Trees are recognized as the critical elements in maintaining sustainable environment for the existence of living beings and in preserving the ecosystem and biodiversity. However, over the years, forests in India have suffered serious depletion. This attributes to relentless pressures arising from ever-increasing demand for fuel-wood, fodder and timber; inadequacy of protection measures, diversion of forest lands to non-forest uses and the tendency to look upon forests as revenue earning resource. The low productivity of forest coupled with ever-increasing demand for wood due to India’s huge and increasing population contributes to the degradation of forest (Gulati and Sharma, 2000). The productivity of forests is also affected by the diseases and insect pests.

Ironically, it may not be feasible to increase forest cover with limited resources, and possible option to bridge the gap is to enhance the productivity through planting genetically improved planting stocks to maximize the returns per unit area (Kumar et al., 2002). There is a worldwide awakening interest and support for research regarding the maintenance, genetic improvement and efficient use of forest resources and products. Therefore, concerted efforts are required to develop package of technology for mass clonal propagation of genetically superior trees as they are always limited in number and their genetic traits can best be maintained through clonal propagation (Barghchi, 1987; Kapoor and Chauhan, 1992). In this context, tissue culture (micro propagation) can play an important role in supply of improved planting stock on mass scale for raising plantations and in boosting productivity. Tissue culture may be defined as the aseptic culture of cells, tissues, organs or whole plants under controlled nutritional and environmental conditions (Thorpe, 2007).

Apart from mass multiplication of elite plants, tissue culture provides the means to multiply and regenerate plants from genetically engineered cells. The promising plants thus produced may be readily cloned in cultures on mass scale (Kapoor and Chauhan, 1992) under aseptic conditions and such plants if produced using meristem as explants will be disease and virus free. In vitro regeneration of plants is a prerequisite for genetic improvement/engineering and mass multiplication.
1.1 General Description of *Dalbergia sissoo* Roxb.

*Dalbergia sissoo* is an erect deciduous tree which is characteristic species of Khair-sissoo (*Acacia catechu-Dalbergia sissoo*) primary seral type dry forest (Champion and Seth, 1968). It is also called Sissoo, Sisu, Shisham, Tahli and Indian Rosewood and is one of the most important timber yielding species of India, Nepal, Pakistan and Bangladesh. It is a major genus of the woody legumes and belongs to the family Fabaceae (old Leguminosae), order Fabales (Shakya and Lakhey, 2007). It is native to the northern part of Indian subcontinent and extends up to Attock in the Indus valley of Pakistan to its western limits of distribution, but does not dominate over any appreciable area (Champion *et al*., 1965). It is naturally distributed in Indus and Ganges river valleys and plains.

In India, it is found in Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Uttarakhand, Bihar, Orissa, Sikkim, Arunachal Pradesh and Assam. In plantation it is being grown in Manipur, Nagaland, Mizoram, Meghalaya, Tripura, Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Nagaland, Tamilnadu, Karnataka, Kerala, Delhi and Rajasthan (Sharma *et al*., 2000). Shisham is found in many parts of India up to 900 m amsl in the sub Himalayan tract and occasionally ascending to 1500 m between latitude 21.17° N to 32.60° N and longitude 74.80° E to 93.43° E. In the sub Himalayan tract, it occurs along rivers and streams, gregariously growing on alluvial soil (Troup, 1921).

It is a medium sized deciduous tree, generally height goes up to 30 m, girth between 2-4 m and characteristically described with a crooked stem. Under favorable conditions, it attains a height of about 40 m and clear bole up to 15 m. It has a long thick tap and ramifying lateral roots. Both tap and lateral roots are commonly covered with nodules in soil inoculated with nitrogen fixing *Rhizobium* sp. bacteria (Troup, 1921). *D. sissoo* trees from different localities have varied characteristics including growth, form, colour, grain, working and strength properties (Sharma *et al*., 2000).

The prominent features that are the characteristic to *D. sissoo* are:

- Propagation is primarily by seeds and suckers.
- Crooked stem, forking and ramicorn branching (one dominant fork partly suppress the other).
• Crooked stem form has high heritability 42 - 46 % (Vidakovic and Ashan, 1970).
• Considerable geographical variation exists among provenances (Suwal et al., 1988).

Shisham is best known internationally as a premier timber species of the rosewood genus, but is also utilized as an important fuel wood, fodder and for shade and shelter. *D. sissoo* is valued for its decorative and often fragrant wood, rich in aromatic oils, multiple products, tolerance of light, frosts and long dry seasons, this species deserves great consideration for tree farming, reforestation and agroforestry applications (Chaturvedi, 2001).

Shisham has been inflicted by a number of diseases, of which some are destructive or lethal. The destructive diseases include root and foliage diseases and diseases caused by phanerogamic parasites and physiological disorders due to water logging and poor drainage etc. (Bagchee, 1952; Bakshi, 1954; Khan et al., 1956; Khan, 1960; Khan and Bokhari, 1972; Yousuf, 2002). Bakshi et al. (1957), during a survey of diseases in *D. sissoo* forests, observed that the wilt and other root diseases are absent in the riverbeds, where *D. sissoo* grows naturally. In some natural forests situated away from the river and also certain plantations, it suffers from root diseases due to *Fusarium solani*, *Ganoderma lucidum* and *Phellinus gilvus* in varying degrees.

1.2 Fusarial wilt

*Fusarium solani* is a facultative parasite inhabiting soil and possesses wide saprophytic colonization ability (Bakshi, 1955). The genus *Fusarium* belongs to the family Tuberculariaceae, order Moniliales (Hyphomycetes) includes a large number of species and many forms within species. Mycelium is extensive and the hyphae are septate and branched. When young, they may be colorless or with a tinge of pink, purple or yellow and become dark colored at maturity. *F. solani* is mainly associated with wilt diseases. Wilting can be caused either by reduction in uptake and transport of water, by mechanical obstruction of the xylem tracts or by increased water loss due to an increase in permeability of cell membranes or to an impairment of stomatal function. Low molecular weight toxins, such as lycomarasmin and fusaric acid, produced by the pathogen and also high molecular weight metabolites and degradation products are also responsible for the wilt in plants. *F. solani* causing
wilt is considered main cause of *Dalbergia sissoo* or shisham mortality (Bakshi, 1954; Kaushik and Singh, 1996).

Shisham mortality causes high economic concern in Indian sub-continent where mature standing trees are dying. The causal agent has been identified as *F. solani* (Mart.) Apple & Wr f.sp. *dalbergiae* (Bakshi, 1954). Bakshi et al. (1954 and 1957) studied the disease in detail with relation to the type of soil and soil mycoflora; and pathogenicity trials conducted on shisham seedlings have confirmed that *F. solani* caused wilting and death of the plants.

**1.2.1 Symptoms of wilt disease**

Wilt disease is systemic in nature which mainly affects the roots and stem. The yellowing and drooping of leaves followed by drying in acropetal succession are the early symptoms. Eventually the entire tree presents a yellow appearance. The leaves are shed rendering the branches bare. The affected trees die within a few months. The bark of infected trees cracks at places and cankers are formed, oozing dark brown to black pitch. The pathogen was isolated from the oozing pitch (Shukla, 2003). Root examination reveals development of pink to reddish stain at the centre, whereas light pale yellowish or greenish white in the roots of healthy plants (Bakshi, 1957; Kumar et al., 2004).

**1.2.2 Possible causal factors**

Wilt on shisham occurs in trees of all age groups but mature trees are highly affected in shisham plantations. Though younger plantations of shisham show high mortality in some areas, particularly where biotic stresses, erratic rainfall and extreme temperature variations/water-logging have occurred during recent years. The Fusarial wilt of *Dalbergia sissoo* is a vascular wilt disease correlated with soil texture and soil moisture. Clayey or stiff soil with water-logged conditions favours the development of the disease. The poor aeration in such soil causes asphyxiation and death of roots. The pathogen produces both macro and microconidia besides terminal and intercalary chlamydospores and attack the tree through drying and weakened roots. The disease is absent in soil with low silt, provided the drainage is good and water logging condition do not exist (Bakshi, 1957). High moisture content associated with heavy soils in the root zone favour the wilt disease in shisham trees. The wilt pathogen has been found associated with dead roots of wilted trees. The fungus grows over a wide range of pH and its growth is optimum at 4.6 pH (Bakshi et al., 1957). The pH in the root zone of
*D. sissoo* lies between 4.6 - 5.2 which is ideal for infection by the pathogen (Bakshi and Singh, 1959).

### 1.3 Ganoderma root rot

Another pathogen causing root rot in shisham is *Ganoderma lucidum* (Leyss.) Karst. The pathogen is a root inhabiting fungus and infects the roots through root contact. It causes white spongy rot in the sapwood. The affected tree exhibits a stag-headed appearance and gradually dies as the root system is decayed. Lateral spread of the pathogen is through root to root contact. The fungus has been reported to be associated with shisham mortality in India (Sharma *et al.*, 2000; Verma and Gupta, 2000; Harsh *et al.*, 2011) and Nepal (Thapa, 1990). The disease can be managed through silvicultural and management practices including removal of stumps and roots from the planting area as this reduces the fungal inoculum and isolation trenching to isolate infected trees from healthy ones to check the spread of the disease through root to root contact.

### 1.4 Phellinus root and butt rot

*Phellinus gilvus* (Schw.) Pat. causes root and butt rot in shisham. It is primarily a wound parasite and occurs in association with *G. lucidum* or *F. solani*. Trees of advanced stages may be infected and they exhibit a stag head appearance. The fungus causes limited decay as white rot in the sapwood and also to an extent in the heartwood. Sporophores of the fungus are developed on root and stem bases. They are annual, sessile, usually reflexed, rarely effuse reflexed, leathery when fresh, drying hard, single or imbricate; upper surface with shades of yellow, brown and red and either coarsely hairy, sub-zonate in forma gilvoides or smooth with concentric zonations in forma licnoides; lower surface yellowish brown, pores round, dissepiments thick, margin sterile (Bakshi, 1971 and 1976).

### 1.5 Dalbergia sissoo (shisham) mortality

*D. sissoo* has been inflicted with two severely damaging diseases viz., wilt and die back. Bagchee (1945) studied wilt and die-back disease of Shisham, Khair and Babul. Shisham mortality has been known from India since 1954 (Bakshi, 1954; Bakshi and Singh, 1954). In last decade, mortality of *D. sissoo* has been reported from different geographic regions of the country which have diverse edaphic and climatic conditions and the factors behind it have been attributed as environmental, hydrological and pathological. The problem of mass mortality of *D. sissoo* has
become a cause of concern in recent 20 years resulting in death of millions of trees ranging from 10-80 % in different areas (Chaturvedi et al., 2002; Kumar and Rai, 2002; Shukla, 2003; Harsh et al., 2011).

In India, the large scale mortality has been primarily observed in Bihar and Uttar Pradesh; however, other affected states include Jammu and Kashmir, Himachal Pradesh, Haryana, Punjab, Delhi, Uttarakhand and West Bengal (Sharma et al., 2000; Verma and Gupta, 2000; Kaushal et al., 2001; Dubey and Mishra, 2002; Gupta, 2002; Solanki, 2002; Dayaram et al., 2003; Shukla, 2003; Kumar et al., 2004). The similar problem has been also reported from other Asian countries like Pakistan, Nepal, Bhutan, Bangladesh and Myanmar (Khan and Bokhari, 1970; Thapa 1990; Shakir et al., 1999; Baksha and Basak, 2000; Joshi and Baral, 2000; Mandhar and Shrestha, 2000; Bajwa et al., 2003; Khan et al., 2004; Hassan, 2005; Webb and Hossain, 2005).

1.6 In vitro testing for disease resistance

Selection for resistance in in vitro conditions must be considered as one of the methods which, in combination with conventional resistance screening and plant breeding methods including biotechnological procedures, may offer plant breeders a new approach to accelerate the development of disease resistant plants (Crino, 1997). Considering the large scale economic loss caused by wilt pathogen (*Fusarium solani f.sp. dalbergiae*) in shisham plantations, it is imperative to find out reason as well as mechanism of disease resistance in shisham using in vitro approaches and subsequently developing resistant or tolerant plants of shisham. Traditional methods of breeding for resistance to pathogen are comprised of screening for genetic variability, searching for natural source of resistance, pathogenicity testing and complemented with inheritance studies. New trends in resistance breeding use a combination of tissue and cell cultures with in vitro selections and classical breeding methods. Somaclonal variability, susceptibility tolerance for resistance to pathogens in plants, offers new possible sources of altered characters. In vitro screening of plants with such features can be exploited on large scale both at cellular level (callus) and at complete plantlet level. The use of tissue culture technique to develop disease resistant planting material would be an attractive alternative. No such studies on shisham mortality have been reported so far.
Aim and scope

An attempt has been made here in *Dalbergia sissoo* for resistance against wilt pathogen *Fusarium solani* f.sp. *dalbergiae* with the following objective:

- To test *in vitro* grown plantlets and callus tissue of *Dalbergia sissoo* for resistance against shisham wilt pathogen *Fusarium solani* f.sp. *dalbergiae*. 