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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (18): 1226-1236, 2009
ISSN 1028-8880

Effect of Gypsum Application on Enzymatic Browning Activity in Lettuce

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Abstract: A comprehensive study to evaluate calcium, in terms of gypsum ($CaSO_4.2H_2O$) by soil dressing application, on enzymatic browning activity of Polyphenol oxidase (PPO) and internal qualities was tested on lettuce var. Grand Rapids under field conditions. A factorial in completely randomized design was arranged with four replications. The results showed that plants-treated with 50 mg kg^{-1} gypsum applied at 40 DAP had the maximal fresh weight of 25.83 g $plant^{-1}$. The internal qualities of the lettuce at harvest showed that plants treated with 50 mg kg^{-1} gypsum had the maximal chlorophyll content (26.80 mg m^{-2}), while all gypsum concentrations applied in this study, had less content of ascorbic acid than the control plants. Plants-treated with 100 mg kg^{-1} gypsum affected to the lowest level of PPO activity at week 3 after transplanting. Furthermore, gypsum application had no effect to biomass, leaf colour, the contents of phenolic and quinone in lettuce at harvesting stage.

Key words: Gypsum, enzymatic browning, PPO activity, lettuce

INTRODUCTION

Lettuce (*Lactuca sataiva*), belonging to the Asteraceae family, is a popular vegetable and considered one of the most important crops all year round in Thailand. In 2007, the total area for growing lettuce in Thailand was 2,119.2 ha with an estimated production of 15,499.87 t ha^{-1} (Cantos *et al*., 2002). Most lettuce is used for fresh consumption as fast food and prepared salads. Lettuce contains significant amounts of biologically active components that can impart health benefits beyond basic nutrition. Lettuce is also a major source of dietary antioxidants, including phenolics, ascorbic acid, carotenoids, tocopherols and glucosinolates, which are known to have a protective effect against various forms of cancer and cardiovascular and cerebrovascular diseases (Lister, 2003; Nicolle *et al*., 2004; Llorach *et al*., 2008; Verlangieri *et al*., 1985). Moreover, lettuce is an excellent source of several nutritional properties that are considered as low-acid food or healthier foods (Ahvenainen, 1996; Dupont *et al*., 2000). Generally, the consumer preference of commercially grown Grand Rapids lettuce is due to its crispiness and attractive bright green colour, but this crop is very vulnerable to enzymatic browning which leads to colour change appearing on the leaf surface; this discoloration has long been considered the main production problem. Colour change may be principally a general indicator of quality because appearance is a major factor that determines lettuce marketability. Altunkaya and Gökmen (2008) cited that lettuce is highly susceptible to enzymatic browning. Thus, leaf browning is a major factor that affects product losses resulting from rejection by consumers, shortening storage life and economic loss (Zhang *et al*., 2001). Little is known about both the origin of this disorder and its physiological mechanism. Enzymatic browning is mainly associated with polyphenoloxidase (PPO). Oxidation of phenolic compounds to o-quinones is the main cause of browning in fresh lettuces (Macheix *et al*., 1990; Nicolas *et al*., 1994). The appearance of this physiological disorders can be observed visually on leaf surfaces (Franck *et al*., 2007) during the preharvest period (Kays, 1999). However, information on tissue browning susceptibility is limited (Castaner *et al*., 1999).

Furthermore, enzymatic browning is a direct consequence of membrane disintegration. Therefore, the causes of browning must be sought in processes which affect the membrane integrity (Franck *et al*., 2007). The advanced maturity stages of plants lead to disruption of the cellular compartments, allowing the substrate and enzymes located in the chloroplast to come into contact (Rocha and Morais, 2001). Enzymatic browning is a direct consequence of membrane disintegration (Kays, 1991; Felicetti and Schrader, 2009). Therefore, the control of browning during plant development, by maintaining the membrane integrity, should be studied to increase the value and quality of harvested lettuce. At present,

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although much is known about the causes of browning disorder, very little is known about the practical method to control browning disorder in lettuce planted in the field. One of the general soil amendment compounds that has been shown to inhibit browning is calcium-releasing compound included in gypsum or $CaSO_4.2H_2O$ which has been widely used as a soil amendment in a range of soils. However, there is very little information available on gypsum concerning this disorder on lettuce production. Thus, the purpose of this experiment was to investigate the effect of exogenous soil amendment, gypsum, on browning disorder, PPO activity and other internal qualities of lettuce grown under field conditions.

MATERIALS AND METHODS

The experiment was carried out at the experimental field, Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the northeast of Thailand in the period between May to July, 2008. 'Grand Rapids' lettuce was planted from seed. The seedlings were transplanted 25 days after planting and grown singly in 2-L pot filled with a sandy loam soil : rice husk : manure ratio 1:1:1 and placed under field conditions. A Factorial in Completely Randomized Design was arranged and composed of two factors: soil application of slow releasing gypsum ($CaSO_4.2H_2O$) at the concentration of 50, 100 or 150 mg kg^{-1} with different three application times (25, 40 or 55 days after planting, DAP) compared with untreated plants (control). Each treatment was carried out in four replicates, ten plants per replication. Plants were harvested at commercial maturity (65 days after planting) and taken to the laboratory within a few minutes for assessments of: (1) Fresh weight (g), (2) biomass was determined by the method of AOAC (1980) and expressed in percentage. (3) leaf colour was measured on the leaf surface with a Hunter Lab Model No. 45/0-L, Serial No. 7092, USA. CIE standard for measuring colour values L*(black = -100 and white = +100), a* (redness) (- = green and + = yellow) and b* (yellowness) (- = blue and + = yellow) were measured to describe the colour of lettuce's leaf. (4) The browning appearance on leaf surfaces were scored for evaluating browning as described in González-Aguilar *et al.* (2004) by determining visually using a scale of 1-5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe and 5 = extreme browning. Degree of browning evaluations was carried out during plant development. (5) Chlorophyll content was determined using a procedure as described by Whitham *et al.* (1986) and expressed as mg m^{-2}. (6) Phenolic content was performed as described by Ribeiro *et al.* (2008). The phenolic content was expressed as absorption at 765 nm/100 g fresh weight of leaf. (7) Quinone content was extracted as described by Pirie and Mullins (1976). Quinone content was expressed as absorbance at 437 nm. (8) Total soluble solid content (TSS) (juices being squeezed from flesh tissue using distilled water at ratio 1:3 between flesh and distilled water, measurement was done by digital refractometer (Atago-Palette PR 101, Atago Co., Ltd., Itabashi-ku, Tokyo, Japan). (9) Titratable acidity (TA) evaluation was made by the use of juices described above in number 8 with the method of AOAC (1990). (10) The measurement of pH values was carried out by using juices described earlier in number 8 on pH meter ID 100D, Singapore. (11) Ascorbic acid content was measured by the use of juices described in number 8 with the method of AOAC (1990). (12) Polyphenol oxidase (PPO). Activity determination was carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS, China. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per 30 sec. The collected data were statistically analyzed using the SPSS Computer Programme, Version 6 (SPSS, 1999).

RESULTS

The results were collected after adding gypsum, with different concentrations of 50, 100 or 150 mg kg^{-1} at various application times (25, 40 or 55 DAP) for lettuce grown under field conditions. The recorded data were composed of:

Fresh weight: At harvest, there was highly significant fresh weight of product (Table 1). Plant-treated with 50 mg kg^{-1} gypsum at 40 DAP had significantly the maximal weight of 25.83 g per plant, while the minimum weight was recorded for 150 mg kg^{-1} at 55 DAP (14.51 g) per plant.

Biomass: The results from Table 1 showed no significant difference in lettuce biomass among treatments with similar mean values of 7.79-14.72%.

Leaf colour: Changes in the leaf colour of the lettuce were monitored by measuring L*, a* and b* at the harvesting stage. From Table 1, there was no significant difference in the leaf colour parameters of L*, a* and b* between the three concentrations of gypsum cooperated with different application times.

Browning appearance: Degree of leaf browning showed an increasing trend with a plant's developmental time. Leaf browning was observed by visual evaluation on

Table 1: External qualities of lettuce as influenced by putting gypsum at different concentrations and time application

Factors	Fresh weight (g)	Biomass (%)	Leaf colour		
			L*	a*	b*
Gypsum conc (mg kg^{-1})					
0	18.07	12.45	49.33	-8.58	28.03
50	21.65	15.22	49.46	-8.79	28.36
100	18.91	9.97	49.12	-8.58	27.97
150	19.04	10.75	48.6	-8.82	28.01
F-test	ns	ns	ns	ns	ns
CV (%)	16.99	15.19	10.76	12.86	9.64
LSD	1.4027	3.6222	0.835	0.1772	0.4285
Time application					
control	18.07	12.45	49.33	-8.58	28.03
25 DAP	20.91	11.17	49.4	-8.78	27.88
40 DAP	20.51	9.74	48.78	-8.77	28.44
55 DAP	18.19	15.03	49.01	-8.64	28.01
F-test	ns	ns	ns	ns	ns
CV (%)	15.03	15.33	10.77	12.9	9.63
LSD	1.4035	3.6283	0.8358	0.1777	0.4278
Conc×time application					
Control	18.07 d	12.45	49.33	-8.58	28.03
50 mg kg^{-1} at 25 DAP	20.34 bc	10.18	50.16	-8.89	28.11
100 mg kg^{-1} at 25 DAP	18.91 bcd	10.59	49.69	-8.28	27.87
150 mg kg^{-1} at 25 DAP	23.48 ab	12.73	48.34	-9.16	27.68
50 mg kg^{-1} at 40 DAP	25.83 a	10.75	49.55	-8.9	28.52
100 mg kg^{-1} at 40 DAP	16.56 cd	7.79	48.35	-8.86	28.18
150 mg kg^{-1} at 40 DAP	19.13 bcd	10.69	48.43	-8.56	28.62
50 mg kg^{-1} at 55 DAP	18.79 bcd	14.72	48.67	-8.59	28.44
100 mg kg^{-1} at 55 DAP	21.27 abc	11.53	49.33	-8.61	27.85
150 mg kg^{-1} at 55 DAP	14.51 cd	8.83	49.03	-8.73	27.73
F-test	**	ns	ns	ns	ns
CV (%)	15.69	18.55	10.88	12.84	9.75
LSD	1.9383	5.144	1.1942	0.2501	0.6124

Letters within columns indicate Least Significant Differences (LSD) at **p = 0.01; NS: Non significant

lettuce at the first week after transplanting. At 25 DAP (week 1), the untreated plants showed significantly lower browning level of 0.15. Table 2 also showed the browning appearance of untreated plants was significantly lower than that of the others after transplanting three weeks of 2.56. Afterwards, there was a similar amount of browning in both gypsum treatments and control samples.

Chlorophyll: The results from Table 3 showed a good correlation between gypsum concentrations and chlorophyll contents. On harvesting date (65 DAP), chlorophyll content from plant-treated with 50 mg kg^{-1} gypsum showed significantly the highest chlorophyll content of 26.80 mg m^{-2}.

Phenolic and quinone content: At harvesting, the total phenolics and quinone contents of the leaf extracts were shown in Table 3. The results revealed that total phenolics and quinone content in lettuce leaves from both plants-treated with different gypsums and control showed the same trend and was not significantly affected by gypsum treatments, ranging from 646.79-1144.11 mg per 100 g and 0.1039-0.2120/mg FW, respectively.

TSS, TA and pH: Gypsum treatment had no significant effect on the internal qualities of the lettuce such as TSS, TA and pH. The mean values of TSS, TA and pH of all treatments at harvesting time ranged from 0.20 -0.28 degree Brix, 0.0320-0.0720% and 6.45-6.55, respectively (Table 4).

Ascorbic acid: Significant differences in ascorbic acid between the plant-treated with gypsum and control were found. All plants-treated with gypsum had the lower ascorbic acid content than control (Table 4).

PPO: The variation in the PPO activity in leaf tissues of lettuce measured at the various developmental stages (week 1, 3 and 5 after transplanting) is shown in Table 5-7. PPO activities in the lettuce leaf were at a very low level at early stage of week 1 after transplanting, then increased obviously in week 3 and finally decreased slightly at week 5. Significant differences ($p>0.05$) of PPO activities were not found between treatments on week 1 and week 5, except for three weeks after transplanting, PPO activity tended to increase progressively and statistical differences among the gypsum concentrations were observed. From Table 6, PPO from lettuce-treated with 100 mg kg^{-1} gypsum showed the lowest activity.

Table 2: Browning scores of lettuce as influenced by putting gypsum at different concentrations and time application

	Scores of leaf browning at different weeks after transplanting				
Factors	1	2	3	4	5
Gypsum conc (mg kg^{-1})					
0	0.15b	1.53	2.56	2.63	2.72
50	0.24ab	1.53	2.69	2.69	2.71
100	0.38a	1.48	2.61	2.62	2.7
150	0.25ab	1.48	2.58	2.67	2.7
F-test	*	ns	ns	ns	ns
CV (%)	15.19	18.02	11.41	12.92	17.79
LSD	0.0502	0.0672	0.0599	0.0597	0.0592
Time application					
control	0.15	1.53ab	2.56 b	2.63	2.72
25 DAP	0.23	1.37b	2.74 a	2.67	2.7
40 DAP	0.32	1.60a	2.59 ab	2.64	2.79
55 DAP	0.32	1.52ab	2.55 b	2.67	2.62
F-test	ns	*	*	ns	ns
CV (%)	15.36	17.56	13.13	18.95	17.49
LSD	0.0504	0.0664	0.0594	0.0598	0.0585
Conc×time application					
Control	0.15	1.53	2.56	1.63	2.72
50 mg kg^{-1} at 25 DAP	0.15	1.42	2.83	2.69	2.63
100 mg kg^{-1} at 25 DAP	0.33	1.28	2.72	2.66	2.75
150 mg kg^{-1} at 25 DAP	0.23	1.42	2.67	2.66	2.72
50 mg kg^{-1} at 40 DAP	0.25	1.64	2.61	2.75	2.91
100 mg kg^{-1} at 40 DAP	0.45	1.69	2.61	2.47	2.72
150 mg kg^{-1} at 40 DAP	0.25	1.47	2.56	2.69	2.75
50 mg kg^{-1} at 55 DAP	0.33	1.53	2.61	2.63	2.59
100 mg kg^{-1} at 55 DAP	0.35	1.47	2.5	2.72	2.63
150 mg kg^{-1} at 55 DAP	0.28	1.56	2.53	2.66	2.63
F-test	ns	ns	ns	ns	ns
CV (%)	15.23	17.62	13.25	18.92	17.57
LSD	0.071	0.094	0.0843	0.0845	0.083

Letters within columns indicate Least Significant Differences (LSD) at *p = 0.05; NS: Non significant

Table 3: The content of chlorophyll, phenolic and quinone of lettuce as influenced by putting gypsum at different concentrations and time application

Factors	Chlorophyll content (mg m^{-2})	Phenolic content (mg/100 g)	Quinone content (mg FW)
Gypsum conc (mg kg^{-1})			
0	18.43b	1039.56	0.1389
50	26.80a	1026.84	0.1226
100	22.21ab	1075.91	0.1593
150	21.79ab	878.5	0.1656
F-test	*	ns	ns
CV (%)	12.41	14.08	15.44
LSD	1.8289	120.9405	0.0286
Time application			
control	18.43	1039.56	0.1389
25 DAP	24.42	939.72	0.1366
40 DAP	23	1068.76	0.1646
55 DAP	23.37	963.4	0.1463
F-test	ns	ns	ns
CV (%)	14.34	14.64	15.68
LSD	1.986	122.9343	0.0291
Conc×time application			
Control	18.43	1039.56	0.1389
50 mg kg^{-1} at 25 DAP	30.65	1018.37	0.1039
100 mg kg^{-1} at 25 DAP	20.72	1154	0.1502
150 mg kg^{-1} at 25 DAP	21.91	646.79	0.1558
50 mg kg^{-1} at 40 DAP	26.5	957.62	0.1507
100 mg kg^{-1} at 40 DAP	21.46	1144.11	0.212
150 mg kg^{-1} at 40 DAP	21.03	1104.55	0.131
50 mg kg^{-1} at 55 DAP	23.26	1104.55	0.1133
100 mg kg^{-1} at 55 DAP	24.44	880.85	0.1157
150 mg kg^{-1} at 55 DAP	22.43	884.15	0.2101
F-test	ns	ns	ns
CV (%)	12.63	14.44	15.86
LSD	2.6119	174.3981	0.0406

Letters within columns indicate Least Significant Differences (LSD) at *p = 0.05; NS: Non significant

Table 4: TSS, TA, pH and ascorbic acid content of lettuce as influenced by putting gypsum at different concentrations and time application

Factor	TSS (°brix)	TA (%)	pH	Ascorbic acid (mg ascorbic acid/100 mL juice)
Gypsum conc (mg kg^{-1})				
0	0.20	0.0640	6.55	14.14a
50	0.23	0.0453	6.50	14.10b
100	0.23	0.0587	6.50	14.09b
150	0.26	0.0827	6.49	14.11b
F-test	ns	ns	ns	*
CV (%)	18.23	12.06	1.54	0.24
LSD	0.0234	0.0203	0.0353	0.0115
Time application				
control	0.20	0.0640	6.55	14.14
25 DAP	0.27	0.0667	6.53	14.09
40 DAP	0.23	0.0747	6.48	14.11
55 DAP	0.23	0.0453	6.48	14.1
F-test	ns	ns	ns	ns
CV (%)	17.58	19.45	1.51	0.24
LSD	0.0229	0.0205	0.0347	0.0118
Conc×time application				
Control	0.20	0.064	6.55	14.14a
50 mg kg^{-1} at 25 DAP	0.25	0.064	6.53	14.09bc
100 mg kg^{-1} at 25 DAP	0.28	0.072	6.53	14.07c
150 mg kg^{-1} at 25 DAP	0.28	0.064	6.53	14.11abc
50 mg kg^{-1} at 40 DAP	0.25	0.040	6.48	14.13ab
100 mg kg^{-1} at 40 DAP	0.20	0.056	6.53	14.11abc
150 mg kg^{-1} at 40 DAP	0.23	0.038	6.45	14.09bc
50 mg kg^{-1} at 55 DAP	0.20	0.032	6.50	14.09bc
100 mg kg^{-1} at 55 DAP	0.20	0.048	6.45	14.07c
150 mg kg^{-1} at 55 DAP	0.28	0.056	6.50	14.13ab
F-test	ns	ns	ns	*
CV (%)	17.9	13.45	1.61	0.22
LSD	0.0329	0.0292	0.0522	0.0156

Letters within columns indicate Least Significant Differences (LSD) at *p = 0.05; NS: Non significant

Table 5: Activity of PPO lettuce as influenced by putting gypsum at different concentrations and period of time one week after transplanting

	PPO acitivity at different times (sec)					
Factor	0	60	120	180	240	300
Gypsum conc (mg kg^{-1})						
0	0.0793	0.0953	0.11	0.1258	0.1383	0.1495
50	0.1044	0.1337	0.1598	0.1828	0.2028	0.22
100	0.084	0.132	0.1533	0.1721	0.1886	0.2028
150	0.1225	0.2089	0.1641	0.1867	0.2092	0.2325
F-test	ns	ns	ns	ns	ns	ns
CV (%)	17.02	16.75	16.2	14.48	13.32	13.05
LSD	0.024	0.0519	0.0306	0.0337	0.0365	0.0397
Time application						
control	0.0793	0.0953	0.11	0.1258	0.1383	0.1495
25 DAP	0.0998	0.1517	0.1783	0.2023	0.2229	0.2408
40 DAP	0.1087	0.1928	0.1453	0.1652	0.1843	0.2053
55 DAP	0.1024	0.1301	0.1534	0.1741	0.1935	0.2093
F-test	ns	ns	ns	ns	ns	ns
CV (%)	18.46	18.09	15.45	13.88	12.82	12.84
LSD	0.0246	0.0527	0.0302	0.0333	0.0362	0.0396
Conc×time application						
Control	0.0793	0.0953	0.11	0.1258	0.1383	0.1495
50 mg kg^{-1} at 25 DAP	0.1055	0.1385	0.1673	0.1932	0.216	0.236
100 mg kg^{-1} at 25 DAP	0.088	0.1775	0.1988	0.2172	0.2328	0.2463
150 mg kg^{-1} at 25 DAP	0.106	0.139	0.169	0.1965	0.22	0.2403
50 mg kg^{-1} at 40 DAP	0.1168	0.1495	0.1793	0.2053	0.2263	0.244
100 mg kg^{-1} at 40 DAP	0.077	0.1055	0.1253	0.143	0.1592	0.1732
150 mg kg^{-1} at 40 DAP	0.1323	0.3235	0.1315	0.1473	0.1673	0.1985
50 mg kg^{-1} at 55 DAP	0.091	0.113	0.1328	0.15	0.1662	0.18
100 mg kg^{-1} at 55 DAP	0.087	0.113	0.1358	0.156	0.1738	0.189
150 mg kg^{-1} at 55 DAP	0.1293	0.1643	0.1918	0.2163	0.2405	0.2588
F-test	ns	ns	ns	ns	ns	ns
CV (%)	12.61	19.19	18.76	16.86	15.76	16.32
LSD	0.0367	0.0754	0.0452	0.0498	0.0542	0.0595

NS: Non significant

Table 6: PPO activity of lettuce as influenced by putting gypsum at different concentrations and period of time three weeks after transplanting

	Acitivity of PPO at different times (sec)					
Factors	0	60	120	180	240	300
Gypsum conc (mg kg^{-1})						
0	0.359	0.4538a	0.5033a	0.5540a	0.6013a	0.6393a
50	0.3008	0.3689ab	0.4314ab	0.4819ab	0.5221ab	0.5541ab
100	0.2273	0.2723b	0.3093b	0.3402b	0.3649b	0.3856b
150	0.3119	0.4085a	0.4838a	0.5407a	0.5847a	0.6190a
F-test	ns	*	*	*	*	*
CV (%)	16.6	14.67	14.28	14.05	13.72	13.34
LSD	0.0373	0.0442	0.0507	0.0559	0.0597	0.0627
Time application						
control	0.359	0.4538	0.5033	0.554	0.6013	0.6393
25 DAP	0.2498	0.3162	0.3693	0.4125	0.4477	0.4763
40 DAP	0.3084	0.385	0.4448	0.4896	0.5243	0.5512
55 DAP	0.2818	0.3486	0.4104	0.4608	0.4997	0.5312
F-test	ns	ns	ns	ns	ns	ns
CV (%)	17.89	17.34	17.78	17.98	17.88	17.68
LSD	0.0386	0.0476	0.0558	0.0623	0.0672	0.0708
Conc×time application						
Control	0.359	0.4538	0.5033	0.554	0.6013	0.6393
50 mg kg^{-1} at 25 DAP	0.2673	0.333	0.3873	0.4325	0.4695	0.4998
100 mg kg^{-1} at 25 DAP	0.2547	0.3068	0.3463	0.377	0.4025	0.4225
150 mg kg^{-1} at 25 DAP	0.2273	0.3088	0.3742	0.428	0.4712	0.5065
50 mg kg^{-1} at 40 DAP	0.315	0.3825	0.4363	0.48	0.5153	0.543
100 mg kg^{-1} at 40 DAP	0.2148	0.2558	0.2925	0.3218	0.3468	0.3672
150 mg kg^{-1} at 40 DAP	0.3955	0.5167	0.6058	0.667	0.7108	0.7435
50 mg kg^{-1} at 55 DAP	0.32	0.3913	0.4708	0.5333	0.5815	0.6195
100 mg kg^{-1} at 55 DAP	0.2123	0.2545	0.2893	0.3218	0.3455	0.367
150 mg kg^{-1} at 55 DAP	0.313	0.4	0.4713	0.5273	0.572	0.607
F-test	ns	ns	ns	ns	ns	ns
CV (%)	16.6	14.44	14.11	14.19	14.13	14.02
LSD	0.0526	0.062	0.0713	0.0794	0.0856	0.0904

Letters within columns indicate Least Significant Differences (LSD) at *p = 0.05; NS: Non significant

Table 7: PPO activity of lettuce as influenced by putting gypsum at different concentrations and period of time five weeks after transplanting

	Acitivity of PPO at different times (sec)					
Factor	0	60	120	180	240	300
Gypsum conc (mg kg^{-1})						
0	0.165	0.212	0.2475	0.2775	0.3013	0.3218
50	0.1961	0.2524	0.3004	0.3412	0.3744	0.4012
100	0.1638	0.2113	0.2525	0.288	0.3177	0.3433
150	0.1888	0.2421	0.2826	0.3193	0.3481	0.3723
F-test	ns	ns	ns	ns	ns	ns
CV (%)	10.74	13.25	14.06	14.78	14.7	14.56
LSD	0.0196	0.0273	0.0331	0.0385	0.042	0.0449
Time application						
control	0.165	0.212	0.2475	0.2775	0.3013	0.3218
25 DAP	0.1951	0.2534	0.2996	0.3405	0.3711	0.3964
40 DAP	0.1742	0.2286	0.2743	0.3128	0.3448	0.3716
55 DAP	0.1795	0.2238	0.2617	0.2952	0.3243	0.3488
F-test	ns	ns	ns	ns	ns	ns
CV (%)	11.24	13.52	14.26	15.08	14.95	14.77
LSD	0.02	0.0276	0.0334	0.0387	0.0423	0.0451
Conc×time application						
Control	0.165	0.212	0.2475	0.2775	0.2695	0.2908
50 mg kg^{-1} at 25 DAP	0.1905	0.2517	0.3025	0.3463	0.3797	0.4045
100 mg kg^{-1} at 25 DAP	0.1805	0.2348	0.2838	0.3233	0.356	0.3858
150 mg kg^{-1} at 25 DAP	0.2143	0.2738	0.3125	0.352	0.3775	0.399
50 mg kg^{-1} at 40 DAP	0.1753	0.2335	0.284	0.324	0.3575	0.3853
100 mg kg^{-1} at 40 DAP	0.1405	0.1833	0.2202	0.2528	0.2798	0.3023
150 mg kg^{-1} at 40 DAP	0.2068	0.269	0.3185	0.3615	0.3973	0.4273
50 mg kg^{-1} at 55 DAP	0.2225	0.272	0.3148	0.3533	0.386	0.4138
100 mg kg^{-1} at 55 DAP	0.1705	0.216	0.2535	0.288	0.3173	0.342
150 mg kg^{-1} at 55 DAP	0.1455	0.1835	0.2168	0.2445	0.2695	0.2908
F-test	ns	ns	ns	ns	ns	ns
CV (%)	10.24	13.79	15.02	16.09	16.03	15.92
LSD	0.0276	0.0393	0.0483	0.0563	0.0617	0.066

NS: Non significant

DISCUSSION

The effect of pre-harvest soil application of gypsum on the development of enzymatic browning and internal qualities of Grand Rapids lettuce was studied. Fresh weight of plants treated with 50 mg kg^{-1} gypsum applied at 40 DAP had the maximal plant weight, but the gypsum had no effect to biomass. This implied that fertilization with gypsum improved only fresh weight. This may be attributed to calcium that can increase the cell wall and the integrity of the plant membrane, regulate ion transport and control ion exchange particularly in meristematic tissue (Rengal, 1992; Gislerod, 1999). Ritchey and Snuffer (2002) reported that gypsum is a readily available calcium amendment that is sufficiently soluble to move rapidly into the soil when surface-applied. The solubility of gypsum, when surface applied, permits a quick release of calcium (Ca^{2+}) and sulfate (SO_4^{2-}) ions into the soil solution (Dontsova *et al*., 2005). Thus, use of gypsum will have a positive effect on lettuce growth. These results were consistent with those reported in blueberry (Wright *et al*., 1993), citrus (Bañuls *et al*., 1991) and maize (Cramer *et al*., 1988). The EPA (2008) reported that gypsum has been recognized as a nutrient source of sulfur, which is essential for many crops. Gypsum also improved soil physical properties and allowed for greater water infiltration in soils (Dontsova *et al*., 2005; EPA, 2008). Some additional benefits of gypsum application may result in increases in the exchangeable Ca/Al ratio (Ritchey and Snuffer, 2002). However, a more likely alternative explanation for yield improvement may be attributed to gypsum enhanced mineral nutrient uptake due to reduced Al phytotoxicity (Stout and Priddy, 1996). However, the appropriate time for gypsum application in this experiment should be taken at 40 DAP because gypsum is considered to be long-lasting effects, which may be directly reflected on fresh weight (Prado *et al*., 2005).

For leaf colour, the results showed that at the harvesting stage, there was not a remarkable difference in leaf colour in terms of L*, a* and b*, while chlorophyll content in the lettuce leaves were increased significantly by application of gypsum treatment to the soil. The reason for this may be derived from exchangeable ions of Ca^{2+} and Mg^{2+} levels increased with the gypsum treatments (Ritchey *et al*., 1995) leading to activate the metabolism of the chlorophyll molecule in lettuce leaves (Heaton and Marangoni, 1996; Heaton *et al*., 1996). Prado *et al*. (2005) reported that liming treatments were most effective in delaying the degradation of the green pigments (chlorophyll) of guava fruit by acting to retard senescent changes.

The results on browning appearance showed that lettuce leaves had the higher scores of browning appearance. Similar results were found by Soares *et al*. (2005) who verified that maturity stages had a strong influence on the incidence of internal browning of pineapple fruit. For gypsum concentration, untreating with gypsum showed a marked difference in leaf browning scores by visual evaluation in only the early stage after transplanting. The opposite result was found by Dilley (1990) and Hewett and Watkins (1991). They reported that calcium application could reduce the incidence of this disorder in apples. It is possible that another variable conditions in the field, especially environmental conditions, could have effect to these variable responses.

For the results on the content of phenolic, quinone, TSS, TA and pH, it was found that all of these parameters in the lettuce leaves were not affected by gypsum application. Unfortunately, there has been a lack of recorded data about these parameters. This may be due to internal qualities of lettuce being influenced by numerous factors, such as environmental factors (Cheynier *et al*., 1998; Liu *et al*., 2007) and degree of maturity (Montealegre *et al*., 2006; Manach *et al*., 2004). These results were similar with the previous researches of Kahkonen *et al*. (1999) who found no relationship between antioxidant activity and phenolic content in some plant extracts. Other authors have reported that browning is not necessarily related to phenolic content in fruit species such as apples (Couture *et al*., 1993; Weurman and Swain, 1955). For quinone content, Aude *et al*. (1990) cited that the o-quinone was quite unstable and highly reactive electrophilic molecules that spontaneously polymerize, leading to the formation of brown pigments responsible for tissue browning (Ke and Saltveit, 1986, 1989; Gawlik-Dziki *et al*., 2008). While Degl'Innocenti *et al*. (2007) found that during the developmental stage, phenol and quinone content did not vary, possibly as a result of continuous conversion between these molecules through an enzymatic spontaneous reaction. Furthermore, no significant difference in the content of TSS, TA and pH were observed in the leaves of lettuce at harvesting stage. These results were in agreement with the results reported by Prado *et al*. (2005) whom reported that liming did not significantly affect on some chemical characteristics of guava fruit. Unfortunately, little information of the internal qualities involving browning appearance present in lettuce has been available during plant development.

The results on ascorbic acid content in plant-treated with gypsum showed lower than control plant. This implied that there was an apparently greater detrimental

effect of gypsum on the ascorbic acid content. The results raised a question on the reason for the differences in ascorbic acid contents between treatments. Unfortunately, little information of the ascorbic acid presented in lettuce has been available during plant development. These are corresponded to the results of de Castro *et al*. (2008) cited that ascorbic acid in plant tissues and calcium may worked together preserving the stability of the membrane and simultaneously decreased ascorbic acid content in plant-treated with gypsum. Thus, it is doubtful that gypsum is associated with still unknown factors and might be an indirect effect to ascorbic acid degradation. Generally, ascorbic acid acts as an antioxidant as an enzyme inhibitor because it reduces the initial quinone formed by the enzyme to the original diphenol before it undergoes secondary reactions, which lead to browning (Rapeanu *et al*., 2006). The content of ascorbic acid in vegetables can be influenced by various factors including preharvest climatic conditions, cultural practices and maturity stage (Lee and Kader, 2000). Our results were consistent with the reports of Lee and Kader (2000) who reported that ascorbic acid was also easily oxidized, especially greatly favored in aqueous solutions of alkaline pH, which is in contrast to Macnish *et al*. (2000), who found that the applied calcium exerts its stabilizing function (Tobias *et al*., 1993), affecting to reduce the rate of ascorbic acid degradation (Conway and Sams, 1983). However, the exact mechanism of gypsum on decreasing the ascorbic acid content is not yet clear. Further work is required to better understand the biochemical mechanism of gypsum-induced ascorbic acid loss in Grand Rapids lettuces because most successful vegetable growers are careful to properly use the fertilizer to obtain good quality and maintain their nutritional value. The results on PPO activities of lettuce plants were quite similar during development and relatively unaffected by treatment with gypsum, except at the third week after transplanting. At this period, activities of PPO in leaves of the control plants were higher than in the plants fertilized with 100 mg kg^{-1} gypsum. This could possibly be due to a role for calcium in gypsum maintaining the stability of membranes in plant cell, thus delaying senescence and controlling physiological disorders in lettuces (Poovaiah, 1986). These corresponded to the results of Zheng *et al*. (2006) who cited that calcium content in apples was characterized by dynamic changes. They showed that the calcium concentration in apple leaves decreased with the developmental stage, thus the control of enzymatic browning was decreased (Poovaiah, 1986) because enzymatic browning is a direct consequence of membrane disintegration (Franck *et al*., 2007). The destruction of cellular compartmentation allows the phenolic substrates to be accessible to PPO which catalyze the phenolic oxidation and form the browning disorder (Mayer, 1987). Thus, the application of gypsum, which is composed of calcium, probably acted to retard senescent changes by preserving membrane integrity (Picchioni *et al*., 1996) and thereby decreased the enzymatic browning by PPO activity (Xuan *et al*., 2001). Similar results concerning change in PPO activity was reported for Feizixiao fruit (Hu *et al*., 2000). This could be explained by, with the extension of development, cells gradually lose the integrity of the membrane system, which results in the loss of compartmentation, allowing PPO enzymes, which solubilized from the plastid to the cytoplasm, act with phenolic compounds leaked from the vacuole leading to quinone formation and polymerization of brown pigments (Zhang *et al*., 2000; De Castro *et al*., 2008). In addition, calcium uptake by root was rapid and linear in the early developmental stage for cell wall stabilization and membrane integrity, but then declined distinctly, continuing until harvest (Cline *et al*., 1991). Thus the later the plant development, the more degree of browning observed. Furthermore, calcium applied to the potting medium could increase calcium levels in tissue susceptible to enzymatic browning during rapid development (Harbaugh and Woltz, 1989). At present, the underlying biochemical factors associated with an enzymatic browning disorder of lettuce focused on gypsum application to modulate PPO enzyme activities are poorly understood. Moreover, there is no report about the efficiency of gypsum for controlling the enzymatic browning in lettuce during development. Thus, it is difficult to ascribe the role of gypsum in modulating PPO activity; however, a role in maintaining cell and membrane integrity has been well established (Poovaiah, 1986). Thus the major role of gypsum affected to PPO in catalyzing the enzymatic browning of lettuce requires further investigation. In any further investigation, the application time may be more suitable if it reduced ten days (i.e., 25, 35 and 45 DAP). Other factors involving browning disorder of lettuce should be examined.

In conclusion, it was found that soil gypsum at 50 mg kg^{-1} applied at 40 DAP had the maximal fresh weight. At harvesting, lettuce-treated with 50 mg kg^{-1} had the highest chlorophyll content. In addition, plants treated with gypsum at different concentrations had lower ascorbic acid content than the control, while 100 mg kg^{-1} gypsum lowered the PPO activity at week 3 after transplanting. Furthermore, treating with gypsum had no effect to the following parameters of biomass, leaf colour, the content of phenolic, quinone, TSS, TA and pH in lettuce.

ACKNOWLEDGMENTS

This research was funded by the Mahasarakham University under project no. 5101074/2551. The authors wish to express their sincere thanks to the Financial Office for financial assistance. We gratefully acknowledge Mr. Paul Dulfer for revising the manuscript.

REFERENCES

Ahvenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol., 7: 179-187.

Altunkaya, A. and V. Gökmen, 2008. Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). Food Chem., 107: 1173-1179.

AOAC, 1980. Official Methods of Analysis of the Association of Official Analytical Chemistry. 13th Edn., AOAC, Washington DC.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analysis Chemists, Washington, DC. USA., ISBN-13: 978-0935584424.

Aude, M., R. Mayer, J. Ralambosoa and J. Philippon, 1990. Roles of o-quinones and their polymers in the enzymic browning of apples. Phytochemistry, 29: 435-440.

Bañuls, J., F. Legaz and E. Primo-Millo, 1991. Salinity-calcium interactions on growth and ionic concentration of citrus plants. Plant Soil, 133: 39-46.

Cantos, E., J.A. Tudela, M.I. Gil and J.C. Espín, 2002. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. J. Agric. Food Chem., 50: 3015-3023.

Castaner, M., M.I. Gil and M.V. Ruiz, 1999. Browning susceptibility of Minimally processed baby and Romaine lettuces. Eur. Food Res. Technol., 209: 52-56.

Cheynier, V., M. Moutounet and P. Sarni-Manchado, 1998. Les Composés Phénoliques. In: Oenologie, Fondements Scientifiques et Tecnologiques, Flanzy, C. (Ed.). Lavoisier Tec and Doc, Paris, ISBN-13: 978-2743002435.

Cline, J.A., E.J. Hanson, W.J. Bramlage, R.A. Cline and M.M. Kushad, 1991. Calcium accumulation in Delicious apple fruit. J. Plant Nutr., 14: 1213-1222.

Conway, W.S. and C.E. Sams, 1983. Calcium infiltration of Golden Delicious apples and its effect on decay. Phytopathololgy, 73: 1068-1071.

Couture, R., M.I. Cantwell, D. Ke and M.E. Saltveit, 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. HortScience, 28: 723-725.

Cramer, G.R., E. Epstein and A. Lauchli, 1988. Kinetics of root elongation of maize in response to short-term exposure to NaCl and elevated calcium concentration. J. Exp. Bot., 39: 1513-1522.

De Castro, E., D.M. Barrett, J. Jobling and E.J. Mitcham, 2008. Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples. Posthar. Biol. Technol., 48: 182-191.

Degl'Innocenti, E., A. Pardossi, F. Tognoni and L. Guidi, 2007. Physiological basis of sensitivity to enzymatic browning in lettuce, escarole and rocket salad when stored as fresh-cut products. Food Chem., 104: 209-215.

Dilley, D.R., 1990. Increasing the calcium content of apple fruits to improve storability and attenuate physiological disorders. Annu. Rep. Michigan State Hortic. Soc., 120: 195-207.

Dontsova, K., Y.B. Lee, B.K. Slater and J.M. Bigham, 2005. Gypsum for agricultural use in Ohio sources and quality of available products. Ohio State University Extension Fact Sheet. School of Natural Resources, http://ohioline.osu.edu/anr-fact/0020.html.

Dupont, S., Z. Mondi, G. Willamson and K. Price, 2000. Effect of variety, processing, and storage on the flavonoid glycoside and composition of lettuce and chicory. J. Agric. Food Chem., 48: 3957-3964.

EPA, 2008. Agricultural uses for Flue Gas Desulfurization (FGD) gypsum. United States Environmental Protection Agency. http://www.epa.gov/osw/.

Felicetti, D.A. and L.E. Schrader, 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and carotenoids. Plant Sci., 176: 78-83.

Franck, C., J. Lammertyn, Q.T. Ho, P. Verboven, B. Verlinden and B.M. Nicolaï, 2007. Browning disorders in pear fruit. Postharvest Biol. Technol., 43: 1-13.

Gawlik-Dziki, U., U. Zlotek and M. Swieca, 2008. Characterization of polyphenol oxidase from butter lettuce *Lactuca sativa capitata* L.). Food Chem., 107: 129-135.

Gislerod, H.R., 1999. The role of calcium on several aspects of plant and flower quality from a floricultural perspective. Acta Hortic., 481: 345-352.

González-Aguilar, G.A., S. Ruiz-Cruz, R. Cruz-Valenzuela, A. Rodríguez-Félix and C.Y. Wang, 2004. Physiological and quality changes of fresh-cut pineapple treated with antibrowning agents. Lebensmittel-Wissenschaft und-Technologie, 37: 369-376.

Harbaugh, B.K. and S.S. Woltz, 1989. Fertilization practice and foliar-bract calcium sprays reduce incidence of marginal bract necrosis of poinsettia. HortScience, 24: 465-468.

Heaton, J.W. and A.G. Marangoni, 1996. Chlorophyll degradation in processed foods and senescent plant tissues. Trends Food Sci. Technol., 7: 8-15.

Heaton, J.W., R.Y. Yada and A.G. Marangoni, 1996. Discoloration of coleslaw is caused by chlorophyll degradation. J. Agric. Food Chem., 44: 395-398.

Hewett, E.W. and C.B. Watkins, 1991. Bitter pit control by sprays and vacuum infiltration of calcium in Cox's Orange Pippin apples. HortScience, 26: 284-286.

Hu, G., D. Chen, P. Li, R. Ouyang, F. Gao and W. Wang, 2000. Changes of pigment, phenol and enzyme activity in litchi fruit peel during the fruit development. J. Fruit Sci., 17: 35-40.

Jiang, Y. and J. Fu, 1998. Inhibition of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. Food Chem., 62: 49-52.

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kulaja and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Kays, S.J., 1991. Postharvest Physiology of Perishable Plant Products. Van Nostrand Reinhold, New York, ISBN-13: 978-1888186536, pp: 532.

Kays, S.J., 1999. Preharvest factors affecting appearance. Postharvest Biol. Technol., 15: 233-247.

Ke, D. and M.E. Saltveit, 1986. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. HortScience, 21: 1169-1171.

Ke, D. and M.E. Saltveit, 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in Iceberg lettuce. Physiol Plantarum., 76: 412-418.

Lee, S.K. and A.A. Kader, 2000. Preharvest and Postharvest factors influencing vitamin C content of horticulture crops. Postharvest Biol. Technol., 28: 207-220.

Lister, C.E., 2003. Antioxidants: A Health Revolution. Institute for Crop and Food Research, New Zealand, ISBN: 978-0478108323, pp: 96.

Liu, L., B. Xie, S. Cao, E. Yang, X. Xu and S. Guo, 2007. A-type procyanidins from *Litchi chinensis* pericarp with antioxidant activity. Food Chem., 105: 1446-1451.

Llorach, R., A. Martínez-Sánchez, F.A. Tomás-Barberán, M.I. Gil and F. Ferreres, 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem., 108: 1028-1038.

Macheix, J.J., A. Fleuriet and J. Billot, 1990. Fruit phenolics. CRC Press, Boca Raton, ISBN: 978-0849349683, pp:392.

Macnish, A.J., D.C. Joyce, P.J. Hofman, D.H. Simons and M.S. Reid, 2000. 1-Methylcyclopropene treatment efficacy in preventing ethylene perception in banana fruit and grevillea and waxflower flowers. Aust. J. Exp. Agric., 40: 471-481.

Manach, C., A. Scalbert, C. Morand, C. Remesy and L. Jimenez, 2004. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr., 79: 727-747.

Mayer, A.M., 1987. Polyphenoloxidase in plants-recent progress. Phytochemistry, 26: 11-20.

Montealegre, R.R., R.R. Peces, J.L.C. Vozmediano, J.M. Gascueña and E.G. Romero, 2006. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. J. Food Compos. Anal., 19: 687-693.

Nicolas, J.J., F.C. Richard-Forget, P.M. Goupy, M.J. Amiot and S.Y. Aubert, 1994. Enzymatic browning reactions in apple and products. Crit. Rev. Food Sci. Nutr., 34: 109-157.

Nicolle, C., N. Cardinault, E. Gueux, L. Jaffrelo, E. Rock and A. Mazur, 2004. Health effect of vegetable-based diet: Lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clin. Nutr., 23: 605-614.

Picchioni, G.A., A.E. Watada, B.D. Whitaker and A. Reyes, 1996. Calcium delays senescence-related membrane lipid changes and increases net synthesis of membrane lipid components in shredded carrots. Postharvest Biol. Technol., 9: 235-245.

Pirie, A. and M.G. Mullins, 1976. Changes in anthocyanin and phenolic content of grapevine leaf and fruit tissue treated with sucrose, nitrate and abscisic acid. Plant Physiol., 58: 468-472.

Poovaiah, B.W., 1986. Role of calcium in prolonging storage life of fruits and vegetables. Food Tech., 40: 86-89.

Prado, M.R., W. Natale and J.A.A. Silva, 2005. Liming and quality of guava fruit cultivated in Brazil. Sci. Hort., 106: 91-102.

Rapeanu, G., A. van Loey, C. Smout and M. Hendrickx, 2006. Biochemical characterization and process stability of polyphenoloxidase extracted from Victoria grape *Vitis vinifera* sp. Sativa. Food Chem., 94: 253-261.

Rengal, Z., 1992. The role of calcium in salt toxicity. Plant Cell Environ., 15: 625-632.

Ribeiro, S.M.R., L.C.A. Barbosa, J.H. Queiroz, M. Knodler and A. Schieber, 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chem., 110: 620-626.

Ritchey, K.D., C.M. Feldhake, R.B. Clark and D.M.G. de Sousa, 1995. Improved Water and Nutrient Uptake from Subsurface Layers of Gypsum-Amended Soils. In: Agricultural Utilization of Urban and Industrial by Products, Wright, R.J. and W.D. Kemper (Eds.). ASA, CSSA and SSSA, Madison, WI., ISBN-13: 9780891181231, pp: 157-181.

Ritchey, K.D. and J.D. Snuffer, 2002. Limestone, gypsum and magnesium oxide influence restoration of an abandoned appalachian pasture. Agron. J., 94: 830-839.

Rocha, A.M.C.N. and A.M.M.B. Morais, 2001. Characterization of polyphenoloxidase (PPO) extracted from Jonagored apple. Food Control, 12: 85-90.

Soares, A.G., L.C. Trugo, N. Botrel and L.F.S. Souza, 2005. Reduction of internal browning of pineapple fruit *Ananas comusus* L.) by preharvest soil application of potassium. Postharvest Biol. Technol., 35: 201-207.

SPSS, 1999. Base 9.0 for Windows Users Guide. SPSS Inc., USA.

Stout, W.L. and W.E. Priddy, 1996. Use of flue gas desulfurization (FGD) by-product gypsum on alfalfa. Commun. Soil Sci. Plant Anal., 27: 2419-2432.

Tobias, R.B., W.S. Conway and C.E. Sams, 1993. Cell wall composition of calcium-treated apples inoculated with *Botrytis cinera*. Phytochemistry, 32: 35-39.

Verlangieri, A.J., J.C. Kapeghian, S. Dean and M. Bush, 1985. Fruit and vegetable consumption and cardiovascular mortality. Med. Hypotheses, 16: 7-15.

Weurman, C. and T. Swain, 1955. Changes in the enzymatic browning of Bramley's seedling apple during their development. J. Sci. Food Agric., 6: 186-192.

Whitham, F.H., D.F. Blaydes and R.M. Devlin, 1986. Exercises in Plant Physiology. 2nd Edn., Van Nostrand Co., New York, ISBN: 9780871509444, pp: 324.

Wright, G.C., K.D. Patten and M.C. Drew, 1993. Gas exchange and chlorophyll content of Tifblue rabbit eye and Sharpblue Southern high bush exposed to salinity and supplemental calcium. J. Am. Soc. Hort. Sci., 118: 456-463.

Xuan, H., J. Streif, H. Pfeffer, F. Dannel, V. Romheld and F. Bangerth, 2001. Effect of pre-harvest boron application on the incidence of CA-storage related disorders in conference pears. J. Hort. Sci. Biotechnol., 76: 133-137.

Zhang, D., P.C. Quantick and J.M. Grigor, 2000. Changes in phenolic compounds in litchi *Litchi chinensis* Sonn fruit during postharvest storage. Postharvest Biol. Technol., 19: 165-172.

Zhang, Z., X. Pang, Z. Ji and Y. Jiang, 2001. Role of anthocyanin degradation in litchi pericarp browning. Food Chem., 75: 217-221.

Zheng, M.W., C. You, Z. Du and H. Zhai, 2006. Dynamic changes in the calcium content of several apple cultivars during the growing season. Agric. Sci. China, 5: 933-937.