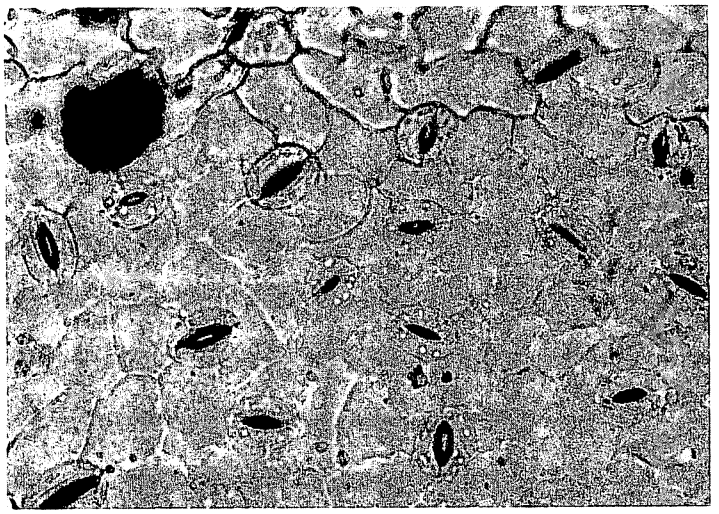

Fig. 3. Leaf peel of diploid *Luffa acutangula* showing the stomatal chloroplasts.

Usually 150 seeds of a cultigen were planted; poor germination and high mortality from colchicine treatment resulted in about 100 plants left for stomatal chloroplast number determination. Of these remaining plants, about 15% were putative tetraploids. An additional 15% were identified as sectoral chimeras and 70% were discarded based on a diploid chloroplast number. Since there is a very low recovery rate of 4× plants from sectoral chimeras, only plants that screen as 4× should be transplanted to the field or greenhouse.

One person screened 100 or more treated plants per day using this technique. More than 100 tetraploids from 25 different watermelon cultigens and 16 tetraploids of one accession of *L. acutangula* have been generated with this system in one year.

Table 2. Stomatal chloroplast numbers of colchicine treated plants and their progeny.

Crop Variety	Putative 4×[y] (no.)	Progeny 2× (no.)	Progeny 2× CN[x]	Progeny 4× (no.)	Progeny 4× CN
Watermelon					
cms[z]	28	6	11.1	14	20.2
Jubilee II	12	6	11.2	3	20.6
Calsweet	12	3	11.1	5	21.1
Picnic	11	3	10.0	7	18.4
Luffa acutangula	26	6	9.0	16	18.4

[z]*cms* = Chinese male sterile.
[y]Screened positive for tetraploid chloroplast number; however, some are known to be periclinal chimeras.
[x]CN = Number of chloroplasts in stomatal guard cells. Avg. no. in 20 cells.

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PRELIMINARY DETERMINATION OF PARAMETERS TO DEVELOP AN OBJECTIVE PROCEDURE FOR ASSESSING TIPBURN IN LETTUCE

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Abstract. **Tipburn resistant varieties of lettuce, *Lactuca sativa* L. provide an important measure of control of the disorder. The lack of a rapid and reliable screening test for tipburn has made selection for resistance time-consuming and difficult. A screening procedure for tipburn was tested utilizing 'Dark Green Boston', a tipburn susceptible line. Plant ages of 21, 38, and 56 days old at the start of testing were evaluated. Prior to testing, plants were grown at 25C and 55% RH, with regular fertilization. During testing, plants were grown in a clear polyethylene tent with high temperature (40C), high humidity (RH > 90%), and high fertility, conditions that provided for rapid plant growth. Tipburn development was assessed daily upon initiation of testing. Tipburn symptoms began appearing within 1 or 2 days after test initiation for 38 and 56 day old plants. Twenty-one day old plants developed tipburn by the third day of testing. Thirty-eight day old plants were most severely affected, having a tipburn index (number of affected leaves/total number of leaves) of 0.58. Twenty-one and 56 day-old lettuce had tipburn indexes of 0.29 and 0.19,**

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respectively. Thirty-eight day lettuce had the most severe tipburn with necrosis of the entire distal end of expanding leaves. This was attributed to the rapid development stage of the 38 day old plants. In this study, tipburn in lettuce is developed rapidly under conditions that promote rapid growth and under high humidity with lettuce that is in a stage of development conducive to rapid growth. This procedure will provide breeders with a rapid test for assessing susceptibility or resistance of lettuce to tipburn.

Introduction

Tipburn of lettuce is a serious problem that can reduce the quality of lettuce. Tipburn is a Ca-related disorder caused by Ca deficiency in young inner leaves of lettuce (Shear, 1975; Thibodeau and Minotti, 1969). Tipburn appears most frequently when lettuce grows rapidly (Tibbitts and Rao, 1968; Thibodeau and Minotti, 1969; Cox et al., 1976; Bangerth, 1979).

Increased air temperature from 30 to 39C (Misaghi and Grogan, 1978) may induce tipburn by increasing the growth rate (Andersen, 1946; Knott et al., 1939; McGinty and Thompson, 1925; Thompson, 1926; Rao, 1966). The minimum temperature for tipburn induction was 24C under laboratory conditions (Misaghi and Grogan, 1978). Increased N fertility is also positively correlated with tipburn incidence (Ashkar and Ries, 1971). The effects of increased temperature and increased N fertility were attributed to an increase in growth rate. Additionally, high humidity can encourage earlier and more severe tipburn symptoms (Bottenberg and Tibbitts, 1968; Read, 1972; Collier and Tibbitts, 1982; Goto and Takakura, 1992).

Varietal differences in the incidence and severity of tipburn of lettuce are well-documented (Newell, 1925; Bohn, 1953; Hume, 1964; Cox and McKee, 1976). Breeding lines of lettuce which have resistance to tipburn will provide a measure of control of the disorder. However, until now no objective tipburn evaluation procedure has been in use. Currently, evaluations are conducted under commercial field or greenhouse conditions, and tipburn resistance is determined by its presence or absence at harvest maturity. Reported here are preliminary experiments for the development of an objective tipburn evaluation procedure. This research utilizes this procedure and investigates whether incidence of tipburn may be assessed at an early age in 'Dark Green Boston', a highly susceptible variety.

Materials and Methods

Tipburn-susceptible lettuce 'Dark Green Boston' was used in this study. Dark Green Boston lettuce seeds were sown in 162 cell seedling flats in peat-perlite mix at 56, 38, or 21 days prior to tenting. The seedlings were thinned to one plant per cell within 14 days after sowing. At 21 days the lettuce were transplanted into 5-inch azalea pots containing the peat-perlite mix. Plants were irrigated and fertilized every other week with 20N-9P-16K fertilizer with micronutrients, but without Ca. Plants were maintained at 25C during the day with an air conditioner in a greenhouse until testing.

At 21, 38, or 56 days of growth, lettuce plants were fertilized and placed in a polyethylene tent located in a pad-and-fan-cooled greenhouse (29C days). Tenting produced high humidity (>90%) and temperatures (>35C day). Testing continued until 100% of the plants developed tipburn symptoms.

Plants were assessed daily for symptoms of tipburn. Numbers of affected leaves per plant were recorded daily. Tipburn indexes were constructed by dividing the number of affected leaves by the total number of leaves. Experiments were arranged in a randomized complete block design (Snedecor and Cochran, 1980) with four replications. The experiment was repeated twice. The experiment was conducted with 8 plants per treatment per replication in the first run and 5 plants per treatment in the second run. Statistical analyses were by analysis of variance (Snedecor and Cochran, 1980; SAS Institute, 1985).

Results

Tipburn developed very rapidly on all plants put into the tents. The 38-day-old Dark Green Boston lettuce plants exhibited symptoms the earliest and most severely with the highest number of leaves affected (Table 1 and 2). Within two days 100% of the 38 day old test plants were affected (Table 1). Twenty-one day old or 56-day-old plants exhibited 100% of the plants affected with tipburn between two and five days. Tipburn symptoms appeared first on 38-day-old plants, about 1 day after tenting (Table 2). For 21 and 56 day-old plants, tipburn appeared an average of 1.5 to 2.25 days after tenting, respectively (Table 2). Twenty-one or 56-day old plants exhibited marginal necrosis and necrotic spots on developing leaves whereas 38-day-old

Table 1. Percentage of 'Dark Green Boston' lettuce plants with tipburn symptoms during five days of testing.

Age of plants (days)	Time in Tent				
	1	2	3	4	5
	Tipburn %				
Exp. 1					
21	3.6	75.0	80.0	ND[z]	100.0
38	82.1	100.0	100.0	ND	100.0
56	46.4	83.3	100.0	ND	100.0
Exp. 2					
21	40.0	100.0	100.0	100.0	100.0
38	75.0	100.0	100.0	100.0	100.0
56	10.0	93.7	91.7	87.5	100.0

[z]Data not collected on this day.

Table 2. Mean number of 'Dark Green Boston' leaves affected with tipburn and the total number of leaves.

Age of plant (days)	Time to tipburn (days)	Leaves with tipburn (no.)	Leaves (total no.)	Tipburn index[z]
Exp. 1				
21	2.25	2.72 b[y]	7-9	0.34 b
38	1.25	6.37 a	10-13	0.56 a
56	1.75	5.56 a	25-29	0.21 b
Exp. 2				
21	1.5	1.79 b	7-9	0.22 b
38	1.0	6.42 a	10-13	0.57 a
56	2.0	5.25 a	25-29	0.20 b

[z]Mean separation within columns by LSD, 5% level.
[y]Number of affected leaves/number of total leaves.

plants exhibited necrosis of the entire distal end of developing leaves and complete necrosis of the growing point in some plants.

On 38-day or 56-day old plants approximately 6.4 or 5.4 leaves per plant (respectively) were affected with tipburn (Table 2). On 21-day old plants significantly fewer leaves were affected, about 2.3 per plant. Thirty-eight day old plants had significantly higher tipburn indexes (0.56 and 0.57 for run one and two, respectively) than the other age plants (Table 2). Twenty-one and 56-day-old plants had significantly lower tipburn indexes ranging from 0.20 to 0.34, respectively. The low index for 56 day old plants is due to the high number of leaves in those plants.

Discussion

Thirty-eight day old Dark Green Boston lettuce plants had the highest tipburn index of the three ages tested. This seems to indicate that there are stages of lettuce plant growth where tipburn development is especially critical. Plants at the 38-day old stage of growth were in first stages of leaf cupping or enclosure and were growing very rapidly. At this point, leaf expansion may be too rapid to allow Dark Green Boston to transport adequate Ca to the expanding leaf margins (Knott et al., 1939; Rao, 1966).

Calcium movement is directed through the plant by transpirational flow (Wiersum, 1966), and tissues which transpire most rapidly will accumulate Ca (Marschner, 1974). When leaves become enclosed by cupping of old leaves over young leaves, transpiration is negligible (Collier and Tibbitts, 1982) so that the age of first leaf cupping or enclosure is critical to tipburn development.

In these experiments, high temperature and fertility in combination with high humidity resulted in the development of tipburn symptoms soon after the three conditions simultaneously were initiated. Although tipburn symptoms were induced in experiments utilizing a low Ca-high NO_3 regime (Ashkar and Ries, 1971) or high humidity (Collier and Tibbitts, 1984) or high temperature (Misaghi and Grogan, 1978) and although tipburn is correlated with accelerated growth rate (Cox et al., 1976) this study represents the first attempt at combining these factors to develop a successful tipburn evaluation procedure. Further testing of additional lettuce cultivars needs to be conducted to evaluate this procedure for screening for tipburn resistance among cultivars.

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