Chapter 6

Effect of water stress on phytotoxic activity of Mexican sunflower

Introduction

Environmental stress such as mineral deficiency, temperature stress, radiation stress and water stress have been reported to influence the productivity and availability of secondary plant metabolites, including allelochemicals (Alsaadawi *et al.*, 1985; Chaves *et al.*, 1997; Einhellig, 1987,1996; Einhellig *et al.*, 1985; Inderjit, 1996b; Kainulainen *et al.*, 1992; Kiemnec and Larson, 1991; Penuelas and Llusia, 1997; Teasdale, 1993). The most common environmental stress on plants is probably water stress (Tang *et al.*. 1995). Water stress not only directly affects plant growth but may enhance or inhibit allelochemical production of the plant (Einhellig, 1995a). Under natural field conditions, Mexican sunflower is also subjected to water stress especially during the dry summer season. Mexican sunflower is a perennial plant that might be subjected to water stress several times along its life cycle. Therefore it is important to determine the effect of water stress on the growth and the phytotoxic ability of Mexican sunflower in terms of understanding the allelopathy. The objective of the study in this

chapter was to investigate the growth of Mexican sunflower grown under different soil moisture levels and the effect of water stress on the production of phytotoxic compound(s) as well as its phytotoxic activity.

Materials and Methods

Growth of Mexican sunflower under water stress

Plastic pots (18 cm in diameter, 19.5 cm in height) containing 2,500 g of air-dried sieved Kannondai soil were planted with a two-week old Mexican sunflower seedling each. The pots were applied with 1,625 ml of tap water to bring them to 65% of soil moisture on a dry soil basis. The original weights of each pot were recorded and the pots were placed in a greenhouse under non-controlled environmental conditions. The pots planted with Mexican sunflower were weighed and the soil was directly applied with water every two days to maintain the soil moisture at 65% as in the beginning level for a 4 week-period. At the end of this period, the plants were subjected to several water stress conditions for a further 4 weeks by controlling the level of soil moisture. The pots were weighed and the soil was directly applied with a proper amount of water every two days without contact of water with the stem or leaves of Mexican sunflower to maintain the soil moisture at levels equivalent to 65, 55, 45, 35, 25 and 15 % of air-dried soil weight. After 4 weeks culture at different

soil moisture levels, the plants were harvested and the soil from each pot was immediately collected. Both the harvested plants and the planted soils, from which the roots had already been separated, were used as the materials for bioassay as described below.

To determine the effect of water stress on the growth of Mexican sunflower, the Mexican sunflower plants grown at different soil moisture levels were harvested and separated into 6 portions; young leaves (unexpanded), mature leaves (fully expanded), senescent leaves (yellow-brown colored), stem and roots. The fresh weight and dry weight of each part as well as plant height and leaf area were measured. The leaf area of the green leaves (including both the young and mature leaves) was measured by using the computer scanner (NIH image software, Macintosh for the Biological Science, Kodansha Scientific, Japan). The fresh plant parts were air-dried at room temperature (25-30℃) for 10 days before weighing.

Effect of water stress on phytotoxic activity of water extract from plant parts of Mexican sunflower grown under water stress

To determine the effect of water stress on the production, content and allocation of phytotoxic compounds in each part of Mexican sunflower, the phytotoxic activity of water extract from each part of Mexican sunflower grown under different soil moisture levels was investigated using the growth of rice

seedlings as the parameter. The air-dried plant materials were ground with an electrical grinder through a 0.5 mm screen and kept at -20℃ until use as described in chapter 1. One gram each of the air-dried plant material powder was separately extracted with 50 ml of distilled water and shaken (120 rpm) at room temperature for 12 hr. The extracts were filtered through a layer of glass fiber in vacuum. The filtrate solutions were considered to be water extracts at the concentration of 20 mg DME/ml. The initial water extracts were diluted with distilled water to the concentration of 5 mg DME/ml and bioassayed with germinated rice seeds in sea sand following the same procedure described in chapter 1. Shoot and root length of the rice seedlings were measured at 4 days after planting and compared with the control seedlings in the sea sand treated with distilled water. The experiment was carried out twice with three replications each time.

Effect of water stress on phytotoxic activity of soil previously planted with Mexican sunflower

To determine the release of phytotoxic substance(s) from Mexican sunflower plant to the soil under water stress condition, the phytotoxic activity of the soil previously planted with Mexican sunflower at different soil moisture levels was investigated on the growth of rice seedlings. The soil from each of the planted pots was immediately collected after the harvesting

of plants. The soils were sieved through a 2 mm screen to separate the roots and adjusted to the maximum water holding capacity (MWHC) by sub-irrigation with distilled water. Seventeen grams of the moisture adjusted soils were put into small glass bottles (8 cm in height and 4 cm in diameter) and bioassayed with five germinated rice seeds using the same procedure described in chapter 2. Shoot and root length of the rice seedlings were measured at 4 days after planting. Soil from the non-planted pots served as the control soils for bioassay.

To investigate the phytotoxic activity of soil-water in the soil previously planted with Mexican sunflower under water stress, the soil-water from the planted soils and the non-planted soils were directly separated by centrifugation as described in chapter 1. Seven milliliters of the soil-water was poured into a glass bottle containing 20 grams of sea sand and bioassayed with five germinated rice seeds in the same procedure as described in chapter 1. Shoot and root length of the rice seedlings were measured at 4 days after planting. Soil-water from the non-planted pots served as the control soil-water for bioassay. The experiments were carried out twice with three replications each time.

Results and Discussion

Growth of Mexican sunflower under water stress

The effect of water stress on the growth of Mexican sunflower was investigated on several growth parameters of the plants at different soil moisture levels. Table 6.1. shows that all growth parameters of Mexican sunflower grown in the low soil moisture levels were lesser compared to those of the plants grown in the high soil moisture levels. A significant reduction on plant height, leaf area, fresh weight and dry weight of each plant part of Mexican sunflower was found at soil moisture levels starting from 45% and lower. The most remarkable reduction in growth was found at the soil moisture level of 15%. This demonstrated that the growth of Mexican sunflower was inhibited in the low soil moisture condition, and suggested that water stress reduced the growth of Mexican sunflower and the degree of growth reduction increased with increasing water stress level.

Effect of water stress on phytotoxic activity of water extract from plant parts of Mexican sunflower grown under water stress

The phytotoxic activity of water extract from each part of Mexican sunflower grown at different soil moisture levels was examined on rice seedling growth in order to determine the effect of water stress on the production and accumulation of phytotoxic compound(s) in Mexican sunflower plant. Generally, shoot and root growth of rice seedlings were inhibited by water extracts from the air-dry powders of roots, stem and leaves of Mexican sunflower (Fig. 6.1). The inhibitory activity of water extract from

the young leaves was greater than the extracts from the mature leaves, senescent leaves, stem and roots, respectively. This result was similar to the finding in chapter 1, the phytotoxic activity of water extract from the leaves and stem of Mexican sunflower grown at a soil moisture level of 65% seems to be greater than that of the extracts from the roots (Fig. 1.1). In this study, shoot and root growth of rice seedlings in sea sand applied with water extract from each part of Mexican sunflower grown under lower soil moisture levels was inhibited more than those in sea sand applied with water extract from the same part of the plants grown under higher soil moisture levels. This demonstrated that the phytotoxic activity of water extracts per dry weight from all parts of water stressed plants was greater than that of the extracts from the non-water stressed, particularly those that were grown at the highest soil moisture level (65%). The results demonstrated that water extracts from the leaves, stem and roots of the water stressed plant showed greater phytotoxic activity than that of the non-water stressed plants and suggested that young leaves, mature leaves, senescent leaves, stem and roots of Mexican sunflower grown under water stress contained more phytotoxic substance(s) than those of the plant grown under non-water stress condition.

Effect of water stress on phytotoxic activity of soil previously planted with Mexican sunflower

The phytotoxic activity of the soil previously planted with Mexican sunflower at different soil moisture levels on rice seedling growth is shown in Fig. 6.2. Shoot and root growth of the rice seedlings in the soil previously planted with Mexican sunflower was considerably inhibited to a similar extent at different soil moisture levels. This suggested that soil planted with Mexican sunflower under water stress condition contained phytotoxic compound(s) in amounts sufficient to inhibit plant seedling growth and that the phytotoxic compound(s) in soil were released from the roots of Mexican sunflower. In this experiment, shoot and root elongation of the rice seedlings in soils previously planted with Mexican sunflower at the soil-moisture level of 15% to 65% of dry soil weight was inhibited to a similar degree. This suggested that the soils planted with Mexican sunflower at these soil moisture levels contained the same amount of phytotoxic substance(s). Since the phytotoxic activity of the planted soil was induced by the phytotoxic substances released from the roots and the amount of roots of the water stressed plant was significantly less than the amount of roots of the non-water stressed plant (Table 6.1). It implied that, even though the root growth of Mexican sunflower was inhibited by water stress, the plant was still able to produce and release phytotoxic substance(s) into soil by root exudation at a greater rate than that of the non-water stressed plant.

The phytotoxic activity of soil-water separated from the soil

previously planted with Mexican sunflower at different soil moisture levels was examined on rice seedling growth in sea sand bioassay. The results in Fig. 6.3. show that shoot and root growth of the rice seedlings treated with the soil-water from the planted soil were less than those treated with the soil-water from the non-planted soil. This suggested that soil-water in the planted soil contained phytotoxic substance(s), confirming the findings in chapter 2 and chapter 5. The phytotoxic activity of the soil-water from the soil previously planted with Mexican sunflower at 65, 55 and 45% soil moisture levels was not different. These results agreed with those in Fig. 6.2 which shows that soil planted with Mexican sunflower at these soil moisture levels have a similar phytotoxic activity. In this experiment, the soil-water from the pots previously planted with Mexican sunflower at the soil moisture level lower than 45% could not be separated to a sufficient amount for bioassay. However, based on the results in the previous experiment (Fig. 6.2), it was assumed that the soil-water in these soils also contained the phytotoxic substance(s) to inhibit rice seedling growth in a similar degree to that of the soil-water from the higher soil moisture treatment pots.

Tang *et al.* (1995) noted that the basic response of plants to water stress is to decrease the growth rate. Water stress condition not only directly influences plant growth but also enhances allelochemical production (Einhellig, 1995a). Tang and coworkers (1995) reported that the growth of purple nutsedge

(*Cyperus rotundus* L.) was decreased by water stress but the amount of phytotoxic compounds in tubers was significantly increased, and that water stress increased the inhibitory activity of root exudates of purple nutsedge against lettuce seed germination and root elongation. The study in this chapter also found that water stress reduced the growth of Mexican sunflower but enhanced the phytotoxic activity of the phytotoxic substance(s) in all parts of the plant grown under water stressed condition. Under natural field conditions, the soil moisture level starts to decrease continually from the end of the rainy season and water stress commonly occurs particularly during the dry season. Generally, the soil moisture level of the top 10 cm of the soil in Mexican sunflower infested areas in Thailand in the dry season is about 20% or lower (Ogawa *et al.*, 1980). Under such conditions, Mexican sunflower plants are subjected to water stress. In this study, the different soil moisture levels in the pots could simulate the degree of water stress under natural field conditions in which the plants grow. It was found that Mexican sunflower could survive under the low soil moisture level but the growth was reduced. In the growth conditions, however, the plant may continually produce and release phytotoxic substance(s) into the soil through exudation from the root and still maintain the phytotoxic activity in the soil to the same degree as that in the non-water stress condition. This may reflect the growth and the phytotoxic ability of Mexican sunflower during the dry season in

natural fields. In the dry summer season which has no or little rainfall, the water leaching of phytotoxic substance(s) from Mexican sunflower leaves and its residues to soil is quite limited. However, if there are some rainfall events during this water stress period, it is reasonable to assume that the phytotoxic compounds in the living and senescent leaves could easily be released into soil by rain water, as described in chapter 4.

Based on these results, it can be assumed that water stress may affect the growth and phytotoxic activity of Mexican sunflower under natural field conditions.

Summary

1) Plant height, leaf area, fresh weight, and dry weight of leaves, stem and roots of Mexican sunflower were reduced with decreasing soil moisture level in the cultural pots.
2) The phytotoxic activity of water extract based on the dry weight from each part of Mexican sunflower grown under low soil moisture levels was greater than that of water extract from the same part of the plants which were grown under high soil moisture levels.
3) Soil previously planted with Mexican sunflower at different soil moisture levels inhibited rice seedling growth. The inhibitory effect on growth was similar in these planted soils.
4) Soil-water separated from the soil previously planted with

Mexican sunflower at different soil moisture levels inhibited rice seedling growth to a similar degree.

5) These results suggested that, under water stress condition, the growth of Mexican sunflower decreased but the plant could produce phytotoxic substance(s) in the leaves, stem and roots in a greater amount than the plant grown under non-water stress condition. The water stressed plants could release phytotoxic substance(s) into soil by the process of root exudation and maintain the phytotoxic activity in soil similar to the non-water stressed plant.

Table 6.1 Plant height, leaf area, fresh weight and dry weight of each part of Mexican sunflower grown under different soil moisture levels*.

Growth parameter	Soil moisture (% of dry soil weight)					
	65	55	45	35	25	15
Plant height (cm)	115.6 a**	112.5 a	95.5 b	77.1 c	66.8 d	54.3 e
Leaf area (cm^2)	1597.5 a	1458.4 a	1110.5 b	1087.1 b	805.5 c	450.5 d
Total fresh weight (g/plant)	155.7 a	141.4 a	121.8 b	99.4 c	74.4 d	44.8 d
Young leaves	10.5 a	9.7 a	6.4 b	5.1 c	5.1 c	2.1 d
Mature leaves	37.3 a	31.4 b	24.3 c	23.9 c	17.6 d	10.1 e
Senescent leaves	12.2 a	11.4 ab	10.5 b	6.3 c	3.8 d	3.1 d
Stem	71.9 a	68.4 a	61.5 b	49.6 c	37.7 d	21.8 e
Roots	23.8 a	20.5 b	19.1 b	14.5 c	10.2 d	7.7 d
Total dry weight (g/plant)	28.5 a	26.7 a	22.4 b	18.7 c	15.7 cd	11.1 d
Young leaves	1.5 a	1.4 ab	1.2 bc	0.9 c	0.9 c	0.4 d
Mature leaves	5.5 a	5.1 a	3.6 b	3.5 bc	2.9 c	1.8 d
Senescent leaves	4.3 a	4.2 a	4.1 a	2.9 b	2.5 bc	2.1 c
Stem	11.1 a	10.3 a	8.3 b	6.9 c	5.3 d	2.9 e
Roots	6.1 a	5.7 ab	5.2 b	4.5 c	4.1 cd	3.9 d

* The two week-old Mexican sunflower seedlings were grown at the 65% soil moisture level for 4 weeks and allowed to grow under the desired soil moisture levels for the succeeding 4 weeks before harvesting.

** Means in a row followed by the same letter are not significantly different at $p < 0.05$ as determined by Duncan's Multiple Range Test.

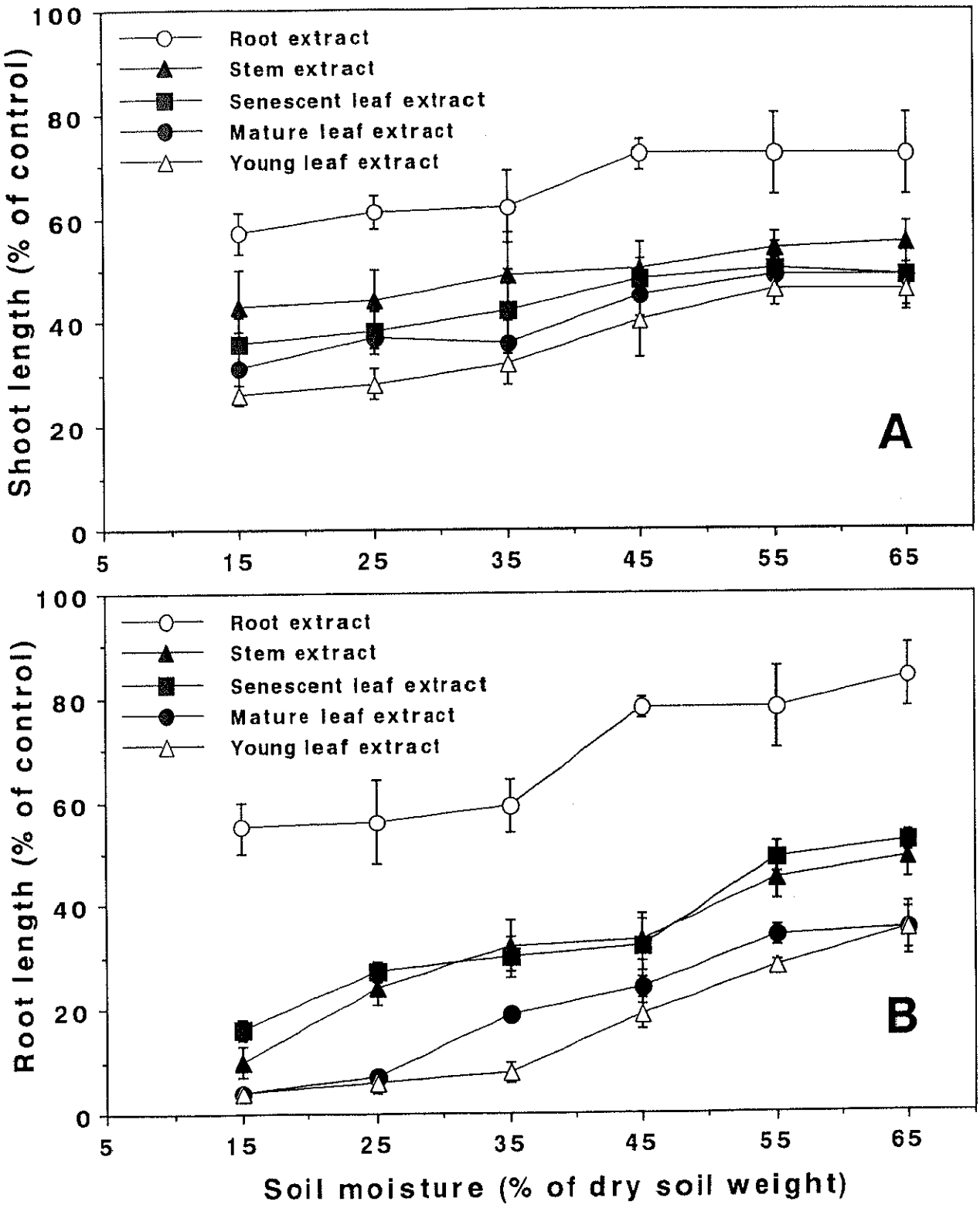

Fig. 6.1 Shoot growth (A) and root growth (B) of rice seedlings in sea sand treated with water extract from roots, stems, young leaves, mature leaves and senescent leaves of Mexican sunflower previously grown at different soil moisture levels. Shoot and root length of the control were 23 ±1 mm and 37 ±2 mm, respectively.

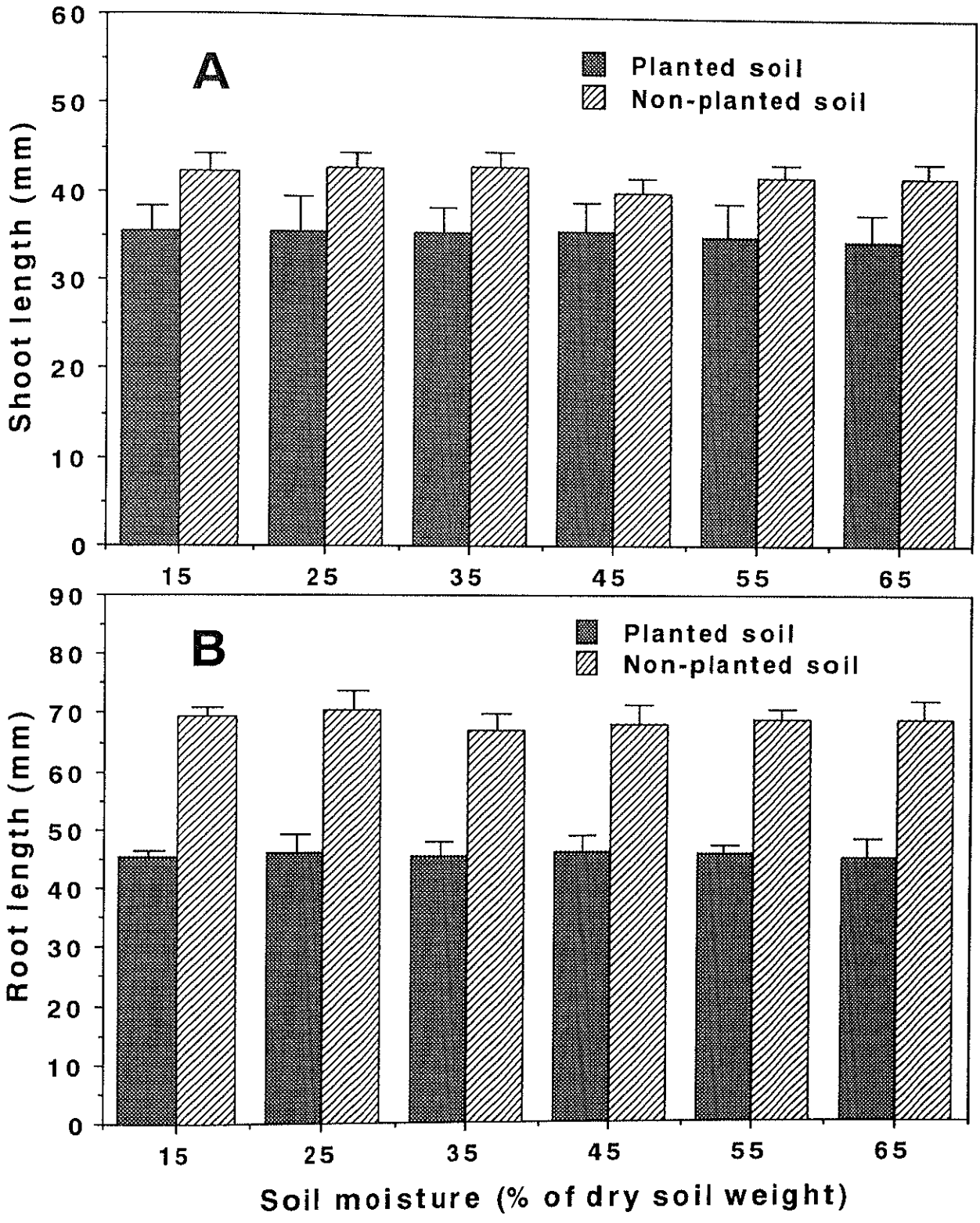

Fig. 6.2 Shoot growth (A) and root growth (B) of rice seedlings in the soil previously planted and non-planted with Mexican sunflower at different soil moisture levels.

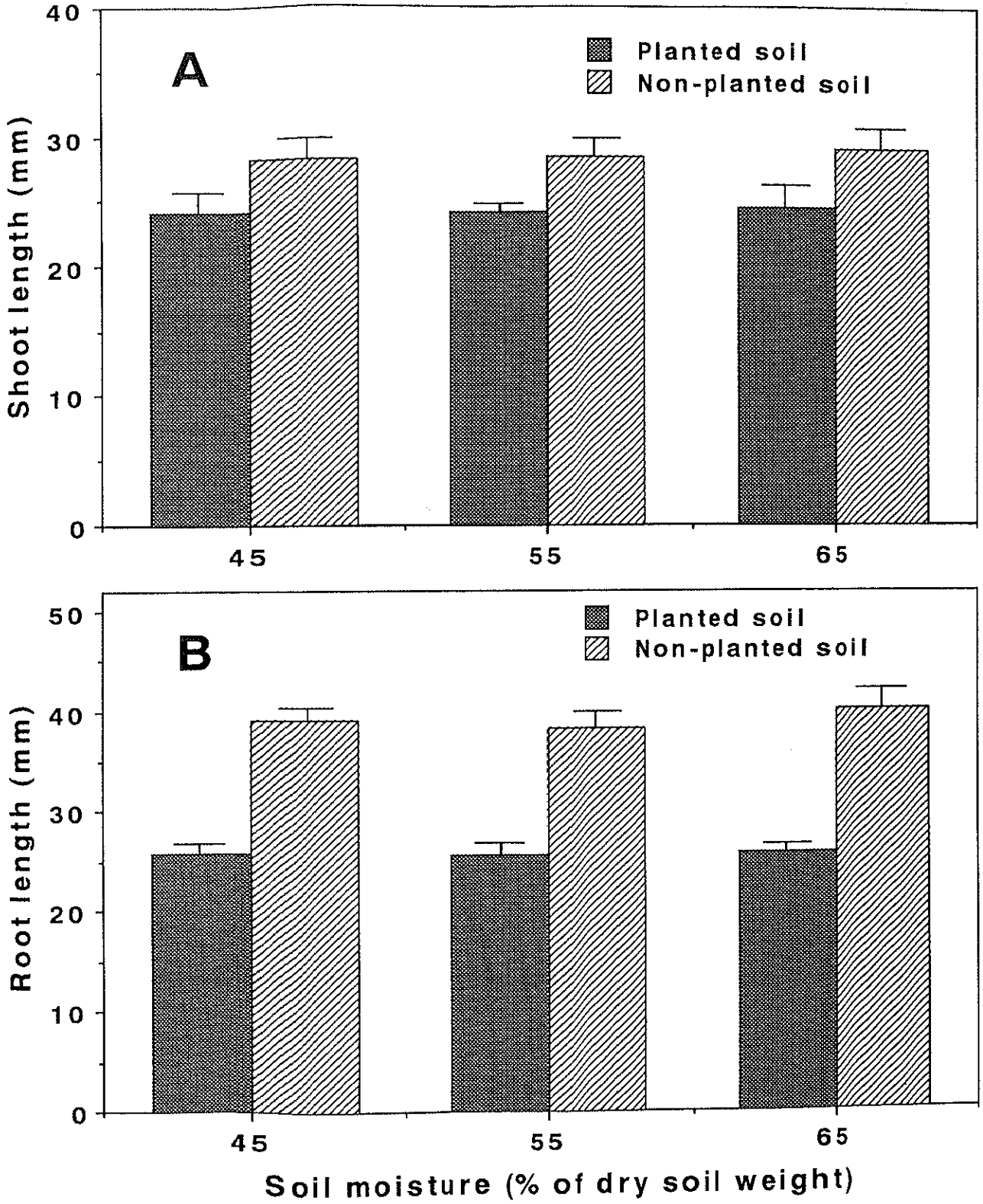

Fig. 6.3 Shoot growth (A) and root growth (B) of rice seedlings in sea sand treated with the soil-water separated from the soil previously planted and non-planted with Mexican sunflower at different soil moisture levels.