DISCUSSION

5.1. Reproductive biology
5.1.1. Phenology
5.1.2. Pollen viability
5.1.3. Stigma receptivity
5.1.4. Mode of pollination
5.1.4.1. Pollen-Pistil interaction
5.1.4.2. Fruit set
5.1.5. Reproductive Success
5.1. Pattern and magnitude of genetic variation
5.1.1. Variability and association of floral and vegetative phenological characteristics
5.1.1.1. Variability
5.2.1.1.1. Analysis of variance
5.2.1.1.2. Performance of floral, leaf and seed characteristics
5.2.1.1.3. Phenotypic and genotypic coefficient of variation
5.2.1.1.4. Estimation of heritability and genetic advance
5.2.1.2. Association of characters
5.2.1.2.1. Correlation coefficient analysis
5.2.2. Variability and association of chemoagronomical characteristics
5.2.2.1. Variability
5.2.2.1.1. Analysis of variance
5.2.2.1.2. Phenotypic and genotypic coefficient of variation
5.2.2.1.3. Estimation of heritability and genetic advance
5.2.2.2. Association of characters
5.2.2.2.1. Correlation coefficient analysis
5.2.2.2.2. Path coefficient analysis
5.3. Genetic divergence (D\textsuperscript{2} analysis)
5.4. Promising selection of populations
5.4.1. Stability analysis
5.4.1.1. Analysis of variance
5.4.2. Stability parameters
Chapter 5
DISCUSSION

*Rauwolfia serpentina* is an important medicinal plant since ancient time. Its roots are traditionally used for treatment of insomnia, insanity, epilepsy, asthma, high blood pressure and snakebite in Ayurvedic system of medicine (Gupta *et al.*, 2005). More than 50 compounds are known in *Rauwolfia* among which the reserpine, racenomin are used for control of high blood pressure, whereas ajamalin and ajamalcin are used for cardiac disease under modern system of medicine (allopathy). *Rauwolfia serpentina* occurs naturally in Himalayan region, Meghalaya, Assam, East U.P., Bihar, H.P., Utrrakhand and Southern India viz. Kerala, Goa and Karnataka. Generally, it is collected from wild and its uncontrolled collection from wild has threatened the survival of the species.

This study was aimed to provide information that would be useful in planning breeding program having as objective the development of *Rauwolfia serpentina* varieties possessing high yield and other chemoagronomic characters. In spite of its importance and utility, very little information is available on its reproductive biology and genetic variation. Present study was therefore, aimed to generate information on reproductive biology and genetic variation of this species. The present study consisted of two sets of experiments. First experiment consisting of 15 populations pertains to study of variation in floral, leaf and seed characters; and different aspects of reproductive biology. In the second set experiments, 25 populations were studied to determine the nature of variation, heritability, genetic advance for yield and yield contributing traits. Inter character association; genetic divergence and genotype x environment interaction were studied. The result of the present study are presented and discussed in the following section.
5.1. Reproductive biology

The experiment was conducted to study the floral characteristics, pollen-pistil interaction, breeding system and variation in floral, leaf and seed characters in a set of 15 populations of *Rauwolfia serpentina*. Towards realizing the objective enumerated in the introduction, a field trial was carried out at TFRI, Jabalpur, MP during January 2007 to December 2008 in 15 populations of *Rauwolfia serpentina* L. originally collected from different ecogeographical regions of India. The seedlings of each of these genotypes were raised in nursery beds and two months old seedlings were transplanted in the field in a Randomized Complete Block Design (RCBD) with three replications at 50 cm x 50 cm spacing during October to November 2006. Five plants in each treatment per replication were selected at random to record observations on 8 morphometric traits of *Rauwolfia serpentina*. The salient findings of this study are summarized below:

5.1.1. Phenology

In *Rauwolfia serpentina*, leaf flushing occurs thrice in a year, that is, during mid February to mid March; mid June to mid July and mid September to mid October. Formation of reproductive buds and flowering is followed little later with leaf flushing. Although each occurrence of flowering overlapped to each other but peak flowering occurs during mid July to mid August. Inflorescences are produced in the terminal part of the twig, each may produce 600-1000 flowers. Most of the plants bloom abundantly and simultaneously; and most of the flowers open at about the same time. Within each inflorescence flowers blooms in 3-6 weeks with an opening rate of 5-25 flower buds per day. Normally, flowering lasts up to 60 days, followed by a fruit maturation period of 105-130 days. Similar results have been reported earlier
also by Ramani (1995) in niger; Sihag and Kaur (1995) in sunflower; Sihag and Priti (1997); Sihag and Wadhwa (2011) in Rauwolfia serpentina. Flowers start opening by 23.00 hour and completely open by nearly 03.30 hour of the following day. Although, anthers dehisce only after sun rise the peak dehiscence was found to occur between 08.0-08.30 hours. However, during rainy days anther dehiscence is delayed by 2-3 hours due to lowering in temperature. This finding is similar to that of earlier finding by Kumari and Pandey (2008) in safflower. The stigma is of wet type and receptive by 06.45-07.00 hours and reaches its peak at 08.30 hours. Fruits develop slowly and they become fully mature in 60-65 days from the day of anthesis. Fruits found hanging in the plant till next flowering season, when they disperse by gravity. Similar observation was earlier recorded by Faegri and Pijl (1979); Sihag and Wadhwa (2011) in Rauwolfia serpentina.

5.1.2. Pollen viability

Pollen viability is considered as an important parameter of pollen quality (Dafni and Firmage, 2000). Pollen longevity is another important factor related to fecundity that might limit seed production (Fritz and Lukaszewski, 1989; Dafni and Firmage, 2000). Dafini et al. (2005) reported that pollen germination success depend on the age of pollen and the humidity to which the pollen grains are exposed prior to the germination test. High pollen viability i.e. > 80% was found in this study and is in agreement with earlier finding by Lopes and Machado (1999) in Rauwolfia grandiflora. Flowers of Rauwolfia serpentina offer visiting insects, pollen as well as nectar as reward. Maximum amount of nectar accumulates in the corolla tube by 09.30 h because of the repeated visits of the pollinators (Faegri and Pijl, 1979). The pollen dimorphism was also observed in this species. Similar finding was found by
Nagrajan et al. (1998) in tamarind. Smaller size of pollen found was possibly due to deficient nutrition.

5.1.3. Stigma receptivity

In many angiosperm species, stigma receptivity last for one to several days. Longer duration of stigma receptivity helps higher pollination success (Nepi and Pacini, 1993; Tangmitcharoen and Owens, 1997; Sornsathapornkul and Owens, 1998; Aleemullah et al., 2000). The results of the present study indicate that stigma receptivity of *Rauwolfia serpentina* lasted for only one day prior to anthesis. At this time stigma is the only functional reproductive parts and the anther remain undehisced. At this time stigma appear light yellowish green in colour, stickier, shiny, fresh, attractive and plum. The anther dehisced next day. At the time of anther dehiscence, stigma loses its receptivity. As a result, pistil usually failed to be pollinated, no matter how high the viability and the density of pollen. Short pistil receptivity act as an adverse factor for seed production in this species. Highest deposition of pollen on stigma was found between 08:45 - 09:15 am. Beyond this time, numbers of pollen were less which shows comparatively less receptivity of stigma. Similar results have been reported earlier by Sihag and Wadhwa (2011) in *Rauwolfia serpentina*.

5.1.4. Mode of pollination

5.1.4.1. Pollen-Pistil interaction

On cross pollination, pollen germinate, pollen tube straightly penetrate the gynostemium (a specialized caplike mass of tissue covering a gynocium) and travel into ovaries. In case of self pollination, germination of pollen was seen on stigma but
penetration showed distortion. Lower to this portion, pollen tubes were found irregular, twisted, very less in number in the mid style, very few in ovary base and often not traceable indicating sporophytic self incompatibility (SI) in the species. A common feature of plant with sporophytic self incompatibility is that inhibition of self pollen is very rapid and occurs at the pollen stigma interface (Newbign et al., 1993).

In this study the pattern of growth of pollen tubes was found similar to that initially seen in a compatible position, but at some stage, growth became irregular, the pollen tube wall became thicker, leading to bursting of the tip. Often, there is large deposition of callose close to the swollen tip. Seed setting in self pollination, indicated that the species is week protogynous. Similar behaviour was noted by Sedgley and Smith (1989) in *Eucalyptus woodwardii*; Sogo and Tobe (2006) in *Casuarinas* and *Alnus spp.*; and Bernardello et al. (2004) in *Sophora fernandeziana*.

5.1.4.2. Fruit set

Low fruit set noticed in *Rauwolfia serpentina* under open pollination is not a rarity; such condition is quite common in many tropical trees (Bawa, 1974). A low fruit set is normally an indication of pollinator limitation (Calvo, 1990). This is severe especially when monoculture is practiced, but can be overcome by introducing captive bee hives (Moncur et al., 1995). Fruit set under bag self pollination was poor (3.9%). Open pollination sets maximum fruits (21%) while 12% of the cross-pollinated flowers set fruits. The results clearly indicate that the species is cross pollinated or rather preferentially cross pollinated. However, seed setting found in bag self pollination indicates the species is week protogynous. Present finding is similar to that of earlier finding by Lopes and Machado (1999) in *Rauwolfia grandiflora* and in contrast with findings of Kaul et al., (2005) in *Withania somnifera*. 
Temporal (dichogamy) separation of pollen availability and stigma receptivity is quite common in species with bisexual flowers. In dichogamous species, protogyny is common. Dichogamy has been traditionally interpreted as a devise to prevent inbreeding. Controlled pollination clearly showed that *Rauwolfia serpentina* is self-incompatible. *Rauwolfia serpentina* is therefore, an example of co-occurrence of protogyny and self-incompatibility which has been reported in a large number of species.

Studies on mating system in species like *Pterocarpus officinalis* (Rivera-Ocasio *et al.*, 2002), *Pterocarpus macrocarpus* (Leingsiri *et al.*, 1998) and *P. indicus* (Changtragoon, 2001) confirm very high outcrossing rates. The breeding system of *Rauwolfia serpentina* is no different from the above species. In present study, high flower, fruit and seed abortions indicate that *Rauwolfia serpentina* is a highly outcrossing species. The values are comparable to teak (Nagrajan *et al.*, 1996). Based on findings of open and control pollination experiments, *Rauwolfia serpentina* is found to be a preferential out crosser. Even though selfed products are generally eliminated through flower abortion, a low proportion does mature into fruits. This behaviour is similar to that of earlier observed by Torres and Galetto (2008) in *Mandevilla pentlandiana* (*Apocynaceae*); Shivanna (2009) in *Adhatoda vasica* Nees. (*Acanthaceae*); Herrera (1991) in *Nerium oleander*; Kumari and Pandey (2008) in safflower.

5.1.5. Reproductive Success

Number of seeds developed in each fruit is generally one (out of four ovules). The number of pollen grains deposited on the stigma is adequate to induce fruit set in all pollinated flowers. There is limited interplant movement of the pollen and the
number of flowers that get cross-pollinated with compatible pollen is less. Thus although *Rauwolfia serpentina* is well adapted to achieve effective pollination, its reproductive success in terms of fruit set is poor due to limitation of compatible pollen. This behaviour is similar to that of earlier observed by Cruden (1977); Cruden and Miller-Ward (1981); Wyatt *et al.* (2000) in milkweeds *Asclepiadaceae*.

5.2. **Pattern and magnitude of genetic variation**

5.2.1. **Variability and character association of floral, leaf and seed characteristics**

5.2.1.1. **Variability**

Genetic variability is a measure of the tendency of individual genotype in a population to vary from one another. Variability of a trait describes how much a trait tends to vary in response to environmental and genetic influences. Genetic variability in a population is important for biodiversity, because without variability, it becomes difficult for a population to adapt to environmental changes and therefore, makes it more prone to extinction. Variability affects an individual’s response to environmental stress and thus can lead to differential survival of organisms within a population due to natural selection of the fit variants. Genetic variability also underlies the differential susceptibility of organism to disease and sensitivity to toxins. Without sufficient genetic variability of the correct types for traits that are of economic interest, an attempt to use genetic principles to improve plant species would be unsuccessful. Therefore, the first thing to do when starting a plant improvement program is to determine the amount, cause and nature of the variance that is present in the species of interest and learn how to use it (Zobel and Talbert, 1984).
5.2.1.1. Analysis of variance

Analysis of variance allows the plant breeder to partition or separate the observed variations into genetic component and environmental component and helps to assess their interactions. It is a statistical technique specially designed to test whether the means of more than two quantitative populations are equal. Basically, it consists of classifying and cross classifying statistical results and testing whether the means of a specified classification differ significantly. Smaller the value of variance, lesser the variability or greater the uniformity in the population.

The analysis of variance was done for 8 floral, leaf and seed characteristics in the populations of 15 different localities. All characters recorded highly significant variation at 1% level of significance at 12 month after planting (MAP) except leaf length, which was significant only at 5% and days to 50% of flowering, indicating that these traits were variable among the 15 populations in *Rauwolfia serpentina*. Moreover, the proportion of variation due to accessions (Populations) to the total variation was considerably higher in all most all the traits.

5.2.1.1.2. Performance of floral, leaf and seed characteristics

The range and mean values recorded among various 15 populations of *Rauwolfia serpentina* with regard to 8 quantitative floral characters viz. days to initiation of flowering, days to 50% flowering, number of flowers per inflorescence, number of fruits per inflorescence, number of inflorescence per plant, leaf length, leaf width and 100-seed weight indicates that these characters were variable among themselves and over the general mean.
Considering the populations, the highest average (305.00) was obtained in case of population A1, followed by A10, both of them are statistically at par for days to initiation of flowering. For the character days to 50% flowering, the highest average (319.80) was obtained in case of population A6, followed by A2, A1, A17, A12, A7, A24, A17, A16, A13, A5, A14, A12, A10 and A7 which being statistically at par. For the character number of flowers per inflorescence, the highest average (999.43) was obtained in case of population A16, followed by A10 and A17, which being statistical at par. For the character number of fruits per inflorescence, the highest average (33.40) was obtained in case of population A23. For the character number of inflorescence per plant, the highest average (7.25) was obtained in case of population A10, followed by A19, A16, A14, A11, A5, A17, A24 and A23 which being statistical at par. For the character leaf length, the highest average (14.47) was obtained in case of population A7, followed by A19, A16, A14, A13, A6, A17, A2, A1, A24 and A23 which being statistical at par. For the character leaf width, the highest average (5.42) was obtained in case of population A19, followed by A16, A13, A2, A1, A24, A23, A10 and A7 which being statistical at par. For the character 100-seed weight, the highest average (5.36) was obtained in case of population A10. This finding is similar to that of earlier findings by Chandra (1956) for number of flowers and fruits per peduncle in *Rauwolfia serpentina*; and Chitra and Rajamiani (2010) in *Gloriosa superba*.

### 5.2.1.1.3. Phenotypic and genotypic coefficients of variation

The best gains are made for characters having a wide range of variability and are strongly under genetic control (Lacaze, 1978). Plant populations are genetically variable and they must be so to survive, grow and reproduce under the disparate
environments that are encountered during generation and over generations (Antonovics, 1971; Eldridge et al., 1993). However, phenotypic variation alone cannot be taken with cognizance, since the influence of environment is also included in that parameter (Allard, 1960). Hence, relatively better and reliable parameter namely GCV which reflects the magnitude of variability for genetic component in the total variability shall be dependable for making selection.

The relative amount of variation expressed by different characters can be properly judged through estimates of phenotypic and genotypic coefficient of variation (Chawhaan et al., 2003). Comparatively higher magnitude of percent coefficient of variability both at phenotypic and genotypic level indicated the presence of high amount of variability. In the present study, for many floral, leaf and seed characters viz. number of fruits per inflorescence followed by number of inflorescence per plant, number of flowers per inflorescence and leaf width studied, high coefficient of variance at phenotypic and genotypic levels were observed. However, the magnitude of phenotypic coefficient of variance, as expected is markedly higher than the genotypic coefficient of variance for all the traits but the difference between these two was almost negligible in case of days to flower and 100-seed weight which indicated the less influence of environment upon these traits. In most of the other traits genotypic coefficient of variance accounted for almost fifty percent of the phenotypic variance coefficients suggesting that these traits are moderately influenced, while other two traits namely days to fifty percent flowering and leaf width are more sensitive to the environmental variations. A number of various studies including Bhagat et al. (1980) in Rauwolphia serpentina; Vashitha et al. (2006) in A. glauca and A. archangelica; Brezinova et al. (2009) in Papaver somniferum are in agreement with these findings.
5.2.1.1.4. Estimation of heritability and genetic advance

The proportion of total variation which is heritable is termed as ‘broad sense of heritability’ (Lush, 1937). Although, narrow sense heritability is more meaningfull, heritability in broad sense may give useful indication about the relative value of selection (Ginwal and Gera, 2000; Chawhaan et al., 2003). However, broad sense heritability represents the upper limit that can be achieved through selection, such estimates when used judiciously, in conjunction with genetic gain is more useful than the heritability alone in predicting the resultant effect for selecting the best genotype (Chawhaan et al., 2003) Heritability has an important place in plant breeding as it provides an index of the relative role of heredity and environment in the expression of various traits (Kaushik et al., 2007; Das et al., 2011).

In the present study, heritability values and genetic advance as percentage of mean respectively recorded high for the traits viz. 100-seed weight, followed by days to initiation of flowering, number of fruits per inflorescence suggesting that environment has comparatively low influence for these traits and the potentially of these traits may be used for future improvement through selection (Table 17).

5.2.1.2. Association of characters

5.2.1.2.1. Correlation coefficient analysis

Correlation of two characters may be either due to similar cause or to similar response to environmental influence. The two components of correlated response may be separated statistically. If the genetic correlation is high, attempts to obtain a positive response in one character by selecting for an associated character shall be highly effective, feasible and profitable. An attempt was made in the present
investigation to study the inter character association at both phenotypic and genotypic level relating to floral, leaf and seed characteristics at 12 months after planting.

It was found that days to initiation of flowering showed significant positive correlation with days to 50% flowering, followed by 100-seed weight, and negative with number of inflorescence per plant, leaf length. Days to 50% flowering showed significant negative correlation with number of inflorescence per plant. Number of fruits per inflorescence showed significant positive correlation with leaf length and negative with 100-seed weight. Number of inflorescence per plant showed significant negative correlation with leaf length. Leaf length showed significant positive correlation with leaf width and negative association with 100 seed weight and indicating that these traits are genetically associated (Table 18). These findings are similar to that of earlier findings by Shivanna et al. (2007) in Solanum nigrum; Kumar et al. (2008) in Chlorophytum borivilianum; and Dalkani et al., (2011) in Carum copticum.

Second experiment was formulated to study the variability, heritability, genetic advance, inter character correlations, path analysis, $D^2$ analysis and stability analysis. Towards realizing the objective enumerated in the introduction, multilocation field trials comprising of 25 populations of Rauwolfia serpentina were carried out at three different locations viz. Chandrapur M.S., Raigarh C.G. and Jabalpur M.P. during March 2008 to December 2009. Rauwolfia serpentina populations were collected from different ecogeographical regions of India representing the wide genetic variation available within the species. The seedlings of each of these genotypes were raised in nursery beds and two months old seedlings were transplanted in to the field in a Randomized Complete Block Design (RCBD)
with three replications at 50 cm x 50 cm spacing during July to August 2008. Five plants in each treatment per replication were selected at random to record observations on 11 chemoagronomic traits of *Rauwolfia serpentina*. The salient findings of this study are discussed below:

5.2.2. Variability and association of chemoagronomical characteristics

5.2.2.1. Variability

Genetic variability is the most essential requirement for any successful crop improvement program. The ultimate goal of plant breeders is to increase the yield potential of a crop. In this study, an attempt has been made to identify various chemoagronomic traits influencing reserpine content in *Rauwolfia serpentina*. This information will be useful in deciding suitable selection criteria to be practiced for genetic improvement of reserpine content.

5.2.2.1.1. Analysis of variance

The analysis of variance was done for 11 chemo-agronomical characters in the populations of 25 different localities. All characters recorded significant variation at 5% level of significance at 18 month after planting (MAP), indicating that these traits are highly variable among the 25 populations in *Rauwolfia serpentina*. The differences between grand mean (over all environments) and mean performance in each of the three environments for the eleven studied traits recorded a wide range and displayed a good distribution within the range. Consequently, the required assumptions for stability analysis are full-filled.

Plant height ranged from 10.25 cm to 53.60 cm in Chandrapur and Jabalpur, respectively. Stem diameter was minimum (0.29 cm) in Chandrapur and maximum
(0.71 cm) in Raigarh. Number of primary stem branch ranged from 1(one) to 3.5 in Raigarh. Number of nodes on main stem varied from 11 to 30 in Raigarh and Chandrapur respectively. Total biomass of plant was found minimum (12.67 g) in Jabalpur and maximum (80.83 g) in Raigarh. Root length ranged from 23.44 cm to 43.17 cm in Chandrapur and Raigarh. Root Diameter ranged from 0.57 cm in Jabalpur to 1.04 cm in Chandrapur. Number of primary root branch varied from 1.33 in Chandrapur to 4.33 in Raigarh. Root yield per plant ranged from 6.33 g to 53.50 g in Jabalpur and Raigarh, respectively. Total alkaloid content in root ranged from 1.11% in Chandrapur to 5.13% in Raigarh. Minimum (0.004%) and maximum (0.122%) reserpine content in root was found in Chandrapur. Present finding is similar to that of earlier findings by Wakhloo et al. (1963); Dhar (1965); Vardarajan (1968); Bhagat et al. (1980); Sethi et al. (1991); Ahmed et al. (2002); Gupta et al. (2005); Kumar et al. (2010) in Rauwolfia serpentina.

5.2.2.1.2. Phenotypic and genotypic coefficient of variation

In the present study, high coefficients of variance at phenotypic and genotypic levels were observed for most of the traits studied. For Chandrapur, maximum phenotypic and genotypic coefficients of variance was recorded for number of primary stem branch, followed by reserpine content, number of primary root branch, plant height, root yield and total alkaloid content. For Raigarh, high phenotypic and genotypic coefficients of variance was recorded for total alkaloid content, followed by number of primary stem branch, reserpine content, number of primary root branch, root yield and total biomass. For Jabalpur, high phenotypic and genotypic coefficients of variance was recorded for number of primary stem branch followed by number of primary root branch, reserpine content, root yield and total alkaloid content. For pooled data of three locations, high coefficient of variation were observed for number
of primary stem branch, reserpine content in root, number of primary root branch, total alkaloid content in root, root yield per plant, total biomass and plant height, which indicated that the influence of environmental upon these traits. High estimates of variation for reserpine content and its components indicated a better scope for selection of desirable combination from segregating populations in successive generations. A number of various reports including Bhagat et al. (1980) in Rauwolfia serpentina; Vashitha et al. (2006) in A. glauca and A. archangelica; Brezinova et al. (2009) in Papaver somniferum; Bhargava et al. (2009) in Chenopodium spp. recorded similar findings.

5.2.2.1.3. Estimation of heritability and genetic advance

Selection can be successful only when the variability in the population is due to genetic causes. Estimation of genetic parameters is therefore a very useful tool in predicting the amount of gain envisaged from clonal and progeny material. The variation among progenies and clones is commonly used as an estimate of total genetic variation and to calculate the degree of genetic control for a particular trait. In the present study, fairly high heritability values and genetic advance as percentage of mean were recorded for most of the traits. High heritability and genetic advance value for a particular trait in one location is also similar in other location although the magnitude of the value differ (Table 21-23). When analysis was done to estimate heritability and genetic advance using pooled data over location, different picture emerged. The value are not high but moderate to low for number of primary stem branch, reserpine content in root, number of primary root branch, total alkaloid content in root, root yield per plant, total biomass and plant height (Table 24). This is utterly obvious as the heritability is a property of the population and the environment under which it grows. Therefore, selection to improve these traits can be practiced for
utilization within the specific location. It is pertinent to point out that the accessions were grown under control irrigated conditions and under more of less same edaphic and weather conditions, hence it is expected that the genotypes expressed their true genetic potential. The genetic constitution of a population of plant reflects the environmental condition under which plants are evolved (Campbell, 1979). The heritability values of a given characteristic in a population often changes but such changes perhaps occur in a predictable fashion as the plantations grow and develop (Namkoog et al. (1972); Namkoong and Conkle (1976); Franklin (1979); Ginwal et al. (2004).

5.2.2.2. Association of characters

5.2.2.2.1. Correlation coefficient analysis

Yield is a complex entity influenced by many components and in many cases these component characters are themselves interrelated. Knowledge of such interrelationship of various yield components is very essential to understand the importance of each factor involved. Correlated quantitative traits are of major interest in an improvement program as the improvement of one character may cause simultaneous changes in other character (Divakara et al., 2009).

An attempt was made in the present investigation to study the inter character association at both phenotypic and genotypic level relating to morphological traits at 18 months after planting. In the present study total alkaloid content exhibited significant positive correlation with reserpine content at phenotypic level in all three locations. Similar values of correlation between the traits at genotypic level were also observed. When correlation values were estimated using pooled data over location,
similar relationship was noticed. The results of this study clearly indicate that not only these traits are related at genotypic level but environmental influence is also use in the development of these characters. Therefore, selection of genotypes for higher alkaloid content will bring about improvement in reserpine content, the trait for which *Rauwolfia serpentina* is valued. This findings is similar to that of earlier findings by Biswas, 1970 in *Rauwolfia serpentina*; Shivanna *et al*. (2007) in *Solanum nigrum*; Singh *et al*. (2008) in *Chlorophytum borivilianum*; Das *et al*. (2011) in *Withania somnifera*; and Dalkani *et al*. (2011) in *Carum copticum*.

5.2.2.2. Path coefficient analysis

The correlation measures the relationship between dependent and independent characters. But such relationship does not provide exact picture of how much a character contributes on its own and through other character on the dependent trait. In this context, path analysis has been found to be an important tool for apportioning the genotypic correlation coefficient into direct and indirect effect of independent variables on the dependent variable (Wright, 1921).

If the correlation between dependent and independent characters is due to the direct effect of the character it reflects the true relationship between them and thus selection can be practiced for such character in order to improve the dependent trait. If the correlation is due to indirect effect through another component trait, then the selection has to be done for the later trait. Hence, a change in the character alerts the relationship with another character and finally reflects on the dependent characters. Path coefficient analysis has been used for understanding the complex traits in breeding program by a number of workers including Dewey and Lu (1959), Frakes *et al*. (1961), Ramanujam and Rai (1963).
In the present study direct effect of total alkaloid content on reserpine content was more in the studied locations viz. Chandrapur, Raigarh and Jabalpur. To be more precise its direct effect was maximum in Chandrapur, Raigarh and forth in case of Jabalpur at genotypic level. Though, traits like plant height, number of primary branches, root length and root diameter exerted more direct effect towards reserpine content in one or the other locations their contribution was not consistent over all the location, and even showed negative effects in some locations. Pooled analysis of data over the locations also emerged out to be similar. None of the other characters had uniform direct effect over locations on the dependent character. Thus, path analysis clearly indicate that this trait can be considered to be reliable for selection as its direct effect on reserpine content in root was maximum, followed by number of primary root branches, and also contributed more indirect effect through all other characters. Moreover these effects are of paramount importance as they are existing at genotypic level. The residual effect was found to be 0.56 for pooled data of locations. It indicates that 44% of variation on yield components was determined by the characters taken for the present study as also reported earlier by Shivanna et al., (2007) in Solanum nigram.

5.3. Genetic divergence ($D^2$) analysis

Genetic diversity in the crop species is a gift of nature. With the advent of scientific progress over the past decades, the botanists and plant breeders have been increasingly cognizant of the importance of genetic diversity in crop plants. An insight into the extent and magnitude of variability present in the crop species is of utmost importance as it forms the basis of selection and thus enables successful execution of any effective crop improvement programm. The quantitative estimation
would indicate the potentiality of the germplasm, from where the selection of desirable types with desirable traits could be done for maximizing the yield potential.

Genetic diversity is the foundation of ecosystem stability and forest sustainability because genetic diversity provides raw material for evolution, survival and adaptation of the species, especially under changed environmental conditions. Genetic diversity needs to be assessed in long term genetic resource collections in breeding populations, in seed orchards or planting materials producing populations and in production populations (Muona 1990). For ecologically and socially sustainable forestry, monitoring genetic diversity in forest species is also important. The exploitation of heterosis and success in getting desirable segregantes in a breeding program, largely depend on the degree of divergence in a chosen population. The genetically diverse parents should possess consistency of genetic divergence under different environments. The more diverse the parents the more are the chances of pronounced heterotic effects and increased spectrum of variability in the segregating generations.

The $D^2$ statistic, which is based on several characters, is one of the powerful tools to assess the relative contribution of different component traits to the total diversity to quantify the degree of divergence between populations to understand the trend of evolution and choose genetically diverse parents for obtaining desirable recombination (Kaushik et al., 2007).

In the present study the application of $D^2$ clustering technique was used to resolve different population in different clusters, cluster mean performance of each trait and its contribution to diversity for each location individually and pooled for all locations.
5.3.1. Chandrapur location

Twenty five populations tested in Chandrapur resolved in to 5 clusters. Among the 5 clusters, cluster I had 21 populations, remaining clusters were having one population each. Maximum intra-cluster distance (69.12) was recorded in cluster I. With regard to inter-cluster distance, cluster III recorded maximum distance from cluster V (635.03) followed by cluster IV (230.54). Cluster III had high mean value for root length (36.67) and for total alkaloid content in root (3.10). The contribution of individual characters to the diversity was worked out in terms of number of times it appeared first. Reserpine content contributed maximum (42.0) to genetic divergence followed by Plant Height (26.67).

5.3.2. Raigarh location

Twenty five populations were identified in to 6 clusters, of which cluster I had 17 populations, followed by cluster II with 4 populations. Remaining clusters had 1 population each. Cluster I recorded maximum intra cluster distance (81.50). Cluster V recorded maximum inter-cluster distance from cluster VI (471.38). Cluster V had marginally high mean value for total alkaloid content in root (5.13). The trait contributing maximum to genetic diversity was total alkaloid content in root (32.00) followed by reserpine content (30.0) and total biomass (18.0).

5.3.3. Jabalpur location

There were 25 populations resolved in to 10 clusters in Jabalpur location with 12 populations in cluster I, followed by cluster II with 5 populations. Remaining cluster had 1 population each. Cluster II recorded maximum intra cluster distance (30.45) followed by cluster I while maximum inter-cluster distance was recorded
between cluster VIII and IX (171.20), followed by cluster IX with X (156.53). Cluster II recorded high mean value for total alkaloid content in root (3.99). The trait contributed maximum genetic diversity was reserpine content (36.67), followed by plant height (19.67) and total alkaloid content in root (11.00).

5.3.4. Pooled analysis over location

Analysis of pooled data over three locations categorized 25 populations into 10 clusters. Cluster I had 11 populations, followed by cluster II with 4 populations and cluster V with 3 populations. Remaining cluster had with one population each. As far the intra cluster distance was concerned, the cluster V recorded maximum intra cluster distance (12.34). With regard to inter cluster distance, cluster V recorded maximum genetic distance from cluster VII (37.56) followed by that of from cluster IX (31.43). As far as the cluster means were concerned, cluster IX had high mean value for total alkaloid content in root (3.37). The trait contributing maximum genetic diversity was root diameter (21.00), followed by reserpine content (16.0).

The cluster composition revealed that the genetic diversity was not related to geographic diversity as the populations of different geographical region fall in the same cluster. In the present study divergence analysis of all three individual locations as well as that of pooled data revealed that the cluster I include majority of the populations of different geographical region. Thus, the populations in cluster I were most heterogeneous and this cluster was best for within group hybridization in breeding programs.

In the present study cluster III showed minimum inter cluster distance of 51.27, 66.36 and 30.90 with clusters II at Chandrapur, cluster IV at Raigarh and
Jabalpur locations respectively. On the basis of pooled analysis also minimum intercluster distance was recorded between cluster III and VI (7.27). Though the cluster membership of these clusters was different at individual location, the populations included were originated mostly from more wet and humid climate. These results indicated that populations in these clusters are closely related. Hence, selection of parents from these two clusters may be avoided. This kind of genetic diversity might be due to differential adaptation, selection criterion, selection pressure and environment (Vivekananda and Subramanian, 1993). However, it has been suggested that three important points are to be considered while selecting genotypes for a breeding programme (i) choice of a particular cluster from which a genotype is to be selected as parent, (ii) selection of a particular genotype from a particular cluster and (iii) relative contribution of characters to the total divergence (Singh and Chaudhary, 1996). It is suggested that the genotypes of clusters that are having more inter cluster distance and high mean values can be crossed to get desirable transgressive segregantes. In present study, cluster V recorded maximum inter-cluster distance from cluster III and VI in Chandrapur and Raigarh locations respectively, whereas clusters VIII and IX were most divergent in Jabalpur. Interestingly these clusters also boasted higher mean values particularly for reserpine content which is the most important trait. This indicates that wide diversity exists between the populations in these groups and selection of parents from such clusters for hybridization program would help to achieve heterotic hybrids. Thus, the genotypes of this cluster can be preferred in crossing programm. These findings were more or less in line with the result obtained by Kumar et al. (2007) in Withania somnifera; and Kumar et al. (2008) in Chlorophytum borivilianum.
5.4. Selection of promising populations

5.4.1. Stability analysis

Information about phenotypic stability is useful for the selection of plant population as well as for breeding programs. The phenotypic performance of a genotype is not necessarily the same under diverse agro-ecological conditions (Ali et al., 2003). Some genotypes may perform well in certain environments, but, fail in several others. Genotype-environment (G x E) interactions are extremely important in the development and evaluation of plant varieties because they reduce the genotypic stability values under diverse environments (Hebert et al., 1995).

Breeder looks for the greater variability in plant with an aim of evolving varieties which would give the maximum consistent economic yield. It is observed that G x E interaction is widely present and contributes substantial to the non realization of expected gain from the selection (Comstock and Moll, 1960). The population which can adjust its genotypic or phenotypic state in response to environmental fluctuation in such a way that it gives high and stable economic return can be termed as “well buffered” (Allard and Bradshaw, 1964). A good method to measure stability was previously proposed (Finlay and Wilkinson, 1963) and was later improved (Eberhart and Russell, 1966). The stability of varieties was defined by high mean yield and regression coefficient ($b_i = 1.0$) and deviations from regression as small as possible ($s^2_{di} = 0$). The stability was defined as adaptation of varieties to unpredictable and transient environmental conditions and the technique has been used to select stable genotypes unaffected by environmental changes (Allard and Bradshaw, 1964). Here, it was emphasized that both linear ($b_i$) and non-linear ($s^2_{di}$) components of genotype-environment interactions are necessary for judging the
stability of a population (Eberhart and Russell, 1966). The objectives of this study were to evaluate the yield and its attributing traits of promising *Rauwolfia serpentina* populations in different environments and to determine their stabilities using stability parameters.

**5.4.1.1. Analysis of variance**

Significant differences among genotypes and environments were found for all studied traits except stem diameter, root length, number of primary root branch and total alkaloid content (%) in root. This reveals not only the amount of variability that existed among environments but also the presence of genetic variability among the populations. Significant genotypes x environments (Linear) interaction was detected for plant height, number of primary stem branch, number of nodes on main stem, total biomass and root yield per plant. This revealed that genotypes responded differently to the different environmental conditions for these traits. This finding suggested the importance of assessment of genotypes under different environments to identify the best genetic makeup for a particular environment.

**5.4.2. Stability parameters**

Yield is the end product of the number of component traits. Stability in yield, therefore were dependent on stability of morphological attributes.

**5.4.2.1. Plant height**

For plant height population 19 was the most stable whereas population 9 possessed fair stability. In contrast, population 24 regarded as sensitive to environmental changes and can be recommended for cultivation under favorable
conditions. Populations 23 and 25 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.2. Stem diameter

In case of stem diameter, population 18 and 21 were the most stable whereas population 16 possessed fair stability. In contrast, population 3 was regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Populations 2 followed by 23 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.3. Number of primary stem branch

Populations 13 followed by 6 were the most stable for number of primary stem branch whereas population 18 followed by 11 possessed fair stability. In contrast, population 2 followed by 3, 14 and 16 were as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Populations 15 followed by 12 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.4. Number of nodes on main stem

Populations 4 followed by 7, 10 and 20 were the most stable for number of nodes on main stem whereas populations 9 followed by 17 possessed fair stability. In contrast, populations 2 followed by 3, 14 and 16 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Populations 5 followed by 11 and 15 populations were insensitive to environmental changes and have adapted to the poor environments.
5.4.2.5.  **Total biomass**

For total biomass, populations 1 followed by 6 and 23 were the most stable whereas populations 2 followed by 3 possessed fair stability. In contrast, populations 5 followed by 21 and 19 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Population 11 followed by 22 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.6.  **Root length**

In case of root length, population 15 followed by 21 for root length possessed fair stability. In contrast, population 12 followed by 20 for root length were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Population 14 followed by 16 populations were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.7.  **Root diameter**

Population 10, followed by 25 were the most stable for root diameter whereas population 2 followed by 20 possessed fair stability. In contrast, population 3 followed by 14 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Populations 5 and 17 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.8.  **Number of primary root branch**

The population 22 for number of primary root possessed fair stability. In contrast, population 2 followed by 11, 14, 19 and 22 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable
conditions. Population 18 was insensitive to environmental changes and have adapted to the poor environments.

5.4.2.9. Root yield

Population 2 followed by 21 were the most stable for root yield whereas population 3 followed by 10, 11, 18, 20 and 23 possessed fair stability. In contrast, population 1 followed by 3, 5 and 19 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Population 22 was insensitive to environmental changes and have adapted to the poor environments.

5.4.2.10. Total alkaloid content

The population 8 followed by 13 for total alkaloid content were fairly stable. In contrast, population 7 followed by 17 were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Population 22 followed by 25 were insensitive to environmental changes and have adapted to the poor environments.

5.4.2.11. Reserpine content

Population 17 followed by 19 for reserpine content were regarded as sensitive to environmental changes and can be recommended for cultivation under favorable conditions. Populations 25 and 22 were insensitive to environmental changes and have adapted to the poor environments.

These results showed that the populations affected differently by different environmental changes and thus their character expressions responded differently too. These findings were more or less in line with the result obtained by Kaicker et al. (1978); Ottai et al. (2006); Dražić et al. (2007).
In last, it is concluded that populations 25 and 22, having higher yielding ability for production of reserpine content (%) and were most suitable for cultivation in wide range of environmental conditions whereas, populations 17 and 19, having also high yielding ability and were most suitable for cultivation in favorable environmental conditions.