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Effects of Foliar Application of Marine Algae Extract on the Growth and Development of Roots and Shoots in Satsuma Mandarin under Plastic House Conditions

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Abstract

The effects of foliar application of marine algae extract (RIC-1) on the growth and development of roots and shoots in satsuma mandarin were studied under plastic house conditions. The 500 times diluted sprays of this extract enhanced the formation of fibrous roots and at higher concentration (300 times diluted), root rotting occurred. While at lower concentration (1000 times diluted), the roots became comparatively thicker. The formation of new branches and their lengths were higher in all treated trees with maximum value at 500 times diluted spray compared to those of control. The number of leaves and their size in terms of length and width were significantly,higher in the treated trees having the maximum output under 500 times diluted treatment. Light microscopic observation revealed the accumulation of numerous starch grains in the mesophyll cells of leaves in the treated trees. The starch grain number/cell increased along the increased concentration of RIC-1 sprays from 1000 times diluted to 300 times diluted. The width of mesophyll cell layer also similarly increased in RIC-1 treated leaves, This study suggested 500 times diluted RIC-1 spray as optimum for the growth and development of roots, branches and leaves of satsuma mandarin trees under plastic house conditions.

Introduction

In Japan, satsuma mandarin cultivation under plastic house conditions has been widely used due to its increased production, steady supply of good quality fruits, early harvesting time and economic feasibility as compared to field cultivation. Fertilizers are generally adddd in different forms in that cultivation process after each harvest. If this is not done, the leaves become smaller, resulting in a decreased yield and lower quality of fruits in the following harvest season (Aguja and Shiraishi, 1998).

Foliar application of nitrogen has been used successfully in many crops (Cook and Boynton, 1952; Wesely *et al*., 1985; Bowman and Paul, 1992; Alva *et al*., 1994). In citrus this practice helped in the recovery of tree vigor and nutritional status (Singh and Singh, 1973; Willis *et al*., 1991) which ultimately increased yield and improved the quality of fruits (Koo, 1979; Mungomery *et al*., 1980), especially in plastic house cultivation. Application of nitrogen to leaves was also considered a better alternative to ground application since the latter appeared to contribute to salinity problems and ground water pollution with nitrate (Alva *et al*., 1994). Most of the relevant works on citrus refer to variations in foliar nitrogen content,its value in the diagnosis of nutritional status (Cameron *et al*., 1952; Jones *et al*., 1968; Wallace *et al*., 1954) and the effects of nitrogen fertilization on yield (Hipp, 1977; Smith, 1970; Stannard, 1973). To the best of our knowledge, the foliar application of compound fertilizer in citrus has not been studied yet. If the compound fertilizer is proved successful it can substantiate the supply of nitrogen and other plant nutrients simultaneously which will be more appropriate and contribute greatly to the citrus industry.

The marine algae extract (RIC-1) contains nitrogen, phosphate, potassium, minerals, amino acids, vitamins, polysaccharides, glycoproteins, giberellin, auxin, cytokinin, ACC and ABA-like materials. The potentiality of this material as foliar spray for plant nutrients in the plastic house cultivation of citrus can therefore be studied.

This experiment was designed to know the effects of marine algae extract (RIC-1) on the formation, growth and development of roots and shoots in satsuma mandarin under plastic house conditions. The optimum concentration of this material as foliar spray solution was also suggested.

Materials and Methods

The experiment was conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University, Japan during the spring seasons of 1995-96. Four-year-old satsuma mandarin (*Citrus unshiu* Marc. cv. Okitsu Wase) trees of uniform vigor and grown in potted mixture of sandy loam soil and granite were used. The pots were irrigated daily with a sufficient amount of water. Six trees were tested for each treatment and were maintained in a plastic house with light intensity of 60,000 lux and a temperature of 23-26°C. The trees were sprayed with three doses of marine algae extract like 300 times diluted, 500 times diluted and 1000 times diluted as three treatments. Another six trees were sprayed with only distilled water as control. Three sprays of each treatment were applied in April 7, 15 and 22 every year. The quantitative data on branch and leaves were taken on May 15 and June 14 every year and average values were used. The chlorophyll content in leaves was measured by chlorophyll meter (SPAD reading) just after sampling of leaves. The data pertinent to the study were analyzed using Dancun's new multiple range test. Leaf samples were also taken then which were fixed in Karnovsky (1965) solution, dehydrated in an alcohol series and embedded in epoxy resin. Sections of 3 μm thickness were cut with dry glass knife on a Sorval (MT-1) ultramicrotome, stained with iodine-potassium-iodide and observed under a light phase microscope.

For the observation of roots, the plants were uprooted on December 5. They were thoroughly washed and the existing roots under different treatments were checked through visual observation.

Results

Formation and Development of roots in satsuma mandarin trees sprayed with marine algae extract (RIC-1): Formation of roots and their development were influenced by the foliar application of RIC-1 (Fig. 1). Under its 1000 times diluted treatment, roots elongated but were comparatively thicker (Fig. 1). The trees sprayed with 500 times diluted RIC-1 solution also showed similar trend of root development. However, in 1000 times diluted treatment, thicker pioneer roots were prominenet and 500 times diluted treatment encouraged the formation of fibrous roots. While under 300 times diluted treatment root shrinking and sometimes root rotting was observed. Under control, new root formation less as a result under this situation the whole root system was weaker than those of the RIC-1 splayed trees (Fig.1).

Growth and Development of branches and leaves in satsuma mandarin under different treatments: The quantitative data on the growth and development of branches and leaves have been summarized in Table 1 and shown in Fig. 2. The number of new branch formation was highest (16.7/tree) in 500 times diluted extract followed by its 1000 times diluted spray (13.0/tree) (Table 1). The branch formation was lower in 300 times diluted spray as well as control treatment. Elongation of branch was significantly enhanced by RIC-1 sprays. Highest elongation (16.56 cm) was recorded in 500 times diluted treatment which was followed by 300 times diluted (15.84 cm) and 1000 times diluted (15.27 cm), respectively in the similar period after spraying. However, all doses of the sprays contributed to branch elongation significantly compared to that of control (10.71 cm).

The new leaf emergence was also favored by RIC-1 sprays (Fig. 2). In all treatments the leaf formation was higher (8.3-8.5/branch) compared to control (6.8/branch) (Table 1).

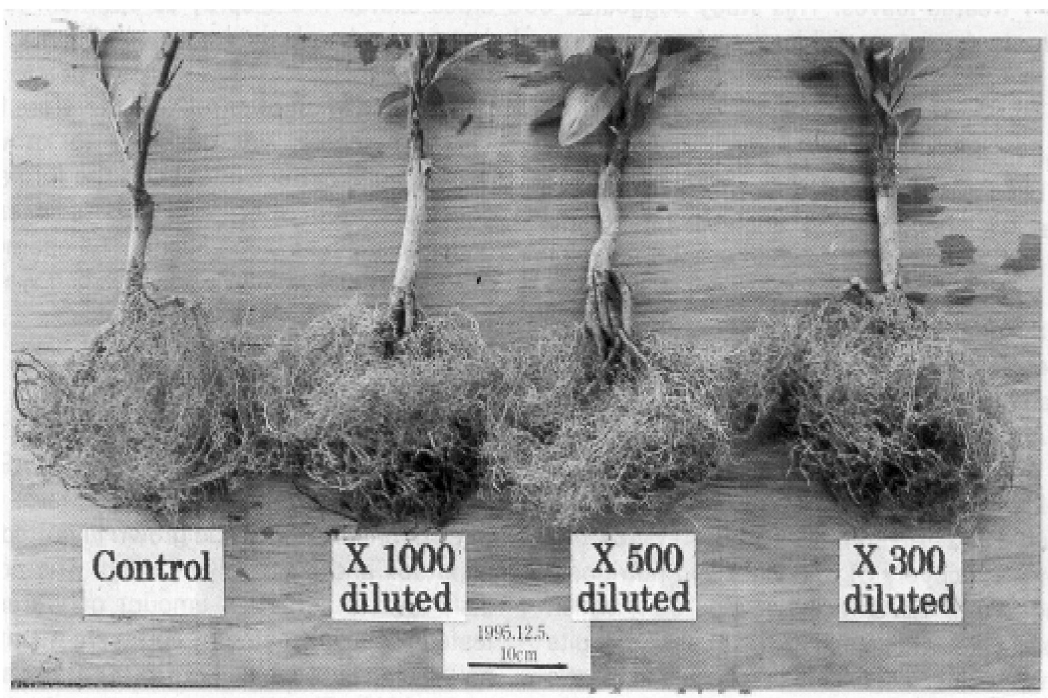

Fig. 1: Formation of roots in satsuma mandarin trees sprayed with different concentrations of marine algae extract (RIC-1). Note: Fibrous roots were abundant under 500 times diluted spray and roots were thicker under 1000 times dilution. While root rot was found under 300 times diluted spray

Table 1: Effects of foliar spray of marine algae extract (RIC-1) on the growth and development of branches and leaves in satsuma mandarin under plastic house condition

Treatment	No. of branch	Elongation of branch (cm)	No. of leaf	Leaf size (mm)			SPAD reading
				Length	Width	Thickness of Mesophyll layer	
300 times diluted	10.5c	15.84c^{z}	8.5a	68.7a	29.4a	0.308bc	37.6^{NS}
500 times diluted	16.7a	16.56a	8.3a	70.6a	30.0a	0.321a	39.4
1000 times diluted	13.0b	15.27b	8.4a	69.0a	30.2a	0.318ab	38.3
Control	12.5b	10.71c	6.8b	41.5b	16.6b	0.302c	37.6

^{Z}Mean separation within column by Duncan's multiple range test, 5% level
^{NS}Mean non significant at 5% level of significance

The size of leaves was increased by RIC-1 treatment. The length of leaf was highest (70.6 mm) in 500 times diluted treatment followed by 1000 times (69.0 mm) and 300 times diluted (68.7 mm) ones (Table 1). However, all these leaf lengths were statistically similar but significantly higher than control (41.5 mm). The width of leaf was highest (30.2mm) in 1000 times diluted spray followed by 500 times (30.0 mm) and 300 times diluted (29.4 mm) treatments (Table 1). This leaf width was very low (16.6 mm) in control. The thickness of mesophyll cell layer was also highest (0.321 mm) in 500 times diluted treatment followed by 1000 times (0.318 mm) and 300 times (0.308) ones. This thickness was lowest (0.302 mm) in control. The SPAD reading indicated that although chlorophyll contents in the treated leaves were higher compared to that of control, these were not statistically significant at 5% level (Table 1).

Starch grain accumulation in the mesophyll cells of leaves under different treatments: Starch grain accumulation in the mesophyll cells of leaves was favored by the foliar application of RIC-1. As the concentration of RIC-1 increased, starch grain number also increased (Fig. 3). However, in control tree leaves, starch grains were found only in the mesophyll cells three cell layers below both upper and lower epidermis (Fig. 3A). Along with the increase of RIC-1 concentration in the spray solution, the mesophyll cells near the epidermis started accumulation of starch grains. Finally at 300 times diluted spray, the mesophyll cells below the epidermis contained many starch grains and these grains were presumed to be larger compared to those of control tree leaves (Figs. 3B-D). Under control, although mesophyll cells contained starch grains, many cells were devoid of grains. While almost all cells possessed starch grains under 300 times diluted spray. The starch grain accumulation mostly occurred just below the cell wall in all treatments and the central portions of cells were completely devoid of starch grain. Even in the case of higher starch grain accumulation the deposition site in the cell remained unchanged.

Discussion

Foliar application of urea has been found to be useful as a source of nitrogen in many crops. In citrus also the application of nitrogen to leaves was beneficial (Embleton *et al*., 1986). From these reports it was assured that plants can absorb nutrients through leaves as well as roots. However, the foliar application of different nutrients is not yet well studied. In our present experiment RIC-1 was sprayed and it was found to be beneficial for root, branch and leaf development in satsuma mandarin. Presently used fertilizer is a mixture of many plant nutrients. It is not clear from this study whether leaves absorbed all sorts of nutrients together through leaves or few selected elements were utilized. To understand this phenomenon, different experiments using different sources of nutrients can be carried out separately. However, the RIC-1 sprays in satsuma mandarin was recorded as useful from our study which might be an important consideration for its use in the plastic house cultivation of citrus.

Although root and shoot development were influenced by the sprays of different concentrations of the presently used material, 500 times diluted spray was found to be optimum. On the other hand, 1000 times dilution paid comparatively lower contribution to the parameters studied. Similarly

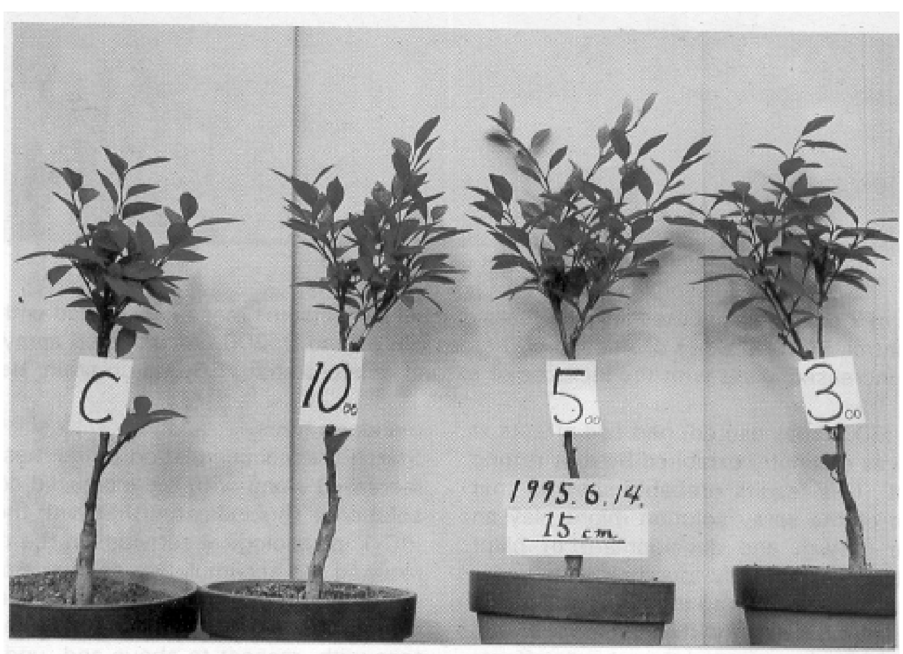

Fig. 2: Effects of foliar application of marine algae extract (RIC-1) on the growth and development of branches and leaves in satsuma mandarin. Both branch and leaf growths were highest under 500 times diluted sprays followed by 1000 times and 300 times diluted ones

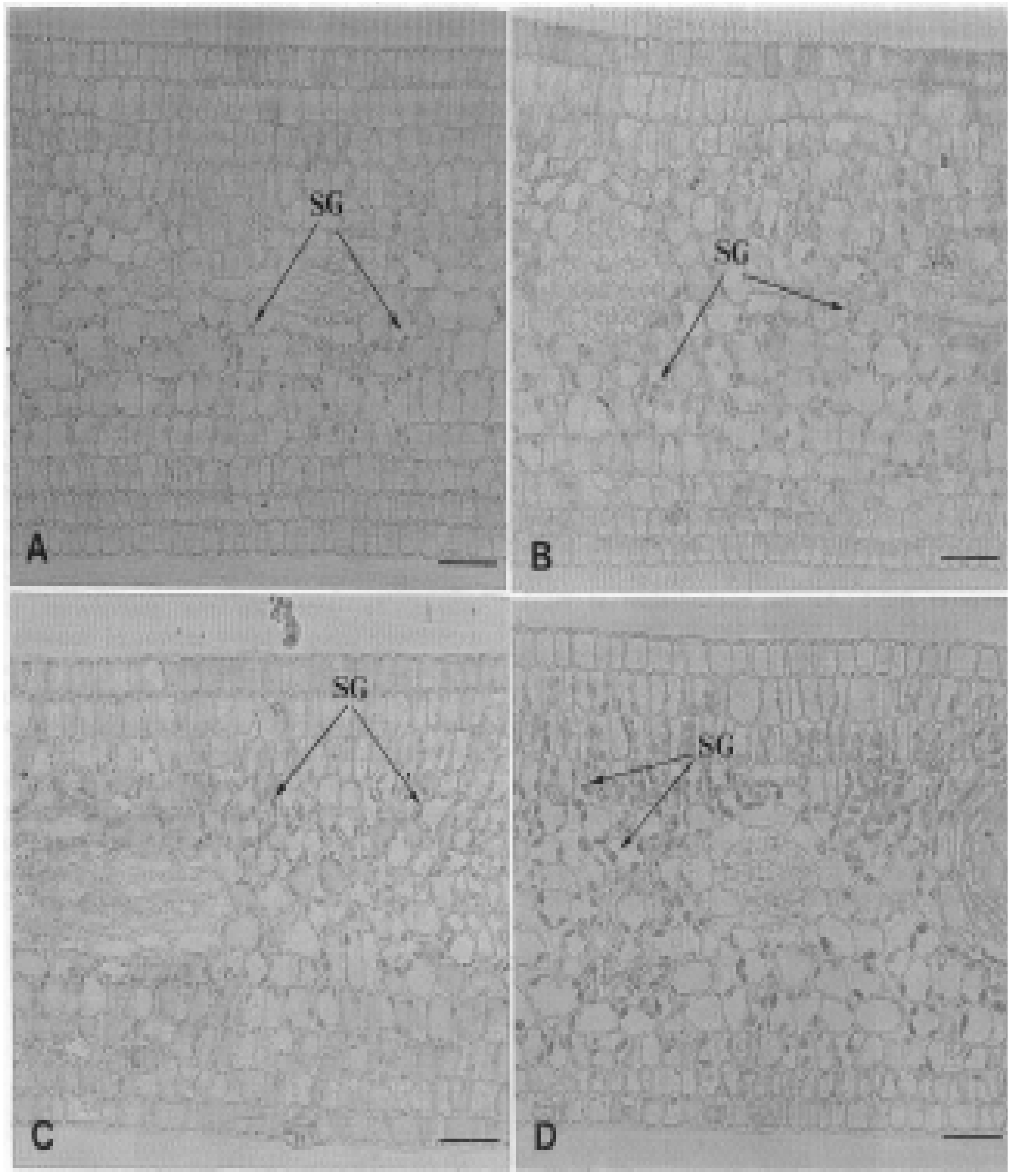

Fig. 3 (A-D): Accumulation of starch grains in the mesophyll cells of leaves in satsuma mandarin sprayed with marine algae extract (RIC-1). A: Control, B: 1000 times diluted, C: 500 times diluted and D: 300 times diluted sprays. Note: Starch grain accumulation increased along with the increase of extract concentration. SG: Starch grain. Bar = 25 µm

higher concentration (300 times diluted) had bad effects in some cases which was distinctly exhibited by root rotting under this treatment. This results probably showed that higher concentration of the spray solution might play an adverse role to the growth and development of plant organs. The root rotting was similarly found recently under higher culture solution pH (Mohammad and Shiraishi, 1999) and higher culture solution salinity (Mohammad *et al.*, 1999). Aguja *et al.* (1996) also reported the adverse effects of higher concentration of urea solution to the growth and cell organelle development in the young leaves of satsuma mandarin trees.

Starch grain accumulation in the mesophyll cells of leaves increased along with the increased concentration of spray solutions. This indicated that with the foliar application of RIC-1, physiological activities in the trees increased which resulted the accumulation of numerous and larger starch grains in the mesophyll cells of leaves. This accumulated grains were subsequently utilized and produced vigorous tees with respect to above and underground parts. This result was consistent with the reports on the relationship between starch grain accumulation and tree vigor in satsuma mandarin by Mohammad and Shiraishi (1998). They also showed that starch grain accumulation in roots was directly related with tree vigor.

Although all kinds of roots absorb nutrients, fibrous roots instantly forms for this purpose. As the formation of this kind of roots was higher under 500 times diluted spray solution, it indicated this concentration as the favorable range for the uptake of plant nutrients. The higher elongation of branches and production of bigger leaves might be resulted from the nutritive value of the spray solution itself and indirectly as well as from this formation of fibrous roots for absorbing nutrients from the growing media.

In conclusion, presently studied marine algae extract (RIC-1) was recommended as foliar spray solution for the enhancement of growth and development of satsuma mandarin trees. This chemical can be used as 500 times diluted spray to obtain the optimum effect. For better understanding of the mechanisms of this effect, the material can be studied in the-cellular and subcellular levels.

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