



SHORT COMMUNICATION

PLANT GROWTH PROMOTING ACTIVITY OF *STEVIA REBAUDIANA* BETRONI ON RICE SEEDLINGS

LOKESH BABAR, R.S. TANWAR, SHASHI B., SINGH AND P. DUREJA

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi-110 012

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Effect of different solvent extracts of dried leaves of *Stevia rebaudiana* Betroni on shoot and root length of rice seedlings in a pot experiment was studied at Indian Agriculture Research Institute, New Delhi. The extracts (hexane, dichloromethane and methanol) when applied at 1.0, 0.5 and 0.25 mg ml⁻¹ concentrations showed significant increase in seed germination, shoot and root length after 15 days of application. Among all the extracts tested maximum increase was exhibited by hexane extract with an increase in root and shoot length 19.14 and 18.12 % respectively as compared to control (9.4 and 13.8 % respectively).

Key words: Growth promotion, rice seedlings, solvent extracts, *Stevia rebaudiana*

Allelopathy is an important mechanism of plant interference caused by the addition of plant-manufactured secondary metabolites in the soil environment. A large number of plants produce inhibitory or stimulatory effects on the germination and growth of neighboring or succession plants by releasing allelopathic substances into soil, either as exudates from living plant tissue or by decomposition of plant residues (Narwal 1999). When the plant-produced substances are beneficial, such association can stimulate plant growth, increase disease resistance, and improves the plant's ability to withstand environmental stress.

Stevia rebaudiana is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is often referred to as "the sweet herb of Paraguay". *Stevia* plant, owing to the presence of stevioside (a sweet-tasting glycoside, 300 times as sweet as sucrose) is considered to be a valuable medicinal plant (Katyama *et al.* 1976).

There are more than 180 species of *S. rebaudiana* that gives the sweet essence (Soejarto *et al.* 1982).

The leaves are the source of the diterpene glucoside, viz. Stevioside, rebaudioside A and C, dulcoside in its leaf tissues (Haung *et al.* 1995, Hanson and de Oliveira 1993). Steviosides is nearly 110-270 times sweeter than sucrose (Uddin *et al.* 2006). They have been used since long as a sweetener in South America, Asia and in different countries of Europe (Kinghorn 2002). In Brazil, Japan and Korea, *Stevia* leaves and its highly refined extracts are officially used as low calorie sweetener (Kim and Kinghorn 2002, Mizutani and Tanaka 2002). Besides its sweetening property it has therapeutic values such as antihyperglycemic, anticancerous (Jeppensen *et al.* 2002, 2003), contraceptive properties and prevention of dental caries. *Stevia* has also been found to inhibit bacterial and fungal growth. *Stevia* leaves also contain diterpenoids of the kaurene series, which have gibberellins like activity. An earlier work has shown the

*Corresponding author, E-mail: pd.dureja@gmail.com

weak activity exhibited by steviol, which is a hydrolysis product of stevioside. In this paper we report the effect of different solvent extracts of dried leaves of *S. rebaudiana* on shoot and root length of rice seedlings

Extraction of *Stevia* leaves: Air dried leaves of *S. rebaudiana* were powdered and extracted with hexane, dichloromethane and methanol using soxhlet apparatus. Hexane, dichloromethane and methanol were purchased from Merck India Ltd. and glass distilled before use. For each extraction 50 g of powdered leaves were taken in a round bottom flask with 100 ml of solvents and extracted for 24 hours. After extraction residue was separated by filtering through a muslin cloth. The filtrates were passed through activated anhydrous sodium sulfate (10g) and evaporated using a rotary vacuum evaporator to obtain dark brown gummy material from each extract. The crude materials obtained from three extracts (hexane, dichloromethane, methanol) were evaluated for plant growth parameters on rice seedlings. Three different concentrations (1000, 500 and 250 ppm) of crude extracts were prepared in acetone/water.

Plant growth activity: A pot experiment was conducted to study the plant growth regulatory activity of the above concentrated solvents extracts. Plastic pots (10x10 cm) were filled with sandy loam soil collected from IARI field having 0.47% organic carbon and pH 8.2. There were three replicates of each treatment along with

control. Water only served as control. Different concentrations (1000, 500 and 250 ppm) of crude extracts were added to each pot at a rate of 5 ml/500g soil. Ten seeds (soaked and pre-germinated) of paddy (*Oryza Sativa* L.) were sown in each pot, irrigated and kept in a net house. The pots were irrigated regularly and observations were recorded after 15 days. After 15 days the pots were filled with water and the seedlings were uprooted carefully, washed with water. The growth of root and shoot elongation was observed and recorded by measuring the length of the root and shoot of each plant in each pot along with control. The fresh weight of plants in each replicated pot was also recorded. The plants were wrapped in a glazed paper and dried in hot air oven at 50°C till constant weight. The dry weight of each replicate was also recorded. The data thus obtained was compared with that of untreated controls.

Results showed in general an increase in root and shoot length was observed when rice seedlings were treated with hexane dichloromethane and methanol extracts of *Stevia* leaves at 250, 500 and 1000 ppm concentrations as compared to control (water). Maximum increase in root length was observed when rice seedlings were treated with hexane extract (Table 1, Fig. 1) rather than dichloromethane and methanol extracts. Increase in shoot and root length was found to be concentration dependent. The rice seedlings when treated with hexane extract at a concentration of 1000

Table 1. Effect of solvent extracts of dried leaves of *S. rebaudiana* on germination, root and shoot length of rice seedlings at different concentrations

| Extracts | Concentration (ppm) | Root length (cm) ± RSD | Increase in root length (%) | Shoot length (cm) ± RSD | Increase in shoot length (%) | Fresh weight g ± RSD | Dry weight g ± RSD |
|-----------------|---------------------|------------------------|-----------------------------|-------------------------|------------------------------|----------------------|--------------------|
| Control (water) | | 9.4 ± 0.7 | | 13.8 ± 0.9 | | 0.062 | 0.018 |
| Hexane | 1000 | 11.2 ± 0.6 | +19.14 | 16.3 ± 1.27 | +18.12 | 0.066 | 0.024 |
| | 500 | 9.8 ± 0.97 | +04.25 | 15.9 ± 1.41 | +15.22 | 0.083 | 0.025 |
| | 250 | 9.6 ± 0.22 | +02.1 | 15.0 ± 0.31 | +13.77 | 0.078 | 0.024 |
| Dichloromethane | 1000 | 8.5 ± 0.27 | -9.57 | 15.0 ± 1.6 | +8.69 | 0.099 | 0.022 |
| | 500 | 8.7 ± 0.27 | -7.44 | 15.5 ± 1.97 | +12.31 | 0.088 | 0.020 |
| | 250 | 8.3 ± 0.65 | -11.70 | 15.1 ± 1.07 | +9.42 | 0.088 | 0.023 |
| Methanol | 1000 | 9.6 ± 1.27 | -8.5 | 15.7 ± 1.60 | +8.69 | 0.092 | 0.02 |
| | 500 | 8.5 ± 0.35 | -9.57 | 14.8 ± 0.42 | +5.79 | 0.090 | 0.019 |
| | 250 | 8.6 ± 0.29 | -8.5 | 14.3 ± 1.41 | +3.62 | 0.086 | 0.018 |

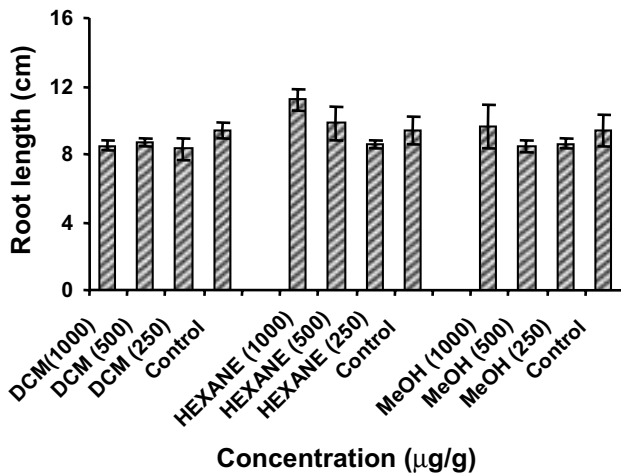


Fig. 1. Effect of solvent extracts of dried leaves of *S. rebaudiana* on root length of rice seedlings at different concentrations

ppm showed 11.2 cm root length, where as only 9.8 and 9.6 cm root length was observed with 500 and 250 concentration respectively (Fig. 1). An increase in shoot length was observed when rice seedlings were treated with different test concentration of hexane, dichloromethane and hexane extracts (Table 1 and Fig. 2). Rice seedling when treated with 1000 ppm concentration of hexane extract showed 16.3 cm shoot length, an increase of 18.12 % as compared to control (13.8 cm). An increase in fresh and dry weight was also observed with treated seeds as compared to control. The control seedling showed fresh weight 0.062 g and dry weight of 0.018g, whereas hexane extract at 1000 ppm

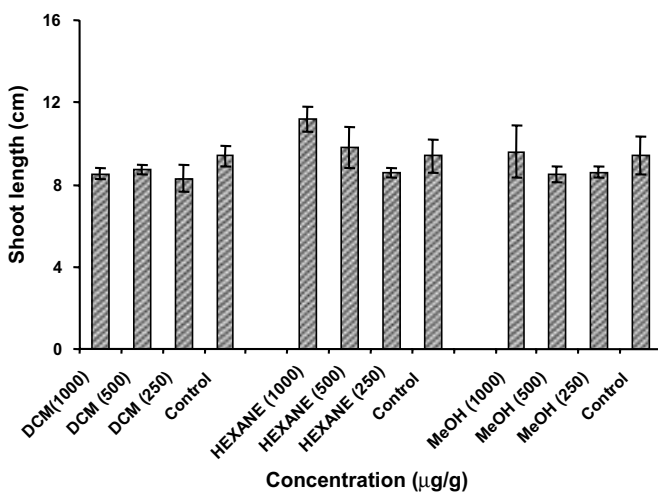


Fig. 2. Effect of solvent extracts of dried leaves of *S. rebaudiana* on shoot length of rice seedlings at different concentrations

showed fresh weight 0.066g and dry weight 0.024 g. A very interesting observation was recorded that an increase in fresh and dry weight at lower concentration rather than at higher concentrations. It was also observed that these parameters registered more enhancements in dichloromethane and methanol extracts than with hexane extract. It has been already reported that stevioside aglycone, steviol possess gibberellin-like activity. It has the capacity to stimulate growth in a single-gene dwarf mutant of *Zea mays* known to respond only to gibberellins (Ruddat *et al.* 1963). Furthermore, steviol has been shown to be a suitable substrate for the biosynthesis of several gibberellins in the fungus *Gibberella fujikuroi*.

The present study therefore, reveals that the hexane extracts of *S. rebaudiana* is highly effective on growth of rice seedlings because of the presence of steviol. There is a need to carry out these studies to test the efficacy of these crude extracts under field conditions and study in detail the mode of action.. Furthermore, the allelochemicals responsible for germination and growth reduction of rice seedlings should be isolated and identified. There is possibility of using these allelochemicals directly or as structural leads for the discovery and development of new plant growth regulators.

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