

CHAPTER 5

IRRIGATIONAL EFFECT ON SOME PHYSIOLOGICAL CHARACTERS AND ROOT AND ALKALOID YIELDS OF *R. serpentina*.

INTRODUCTION

R. serpentina grows wild in forests under high rainfall conditions of about 1,000 mm to 1500 mm per annum. The natural habitat of the plant suggests that it is grown in partial shaded areas as an undergrowth. As a result, plant water deficit usually increases less quickly and photosynthesis proceeds for longer period in shelter, so allowing better dry matter production and increased efficiency in water use. A prerequisite for the field cultivation of *R. serpentina* is to create forest microclimate that prevails at the habitat of the plant. The moist humid micro-climate favourable and necessary for this undergrowth in forest can be provided in the open field by means of adequate irrigation.

Rainfall is the major component of water resources in India and provide a major share of the crop-water requirements during the wet season and partially during the dry season through storage and soil layers. During the winter season rainfall occurs very rarely. Therefore, if *R. serpentina* field is not irrigated, growth is restricted due to water stress. Information is needed regarding the physiological and morphological responses of *R. serpentina*. Little information is available on the irrigation and water requirement of *R. serpentina* as a plantation crop. Increasing

existing knowledge of the nature of the response to drought and / or irrigation will promote higher production potential and more efficient water use in *R. serpentina*.

Developmental factors influencing the accumulation of dry matter and subsequent partitioning of assimilates are of great importance in determining the final yield of crop plants (Watson, 1971; Wareing and Patric, 1975). Water is one of the most essential factors influencing plant growth and development. A number of workers reported significant increases of dry matter by water application in a number of crop plants [Krogman and Hobbs (1975) and Kundu and Paul (1995-96) in rape, D'Souza and Coulson (1988) in *Phaseolus vulgaris*, Mondal and Paul (1992) and Begum and Paul (1993) in mustard and Sarker and Paul (1998), Nahar and Paul (1988) in wheat]. Several workers have reported that irrigation had positive influence on growth parameters such as RGR, NAR, LAR, RLGR, SLA and LWR in a number of crop plants (Sarker and Paul, 1998; Nahar and Paul, 1998; Sarker *et al.*, 1996; Nerkar *et al.*, 1981; Mondal and Paul, 1995; Saha and Paul, 1995; Mondal and Paul, 1994).

Fischer (1973) found that relative water content was directly related to soil water content and suggested that relative water content might also be used to indicate soil water content. Free proline accumulation occurs in the leaves of crop plants when exposed to moderate to severe water stress (Palfi *et al.*, 1973). Free sugars are also accumulated in the stressed plants (Stewart, 1971; Narashima Rao and Shiv Raj, 1985). It has been suggested that proline and sugar accumulating potentials could serve as indices of drought resistance. However, the physiological significance of metabolic response to water stress is contentious.

Since scientific data on this aspect of drought resistance are meagre, an attempt was made to study the effect of soil moisture on relative leaf water content

(RLWC) and some biochemical parameters, such as chlorophyll, proline and sugar contents of *R. serpentina*. Soil moisture effect on root yield and alkaloid content was also studied.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of North Bengal University. The field was prepared after repeated ploughing. A basal dose of urea (80 kg/ ha), TSP (50 kg/ ha) and MP (40 kg/ ha) were added to the field. Uniform and healthy seedlings of *R. serpentina* of 30–35 days old were transplanted in the field by the end of September, 1998. A spacing of 30 cm x 30 cm was followed and three were 3 replications.

Three levels of irrigation treatment were adopted, viz., (i) rainfed (I_0 = no irrigation), (ii) irrigation once in every month (I_1) and (iii) irrigation twice in every month (I_2). On each occasion, 20 mm of irrigation water were uniformly added over the irrigated plots with sprinklers. Irrigation started from November, 1998 and continued upto May, 1999.

Growth Attributes

For growth analysis, four harvests were taken at equal interval of 15 days. Three plants/ treatment/ replication were taken on each occasion. The first harvest was taken at 60 days after transplanting (DAT). At each harvest, plants were cut at the ground level and the tops were separated into leaves, petioles and stems. The dry weights of different plant parts were recorded after oven-drying at about 85°C for 24

h till they reached constant weight. For leaf area measurement, disc method was followed. From leaf area and dry weight data, different growth attributes were calculated as in Chapter 4.

Relative Leaf Water Content (RLWC)

Relative leaf water content was determined from the fully matured leaves. The leaves were collected at 8 a.m., 12 noon and 4 p.m. Three leaves were taken from each replication of each treatment. Their fresh weights were taken immediately and were sunk into water kept in beaker for 4 hours. After 4 hours, when the cells of the leaves became fully turgid, they were taken out from water and after drying with blotting paper their turgid weights were determined. Then the leaves were dried in an oven and weighed. The RLWC was calculated from the following formula (Barrs and Weatherley, 1962) :

$$\text{RLWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Chlorophyll Content

Chlorophyll content of the matured leaf was determined as in Chapter 4.

Estimation of Proline

Proline estimation of the matured leaves was done according to Bates *et al.* (1973). For estimation of proline, the reagents required were (1) 3% aqueous sulphosalicylic acid, (2) Glacial acetic acid, (3) Toluene, (4) Proline (AR) and (5) Acid ninhydrin reagent [6M orthophosphoric acid (20 ml) + glacial acetic acid (30 ml) + ninhydrin (1.25 g)].

At first 0.5 g fresh leaf was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and centrifuged it for 6 minutes. The clear solution was separated and 2 ml of it was reacted with 2 ml of acid ninhydrin and 2 ml glacial acetic acid in a test tube. Then it was boiled in a boiling water bath for 1 hour and the reaction was terminated in an ice bath. The reaction mixture was then extracted with 4 ml of toluene mixed vigorously with stirring for 15 – 20 seconds. The chromophore containing proline-toluene was separated with a separating funnel and warmed to room temperature. The optical density (O.D.) at 520 nm was read using toluene as blank. Proline content was determined from the standard curve.

Stock solution of 1 millimole proline was prepared by dissolving 0.1151 g of proline (AR) in distilled water and made to 1000 ml. By successive dilution 2 ml of solution containing 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 micromole concentrations were prepared and reacted with acid ninhydrin and glacial acetic acid as described earlier. The colour was read at 520 nm and the O.D. was plotted against concentration (Figure 15).

Determination of Sugar Content

Extraction of sugar from matured leaves was done following the method described by Jayaraman (1975).

One gram of leaf was thoroughly crushed in a mortar with a pestle and 10 ml of ethyl alcohol were used for leaf crushing. The extract was filtered through two layers of muslin cloth. The volume of the extract was evaporated to about one-fourth of the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to a 100 ml volumetric flask and made up to the mark with distilled water. One ml of the diluted solution was taken into another 100 ml volumetric flask and made up to the mark with distilled water.

Aliquot of 1 ml of the extract was pipetted into test tubes and 4 ml of anthrone reagent was added to each of these solutions and mixed well. Glass marbles were placed on top of each tube and the test tubes were heated for 10 minutes in boiling water bath and then cooled. A reagent blank was prepared by taking 1 ml of water and 4 ml of anthrone reagent in a tube and treated similarly. The absorbance of the blue-green solution was measured at 680 nm in a spectrophotometer.

A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0.0, 10, 20, 40, 60, 80 and 100 μg of glucose, respectively and made the volume up to 1.0 ml with distilled water. Four ml of anthrone reagent were added to each tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 640 nm using the blank containing 1 ml of water and 4 ml of anthrone.

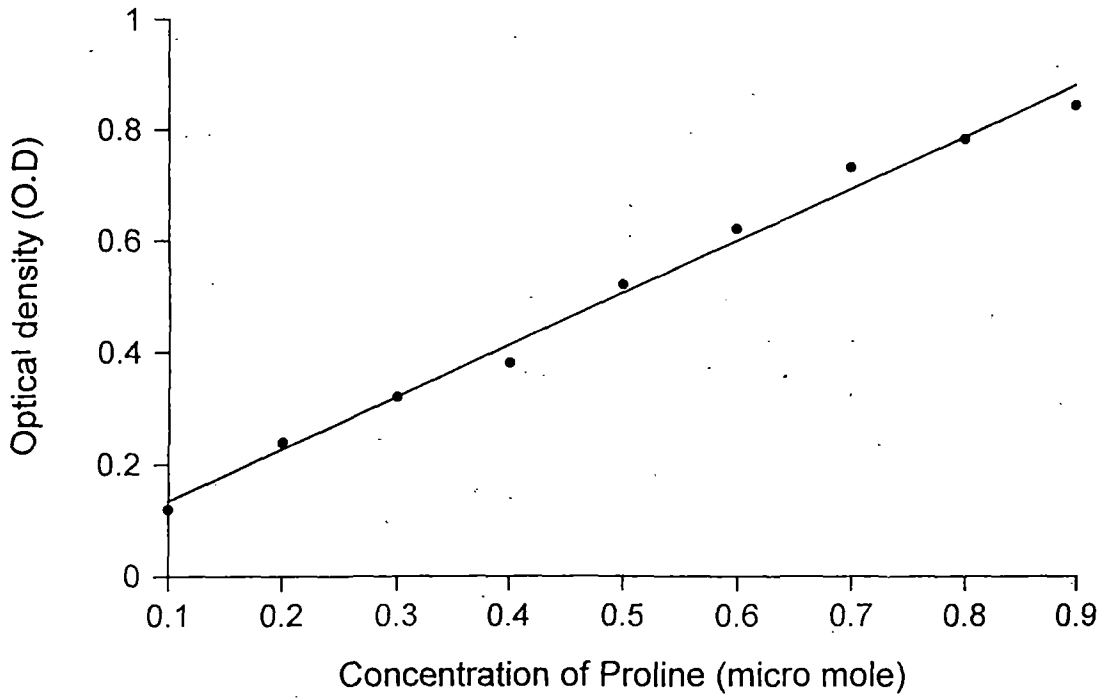


Fig. 15 Standard curve for proline

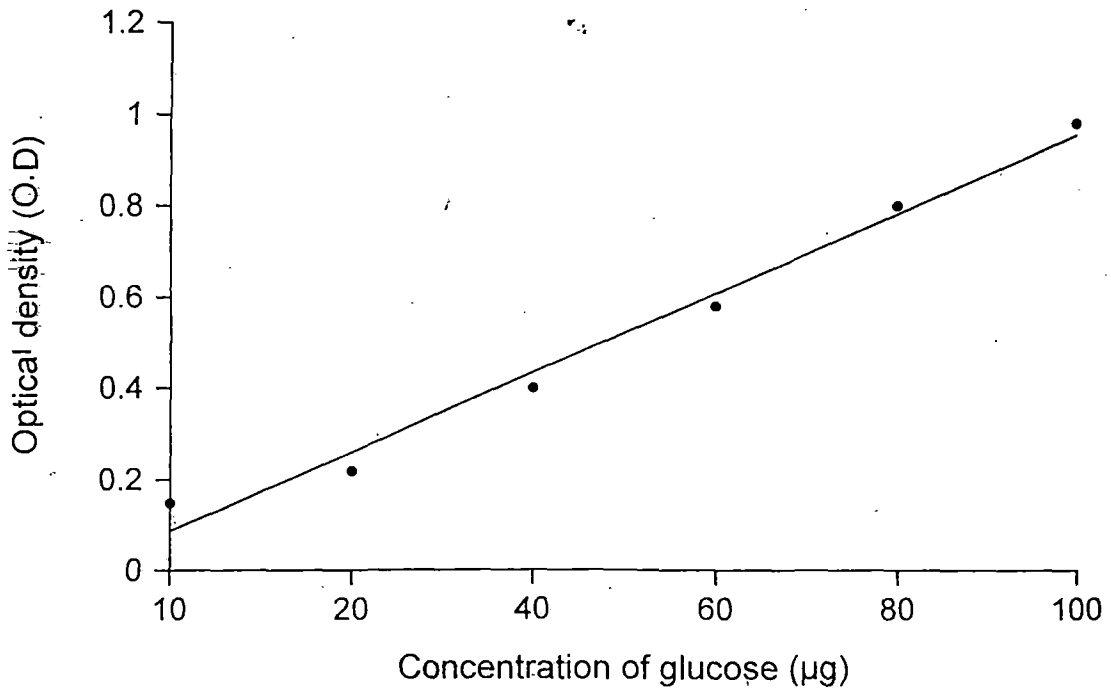


Fig. 16 Standard curve for glucose

The amounts of free sugar were calculated from the standard curve of glucose (Figure 16). Finally the percentage of free sugar present in the leaves was determined using the formula given below:

$$\text{Percentage of free sugar} = \frac{\text{Weight of sugar obtained}}{\text{Weight of leaf dust}} \times 100$$

Sugar content was determined at 3 times i.e. at 120, 180 and 240 days after transplanting.

Root Yield and Alkaloid Content

After 18 months of planing, plants were dug out in March, 2000 and the roots were separated and washed in the running tap water. The roots were air-dried to a constant weight. Six randomly selected plants from each treatment and replication were used for this purpose. Composite root samples were analysed for total alkaloid and reserpine contents as in Chapter 2 of this study. Data were analysed statistically.

RESULTS AND DISCUSSION

Leaf Area and Dry Matter

Effect of soil moisture on leaf area and total dry matter of *R. serpentina* is shown in Figures 17 and 18, respectively. Both leaf area and dry matter were significantly affected by soil moisture. Compared to control, I₁ and I₂ treatments had greater values at all the stages of growth. Similar result was reported in several plants

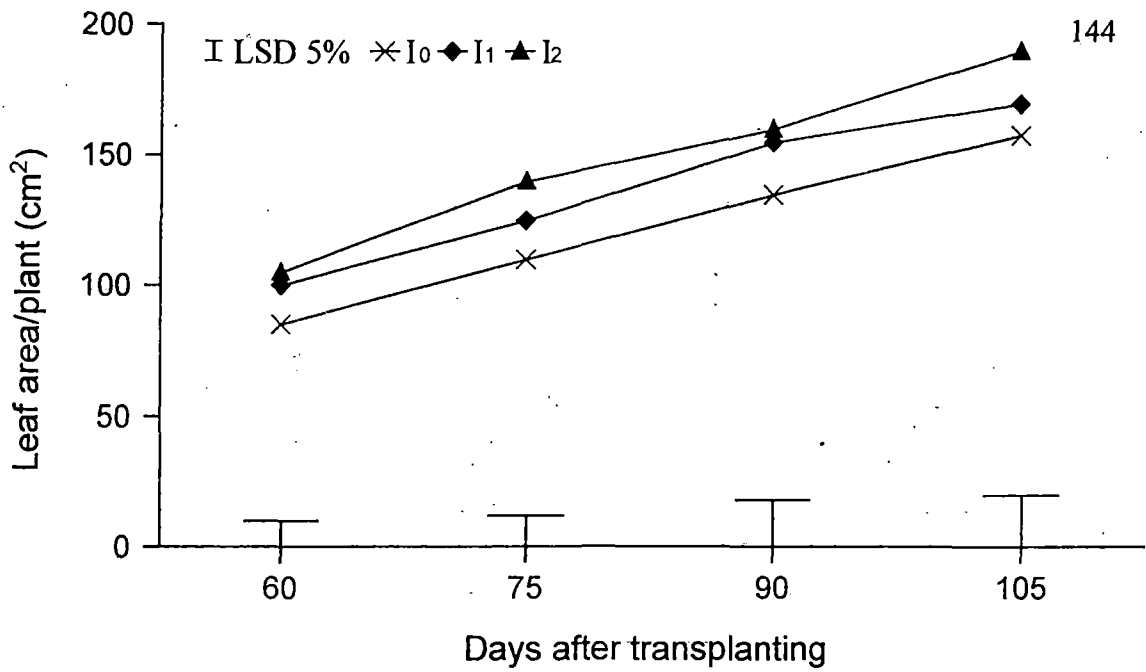


Fig. 17 Influence of soil moisture on leaf area of *R. serpentina*

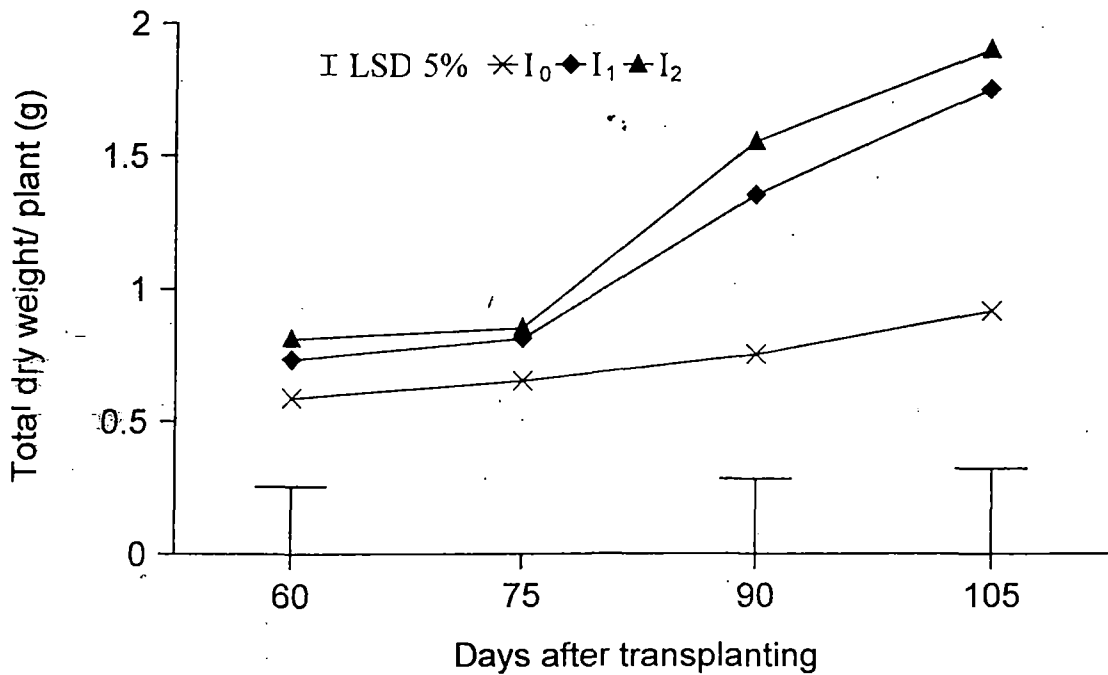


Fig. 18 Influence of soil moisture on total dry weight of *R. serpentina*

like barley (Kirby, 1969), sorghum (Constable and Hearn, 1978; Sivkumar *et al.*, 1979 and Rabindranath and Shiv Raj, 1983), mustard (Mondal and Paul, 1992, 1994, 1995; Begum and Paul, 1993), in rape (Clark and Simpson, 1978; Paul and Kundu, 1991) and in wheat (Rahman and Paul, 1998; Nahar and Paul, 1998; Sarker and Paul, 1998).

Leaf area of plant may be varied due to change either in leaf number or leaf size (Arnon, 1975). The increase of leaf area occurred due to increase of the leaf expansion in the irrigated plants. Soil moisture increased turgor pressure in the cells and turgor forces played a part in the process of leaf expansion (Hsiao and Acevedo, 1974). With the increase in the frequency of irrigation, uptake of nutrients was more, hence more expansion of leaf took place (Mandal *et al.*, 1986).

Soil moisture increased the relative leaf water content, which increased cell expansion and ultimately leaf area increased. Mare and Palmer (1976) found that the total number of leaves produced by the primary stem of sunflower was reduced due to water stress. This may be due to the inhibition of the initiation and differentiation of leaf primordia due to water stress.

Growth Attributes

Compared to control, RGR of the irrigated plants was higher at the initial and the final harvest intervals, but lower at the middle harvest interval (Figure 19). Saha and Paul (1995) studied the effect of soil moisture on growth attributes of wheat and reported that RGR was unaffected by soil moisture and it decreased with plant age. Richards (1978) found that RGR was reduced in more severe drought treatment in rape.

RGR declined with increasing age and plant dry weight. Similar results were reported for RGR in barley (Thorne, 1960) and in wheat (Sarker and Paul, 1998; Nahar and Paul, 1998; Sarker *et al.*, 1996). It had been suggested that the decrease in RGR could be attributed to shading of lower leaves by upper leaves (Thorne, 1961). The decreasing trend in RGR with age was mainly due to decline of LAR (Chanda *et al.*, 1987).

Irrigation increased NAR (Figure 20). Higher NAR due to higher soil moisture was found by El Nadi (1969) and Nerkar *et al.* (1981) in beans and Rabindranath and Shiv Raj (1983) in sorghum. Unlike RGR, NAR in all the treatments increased with plant age and weight. As *R. serpentina* is a long duration crop, NAR did not decrease up to 105 DAT. But LAR decline steadily with increasing plant age (Figure 21). Similar results were reported in dry beans by Wallace and Munger (1965), in wheat by Sarker and Paul (1998) and Nahar and Paul (1998), in mustard by Begum and Paul (1993) and Mondal and Paul (1994). In the present investigation, irrigated plants had lower LAR than the rainfed control. But Sarker and Paul (1998) observed slightly higher LAR in the irrigated treatments. Kirby (1969) also reported that LAR increased with increasing level of irrigation in barley. Similar result was reported in bean by Nerkar *et al.* (1981). However, no significant effect of irrigation on LAR was found by Mondal and Paul (1992), but Paul and Kundu (1991) and Mondal and Paul (1995) reported decreased LAR in rape and mustard, respectively.

The plants under irrigated condition had higher RLGR than the rainfed ones (Figure 22). Similar result was reported in wheat (Saha and Paul, 1995; Sarker and Paul, 1998). But Mondal and Paul (1995) did not find any significant effect of soil moisture on RLGR of mustard. RLGR in all the treatments declined with increasing

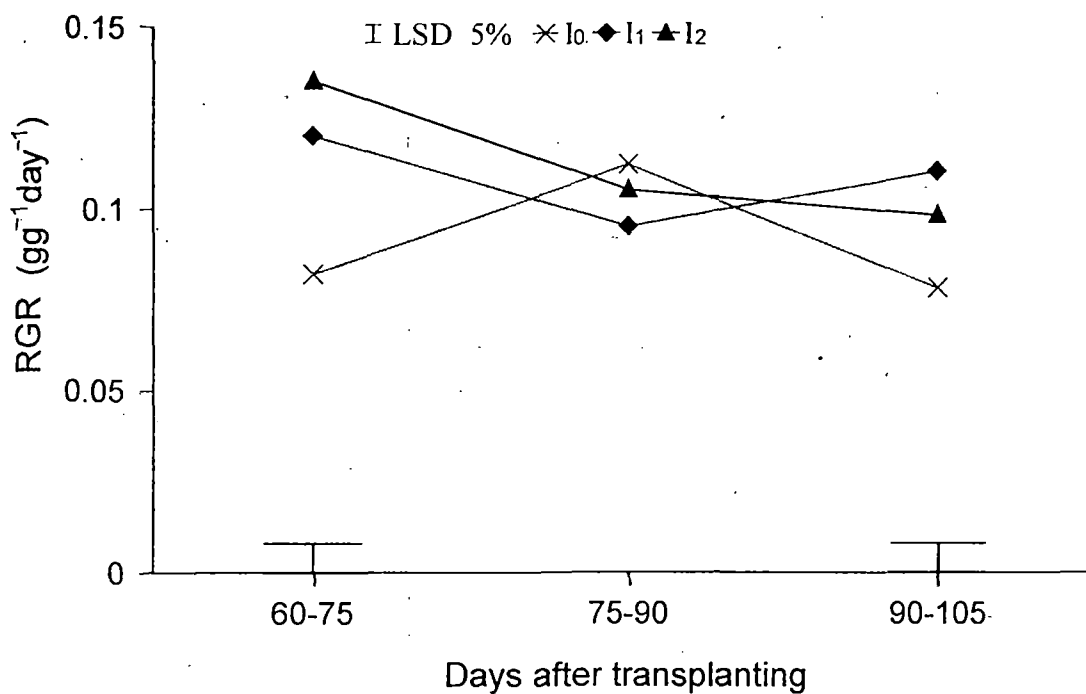


Fig. 19 Influence of soil moisture on RGR of *R. serpentina*

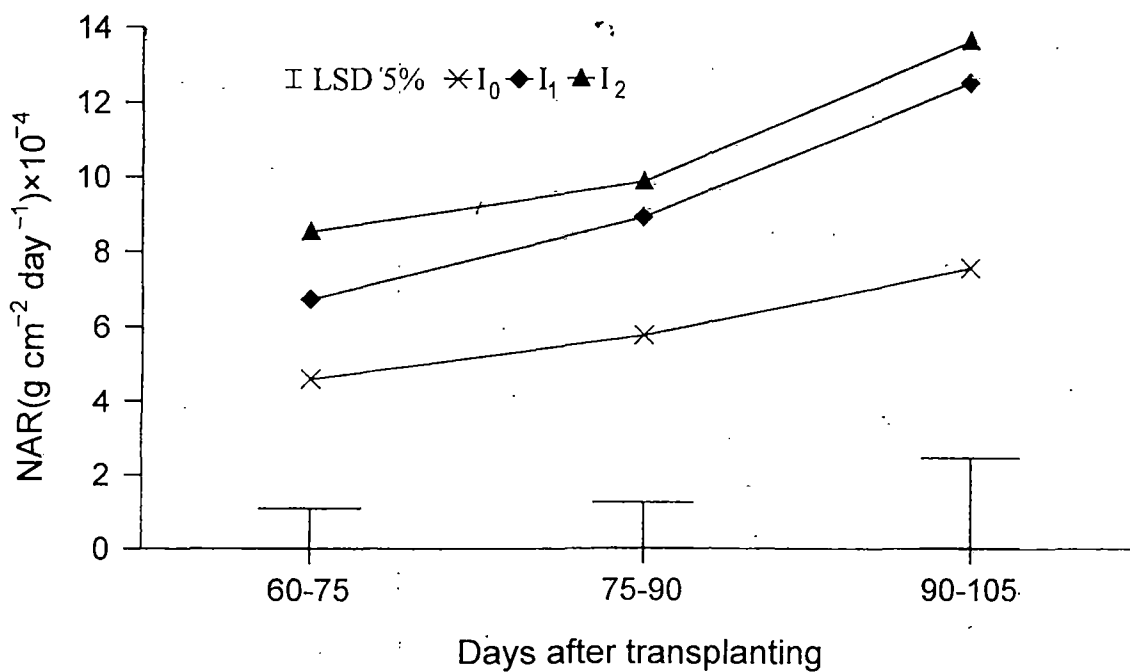


Fig. 20 Influence of soil moisture on NAR of *R. serpentina*

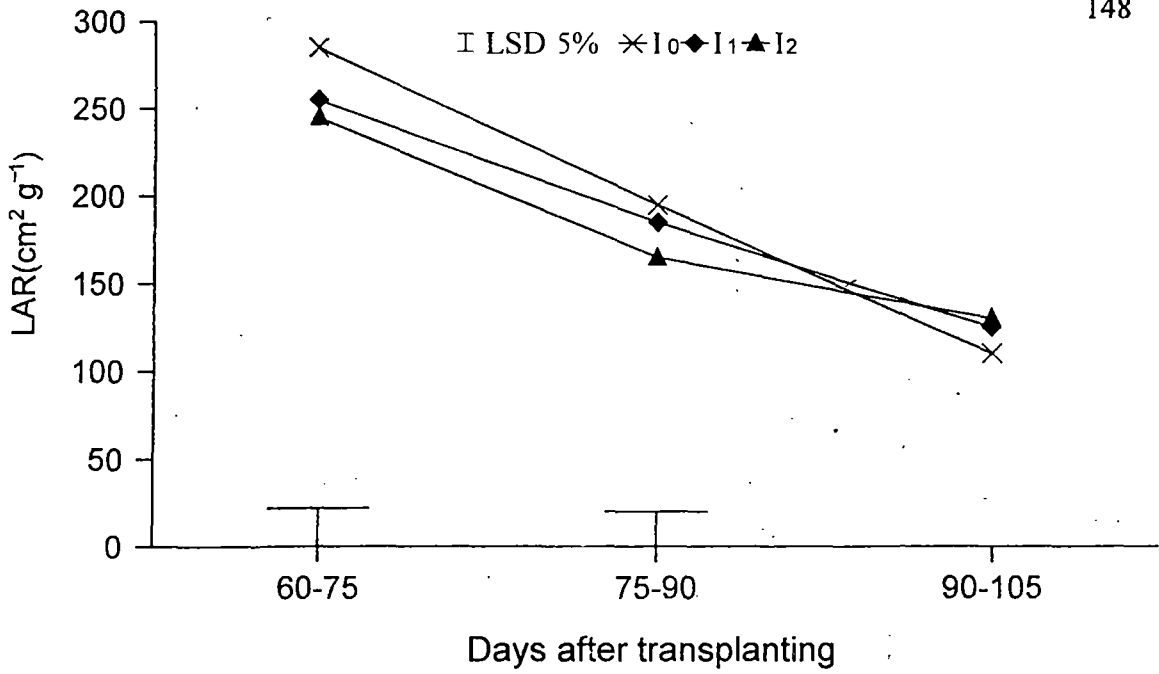


Fig. 21 Influence of soil moisture on LAR of *R. serpentina*

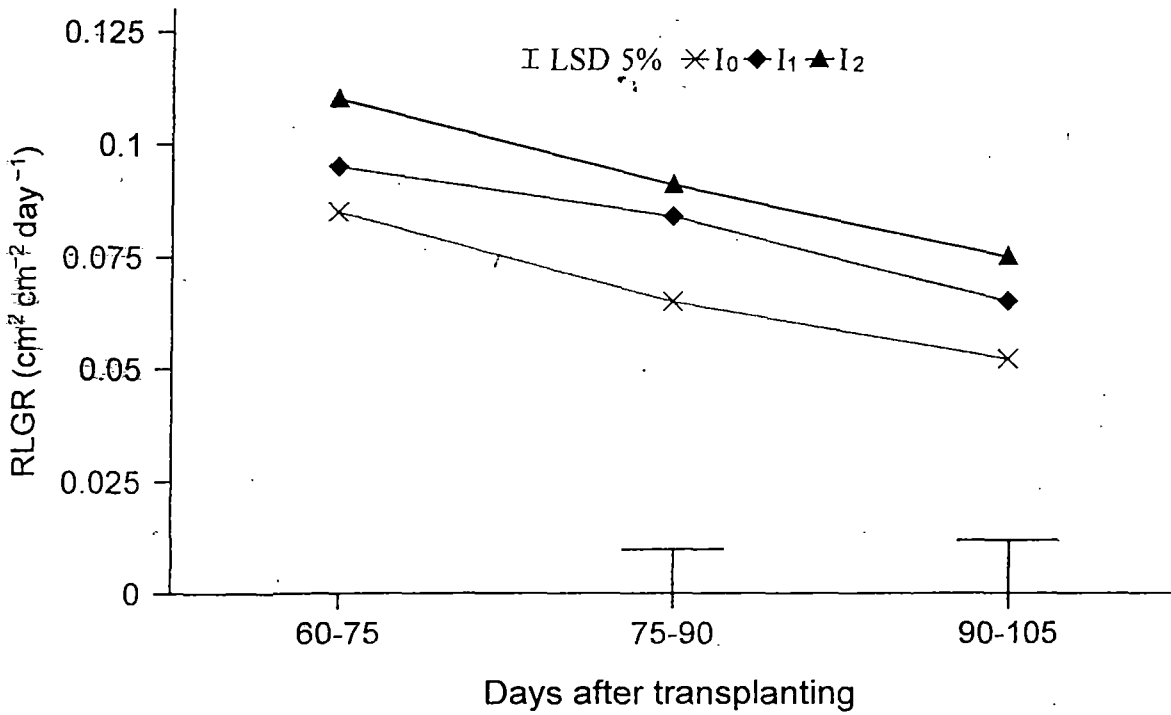


Fig. 22 Influence of soil moisture on RLGR of *R. serpentina*

plant age. Similar decline in RLGR with plant age was reported by Pandey *et al.* (1978) in blackgram and Chanda *et al.* (1987) in pearl millet.

There were no clear patterns of soil moisture effect on SLA (Figure 23), although there was some tendency of increase of SLA with increasing soil moisture. Saha and Paul (1995) and Nahar and Paul (1998) indicated that SLA was decreased by irrigation. Paul and Kundu (1991) found that SLA decreased by irrigation in rape. But Mondal and Paul (1995) did not find significant effect of irrigation on SLA. In the present investigation, SLA gradually decreased with plant age. Decrease of SLA with plant age was reported in wheat (Saha and Paul, 1995; Sarker and Paul, 1998 and Nahar and Paul, 1998), in mustard (Islam and Paul, 1986), in jute (Hussain and Paul, 1984) and in pearl millet (Chanda *et al.*, 1987).

Like SLA, there was no clear pattern of soil moisture effect on LWR (Figure 24). But Sarker and Paul (1998) in wheat and Mondal and Paul (1995) in mustard reported that LWR was higher in the well-watered plants. The values of LWR showed downward drifts with age (Figure 24). Similar result was reported by Mondal and Paul (1995) in mustard, Sarker and Paul (1998) in wheat and Chanda *et al.* (1987) in pearl millet. Similar results were also reported in sweet potato (Shamsuddin and Paul, 1988) and in jute (Hussain and Paul, 1984). Kundu (1992) stated that the sharp decrease of LWR at the later stages might be due to sharp increase of total dry matter towards the later stages.

Relative Leaf Water Content

Relative leaf water content (RLWC) of the irrigated plants (I_1 and I_2) was significantly higher than that of the rainfed plants (I_0) (Table 15). Similar result was

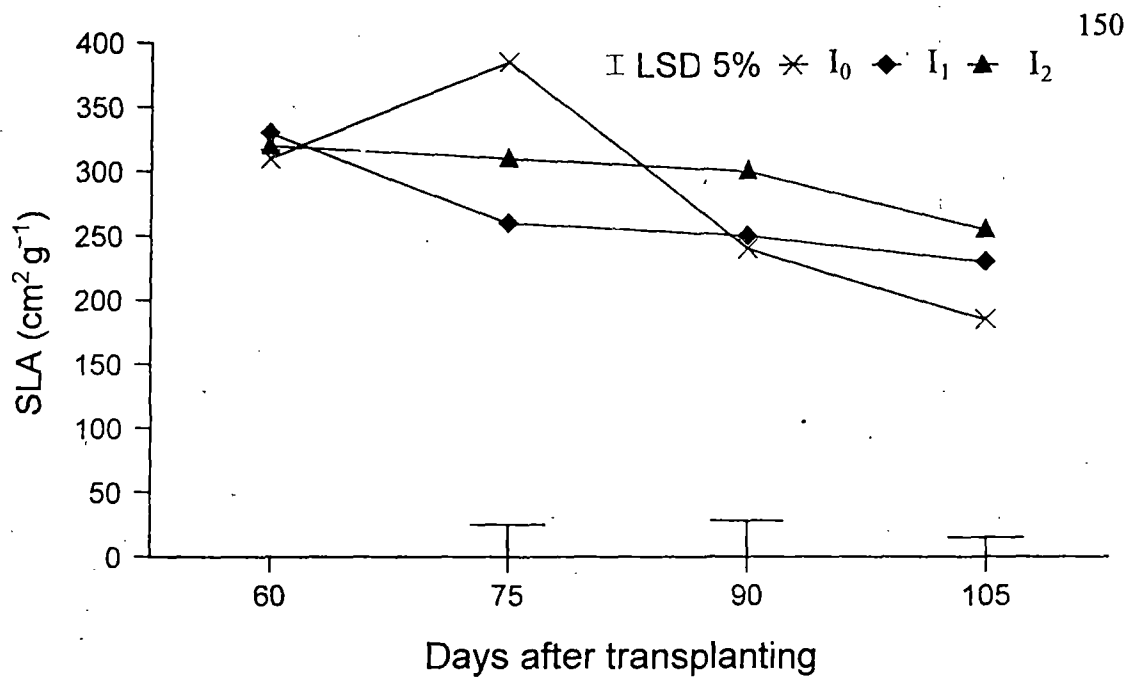


Fig. 23 Influence of soil moisture on SLA of *R. serpentina*

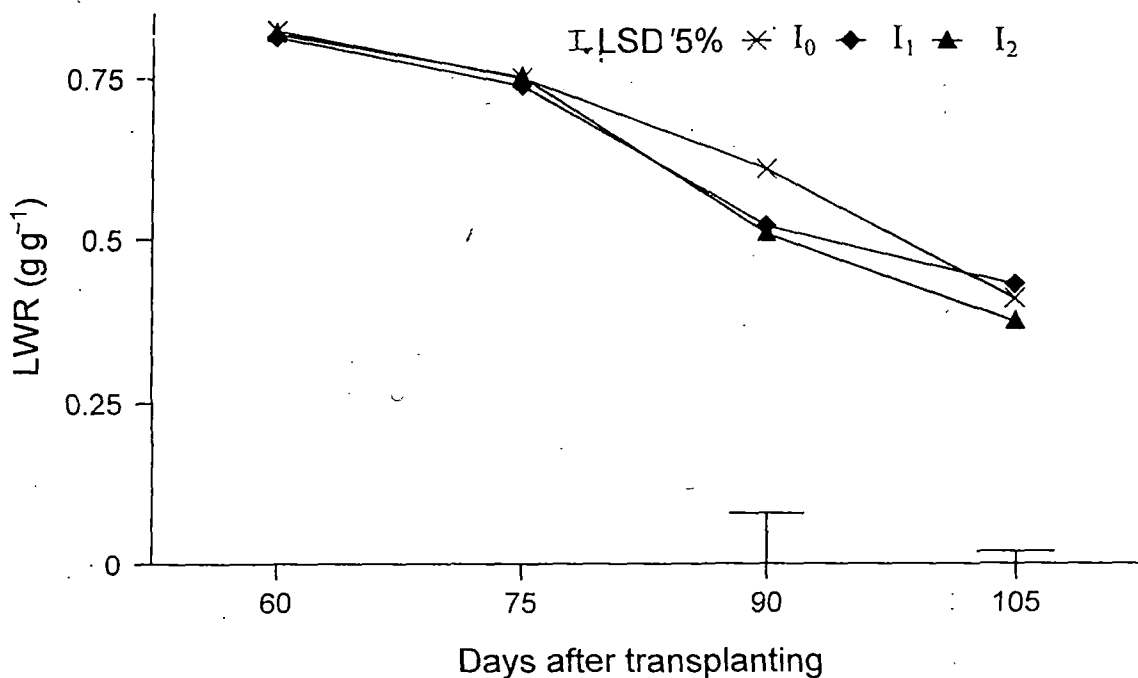


Fig. 24 Influence of soil moisture on LWR of *R. serpentina*

reported in wheat by Rajagopal et al., (1977), Nayak et al. (1983), Sarker et al. (1999), Schonfeld et al. (1988), Rahman and Paul (1998), in rape by Raja and Bishnoi (1990) and Kundu and Paul (1996) and in mustard by Sharma and Kumar (1989) and Begum and Paul (1993).

Table 15 : Relative leaf water content(%)of *R. serpentina* at different times of the day as influenced by soil moisture.

Treatment	Time		
	8 a.m.	12 noon	4 p.m.
I ₀	88.0	75.0	75.8
I ₁	93.2	79.3	82.0
I ₂	95.7	80.0	84.7
LSD 5%	3.2	2.7	4.0

In all the three treatments, RLWC was higher towards the morning, got reduced at noon and again showed some recovery towards the afternoon (Table 15). This behaviour was related to the environmental conditions and is in agreement with earlier reports (Begum and Paul, 1993; Mondal and Paul, 1996; Sarker and et al., 1999). At midday, RWLC of the rainfed plants (I₀) decreased more than that of the irrigated plants (I₁ and I₂). Cortes and Sinclair (1986) also found more negative leaf water potential of stressed soybean plants than that of the irrigated ones at midday. Higher RWLC is associated with higher dry matter production rates of the irrigated plants because cell turgidity is important in relation to the opening and closing of stomata, expansion of leaves and movement of nutrients of various parts of the plant (Kramer, 1969).

Chlorophyll Content

Chlorophyll a and b are the most important pigments active in the photosynthetic process. Increased chlorophyll contents absorb higher quantity of light and hence increased photosynthesis. Several workers have reported that the rate of photosynthesis in leaves is positively associated with chlorophyll (Muramoto *et al.*, 1965; Kariya and Tsunoda, 1971). Hesketh (1963) showed that species could vary greatly in rate of photosynthesis and this variation was not related to chlorophyll content. Buttery and Buzzell (1972) reported that many crop plants were never light saturated even in full sunlight and that a higher level of chlorophyll could further increase light absorption and photosynthetic rate only if sufficient light was provided.

In the present investigation, soil moisture effect was found to be significant for chlorophyll a, b, and total chlorophyll (Table 16). A reduction of chlorophyll formation due to water stress was also reported by Mondal and Paul (1992), Begum and Paul (1993), Kundu and Paul (1997) and Sarker *et al.* (1999). However, Saha and Paul (1997) did not find any significant effect of soil moisture on chlorophyll content of wheat.

Table 16 : Mean values of chlorophyll content (mg dm^{-2}) of *R. serpentina* leaf as influenced by soil moisture.

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll
I ₀	3.07	0.72	3.79
I ₁	3.62	0.87	4.48
I ₂	3.71	0.90	4.61
LSD 5%	0.13	0.09	0.14

Proline Content

Proline works as a source of energy, carbon and nitrogen and also protects several enzymes against the inactivating effects of heat during water stress (Paleg *et al.*, 1981). Hence, possibly proline accumulation under water stress helps the plant to resist the drought. In this study, the rainfed plants (I_0) had significantly higher proline content than the irrigated plants (I_1 and I_2) (Table 17). Similar result was also reported in wheat (Saha and Paul, 1997; Rahman and Paul, 1998; Sarker *et al.*, 1999), in rape (Paul and Kundu, 1991; Kundu and Paul, 1997), in mustard (Begum and Paul, 1993), in sorghum (Blum and Ebercon, 1976) and in foxtail millet (Narashima Rao and Shiv Raj, 1985). Total free amino acids are increased in water stressed leaves and proline being the most pronounced (Hsiao, 1973).

Table 17 : Proline content ($\mu\text{g/g}$ fresh weight) of *R. serpentina* leaf as influenced by soil moisture at different stages of growth.

Treatment	Days after transplanting		
	120	180	240
I_0	249	265	272
I_1	166	170	180
I_2	148	174	191
LSD 5%	24	32	28

Blum and Ebercon (1976) suggested that the accumulated proline could also serve as a readily available energy and nitrogen source for use upon the relief of stress. Accumulated proline may be oxidized and serve as a source of energy, especially when carbohydrate content is low (Wang, 1968; Oks *et al.*, 1970; Stewart, 1971). There is also evidence that proline may confer protection on some mitochondrial and solubilized enzymes against heat instability (Nash *et al.*, 1980).

In the present investigation, accumulation of proline in all the treatments increased with the advancement of plant age (Table 17). Similar result was reported by Kundu and Paul (1997) in rape. This increase might be due to increased atmospheric and soil drought at the later stages of growth.

Total Sugar Content

Total free sugar content was significantly (except at 240 DAT) higher in the rainfed plants at all the three stages of growth (Table 18). Turner *et al.* (1985), Drossopoulos *et al.* (1987), Kundu and Paul (1997) and Sarker *et al.* (1999) obtained similar results. As the stress increased at the later stage, amount of sugar also increased in all the three treatments (I_0 , I_1 and I_2), but the amount was much greater in the rainfed plants.

Plants accumulated more sugar during stress which can be utilized when stress is released (Stewart, 1971). Easten and Ergle (1948) suggested that starch would be converted into sugar under stress. Sugar is also known to stabilize protoplasmic membranes (Larson, 1975).

Table 18 : Total sugar content (mg/ g dry weight) of leaf of *R. serpentina* at three growth stages as influenced by soil moisture.

Treatment	Days after transplanting		
	120	180	240
I ₀	12.6	13.5	14.8
I ₁	11.7	11.5	12.4
I ₂	9.5	9.8	11.5
LSD 5%	1.7	2.5	NS

The present study suggests that moisture stress had an inhibitory effect on RLWC and chlorophyll content, but proline and sugar contents of leaves increased markedly as a result of soil dryness. High proline and sugar accumulation of *R. serpentina* in drought conditions might be an adaptation for tolerance of drought which helps the crop to survive and continue production during the dry season. The above parameters may also be used to assess crop water status.

Root Yield and Alkaloid Content

Effects of soil moisture on root yield and alkaloid contents of *R. serpentina* are shown in Table 19. Results indicated that main root length, total alkaloid and reserpine contents were unaffected by soil moisture, but air-dried root yield gradually increased with the increase of irrigation frequency.

Table 19 : Effect of soil moisture on root yield and alkaloid contents of *R. serpentina*

Treatment	Main root length (cm)	Air-dried root weight/ plant (g)	Total alkaloid content (%)	Reserpine content (%)
I ₀	50	32	1.62	0.38
I ₁	52	37	1.83	0.42
I ₂	47	44	1.74	0.40
LSD 5%	NS	3	NS	NS

Little information is available on the irrigation and water requirement of *R. serpentina* as a plantation crop. Sahu (1972) reported a field experiment at the State Research Station, Bhubaneswar, Orissa. Irrigation started from January when the plantation was about four and a half month old. The results revealed that the longest root of 55 cm length was noticed in plants receiving no irrigation and the shortest root of 45 cm length was in plants receiving irrigation every month. Plants received irrigation in alternate month showed intermediate growth of 50 cm long. He further reported that overall growth of *R. serpentina* plantation diminished with increasing water stress but root growth was less influenced than was shoot growth. Root yield of the crop grown without irrigation was less than the irrigated ones. Irrigating the plantation once in every month from January to May raised the root yield by about 54.3%. Irrigation in alternate months also increased root yield by about 22.6%. Withholding irrigation in February, the yield of roots was decreased by 8.36% in comparison with the yield of the crop receiving irrigation in that month. He further reported that for a 18 months old *Rauwolfia* plantation, 139 mm water provided by 5 irrigations is needed to get high root yield.

The results of the present investigation indicated that better growth of *R. serpentina* is possible in well watered condition which ultimately considered as the basic need for satisfactory root yield. The results also suggest that during the dry season, irrigation of *R. serpentina* once in a month will assure high root yield.

The overall results of the present study suggested that leaf area and dry matter production were significantly increased by soil moisture. Higher dry matter and root yield of the irrigated crop were due to the increase of net assimilation rate. Increased chlorophyll content also contributed to higher NAR of the irrigated crop. Relative leaf water content (RLWC) of the irrigated crop was higher than the rainfed crop. The RLWC was associated with higher dry matter production rates because cell turgidity is important in relation to the opening and closing stomata, expansion of leaves and movement of water and nutrients to various parts of the plant. Physiological criteria like NAR can be taken as a criterion for root yield improvement of *R. serpentina* under irrigated condition.

SUMMARY

Information is needed regarding the morphological and physiological responses of *R. serpentina* to water stress for cultivation as plantation crop. The effect of soil moisture on growth parameters, relative leaf water content (RLWC), chlorophyll, proline and sugar contents and root and alkaloid yields was studied. Three levels of irrigation treatment were adopted: rainfed, irrigation once in every month and irrigation twice in every month.

Both leaf area and dry matter/ plant were significantly increased with increase of soil moisture. Compared to the control, RGR of the irrigated plants was higher at the initial and the final harvest intervals, but lower at the middle harvest interval.

Irrigation increased NAR. The plant under irrigated condition had higher RLGR than the rainfed ones. There were no clear patterns of soil moisture effect on SLA and LWR.

Relative leaf water content of the irrigated plants was significantly higher than that of the rainfed plants. Chlorophyll content was higher in the irrigated plants but proline content was higher in the rainfed control. Total free sugar content was also significantly higher in the rainfed plants at all the three stages of growth.

Effect of irrigation on main root length, total alkaloid and reserpine contents were unaffected by soil moisture, but air-dried root yield increased with the increase of irrigation frequency.