

ABSTRACT

The present investigation was carried with *Rauvolfia serpentina* which grows abundantly in the ecological conditions of Darjeeling District, West Bengal, India and yields highest amount of alkaloids as compared to other species of *Rauvolfia* available in India. It has been the objectives of the study to investigate some of the chemical and botanical aspects for its purposeful utilization in our society.

An important indole alkaloid reserpine was isolated, purified and characterised from root of *R. serpentina*. Dry powdered bark of *R. serpentina* was extracted with chloroform and was purified under column chromatography. The chemical was identified by paper chromatography and finally identification and characteization was done by IR spectrum.

For quantitative estimation of reserpine from root bark of *R. serpentina*, a new, easy and rapid colorimetric method was developed.

Owing to the hard sclerotic endocarp, the seeds of *R. serpentina* require longer time to germinate and the germination percentage is low. Effectiveness of different treatments for germination percentage of seeds of *R. serpentina* was studied.

Seeds scarification with sand paper was able to increase germination to some extent. But grinding of seeds with stone or nicking with a needle were not effective. The concentrated sulphuric and nitric acids and hot water treatment could not prove to be effective in increasing germination.

Pre-sowing seed treatment with chemicals did not improve germination. However, potassium nitrate and thiourea improved germination to some extent.

Although cultivation of *R. serpentina* through seeds is the most economic method, but it is extremely difficult to obtain the required supply of seed from natural sources. Moreover, germination percentage is very low and cannot be overcome by physical and chemical methods. So, there seems no other alternative than to undertake propagation by stumps, root and stem cuttings, which are more easily available.

The results of the present investigation indicated that stump cuttings had the highest root formation activity and it was followed by root and stem cuttings. 2,4-D at very low concentration and IBA and NAA at low concentration stimulated root formation of stem cuttings. The highest activity (100%) was observed at 5 ppm of 2,4-D and at 100 ppm. it totally inhibited rooting activity of the stem cuttings.

The effect of time of planting and type of cutting on the success of cutting and subsequent growth of *R. serpentina* was studied. Both type of planting and type of cutting had significant effect on percentage of success, plant height, number of roots per plant, length of longest root and air-dried root weight. Planting on 15 June produced the highest percentage of success and plant height, maximum number of roots and higher root weight per plant. 15 March planting produced the lowest root yield. Root cuttings produced significantly higher root weight than the stem cuttings.

Under planting condition, regular supply of nutrients should be maintained for proper growth and higher yields of root and alkaloid of *R. serpentina*. Effect of different combinations and levels of NPK fertilizers on growth and yield of *R. serpentina* was studied. Five combinations of NPK fertilizers were T₀ (N₀P₀K₀), T₁ (N₃₀P₂₀K₁₅), T₂ (N₆₀P₄₀K₃₀), T₃ (N₉₀P₆₀K₄₅) and T₄ (N₁₂₀P₉₀K₆₀). Three levels of each of the fertilizers were N₃₀, N₆₀, N₉₀, P₂₀, P₄₀, P₆₀, K₁₅, K₃₀ and K₄₅.

Leaf area per plant and total above-ground dry matter were increased with different combinations and levels of NPK fertilizers. Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and relative leaf growth ratio (RLGR) responded much by the application of N, P and K, but on specific leaf area (SLA) and leaf weight ratio (LWR) were less pronounced. NAR had positive correlation with RGR and LAR. LAR had positive association with both its components, SLA and LWR. Root yield had positive correlation with RGR and NAR.

Chlorophyll content was increased by NPK fertilizers. Compared to P and K, the different levels of N had more effect on chlorophyll content. Root yield and alkaloid content were increased by NPK fertilizers.

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 attributes, relative leaf water content, chlorophyll, proline and sugar contents and root and alkaloid yields was studied. Three levels of irrigation treatment were rainfed, irrigation one in every month and irrigation twice in every month.

Both leaf area and dry matter per plant were significantly increased with the increase of soil moisture. Compared to control, RGR of the irrigated plants were higher at the initial and the final harvest intervals, but lower at the middle harvest interval. Irrigation increased NAR. The plants under irrigated condition had higher RLGR than the rainfed ones. There were no clear pattern of soil moisture effect on SLA and LWR.

Relative leaf water content of the irrigated plants was significantly higher than the rainfed plants. Chlorophyll content was higher in the irrigated plants but proline and total free sugar was higher in the rainfed control.

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.

As *R. serpentina* has poor seed viability and low germination percentage, tissue culture methods for propagation offer an effective and quicker way to overcome the obstacles for production of a large number of propagules. Tissue culture methods would be a valuable alternative for rapid propagation and conservation of this valuable threatened plant species.

In the present investigation, various *in vitro* aspects of tissue culture have been tried with different explants of *R. serpentina*. Young branches of field grown plants were used for primary establishment of cultures. For shoot proliferation, shoot tips and nodal explants from 8-9 months old field grown plants were used as explant sources.

It was observed that frequency of callus formation was greater in cultures of shoot tips than nodal explants. Formation of base callus significantly decreased the frequency of multiple shoot formation, number of shoots per explant and also shoot length.

By increasing the concentration of BA, the percentage of explant produced shoots increased and the optimum concentration was 2.0 mg/l irrespective of concentrations of NAA used. GA₃ with BA and K_n did not increase the number of shoots per explant like BA with NAA and K_n with NAA. Optimal concentration of GA₃ was 0.1 mg/l with 2.0 mg/l BA and 0.5 mg/l with K_n.

MS medium supplemented with different auxin-cytokinin combinations was used to study the callusing response. Field grown plants and *in vitro* grown plants were used for this purpose. It was observed that callus proliferation strictly depended on exogenous hormone supplementation. In absence of exogenous

hormone, explants failed to induce callus and became necrotic and died within a few days. Among the four auxins used, 2,4-D was found to be the best in respect of callusing response. On the other hand, BA was superior to K_n for callus growth when supplemented with 2,4-D.

The combination of 2,4-D and BA was the most effective formation for both explants (internode and leaves) used in the induction of callus. But IBA alone or either with BA or K_n was the least effective to induce callus formation.

Field grown explants failed to induce root formation when placed in a culture medium. However, shoot obtained from first and second subcultures failed to induce root formation. Rooting took place only when explants were taken from third sub-cultures. NAA was superior to other auxins when used singly or combinedly with other auxins for rooting. Further the percentage of cultures responded to rooting was always higher in shoot tip than nodal explants having one axillary bud.

The results clearly show that both the shoot tips and nodal explants of *R. serpentina* plant are capable of producing multiple shoots *in vitro* and subsequently root to form complete plantlets.

In order to know and compare the content of reserpine in shoots and leaves of *in vitro* grown plants, reserpine content of field grown plants were also studied as a control. It was noted that *in vitro* grown plant parts possessed greater amount of reserpine compared to those in field grown plant parts. Almost 4 times reserpine were present in roots compared to shoot and leaves. Culture media also possessed some amount of reserpine.