5.0 DISCUSSION

After the recommendation by the World Health Organization in 2002, the global demand for artemisinin-based combination therapies (ACTs) has grown sharply, because it is safe and effective against both chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium* sp. It is also useful in cerebral malaria treatment (Luo and Shen, 1987; Klayman, 1985). Unfortunately, due to their high cost, these therapies remain too expensive for the patients especially in developing countries, where there is excessive population load. Despite substantial efforts by governmental, non-governmental organizations, and pharmaceutical companies to make ACTs available to malaria patients, ACTs remain beyond the reach of the majority of people in these endemic countries. The major contribution to the high price of ACT combinations is the production price of artemisinin, the core ingredient.

The content of artemisinin in *A. annua* L. plants is limited, ranging from 0.01 to 1.0% of the dry weight (dw), which makes its extraction expensive (Weathers et al., 2011). Artemisinin can be chemically synthesized. Its chemical synthesis/semi-synthesis is, however, complicated and economically unviable due to low yields (Zeng et al., 2012). Conventional breeding, cell suspension and hairy root cultures, and other biotechnological techniques showed potential for future development, but improvements delivered by them so far have not met the global demand and unable to reduce the cost of ACTs. Transgenic technology is also one of the promising approaches to increase the artemisinin content in *A. annua* L. plants. Under field conditions, the artemisinin content in *A. annua* L. plants is, however, influenced by variations in environmental conditions such as temperature, humidity and sunlight during the relatively long agricultural timeframe. These factors together limit the large scale extraction of the drug from the natural source for use by the pharmacological industries. Therefore, in the current scenario where the conventional methods of large scale plantation of *A. annua* L. have failed to fulfill the increasing demand, the *in vitro* techniques offer an alternative means of propagation which had already proven their worthiness for micropropagation, conservation and production of secondary metabolites from the medicinal plants.

The tissue culture techniques are being increasingly exploited for *in vitro* propagation of medicinal and aromatic plants and for commercial exploitation of valuable plant derived...
pharmaceuticals (Faisal et al., 2006b; Veeresham et al., 1998; Pattnaik and Chand, 1996; Purohit et al., 1994; Arora and Bhojwani, 1989; Bhojwani et al., 1989; Bajaj et al., 1988). The prime concern of tissue culture studies is to develop reliable protocols that are simple, efficient, reproducible, cost effective, adaptable and offer a better platform for genetic manipulation of a wide variety of plants (Ahuja, 1987). Keeping in view these facts, the present study was undertaken with the objective to develop a protocol for efficient regeneration, in vitro growth and multiplication of A. annua L. plants. Also, we aimed to develop hardening protocol using various physiochemical and biological factors for optimization of survival and better growth of these in vitro raised plants under natural conditions in the field. The results obtained during this study have been discussed in the light of existing literature under following sections:

5.1 Effect of PGRs (NAA, BAP, 2, 4-D and TDZ) on the callus induction and its growth in the leaf explants from the A. annua L. plants

This study was conducted to evaluate the effect of various combinations and concentrations of PGRs on callus induction in the leaf explants for micropropagation of A. annua L. The initiation of a proliferating callus culture from the explants involves profound changes in the developmental stages of the tissue and results in alteration in basic architecture of cell and tissues leading to activation of even quiescent or fully differentiated cells. Clonal multiplication through various explants is advantageous over conventional propagation methods as large number of plants can be produced within short span of time. According to Huang and Murashige (1977), the in vitro differentiation in excised tissue depends on the composition of medium. One of the most influencing factors in multiple shoot regeneration is the modulation of endogenous auxin to cytokinin balance (Thorpe, 1980; Skoog and Miller, 1957). Morphogenic responses exhibited in the form of shoots or roots are thus correlative to specific auxin and cytokinin ratio. Generally, high concentrations of auxins and low concentrations of cytokinins in medium promote abundant cell proliferations with the formation of callus (Chawla, 1987; Bennici, 1988). The tissue culture of A. annua L. is largely focused on improving artemisinin production. In vitro cultures of this species have been the object of great amount of research made by various groups (Janarthanum et al., 2012; Mannan et al., 2010; Sujatha et al., 2007; Liu et
The specific growth hormones at appropriate concentrations can play a major role to induce callus besides the other factors (Ananthi et al., 2011). Therefore, it is important to study callus induction and morphogenic response at different combinations and concentrations of the growth regulators. In our study, we have observed the effect of different concentration and combination of PGRs (BAP, NAA, 2, 4-D and TDZ; Table 3) on the callus growth, morphology, biomass and artemisinin content in both transgenic and non-transgenic *A. annua* L. leaf explants.

### 5.1.1 Callus induction, morphology and biomass accumulation in the calli of transgenic and non-transgenic *A. annua* L. plants.

High auxins in combination with low cytokinins promote cell proliferations, cell division, cell elongation and vascular tissue differentiation (Park et al., 2010; George et al., 2008; Chawla, 2002; Bennici et al., 1988; Chawala and Wenzel, 1987) leading to the enhanced biomass of the developed callus. The callus induction in *Artemisia annua* L. leaf explants was observed in 7 days in MS+BAP (1.0 mg/l) + NAA (2.0 mg/l). This combination of PGRs in Ms medium also gave the maximal response (Table 7). It may be explained that the specific growth hormones at appropriate concentrations can play a major role to induce callus. Further, MS medium fortified with BAP (1.0 mg/l) and NAA (2.0 mg/l) caused maximum callus induction in leaf explants followed by MS medium fortified with BAP (1.0 mg/l) and 2, 4-D (0.5 mg/l) or with TDZ (0.5 mg/l) and 2,4-D (1.0 mg/l) (Table 7). The variation could be due to the nature of tissue, degree of totipotency and composition of medium with respect to micronutrients and hormones. Variation in distribution of endogenous levels of growth regulators could also be reason for the variation in response of leaf explants in terms of callus initiation (Senthilkumar and Paulsamy, 2010b; Baskaran and Jayabal, 2005; Farternale et al., 2002).

The combinations of BAP and NAA supplemented media induced compact calli in the leaf explants of transgenic plants, but varying in colour and growth patterns. The dark brown, creamish green and green coloured calli with good growth were observed under light regime on MS medium supplemented with BAP (0.1 mg/l) and increasing concentrations of NAA (0.5-2.0 mg/l), while on similar combinations and concentrations of other PGRs viz.
2, 4-D, TDZ, brownish, light green and creamish green calli with moderate growth were produced under the light and dark regimes (Table 8-11). This is because of the fact that NAA is less inhibitory to the chlorophyll biosynthesis than TDZ, and 2, 4-D makes the calli greener (Afshari, 2011; Van der Valk, 1995), ultimately leading to higher biomass because of high photosynthetic potential.

In our study, the biomass of transgenic calli was increased by 1.5 times on MS medium supplemented with BAP (1.0 mg/l) and NAA (2.0 mg/l), when compared with the transgenic calli cultured on MS medium supplemented with BAP (1.0 mg/l) + 2, 4-D (0.5 mg/l). The results in our study indicated that the callus formation in *A. annua* L. plants has higher requirement of auxin and lower requirement of cytokinins (BAP and TDZ). The earlier reports also suggest that the high concentration of NAA with low concentration of BAP generally has the higher efficiency of abundant cell proliferations with the formation of callus in leaf explants (Senthilkumar and Paulsamy, 2010a; Karappusamy and Pullaiyah, 2007; Bennici et al., 1988; Chawala and Wenzel, 1987). Similar observations were also made by several other investigators in *P. vera* and *P. atlantica* subsp. kurdica (Aghaei, 2013; Tilkat and Onay, 2009). Our results are also supported by recent reports that show that light has a significant effect on increasing the biomass of calli in terms of fresh weight in different explants derived calli such as leaf, hypocotyl, cotyledon, etc. (Aghaei, 2013; Afshari, 2011; George et al., 2008).

5.1.2 Artemisinin concentrations in the calli induced in leaf explants of *A. annua* L. plants by different combinations and concentrations of auxins and cytokinins

Secondary metabolites production from undifferentiated plant cell cultures has been intensively studied. There are, however, only few compounds that have been commercially manufactured *in vitro* (Santoro et al., 2013; Vanisree et al., 2008; Verpoorte et al., 2002; Bourgaud et al., 2001). The artemisinin concentration, ranging from 0.78 to 1.13 mg g⁻¹ dw, was reported earlier in *A. annua* L. calli that were cultured on MS media supplemented with different concentrations of BAP, NAA and 2, 4-D, but it was declined to zero after three subcultures (Paniego and Giulietti, 1994). The nature and the types of calli, and the phytohormones concentrations and combinations used were, however, not mentioned. Further, the yield of artemisinin was quite low or sometimes even not detectable in the dedifferentiated cells (Yoshimatsu, 2008; Brown, 1992; Woerdénbag et al., 1992, Jha et al.,
In our study, we have observed 0.006% artemisinin on dry weight basis in the transgenic calli of *A. annua* L. plants cultured in light on MS medium supplemented with BAP (1.0 mg l⁻¹) and NAA (2.0 mg l⁻¹). These calli were, however, undifferentiated, friable and green. Such calli can be used to develop photosynthetically active cell suspension culture for the production of artemisinin (Zia et al., 2007; Aslam et al., 2006; Nin et al., 1996).

There are many reports showing that light is an important physical factor influencing the growth of calli and formation of primary and secondary metabolites. Besides, illumination was also found to affect the composition of sesquiterpenes in callus culture of *Marticaria chamomile* (Anasori and Asghari, 2009; Rao and Ravishankar, 2002; Krewzaler and Hahlbrock, 1973). The results of these studies support our results, where we observed higher artemisinin content in the transgenic calli grown in light (1.5 times higher) than the transgenic calli grown in the dark.

The friable, green, autotrophic calli of *A. annua* L. developed by us could be used for sustainable calli-based platform for the production of artemisinin. This callus culture may also be used to initiate cell cultures that could be extrapolated to the photo-bioreactor level for artemisinin extraction in the near future. This system could also be used for the clonal propagation after shoot induction of high artemisinin yielding strains of *A. annua* L. for large scale cultivation.

### 5.2 Regeneration and Multiplication of *A. annua* L. Plants

#### 5.2.1 Shoot regeneration

The leaf explants from 30 days old seedlings of *A. annua* L. were cultured on shoot induction medium having different concentrations and combinations of PGRs (BAP and NAA) in our study. Of all the 24 treatments, only eight treatments of BAP and NAA have shown the response to shoot induction in the leaf explants from both transgenic and non-transgenic *A. annua* L. plants (Table 12). The maximum shoot regeneration frequency (91%) and the number of shoots per explant (40±2.15) were, however, recorded with T11 treatment [MS + BAP (1.5mg/l) + NAA(0.075 mg/l)] in leaf explants of *A. annua* L. plants followed by the T2, T9, T10, T12, T13, T19 and T20 (Table 13). Further, the multiple primary shoots were emerged in a cluster from the leaf explants. It is interesting to note that T11 had high concentration of cytokinin and low concentration of auxin, thus suggesting
that the cytokinin (BAP) is influencing shoot regeneration in the leaf explants of *A. annua* L. plants. The earlier reports have also shown that cytokinin is the major growth hormone and at higher concentrations, it is involved in shoot induction and multiplication in many plant species (Sunder and Jawahar, 2011; Senthilkumar and Paulsamy, 2010a; Roy et al., 2008; Kumari et al., 2001). In contrast, when the leaf explants were grown on culture media containing cytokinins and auxins, axillary shoots developed precociously, which proliferated to form clusters of secondary and tertiary shoots. The role of BAP in bud breaking and shoot formation from nodal explants has been reported in many plants such as *Anemopaegma arvense* (Pereira et al., 2003), *Feronia limonia* (Hiregoudar et al., 2003), *Annona squamosa* (Nagori and Purohit, 2004), *Davidsonia* sp. (Nand et al., 2004), *Bupleurum raoi* (Chen et al., 2006), *Artemisia vulgaris* (Sujatha and Kumari, 2007), *Sida cordifolia* (Sivanesan and Jeong, 2007a), *Pongamia pinnata* (Sugla et al., 2007) and *Ulmus parvifolia* (Thakur and Karnosky, 2007).

In the present study, we have also found that BAP at the concentration of 1.5mg/l and NAA at 0.075 mg/l were optimal for maximum shoot induction in the leaf explants of *Artemisia annua* L. The increase in concentration of BAP and NAA beyond optimal level had a negative effect and the shoot exhibited a stunted nature with the reduction in number of shoots generated from each explant. These findings are in consonance with the results obtained earlier in *Withania somnifera* (Sen and Sharma, 1991), *Kaempferia galanga* (Vincent et al., 1992), *Vitex negundo* (Sahoo and Chand, 1998), *Rauwolfia tetraphylla* (Faisal and Anis, 2002), *Tylophora indica* (Sharma and Chandel, 1992), *Liquidambar styraciflua* (Kim et al., 1997), *Psoralea corylifolia* (Anis and Faisal, 2005), *Aloysia polystachya* (Burdyn et al., 2006), *Dioscorea nipponica* (Chen et al., 2007), *Sida cordifolia* (Sivanesan and Jeong, 2007a), *Erigeron breviscapus* (Liu et al., 2008) and *Pogostemon heyneanus* (Hemborn et al., 2006).

Based on the results of our study, it can be concluded that the growth regulators, BAP and NAA ensure *in vitro* regeneration of shoots from leaf explants and synergism of BAP and NAA in proper concentration is extremely favourable for the shoot multiplication.

### 5.2.2 Root regeneration

In our study, the rooting of *A. annua* L., while sub-culturing the shoots, was well pronounced in the MS medium supplemented with either NAA or IBA alone at
concentrations ranging from 0.1 to 1.0 mg/l. 0.5 mg/l NAA initiated root formation in 100% shoots cultured on rooting medium followed by MS medium supplemented with 1.0 mg/l IBA (80.6% shoots rooted) (Table 14). The number of roots per shoot were also observed to be higher (16.6 roots/shoot) in the MS medium containing 0.5 mg/l NAA in *A. annua* L. plants. All these facts showed that the NAA and IBA are the most required growth regulators for rooting in the present study. It agrees with the concept that both endogenous and exogenous auxins are the plant hormones that induce root formation in the shoots of majority of plant species (Loc et al., 2011; Mungole et al., 2011; Rajput et al., 2011; Mahesh et al., 2010; Mallikadevi and Paulsamy, 2009; Van Eck and Kitto, 1992).

*In vitro* rooting response is perhaps related to the endogenous auxin or cytokinin levels. Control over the type of organ produced in culture is governed by the balance of exogenous and endogenous growth regulators. Cells of the same plant can have varying levels of endogenous plant growth regulators, and, therefore, it is reasonable to postulate that responses to exogenous or endogenous auxin also vary during *in vitro* rooting. Further, differences in rooting responses may also provide progress towards improving rooting efficiencies of recalcitrant genotypes (Kim et al., 1997).

### 5.2.3 Biomass, chlorophyll, protein and artemisinin concentration in inoculated and non-inoculated *Artemisia annua* L. plantlets with *P. indica* in *in vitro* culture

The artemisinin concentration increased by 206% and 83.67%, respectively, when compared with non-inoculated non-transgenic *A. annua* L. plants. Further, the biomass, chlorophyll and soluble protein contents were also increased by 60.87, 53.18 and 82.62%, respectively in transgenic plants, when compared with non-inoculated non-transgenic *A. annua* L. plants after 6th week of the culture on rooting medium (Table 17, 18, 19). The enhanced artemisinin and other physiological parameters in *A. annua* L. colonized with *P. indica* could be due to more uptake and assimilation of mineral nutrients that could have led to higher carbon assimilation and increased flux of carbon towards the artemisinin biosynthesis and its production. Our results are supported by Sharma et al. (2013) and Das et al. (2012), who also observed a significant increase in secondary plant metabolites contents and chlorophyll as well as protein contents that were inoculated with *P. indica* in medicinal plants.
More increase in artemisinin concentration in inoculated transgenic *A. annua* L. plants as compared to the inoculated non-transgenic *A. annua* L. plants could possibly be due to higher genetic and biosynthetic potential of the former to synthesize more mevalonate and higher carbon supply for artemisinin biosynthesis because of over-expression of *hmgr* and higher activity of the enzyme encoded by this gene (Nafis et al., 2011; MaujiRam et al., 2010).

5.3 Assessment of Genetic Fidelity in Rooted Plantlets of *A. annua* L.

Micropropagated plants derived from different explants of various plant species through caulogenesis are generally considered to be at low risk for genetic stability (Samantaray and Maiti, 2010; Yang et al., 2008; Tyagi et al., 2007; Hembrom et al., 2006). To ensure genetic fidelity of micropropagated transgenic and non-transgenic *A. annua* L. plants, we used RAPD markers for amplification of their genomes and compared the banding patterns with that of their respective mother plants. The results of the RAPD analyses showed no evidence of polymorphism between the *in vitro* raised mother and transgenic as well as the non-transgenic plants and these were true clones of their respective mother plants. Similar kind of studies were also reported in other plant species such as *Chlorophytum borivilianum, Populus deltoids, Picea marina, Festuca pratensis, Liriodendron tulipifera* (Ghosh et al., 2013; Samantaray and Maiti, 2010; Rani et al., 1995; Isabel, 1993; Valles et al., 1993; Merkle et al., 1988).

5.4 Acclimatization and Hardening of *In vitro* Raised Transgenic and Non-transgenic *A. annua* L. Plantlets in Polyhouse Conditions

Plant tissue culture relies on growing microbe-free plant material in a sterile environment, in conjunction with defined media containing nutrients, growth regulators, and carbohydrate sources. Typically plant tissue culture is carried out on a gelled medium within an enclosed clear or translucent culture vessel with limited ventilation, and placed under fluorescent lighting. To negate the need for plants to be photosynthetically active, or even photosynthetically competent under artificial lighting, sucrose is added to the medium as a carbohydrate source. Major differences exist between the environments of plants growing in tissue culture and those in a greenhouse. These include differences in lighting (both quantity and quality), relative humidity, nutrients and other growth promoters, the gaseous composition, and the medium substrate. In addition, the rooting procedure differs
markedly. In greenhouses, a high-auxin quick dip is used for rooting cuttings. Excess auxin is flushed away in the free-draining and aerated potting medium. This contrasts to in vitro rooting, where a low auxin concentration is available over several weeks in a poorly aerated, gelled medium. Therefore, it is not surprising that the transfer of rooted plantlets from the tissue culture environment to the greenhouse causes tissue stress and is often associated with slow growth and significant plant losses. This period of plant stress often coincides with a change in plant ownership following the sale of the plants by the tissue culture laboratory to the end or intermediate user. Consequently, the tissue culture conditions that the plantlet has been grown in and the conditions the plants are to be transplanted into are often poorly understood by the two parties. Differences between the two environments and their effects on plants have been recognized in numerous studies that aim to understand the factors involved in the transition and establishment of tissue culture plantlets into a standard greenhouse environment and improve the success rate (Preece and Sutter, 1990).

During the last three decades, plant micropropagation has developed from a laboratory curiosity to a real industry. However, its wide spread use is restricted for several reasons, one of these being that a high percentage of micropropagated plants are lost or damaged during transfer from the culture vessel to soil. Due to the special environment in vitro, it is difficult to produce plants which can be acclimatized to the outside environment (Kozai, 1991). High humidity of the environment in in vitro condition does not allow synthesis of cuticle and epicuticular wax on the epidermis of leaves of regenerated plants. Consequently, when such plants are transferred to a relatively less humid external environment, they undergo desiccation and death (Selvapandiyan et al., 1988). Malik et al. (2005) used medium with reduced mineral salts and sucrose concentration for hardening of in vitro raised shoots of Garcinia indica as it probably forced the regenerants to rely their own photosynthetic apparatus for nutrition (Kozai and Sekimoto, 1988). A lengthy and cumbersome acclimatization, procedure is required for such cultured plants during which they develop thickening, epicuticular wax and the mechanisms of closure of stomata become operative. In order to solve these problems, we have standardized the acclimatization technique that not only reduces the time of acclimatization, but also allowed a high survival rate in both transgenic and non-transgenic A. annua L. plants. To
ensure high humidity, the transplanted *in vitro* raised plantlets of *Artemisia annua* L. were covered with clear polyethylene bags individually and maintained in environmentally controlled polyhouse (Temperature 25 ± 2°C; relative humidity 90-50%) initially for 2 weeks, but with gradual removal of polybags in order to acclimatize them. The plants were found to be acclimatized in 45 days under polyhouse conditions. Thereafter, they were transferred in open environment in net house. In order to harden the micropropagated *A. annua* L. plants, the biotic and abiotic factors were used. The effects on survival of these plants under natural conditions were evaluated. The results of our findings are discussed in the following subsections:

### 5.4.1 Effect of biotic (*P. indica*) and abiotic (ABA) factors on acclimatization and hardening of *A. annua* L. plants

*In vitro* co-culture of plant tissue explants with vesicular arbuscular mycorrhiza induces developmental and metabolic changes in the derived plantlets, which enhance their tolerance to abiotic and biotic stresses. Hao et al. (2010) reported that treatment of suspension cells of *Ginkgo biloba* with fungal endophytes resulted in enhanced production of flavonoids as well as endogenous ABA and the activation of phenylalanine ammonia lyase. In addition to these fungi, many symbiotic/free living bacteria belonging to the genus *Bacillus* and *Pseudomonas* also proliferate around and inside the plant tissues in a uniform environment free from fluctuations of temperature and moisture conditions. They improve not only the growth of plants, but also their survival under stress environment. Trivedi and Pandey (2007) also reported that inoculation of micro shoots of *Picrorhiza kurrooa* by the three plant growth promoting rhizobacteria (*Bacillus megaterium*, *Bacillus subtilis* and *Pseudomonas corrugata*) was effective in improving the survival (94.5%) of micropropagated plants after transfer to soil. Consequently, we have also used *P. indica* in hardening process and achieved a high degree of survival of transgenic (95%) and non-transgenic (85%) *A. annua* L. plants in polyhouse conditions after 45 days of transplantation. The survival rate of non-inoculated transgenic as well as non-transgenic plants was, however, only 75% (*Table 20*).

Abscisic acid (ABA), a naturally occurring plant hormone critical for plant growth and development, plays an important role in plant water balance and in the adaptation of plants to stress environments including low temperature (Finkelstein and Gibson, 2002;
Hetherington, 2001). It is transported via xylem to the shoot, where it regulates transpiration and leaf growth (Hronkova et al., 2003). Role of abscisic acid on tolerance to abiotic stresses has also been studied by Aguilar et al. (2000) in Tagetes erecta in controlling leaf water loss, survival and growth of microshoots, when transferred directly to the field. Keeping in view the above studies, we have also used ABA to harden the micropropagated A. annua L. plants. We have found a high degree of survival in transgenic (90%) and non-transgenic (80%) A. annua L. plants that were treated with ABA, when compared with the non-treated plants (75%; Table 20). Our results are also supported by other studies in Tagetes erecta L. (75% survival) and Aronia arbutifolia (100% survival) treated with ABA (Aguilar et al., 2000; Colon-Guasp, 1996).

5.5 Physiochemical Changes in Artemisia annua L. Plants as Influenced by Biotic and Abiotic Factors in Polyhouse Conditions

5.5.1 Effect of biotic factor (P. indica)

P. indica is a wide-host root-colonizing endophytic fungus, which allows the plants to grow under extreme physical and nutrient stresses. Further, it is shown to stimulate excessive production of biomass at pre-flowering stage and a potential microorganism imparting biological hardening to tissue culture-raised plants (Das et al., 2012; Yadav et al., 2010; Verma et al., 1998). The growth promoting effect initiated by P. indica is accompanied by a co-regulated stimulation of enzymes involved in nitrate, starch, phosphorus and sulphur assimilation in Arabidopsis, tobacco, maize and Coleus forskohlii plants, that ultimately leads to higher biomass production in these plants (Das et al., 2012; Verma et al., 2012; Yadav et al., 2010; Sherameti et al., 2008).

In our study, the in vitro raised rooted plantlets of A. annua L. inoculated and non-inoculated with P. indica on MS rooting medium showed differential physiological responses in their growth and development. After 15-45 days of inoculation, the leaf biomass, stem biomass, leaf:stem ratio and artemisinin concentration as well as yield were increased gradually (Table 21a, b, c). The maximum increase in leaf biomass (68.22%), stem biomass (33.33%), leaf:stem ratio (28.52%), artemisinin concentration (75%, 0.35±0.01 mg/g dw) as well as yield (148.03%) was recorded in inoculated transgenic plants, when compared with non-inoculated A. annua L. plants after 45 days of inoculation (Table 21c). The enhanced biomass accumulation and increase in other
physiological parameters in *A. annua* L. colonized with *P. indica* could be due to more uptake and assimilation of mineral nutrients that could have led to higher carbon assimilation and increased flux of carbon towards the artemisinin biosynthesis and production.

**5.5.2 Effect of abiotic factor (ABA)**

To assess the effect of abiotic factor i.e. ABA, the micropropagated *A. annua* L. plants were treated with 1.5 mg/l ABA during acclimatization and hardening. Various physiological responses were thereafter evaluated (Table 22a, b, c). The ABA treated transgenic *A. annua* L. plants had shown increased leaf biomass (80%), stem biomass (33.47%), leaf:stem ratio (35.29%), and artemisinin concentration (50%, 0.30 ± 0.01 mg/g dw) as well as yield (143%, 4.86 ± 0.03 mg/plant), when compared with non-inoculated *A. annua* L. plants after 45 days of transplantation (Table 22c). These attributes could be due to the higher survival of the plants and the modulation of expression of ABA related genes or transcription factors associated with their better growth as well as development and artemisinin biosynthesis. This inference is supported by earlier studies, where sufficient evidences exist to show that there is probably a crosstalk between the ABA signaling pathway and artemisinin biosynthetic pathway (Qian et al., 2007; Barrero et al., 2006; Verslues and Zhu, 2005; Qureshi et al., 2005).