had the highest quinone content (3.52 g FW). While plants received the shading under 60% sunlight had the lowest quinone content of 1.42 g FW, at harvest stage (60 DAP).

Leaf color: Changes in the leaf colour of lettuce were monitored by measuring L*, a* and b* at harvesting stage.

L*: The results from Table 2 showed that shading supplements had no effect to the brightness of leaf color in term of L* value. The results revealed that L* values in lettuce leaves from both plants-treated with shadings showed the same trend as control plants, ranging from 49.34-55.07 at 60 DAP.

a*: The results showed that significant differences of leaf colour, measured by monitoring in terms of a* values were observed at 60 DAP. From Table 2, plants treated with 60% shading showed the lowest a* values of -9.30. These results implied that shading condition at 60% sunlight could promote the maximal green color in lettuce leaf.

Table 3: Activity of PPO after different shading at harvest

	PPO activity (Unit/mg FW)					
Treatment	0	60	120	180	240	300
Outdoor	0.577	1.004[a]	1.123[a]	1.162[a]	1.157[a]	1.106[a]
Shading 50%	0.399	0.700[ab]	0.768[ab]	0.776[b]	0.758[bc]	0.708[b]
Shading 60%	0.499	0.596[b]	0.661[b]	0.696[b]	0.693[c]	0.678[b]
Shading 70%	0.587	0.867[ab]	0.995[ab]	1.062[ab]	1.096[ab]	1.121[a]
Shading 80%	0.411	0.616[b]	0.696[b]	0.685[b]	0.648[c]	0.622[b]
F-test	ns	*	*	*	*	**
C.V. (%)	6.41	3.72	2.71	8.63	8.40	9.59
LSD	0.22	0.27	1.27	0.28	0.28	0.28

Same letters within columns indicate Least Significant Differences (LSD) at p** = 0.01, p* = 0.05, ns: Non significant

b*: The results from Table 2 showed that nonsignificant differences of b* values from plants applied with different shadings were observed. These results revealed that shading application had no effect to leaf yellowness in harvested lettuce.

Browning appearance: For content of browning disorder, through the all recorded data, the control plants showed significantly the greatest browning appearance of 35.12% at harvesting stage. While the application of 50 and 60% shading showed the lowest browning incidence accounting for 15.37 and 14.37%, of observations, respectively at harvest (Table 2).

PPO activity: The variation in PPO activity in the lettuce received from different shadings measured at the harvesting stages (60 DAP) is shown in Table 3. At harvest (60 DAP), plants exposed to full sun and plants grown under 70% shading showed clearly the highest PPO activities while plants grown under 50, 60 and 80% shading showed the significant lowest activities of PPO enzyme.

The effect of different shading on the internal characteristics related to the contents of phenolic and quinone, leaf color, level of browning incidence and PPO activity in 'Grand Rapids' lettuce was studied. For phenolic contents, the results revealed that reducing sunlight to 60 and 80% had remarkable impact on the phenolic content of lettuce leaf. Minimum amounts of phenolic content in lettuce leaf after shading under 60 and 80% sunlight were observed at harvest while plants grown under sunlight conditions and 50% shading had the maximal phenolic contents of 0.76 and 0.79 g/100 g FW, respectively at the harvesting time. These results are also supported by the findings of Podsedek (2007) who cited that the higher content of phenolic compounds in vegetables can be influenced by the higher temperature during plant growth. Similarly, Zhang and Bjorn (2009) reported that the production of phenolic compounds could be stimulated by Ultraviolet radiation. Likewise Carbonaro and Mattera (2001) found that phenolic substances in callus cultures of tea plant were increased by UV-B radiation. These results were similar with the previous researches of Amiot *et al.* (1992) who indicated that sunlight has been demonstrated to affect phenolic metabolism. In addition, Chutichudet and Chutichudet (2009) indicated that the production of phenolic compounds contributed to plant resistance to stress. A significant increase in the total phenolic content was noted immediately after receiving the stress treatments (Oh *et al.*, 2009). Therefore, the greatest accumulation of phenolic compounds in lettuce in response to the high light stresses were observed, especially under full sunlight (Oh *et al.*, 2009). These findings are not in agreement with previously reported studies of Tsormpatsidis *et al.* (2008) who found that total phenolic content in Lolo Rosso lettuce was curvilinear related to degree of UV radiation cutoff. Future research should be focused on the link of internal mechanisms of shading treatment to the change of phenolic compounds in lettuce plant.

With regards to the effect of shading application on quinone content, the results showed that this chemical substance in lettuce was also affected by eliminating sunlight application. Control plants and plants held under 70% shading had the highest quinone contents of 3.52 and 3.40 g FW, respectively. Generally, as enzymatic oxidation of phenol compounds by PPO proceeded, an increase in the content of quinone was observed (Khumjing *et al.*, 2011). Both increases in phenolic and quinone content in these two above treatments may be due to lettuce being subjected to stress conditions. This could be as a result of the increase in both these two substances levels protecting themselves against photoinhibition (Tsormpatsidis *et al.*, 2008) while the highly significant lowest concentrations of quinone was observed in plants treated with 60% shading. The prominent results shown in Table 1 indicate that the shading treatment, especially 60% shading significantly affected similar reduction in both the phenolic and quinone contents of lettuce when compared with control samples. However, the significance of the involvement of phenolic and quinone alteration following the different shading has not been adequately documented.

For the results of leaf color measuring based on L* (brightness/darkness), a* (redness/greenness) and b*(yellowness/blueness) values, the results showed that various shading treatments had no effect on leaf color in terms of L* and b* in lettuce following the shading application. Thus, color parameter in terms of brightness and yellowness characteristics of the leaf showed similar values among the treatments. For a* values, the results showed that the parameter a* from plants treated with 60% shading decreased to be at the lowest level of -9.30 at harvesting stage. These results indicate that pre-harvest application of 60% shading was the most effective in promoting the greenness of leaf color. These results corresponded to Matile *et al.* (1999) and Hortensteiner and Feller (2002) who reported that stress response leading to membrane breakdown occurs and this leads to loss of sub-cellular compartmentalization, intermixing of many enzyme systems and their potential substrates thus leading to oxidation of chlorophyll and declining green characteristics. These findings were also consistent with those reported by Limbo and Piergiovanni (2006) who cited that photodestruction of the green pigments in leaf may occur when plants are grown under inappropriate condition. In addition, Olarte *et al.* (2009) obtained better chlorophyll retention in cabbage stored at low light intensities compared with samples stored in darkness. However, these results were not in agreement with the previous findings of Lin and Jolliffe (1996) who found that low light intensity resulted in a reduction in the total chlorophyll content of English cucumber skin. Nevertheless, changes in colour associated with shading treatment are rather complex and poorly understood. In the present, information on promoting the green leaf color in lettuce after receiving shading is still lacking.

For degree of leaf browning, the results suggested a link with light intensity and browning effects. The highly significant differences among control and lettuce treated with different shading were found. At harvest, maximum browning incidence tended to occur in control plants accounting for 35.12% of observations. These results were in agreement with the previous findings of Chutichudet and Chutichudet (2009) who found the occurrence of browning disorder depended upon PPO enzymes associated with phenol substrate concentration. Bowning reactions have generally been assumed to be a direct consequence of PPO action on polyphenols (Martinez and Whitaker, 1995). Altunkaya and Gokmen (2009) cited that browning severity was related to phenolic compounds which have a significant role as substrates in the oxidation processes leading to browning reactions (Robards *et al.*, 1999). Moreover, in artichoke, Brecht *et al.* (2004) cited that the content of phenol is associated with the sensitivity to enzymatic browning. Thus, it may be possible that the highest content of phenolic substance in sun plants was positively responsible for promoting the maximal browning incidence in lettuce. These observations are consistent with the results of Chutichudet and Chutichudet (2009) who proposed that the altered phenol metabolism is thought to be involved in browning incidence. Phenolic compounds can be oxidized by enzyme PPO to form quinones that spontaneously polymerize leading to form the brown pigments responsible for tissue browning (Khumjing *et al.*, 2011). Furthermore, the susceptibility to browning disorder is also influenced by environmental conditions (Wissemeier *et al.*, 2000; Chow *et al.*, 2004). Saure (1998) cited many instances of greater browning occurrence and severity in vegetables with increasing light intensities. For waratahs grown under strong sunlight and heat stress, Nixon (1998) reported that these conditions are a trigger for inducing bract browning disorder. Some studies indicate that fruit browning is associated with the level of the browning-related substrate (Chutichudet *et al.*, 2008). Hodges and Toivonen (2008) reported that phenolics can be oxidized by PPO to quinones which ultimately polymerize to produce the browning appearance (Degl'Innocenti *et al.*, 2005). In the case of lettuce, oxidation of the accumulated polyphenols by PPO results in the formation of brown pigments. This might be a result in both cases of the greater the severity of tissue injury, the more intense the browning (Ke and Saltveit, 1989). Similar results were obtained by Kawai *et al.* (1992) who pointed out that an increase in the severity of the browning disorder in *Raphanus* roots is accompanied by a related increase in the amount of phenolic compounds present. Thus, oxidation of the higher accumulated polyphenols in sun plant resulted in the formation of brown pigments appearing on leaf surfaces. In contrast, the lowest extent of browning disorder in lettuce plant was obtained when 50 and 60% shading were applied. Thus, the best result for controlling the browning disorder was obtained from plants grown under 50 and 60% shading (15.37 and 14.37 %, respectively). This is in accordance with Saure (1998) who observed that browning appearance seemed to be rather a stress-related disorder. Environmental conditions may cause stress, or they may increase the susceptibility to stress. Direct effects of high temperature stress include damage to cellular membranes, proteins and nucleic acids (Kays, 1999). After membrane damage occurred, the phenolic substrates may be enzymatically oxidised to o-quinones and, eventually, brown coloured polymers that are responsible for the actual browning symptoms. This might explain why plants grown in the outdoor condition are more susceptible to browning disorders than plants grown under shading (Zerbini *et al.*, 2002). Similar observations have been reported by Wissemeier *et al.* (2000) who found that decreasing light intensity, such as shading, reduced browning occurrence. These results are also in agreement with the previous findings of Martyn *et al.* (2007) who revealed that 50% shading significantly reduced the bract browning in potted and commercially grown plants. They explained that the shade condition altered the microclimate experienced by plants, as well as decreasing light intensity. The mean daily maximum temperature was significantly higher by 5°C in full sun than when under shade cloth. Even in temperate regions, apple fruit flesh temperatures under direct sunlight can reach in excess of 40°C (Ferguson *et al.*, 1998). Frequent exposure of apple fruit to such temperatures will result in damage, such as sunburn (Bergh *et al.*, 1980). In addition, the decrease in the browning severity of lettuce plants grown under 60% shading could be due to the reduced content of browning substrate, namely phenolic and quinone as well as decreased PPO activity at harvest (Jiang *et al.*, 2002; Shi *et al.*, 2008; Sun *et al.*, 2006; Chutichudet *et al.*, 2009). The decrease in phenolic and quinone content from plants grown under 60% shading may be attributed to a decrease in PPO activity and browning incidence. The results from this experiment could provide the suitable preharvest treatments to control effectively leaf browning of lettuce by using shading at 60%. However, the exact mechanism of shading supplement on reducing the browning occurrence in lettuce is not yet clear. Unfortunately, there is no study that reports the application of shading sunlight for controlling the browning appearance in lettuce. The link between leaf browning in shaded plant will be investigated in further studies.

For PPO activity, at harvest, highly significant differences of PPO activities under various shadings were observed. The results revealed that shading application to cut off irradiation to 50, 60 and 80% was effective in the lowering activity of PPO enzyme. While a pronounced increase in PPO activity in lettuce plants occurred, it was dramatically enhanced by outdoor conditions. This could be explained by the fact that stress affects the degradation of the plant membrane and thus enhances PPO leakage which contacts the phenolic substrates initiating the browning reaction (Huang *et al*., 2005). Thus, the severity of browning in the control plant is highly related to the high activity of PPO. Similar results were reported by Degl'Innocenti *et al*. (2007) who found that the induction of PPO enzymes have been known to be increased, especially sunlight stress. Additionally, the result from this experiment indicated that PPO activity in lettuce is very sensitive to full sunlight conditions. This may be partially explained by another indirect manner, higher temperatures can affect to increase leaf temperatures and, thus, influence stress in plant tissues (Lloyd and Farquhar, 2008). This is in agreement with the result of Franck *et al*. (2007) who found that high temperatures can accelerate the rate of biochemical reactions catalyzed by enzymes which cause physiological disorders. Similarly, apple fruit exposed to direct sunlight will result in sunburn damage (Bergh *et al*., 1980). These results were also consistent with the observation of Dogan *et al*. (2005) who cited that the activity of PPO is rather active in sunlight. Thus, enzymatic browning was also promoted by an increase in polyphenol oxidase activity when lettuce plant was grown in the outdoor condition. The opposite results were reported by Manzocco *et al*. (2009) who found that the higher the intensity of UV-C irradiance, the greater the reduction of PPO activity. Although the mechanisms of changes in phytochemicals under different shading are not well known, the results demonstrated that supplemental shading to 60% could be practically used to reduce leaf browning of 'Grand Rapids' lettuce. Furthermore, these results suggested that this disorder is affected not only by phenolic and quinone content but by enzyme PPO activities. However, at present, the underlying biochemical factors associated with an enzymatic browning disorder of lettuce, focused on eliminating sunlight to control PPO enzyme activities, are poorly understood. Further studies are needed to understand the biochemical factors involved in browning appearance after shading application.

CONCLUSION

In conclusion, after various shading treatments to Grand Rapids lettuce seedlings at 15 DAP, the content of phenolic and quinone substances, leaf color, browning damage and PPO activity of the lettuce plants was significantly affected by cutting off sunlight treatments. At harvest, both content of phenolic compounds and quinone substance from plants grown under 60% shading decreased to their lowest amounts of 0.35 g/100 g FW and 1.42 g FW, respectively. In addition, plants treated with 60% shading gave the highest green color present in the lettuce leaf in terms of a* value. Furthermore, holding the plants at 60% shading also resulted in reducing leaf browning incidence and PPO activity to their lowest level. Thus, the preharvest of 60% shading sunlight to lettuce plant at 15 DAP was effective for controlling the leaf browning damage in Grand Rapids lettuce.

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