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〈論 文〉

Effect of Boron in Nutrient Solution on Root Development and Freezing Tolerance of Mulberry

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Abstract

The experiment was carried out to define the effect of the boron in nutrient solution on the development of mulberry root and cold damage. The length of shoots only attained about 50 cm and then stopped in case of boron deficiency, while the mulberry trees developed vigorous and attained over 190 cm long under boron supply condition. When boron is deficient, the measles appeared on surface of the stem and the necrosis appeared on the petiole, midribs and veins of leaves. At 70 days after planting, almost all new roots and old roots changed to brown, the lateral roots became necrosis by boron deficiency. After three days of stopping boron supply, many new roots changed to brown and after two days of boron resupply, new roots began to grow out and then new roots normally developed. Content of boron in leaves and barks increased by the increase of boron concentration in nutrient solution and maximum boron content in leaves and barks was obtained with boron-sufficient treatment of 0.5 ppm. Treatment of 0.5 ppm boron supply indicated that the leaves and barks contained more phospholipid, protein, sugar, RNA and proline than treatment of 0.01 ppm boron supply in nutrient solution. The cuttings grown in boron supply nutrition have a sufficient tolerance at −10℃ and −15℃ for 24 hours while cuttings grown in boron-deficient nutrition have a weak tolerance at the same condition. As mentioned above, we can conclude that the effect of boron deficiency on root development is much severe and the relationship between the lack of boron and cold damage is very closely related.

Key words : Mulberry root, cold damage, boron, phospholipid, protein, sugar, RNA, proline

INTRODUCTION

Like other crops, beside three major elements as nitrogen, phosphorus and potasium, for normal development and growth the mulberry tree also takes other elements such as calcium, magnesium, boron, iron, manganese, copper, zincs and molybdenum. Though these elements are required in small quantities, but they are necessary to obtain high productivity and high quality of mulberry leaves. The lack of any one of these elements will affect to normal development and protein synthesis and among them boron is the most important. Boron plays an essential role in higher plant (Warington 1923). About the role of boron in the growth of plant, there are many researchers as Gauch (1972), Takahashi (1971) and in recent years, the influence of boron deficiency in root growth has been conducting. Studies on the reason causing non-sprouting of buds in national scale of Korea in spring of 1983 showed that the deficiency of boron was one of the main reason (Ryu 1984, 1986 and Lee *et al.* 1985) and generally the soil of reclaimed land usually lacks in organic matter and boron content (Ryu 1974).

In Korea, over 70 percent of the land was derived

from graint which is commonly deficient of boron (Michel 1955, Page 1954, Alina 1985). Its boron content is only 15% to 25% compared with standard content, so 3 kg to 6kg of borax per 10a of mulberry field should be recommended for application annually (Ryu 1974, 1984, 1987, 1992).

According to Johnson and Albert (1967), Albert (1975), they showed that decrease of RNA content was the first symptom of boron deficiency in tomato roots after the cessation of root growth but without any observation of the changing the content of DNA. Cresswell and Nelson (1973), Rajaratan and Lowry (1974), Sherstnev (1970) have reported similarly the reduction of RNA in boron-deficient plants. Sherstnev (1974) also reported that in boron deficient tissue, the utility of amino acids in protein synthesis was limited.

In addition, Kalichave *et al.* (1974) suggested that there was 30% reduction of the N-terminal alanine residue occurring in ribosomes from boron deficiency plants. Sakai (1968) and Siminovich *et al.* (1968) reported that the carbohydrate, protein, RNA and phospholipid in plant are the materials which strengthen their ability to freezing tolerance. Bogges et al. (1976) reported that the proline is easiest soluble amino acid which strengthens cold resistance in plant. Under boron deficiency condition, frost resistance is weak because carbohydrate content is low (Obelly 1966). Cooling and Jones (1970) concluded that the lack of boron is very easy to make frost damage of eucalyptus and adequate boron content increase the frost resistance. Carbohydrate and RNA content in mulberry tree were closely related with freezing tolerance (Kim 1980).

Kevresan *et al.* (1977) reported that the boron deficiency inhibited the biosynthesis and stimulated the degradation of nucleic acid, especially ribosomal RNA in sunflower and resulted in a significant deficiency of proline and a large decrease in N-terminal analine residues (Kalichava and Sherstnev 1974, Sherstnev 1974).

According to Kouch and Kumazawa (1975), Lovatt (1981), the root elongation was ceased rapidly within six hours after squash plant was transferred to boron-free medium. Root and apical meristem of sunflower plants grown under boron-deficient conditions contained lower than normal amounts of phospholipid and cell structural organization (Augsten and Eichhorn 1976, Mengel and Kirkby 1978). Other researchers (Gauch and Dugger 1954, Bonilla 1980) reported that adequate boron supply is necessary for sugar synthesis.

As mentioned above, boron is very closely related to the synthesis of proline, amino acid, RNA, protein, carbohydrate and phospholipid in plant. Though boron element is required in a small quantity, lacking of boron make new roots develop slowly and poorly, and the nutritive absorption unbalanced.

There are many reports related to the effect of boron on the growth and develpoment of plant. However, there is no information about the effect of boron deficiency on the development of mulberry root and relation between lacking of boron and the cold damage. This experiment was conducted to define the effect of boron deficiency on development of mulberry root and cold damage.

MATERIALS AND METHODS

1. Variety and cultivating method

The experiment was conducted in the laboratory of mulberry science of Sericultural Department of Kyungpook National University from April 1992 to December 1993.

The Chungilppong (*Morus alba L.*) was used in this study and the mulberry tree was planted in each 3-liter pot containing the nutrient solution. Illumination was adjusted to 6,000~7,000 lux with Halogen light. The nutrient solution was aerated continuously and renewed every three to ten days depending on growth of mulberry tree.

Stem cuttings of mulberry trees in winter dormancy were used for this study.

Stems were cut into pieces about 10 cm with 3 buds and they were preserved at -10℃ and -15℃ for 24 hours and then they were preserved in growth cabinet at 25℃ and light 850~900 lux for sprouting test.

2. Analysis of mulberry

1) Analysis of boron

Table 1. *Composition of the nutrient solution*

Element treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Mo	B (ppm)
Boron supply (+B+)	50	10	50	40	15	2	0.5	0.1	0.1	0.1	0.5
Low concentration of boron (+B+)	50	10	50	40	15	2	0.5	0.1	0.1	0.1	0.01
Boron deficiency (−B−)	50	10	50	40	15	2	0.5	0.1	0.1	0.1	0.00
−B+	Non-boron supply and then boron supply										
+B−	Boron supply and then stop boron supply										

Curcumin oxalic acid method was used for the determination of boron in solution. 0.5 g of dry leaves or barks was mineralized at 550℃ for over one hour. Ashes dissolved in 1 N-HCl and 1 m*l* of sample solution was pipetted into a plastic beaker and 4 m*l* of curcumin solution was added, and was evaporated to dryness at 50± 3℃ in water bath for over one hour. The residue was dissolved in alcohol 95% and the boron determined by spectrophotometer.

2) Extraction and determination of soluble protein, total sugar and RNA

Leaves and barks were separated from wood of one-year-old shoots, cut into small pieces and weighed immediately. Three gram of the fresh sample of the leaf or the bark was ground in mortar at 0℃ to 4℃ in the presence of 3 g sea sand, 0.6 g polyvinylpolypyrrolidone (PVP) and 10 m*l* of 0.2 M solution of phosphate buffer (pH 7.2). The extract was filtered through four layers of cheese-cloth. The combined extracts were transfered to a centrifuge tube and centrifuged at 2,800 g for 10 min and the supernatant collected. The supernatant was used as the sample solution. This solution was frozen and stored until analysis.

Protein concentration was measured by the Lowry method using bovine serum albumin (BSA) as a standard.

Total sugar concentration was measured by the Phenol-H_2SO_4 method using glucose as a standard.

RNA concentration was measured by the modified Schmidt-Thannhauser-Schneider method of Fig. 1.

3) Proline determination

one gram of the fresh sample of the leaves or the barks was used for extraction. The samples were ground in a mortar at 0℃ to 4℃ in the presence of 1 g sea sand and 10 m*l* solvent solution (methanol : chloroform : H_2O = 12 : 5 : 1). The extract was filtered through four layers of cheese-cloth. The combined extracts were transferred to a centrifuge tube and centrifuged at 1,000 g for 10 min and the supernatant collected. The sample solution was frozen and stored until analysis.

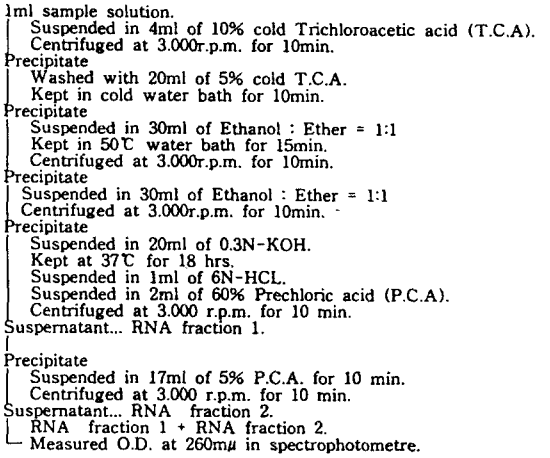

Fig. 1. *Procedures of RNA determination of mulberry tissue.*

Proline analysis was carried out by the spectrophotometric method in a Pharmacia KLB spectrophotometer (the absorbance of 520 nm). Proline concentration was measured by Ninhydrin method using L-proline a standard.

4) Thin layer chromatography for analysis of phospholipid

1 g of the fresh sample was homogenized in 10 m*l* of chloroform : methanol (C/M, 2 : 1) solution, pla-

ced at room temperature for about 5 hours and 2 ml of the distilled water was added. Then vortexed and centrifuged at 2,000r.p.m. for 10 min. Low phase (chloroform) was taken and put it into another test tube which was weighed for total lipid. Chloroform was dried up by N_2 gas and the residue was dissolved in 2 ml of C/M (2:1) solution. Sample solution was pipetted and spotted on a T.L.C. plate. Plate was developed in the solvent, dried and sprayed with 2, 7 dichlorofluorecein. The plant was placed in an ammonia chamber for about 2~5 seconds and phospholipid was identified in UV light.

Phospholipid was scraped and transferred to a test tube and 20μl of interal standard (15 : 0 fatty acid), 4 ml of 6% methanolic sulfric acid were added. Tube was vortexed and placed in a water bath at 50℃ for 1 hr 30min. Petroleum ether (2 ml) and distilled water (1 ml) was added to the tube. Upper phase transferred to a minivial, solvent was dried up with N_2 gas. After adding hexane phospholipid was determined by gas liquid chromatography.

RESULTS AND DISCUSSION

1. The boron deficiency symptoms of mulberry tree

The boron deficiency symptoms appeared first at the growing points and younger leaves, but boron deficiency did not immediately result in visible symptoms at first. Growth of mulberry tree in boron deficiency gradually decreased and stopped. Necrosis spots were formed which were caused by auxin accumulation (Coke and Whirrington 1968) and the movement of boron along with the transpiration stream also explains the fact that boron deficiency also begin at the growing point. It is generally held that boron like Ca is not mobile or mobile to only a very limited extent in the phloem (Raven 1980, Dugger 1983).

The younger leaves of boron deficiency were misshapened and wrinkled, the measles appeared in surface on the stem and necrosis appeared on the petiole, midribs, veins of leaves and then the leaves and stems become brittle. As the deficiency progresses the terminal growing point died. The original cause of plant death during boron deficiency is an accumulation of phenolic compounds in plants (Shkol'nik 1974). The similar results were reported in tomato studies by Brown (1979).

2. Effect of boron concentration on the length of shoots and root development

1) Effect of boron concentration on shoot development

As the data shown in table 2, the length of shoots attained from 191.4 cm to 209.0 cm in case of continuous supply of boron. The lack of boron in nutrient solution caused an extreme inhibition of growth and the length of shoots only attained about 50 cm long.

Figure 2 shows that about 70 days after planting the mulberry trees began the symptom of stopping growth and then completely stopped within a few days.

2) Effect of boron concentration on root development

There is a close relationship between the growth of branches, leaves and development of roots. Experimental results showed that the boron deficiency had an influence not only on the growth of shoots and leaves but also on the development of root.

In the treatment of the +B+ the root system developed well, their new roots were stronger, thicker and root color was bright yellow compared with the −B− (boron-deficient). The root system of lacking boron developes very weak, root color is dark yellow (Fig. 3).

In the −B+ the new roots reappeared after supplying boron two days and then normally developed with formation of many new roots.

In the +B−, after three days of stopping supply boron, the color of many new roots changed to brown, the lateral roots became necrotic and the old root system decreased significantly, similar to the results of Bussler (1960, 1964), Albert (1975) and Yih, Clark (1964) in sunflower, squash plant and tomato plant.

Figure 4 showed that formation of new root normally appeared and increased vigorously in the treatment of 0.5 ppm boron supply. In the −B+ which the first period was boron-deficient, the new root developed slowly, after planting about 70 days

Table 2. *Effect of boron deficiency on length of shoots (cm)*

Treatment	Days after cultivating								
	20	30	50	70	90	110	130	150	170
+B+ (0.5 ppm)	11.5	25.5	48.2	61.4	84.8	96.4	130.8	160.0	209.0
+B+ (0.01 ppm)	13.5	27.5	52.4	61.8	88.2	97.2	134.0	150.0	191.0
−B+	12.5	23.5	30.0	50.0	72.5	87.5	116.5	149.0	200.0
+B−	13.5	29.5	55.5	60.5	stop	−	−	−	−
−B−	12.5	20.5	40.5	50.5	stop	−	−	−	−

Note : +B+ 0.5 ppm boron supply
+B+ 0.01 ppm boron supply
−B+ Non-boron supply and then boron supply
+B− Supply boron and then stop boron supply
−B− Boron deficiency

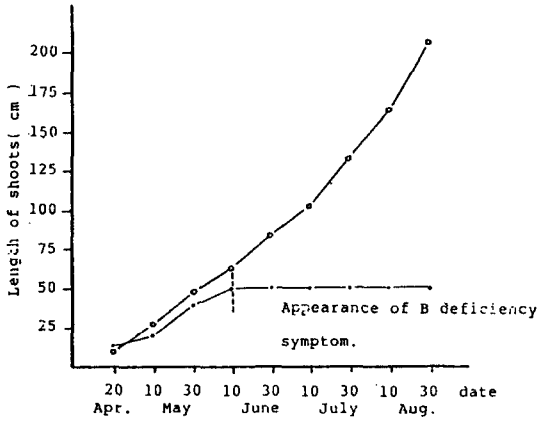

Fig. 2. *Effect of boron deficiency on developing of shoots and appearance of boron deficiency symptom. −Boron supply +B+(0.5 ppm): (○−○) −Boron deficiency −B−: (●−●)*

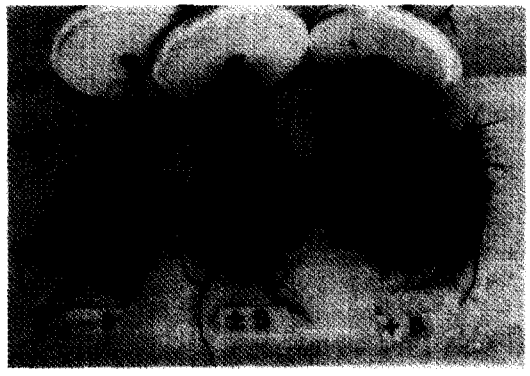

Fig. 3. *The effect of boron deficiency on root development*
− −B : Boron deficiency
− ±B : Supply boron and then stop boron supply
− +B : Boron supply

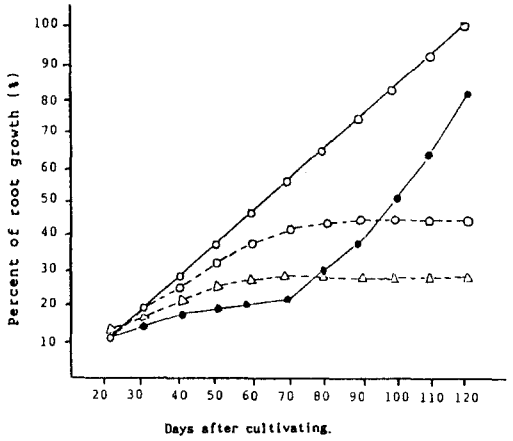

Fig. 4. *Effect of boron on the formation of new root. −Treatment of 0.5 ppm boron supply +B+ (○−○), −Non-boron supply and then boron supply −B+ (●−●), −Sdupply boron and then stop boron supply +B− (○---○), −Boron deficiency −B− (△---△)*

almost all the new roots did not grow out. After boron was resupplied, the new root reappeared and developed rapidly. The percent of root growth attained over 80% compared with 100% of the +B+.

In the +B− which the first period was boron supply, the root system normally developed, and after stopping boron supply the new root developed poorly, root color turned dark yellow. The percent of root growth of the +B− was 43.0% and that of the −B− was 25.0%. Continuous supply of boron is required for the maintenance of meristematic activity, and for the synthesis of uracil. Uracil is an essential component of RNA and if it is absent RNA contaning assemblies such as ribosomes can-

not be formed, thus affecting protein synthesis. Ribonucleic acid synthesis, ribose formation, and the synthesis of protein are most important processes in meristemetic tissues. If they are disturbed by a lack of boron the entire process of meristemetic growth is impaired. The cessation of root elongation brought about by boron deficiency was caused by a failure of cell division in merismatic cells, and not by cellular elongation (Cohen and Lepper 1977, Hirsch *et al.* 1982). This is the main reason why formation of new roots decreased and stopped rapidly under boron-deficient condition (Albert 1968, Birnbaum *et al.* 1977, Gupta 1979).

3. Effect of boron concentration on the content of boron in mulberry leaves and barks

Boron content in leaves was significantly higher than that in barks. Boron content of leaves in the −B− was 9.0 ppm. But it increased vigorously in the +B+ (0.01 ppm) and +B+ (0.5 ppm), resulting in 35.08 ppm and 98.25 ppm, respectively. Meanwhile in case of mulberry bark, boron content of −B−, +B+ (0.01 ppm) and +B+ (0.5 ppm) was 12.25 ppm, 17.0 ppm, 22.75 ppm, respectively.

As the results shown in table 3 both in the leaves and barks, there are significant in these treatments but the content of boron in the mulberry leaves was generally higher than that in the mulberry bark, similar with results of Michale (1969), Jones (1970), Syworotkin (1958) of the studies on tabaco and opium poppy.

4. Relationship between boron concentration and cold tolerance

The damage caused by boron deficiency was enormous as mentioned above. Moreover boron could strengthen resistance to cold. The results of studies of the effect of boron deficiency on cold damage were as follows:

As shown table 4 the sprouting percent of winter bud at −10℃ was 76.72% in the +B+ (0.5 ppm), 44.41% in the +B+ (0.01 ppm), 58.18% in the +B−, 6.40% in the −B− and at −15℃ it was 37.35 % in the +B+ (0.5 ppm), but not sprouted completly in the +B+ (0.01%), the +B− and −B−.

This result indicated that boron is nutritive element which strengthen their ability of freezing tolerance and lack of boron is very easy to make cold damage. Under boron deficiency conditions, the frost resistance of mulberry is very weak. Experimental data showed that cold tolerance of the mulberry tree which contained enough boron was higher than that of the mulberry tree without boron.

Contents of sugar, protein, RNA and phospholipid have a close relation to cold resistance of plant and proline also strengthens cold resistance of plant (Albert 1975, Bogges *et al.* 1976, Gauch and Dugger 1954, Keversans *et al.* 1977, Sakai and Yoshida 1968). This is the reason why these components mentioned above were analyzed.

The content of soluble protein in mulberry leaf of +B+ (0.01ppm) and +B+ (0.5 ppm) was 1.22 mg/g·FW and was 0.64 mg/g·FW, 0.68 mg/g in the bark, respectively. However, there is no significant of two treatments but the leaves and the barks of mulberry with +B+ (0.5 ppm) had more boron content than with +B+ (0.01 ppm). Boron is very important element affecting on the translocation of

Table 3. *The content of boron in mulberry leaves and barks*

Items	Leaves (ppm)				Barks (ppm)			
Treatment	I	II	III	Mean	I	II	III	Mean
+B*+	99.00	98.25	97.50	98.25	23.25	22.50	22.50	22.75
+B+	33.75	33.75	37.75	35.08	18.00	17.25	17.75	17.00
−B−	9.75	7.50	9.75	9.00	12.00	12.75	12.00	12.25
L.S.D. 0.05				3.60				1.50
C.V. (%)				3.20				3.80

Note : +B*+ 0.5 ppm boron supply
+B+ 0.01 ppm boron supply
−B− Boron deficiency

Table 4. *Effect of boron concentration on sprouting of cutting treated at lower temperatures*

Treatment		Total of buds	No. of sprouting buds	%	No. of green buds	%	No. of sprouting buds	%
−10℃	+B*+	232	178	76.72	15	6.47	39	16.81
	+B+	98	44	44.41	8	8.16	46	46.94
	+B−	55	32	58.18	13	23.64	8	18.18
	−B−	31	2	6.4	12	38.70	17	54.85
−15℃	+B*+	265	99	37.35	85	32.08	81	30.57
	+B+	102	0	0.00	1	0.98	101	99.02
	+B−	62	0	0.00	59	95.16	3	4.84
	−B−	34	0	0.00	10	29.40	24	70.60

Note : +B*+ 0.5 ppm boron supply
+B+ 0.01 ppm boron supply
+b− Supply boron and then stop boron supply
−B− Boron deficiency

Table 5. *Effect of boron concentration on protein, sugar, RNA, proline and phospholipid content in leaves and bark*

Item	Soluble protein (mg/g.FW)		T-sugar (mg/g.FW)		RNA (μg/g.FW)		Proline (ppm)		Phospholipid (μmol/g)	
Treatment	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
+B+	1.22	0.64	3.81	6.72	11.7	73.77	32.27	32.37	984.69	637.5
+B*+	1.59	0.68	5.02	6.34	11.6	98.16	43.80	36.37	1,123.60	720.9
L.S.D. 0.05	N.S	N.S	0.5	N.S	N.S	4.8	6.6	N.S	N.S	75.8
C.V. (%)	16.0	11.5	3.3	7.7	0.7	3.5	3.5	5.5	9.5	3.2

Note : +B*+ 0.5 ppm boron supply
+B+ 0.01 ppm boron supply

sugar because the borate-polyhydric complex is more mobile than polar sugar molecules. There were many reports related to effect of boron on the metabolism of plant and adequate boron supply is necessary for sugar synthesis. Experimental data showed that the total sugar content in leaves increased by continuous supply of boron (0.5 ppm) in nutrient solution, total sugar content in leaf of +B+ (0.5 ppm) and +B+ (0.01 ppm) was 5.02 mg/g·FW and 3.81 mg/g·FW, respectively. It can be said that there is a significant difference between the two treatments. Meanwhile, in case of mulberry bark, total sugar content of +B+ (0.5 ppm), +B+ (0.01 ppm) is 6.34 mg/g·FW and 6.72 mg/g·FW, respectively but no siginficant difference of two treatments, because total sugar, soluble protein and proline have probably not yet translated from the leaves to the barks due to an earlier harvesting (On 6, October) of bark samples for analysis.

The RNA content of leaves and barks was different with the boron concentration in nutrient solution. In the barks, the 0.5 ppm boron supply had more RNA content than that of 0.01 ppm boron supply. However, in the leaf there was no difference between the two treatments.

Proline content in the leaves and the barks of +B+ (0.5 ppm) was higher than that of +B+ (0.01 ppm) (Table 5). In case of mulberry leaf, proline content of +B+ (0.5 ppm) and +B+ (0.01 ppm) was 43.80 ppm, 32.37 ppm, respectively. Two treatments were significantly different. Meanwhile, in case of mulberry bark, proline content of +B+ (0.5 ppm) and +B+ (0.01 ppm) was 36.37 ppm, 32.37 ppm, respectively but there was no significant

difference between the two treatments. Kalichava and Sherstnev (1974) have reported a similar proline reduction in boron-deficient plants.

Boron concentration in the nutrient solution affected the phospholipid content in leaves and barks. The leaves and barks of +B+ (0.5 ppm) contained more phospholipid (1,123.64 μmol/g, 720.90 μmol/g) than (984.69 μmol/g, 637.50 μmol/g) those of the +B+ (0,01 ppm), similar to the results of studies on sunflower (Augsten and Eichhorn 1976, Dugger 1973, Mengel and Kirkby 1978).

It was concluded that boron is implicated directly in the control of membrane function. Phospholipid is the major component of cell membrane and it is closely related to the cold resistance. It plays a very important role to overcome at freezing temperature during the winter season in mulberry trees. So, treatment of 0.5 ppm boron supply was higher cold tolerance than treatment of 0.01 ppm boron supply in the nutrient solution.

摘 要

붕소 결핍이 식물의 뿌리 발육에 큰 영향을 미치고 있으며, 특히 붕소가 결핍하면 식물체의 내동성 물질로 알려진 당, 가용성 단백질, RNA, proline 및 인지질 등이 감소하기 때문에 붕소와 뽕나무의 뿌리 발육 및 내동성과 깊은 관계가 있을 것으로 예상하고 이에 대한 실험을 실시하여 다음과 같은 결과를 얻었다.

붕소를 주지 않은 뽕나무의 가지는 50 cm 가량 밖에 자라지 않았으며, 붕소를 준 뽕나무는 190 cm 이상 자랐다. 붕소를 주지 않은 뽕나무의 가지에 measles 현상이 보였으며 잎의 엽맥은 necrosis현상이 심하였다.

붕소를 주지 않은 뽕나무는 식재 초기에 새 뿌리가 나오다가 70일 후 부터는 새 뿌리와 묵은 뿌리 모두 갈색으로 변하였으며, 붕소시용 뽕나무의 뿌리는 새 뿌리가 왕성하게 발육하였다.

붕소를 주다가 붕소를 주지 않으면 3일 후부터 새 뿌리가 갈색으로 변하였으며, 붕소를 주지 않다가 붕소를 시용하면 2일 후부터 새 뿌리가 나기 시작해서 이것이 왕성하게 자랐다.

고농도 붕소 영양액 (0.5 ppm)으로 키운 뽕나무의 잎과 줄기 피층부에는 저농도 붕소 영양액 (0.01 ppm)으로 키운 뽕나무의 잎과 줄기 피층부에서 보다 붕소함량이 많았다.

고농도 붕소 영양액 (0.5 ppm)으로 키운 뽕나무의 잎과 줄기 피층부에는 저농도 붕소 영양액 (0.01 ppm)으로 키운 것보다 인지질, 단백질, 당, RNA 및 proline 함량이 많았다.

뽕가지를 −15℃에서 24시간 처리한 결과 붕소를 준 뽕나무는 정상적으로 발아 하였는데, 붕소를 주다가 주지 않은 뽕나무와 처음부터 붕소를 주지 않은 뽕나무는 전혀 발아가 되지 않았다. 이상의 결과로 보아 붕소는 뽕나무의 뿌리 발육에 지대한 영향을 미치며 나아가서는 뽕나무의 내동성과도 관계가 있음을 알 수 있었다.

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