Chapter - 2
Review of literature
CHAPTER II
REVIEW OF LITERATURE

2.1 TYPES OF MUTAGEN

2.1.1 Physical mutagen [Gamma irradiation]

In mulberry, researchers have carried out enormous mutation induction studies, mostly using irradiations (X-rays and gamma rays). These workers have tried irradiations, for studying the effectiveness and efficiency of mutagens to evolve promising mulberry varieties [Kuchkarov 1964, Hazama et al., 1968a, 1968b; Tojyo 1969, 1979, 1980; Das, 1970; Nakajima 1972; Fujita and Nakajima, 1973; Fujita and Wada 1982; Katagiri and Nakajima 1980; Rao J.P. et. al., 1984; Jayaramaiah and Munirajappa 1987; Kuchkarov and Ogurtsov, 1987; Ramesh, 1997 and Reddy, 2002]. Here, the available work related to physical mutagenesis in mulberry is reviewed and presented as below.

Kuchkarov [1964] reported a large leaves mutant and other valuable mutants by irradiating the seeds of *Morus alba* with 5-35 kR gamma-rays. Doses of 25 kR and 35 kR were particularly effective, inducing 1.95 and 1.96 percent mutation respectively in the *M₁* generation.

Swaminathan [1964] at the Indian Agricultural Research Institute, New Delhi initiated a major program on mutagenesis in different crops including mulberry plant. These studies were broadly aimed at understanding the process of mutation, testing the effectiveness of various mutagens, identifying optimum dose and the best method of treatment for different crop species.

Hazama et al. [1968] studied the varietal differences of mulberry to radio-sensitivity and reported bud mutation.
They found the inhibition of plant height, growth and induction of fasciations among 15 mulberry varieties of Japan due to subsequent gamma irradiation. They also reported that mutation rate/plant was quite high at 3 kR to 10 kR.

Hazama [1968a] discussed the breeding new varieties selecting from F₁ seedlings, raising polyploidy or induced mutation.

Hazama [1968b] irradiated 50 plants of variety [Kairyonezumigheshi) KNG with gamma rays (5 kR-15 kR) and secured one mutant possessing both the types of entire and dissected leaves. The mutant showed increased leaf yield over the control. The irradiated clones were subjected to pruning for 3 successive years in order to widen mutation spectrum and to increase mutation frequency.

Tojyo [1969] carried out an experiment in which mulberry cuttings preserved at 3-5°C were irradiated with different doses of gamma rays (2.5 kR -10 kR). In the plants developed from irradiated cuttings, growth of flower buds was inhibited, size of the pollen grains was varied and meiotic irregularities were encountered.

Das [1970] studied the effects of gamma irradiation on germination and seedling development in mulberry. Seeds and seedlings of Morus alba var. “Ichinose” were irradiated with gamma rays (2.5, 5, 7.5, 10 and 15 kR). He reported that 10 kR is lethal dose for growth and development of mulberry seedlings.

Fujita and Takato [1970] found a valuable mutant having entire leaves by irradiating gamma irradiation of the variety “Ichinose”. The mutant has smaller leaf with slightly more dry matter and less water than the original variety.
Katagiri [1970] studied the varietal differences of mutation rate and mutation spectrum of nine varieties exposed to acute gamma ray. He found that there were apparent differences among varieties as to mutation rate and a total of 9 kinds of mutation were produced in the 9 varieties. Kinds of mutations and the number of mutated shoots showing each mutation were ‘elongated leaf’,52’; ‘marginally curled leaf,5’; variegated leaf,5’; ‘entire leaf, 3’; ‘centrally pale green leaf,2’; ‘multi-lobed leaf,2’; ‘small leaf, 2’; and ‘yellow leaf,1’. A variety with a high mutation rate is likely to give a wider range of mutation spectrum.

Nakajima [1972] recovered a beneficial mutant in mulberry by gamma irradiation. He secured a promising mutant “3183” by irradiating one year old grafts of “KNG” variety with 5 kR gamma rays. This mutant showed changed leaf morphology i.e., entire leaf (unlobed) in contrast to 5-lobed leaf of control. It also revealed 7% increase in leaf thickness, 20% reduction in inter-nodal distance and contributed to 10% increase in leaf yield.

Nakajima [1973] in his study of gamma irradiation of three mulberry cultivars namely “KNG”, “Kenmochi” and “Ichinose” has resulted in the production of mutants showing increased leaf yield from “KNG” and “Ichinose” and several mutants resistance to Diaporthe nomurai disease [die-back disease] from “KNG” and “Kenmochi”.

Fujita and Nakajima [1973] subjected the induced mutants to re-irradiation with 10 kR gamma rays. The shoots of re-irradiated plants were pruned repeatedly. They observed that mutant “3198” and “IRB 240-I” exhibited higher mutation frequency than the parent variety “Ichinose”. Further, they recorded the reversal of leaf shape character due to re-irradiation.
Katagiri [1973] studied on radiation damage in winter buds and relation of shoot cutting-back to mutation frequencies and spectra in acutely gamma-irradiated mulberry. He highlight on radiation damage in shoot tips and axillary buds irradiated at the dormant stage, and mutation frequencies and spectra in secondary and tertiary shoots derived from the shoots from main buds irradiated at the stage of just before sprouting.

Katagiri [1976] irradiated the vigorously growing shoots of mulberry var. "Ichinose" with 6 kR (120 Rad/h) and 7.5 kR (150 Rad/h) gamma rays during early July, August and September seasons. Irradiated shoots of early September season produced deformed narrow leaves and failed to grow further. Whereas, at lower doses (6 kR), the irradiated shoots of early July and August seasons exhibited normal growth and development. However, in these seasons shoots exposed at higher doses failed to develop. Based on these results he reported 7.5 kR as LD50 dose.

Katagiri et al. [1976] reported that mutation was induced by chronic gamma irradiation using 4 varieties. Four resistant mutants were obtained from var. "Kenmochi" through screening test for die back resistant in heavy snow fall region. The mutation is also induced by colchicine treatment with var. "Kenmochi". Gamma rays and colchicine considered to be careful tools for induction of dieback resistant mulberry mutations.

Nakajima [1977] subjected the irradiated plants for repeated pruning in order to force the development of wholly mutated shoots from the vegetative buds. He reported that the entire leaf of mutant reverted back to normal lobed leaf due to re-irradiation.
Fujita et al. [1980] re-irradiated the mutant line “3198” of “Ichinose” by 10 kR gamma rays. The mutant was secured originally by irradiated the cuttings of variety “Ichinose” with 10 kR gamma rays. They observed the increased frequency of mutations in the mutant (7.1%) over the control (0.5%) due to re-irradiation. 8 mutants had improvement in leaf and shoot characteristic feature compared with “Ichinose” and five mutants gave better leaf yield.

Katagiri and Nakajima [1980] irradiated the mulberry trees with 5kR gamma rays at the rate of 0.2kR/hour and 5kR/h. The irradiated trees were allowed to grow and subjected to pruning three times in two growing seasons. The frequency of tetraploids induced was much higher than that of mutations.

Tojyo [1980] studied on gamma rays irradiation on the autotetraploids of mulberry tree.

Fujita and Wada [1982] re-irradiated and induced mutant of “Ichinose” variety with gamma rays (10 kR). They recorded the increased mutation frequency and wider mutation spectrum due to re-irradiation. They obtained a mutant with entire leaf by irradiating a cultivar possessing 5-lobed leaf.

Sastry et al. [1983] studied the differential sensitivity of physical and chemical mutagens on sprouting, survival and injury in three varieties of mulberry, “S30”, “S36”, and “Kanva-2”, by treating the buds with aqueous solutions of sodium azide (0.005%, 0.01%, 0.015%), diethyl sulphate (250, 500, 750 ppm) and 5,10 & 15 kR gamma rays and found that the injury was directly proportional to the concentrations and doses of the mutagens used.
Rao J.P. et al. [1984] studied the effects of different doses and concentration of gamma irradiation and hydrazide respectively on “M₅” and “Local” mulberry varieties. They recorded promising results with regard to sprouting and survival percentage, plant height, branching pattern, leaf size and total yield at M₁ generation. Based on the observed results, they concluded that gamma irradiation is a very effective tool to induce variation and to improve mulberry cultivars.

Yang and Tang [1984] studied on radiation breeding in mulberry (Morus alba L.). The mutant strain was induced by gamma ray irradiation of one-year old grafts of cultivar “Shinichinose” with the dose of 6 kR at the exposure rate of 2.2kR/h; it is characteristics of polyploidy mulberry. Its leaves become larger, leaf width increases about 23 %, leaf thickness increases about 20%, and inter-node length decreases, but it shows slight decrease in number of branches and in length of branches.

Yang et al. [1984] studied on effect of radiation on the improvements of leaf quality and disease resistance. The result indicates that the radiation has an effect on the improvement of the leaf quality and disease resistance in mulberry.

Jayaramaiah and Munirajappa [1987] conducted irradiation studies on “Mysore Local” mulberry variety. They irradiated the cuttings with different dosages of gamma rays (1kR to 10 kR). They studied various parameters like survival percentage, sprouting ability, vigor of plant and cytological features at R₁ and R₂ generations. They secured mutants with visible morphological traits like shortened internode, thick, succulent and dark green leaves, opposite phyllotaxy, etc. Further, they also reported the meiotic anomalies like clumping of chromosomes, laggards and anaphase bridges in mutants.
Kuchkarov and Ogurtsov [1987] studied the different forms of mulberry mutants exhibiting joined snake like shoots, rolled leaf blades and weeping habit.

Dandin et al. [1989a] studied the induction of variability by physical mutagens. They treated cuttings of four mulberry genotypes with 5-8 kR gamma rays. Progeny of “S30” and “S36” treated by gamma rays were screened and 40 variants were isolated, out of these 10 showed 80% rooting. Variants were crossed to “Ber.C763”. Seeds of 6 accessions were treated with different doses of gamma rays and planted in pots. Dose rate @ 7.5, 10, and 12 kR found to be lethal. Higher dose rate of 20, 25, 30 kR were found injurious to seeds. Cuttings of “K2”, “Local” and “S30” were treated with 3, 4 and 5 kR and seeds of [oph] “S30”, “Local” and “K2” were treated with 6, 9,12 and 15 kR and found that 3 kR for cuttings and 6kR for seeds were found to be most suitable.

Dandin et al. [1989b] studied induction of mutation through interspecific grafting and found that length of mutant branch; internodal distance and leaf area are reduced when compared to those of non-mutant stock and scion.

Hanumantha Raju et al. [1992] studied on genetic variability, correlation and path analysis in M1 generation in mulberry (Morus indica L.). Open pollinated seeds of “Ms” cultivar were treated with different doses of gamma rays and different concentrations of Ethyl Methane Sulphonate (EMS) to study their effect on various biological parameters.

Dandin et al. [1996] studied on different forms in mulberry. He classified mutants into two groups, i.e. stem mutants and leaf mutants.
Ramesh (1997) investigated the effect of gamma irradiation on few mulberry varieties and secured promising mutants in “S54" and “Ms” varieties at 7kR and 4kR respectively showing shortened internodal distance, increased leaf area and yield.

Hanumantha Raju et al. [2000] studied on the mutation of mulberry tree induced by radiation, the relation between the dose rate of γ rays and the bud mutation. The result revealed that the LD50 dose for seedling survival was about 28 kR of gamma rays. Occurrence of chlorophyll deviants was directly proportional to the increase in dosage.

Reddy Muniswamy [2002] studied on effect of gamma irradiation on S13 and S34 mulberry varieties. The different doses of gamma rays from 4kR to 10kR had been used on hard wood stem cuttings of the above taxa. The present investigation suggested that the promising mutants of “S13” and “S34” obtained through gamma irradiation might prove to be of immense value in the Sericulture industry where mulberry is grown under rainfed conditions on account of their commercial morpho-economic traits.

Chopra [2005] have discussed effect of ploidy on mutation, induced mutation in phylogenetic studies, radiomimetic effect caused by irradiated media in Barley, Potato, wheat, Morus alba, E-coli and Drosophilla etc. to reveal the number of released mutant varieties in different crop species in India. He has also specifically mentioned the role of biotechnology and functional genomics in crop improvement program.
2.1.2 CHEMICAL MUTAGEN [EMS SOLUTION]

Not much work has been carried out in mulberry breeding, using the chemical mutagens, but the beneficial mutants are also obtained in mulberry with the application of various mutagenic chemicals. Main chemicals for the purpose are ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphonate (DES), etc. According to the research in India EMS is especially effective in giving a wider spectrum of morphological characters than physical radiation. Available work related to chemical mutagenesis in mulberry is reviewed and presented as follows.

Gustafsson [1960] reviewed the work on chemical mutagenesis in higher plants and summarized various chemicals that have been tried. According to him ethyl methane sulphonate is very potent mutagen because it induces mutation rate higher than those obtained with physical mutagens in some plant species.

Konzak et al. [1965] reported that the potency of any mutagen is dependent on its mutagenic efficiency and effectiveness and EMS has been found to possess both the qualities.

Wada [1968] reported the sex conversion in mulberry treated with colchicicine.

Kaicker and Swarup [1972], Kolontaev [1974a, 1974c] while working on mutagens like DES, NMU, EI, X-rays and Gamma rays concluded that the injury caused by the mutagens was proportional to the dose rate and concentrations.
Sastry et al. [1974] treated the seeds of true breeding monoecious Berhampore variety of mulberry with aqueous solution of EMS in different concentrations [0.1%, 0.15%, 0.3%, 0.45% and 0.6%] for six hours, 12 hours and 24 hours at 5°C and 20°C. They reported that maximum survival rate at 12 hours duration at 5°C. Beneficial mutants “S₃₀”, “S₃₆”, “S₄₁” and “S₅₄”, superior to “Kanva-2” were recovered. The variety “S₅₄” and “S₃₆” were released for cultivation at farmer’s level.

Aliev [1977] used chemical mutagens Ethyl methane sulphonate, Diethyl methane sulphonate and N-nitrosomethyl urea in combination with hybridization techniques in few varieties of mulberry to evolve beneficial genotypes.

Mulov and Kovalik [1977] used chemical mutagens for production of mutants; by treating the hybrids after inter specific hybridization among open pollinated mulberry genotypes.

Kozikova [1979] evolved four promising forms of mulberry variety resistant to cylindrosporium with several other useful characters at M₂ generation by treating air dried seeds of some hybrids with N-nitroso methyl-N-ethyl urea, Dimethyl sulphate, ethylene imine, and 1,4-bisdi-azoacetylbutane (DAB). Cylindrosporium resistant forms were obtained from DAB treatment.

Kozikova [1980] recovered useful mutants of mulberry genotypes for breeding program at M₂ generation, by inducing significant morphological deviations with chemical mutagens.
Gatin and Ogurtsov [1981] reported changes such as fasciations of stems and inflorescence, twisting of stems, abnormal development of apical parts, changes in growth pattern of leaves and inflorescences associated with considerable branching of inflorescence and increased flower number, in mulberry varieties “Letnii” (summer), “Ussurjiskaya”, “Tashkentii” and “K2”, which were treated with 0.01%, 0.05% and 1% of N-nitroso-N-methyl urea solutions. High mutation frequency in the monoecious “K2”, in addition to the normal male and female inflorescences, hermaphrodite inflorescence and cytological abnormalities in the cells of apical meristem of treated shoots were noticed. Further they reported that 0.01% and 0.05% treatments showed high range of mutations.

Zu [1982] has reported the superior varieties of mutants from Morus alba, with bigger and thicker leaves resulting in high yield.

Sastry et al. [1985] studied on induction of variability by physical and chemical mutagens. Three budded cuttings and rooted saplings of “K2”, “S30”, and “S36” were irradiated with different doses of gamma rays [1-15kR] and chemical mutagens. Results showed that the injury was directly proportional to the concentration and doses of mutagen used. Several morphological variations such as stem fasciations, stem bifurcation, malformation of leaves were observed. Sprouting percentage increased over control at lower concentration of diethyl sulphate and sodium azide. Rooting percentage increased in all concentration of mutagens except in sodium azide. No variation was found after pruning the plants treated with chemical mutagens.
Dandin et al. [1987] studied the induction of variability by chemical mutagens. Selfed seeds of “Acc.139” and “Acc.142” were treated with diethyl sulphoate and sodium azide. M₁ and M₂ generations were raised.

Anilkumar [2008] studied the var. “RFS₁₃₅” and “BC₂₅₉” by using 0.1%, 0.3%, and 0.5% concentrations of EMS. After six months he found various beneficial morphological traits, such as increase in leaf area and girth of the stem, change in shape and texture of leaf, enhanced number of leaves per branch and reduced the internodal distance.

2.2 PROPAGATION STUDIES

Mulberry mainly cultivated for leaf production as it is the sole foods plant of silkworm Bombyx mori L. In sericulture industry production of quality mulberry leaves plays a crucial role for production of silk. Among the different factors, the mulberry leaf contributes more than one third (33%) for cocoon production. The procedures of mulberry sericulture begin with cultivation of suitable and best fit mulberry varieties for an ecozone. Vegetative propagation efficiency is one of the desirable traits of superior varieties. Here, the available work related to propagation studies in mulberry is reviewed and presented as below.

Yokoyama [1962] reported that the quantity of crops of mulberry leaves depends upon the number and length of shoots, internodal distance and number and weight of leaves per plant.

Kasiviswanathan and Iyengar [1966] studied three different methods, viz. planimeter method, simple method \[ A = F \times L \times W \] and
Linear regression method to determine leaf area in mulberry and found that there was no significant difference amongst methods, simple relationship utilizing the factor F will be preferred since it will be easy of computation.

Dzhafarov and Abbasov [1967, 1970] studied the propagation characteristic features of diploid and tetraploid mulberry genotypes in nursery conditions. They found that saplings of triploid possess larger and heavier leaves and grow more quickly than those of diploid and tetraploid genotypes. They also reported that triploid seedlings were taller and heavier than diploid and tetraploids.

Cafarov and Abbasov [1977] studied the propagation characteristics in mulberry genotypes of different ploidies. They found that the best source material for providing cuttings was the triploid hybrids.

Murakami [1980] showed that the cuttings from the medium portion of the branches were more suitable in producing larger amount of roots and shoots than the bottom and top ones.

Nakagawa and Naoi [1983] carried out the experiment to clarify the relationship between growth of hard wood cutting of mulberry tree "Ichinose" and moisture tension, using volcanic ash soils and red-yellow soil. They revealed highest percentage of root initiation under higher soil moisture conditions.

Sarkar et al. [1983] reported that the yielding ability of initial diploid, its triploid and tetraploid derivatives was assessed through yield trial and found that triploid mulberry produced more leaves than both the diploid and the tetraploid in pre monsoon and post monsoon seasons.
It has been also concluded that the bivoltine silkworms respond more favorably to triploid mulberry.

Verma et al. [1984] crossed induced tetraploids with diploids to evolved triploid mulberry. They found that triploids are superior to other varieties in leaf yield and nutritive value, qualities which are of prime importance in silkworm feeding.

Shamachary and Jolly [1988] studied a simple device for quick determination of mulberry leaf area in field.

Dwivedi et al. [1989] observed low rooting percentage in colchicines induced autotetraploids of “S₃₀” and “S₃₆” varieties when compared to diploid genotype.

Dandin and Kumar [1989] reported that Mulberry, a sole food source of silkworm *Bombyx mori* L. is grown for its foliage. Hence, the major objectives of mulberry breeding under tropical conditions are production of more foliage of good quality throughout the year, amenability of a genotype to vegetative propagation with fast regeneration capacity. They classified various parameters in four groups to achieve the above two major objectives.

Bari et al. [1990] studied on leaf yield performance of six open-pollinated selections and two improved varieties under four environmental conditions (seasons) in order to evaluate their relative stability and response to the environmental fluctuations. Both the genotypes and seasons were highly variable among themselves.

Yang and Yang [1991] and Dwivedi [1992] reported that the triploid hybrid is superior by having a higher number of branches and
rooting behavior but exhibits morphologically features intermediate between diploids and autotetraploids.

Baksh et al. [1992] screened twenty seven mulberry genotypes comprising 18 tropical and 9 sub-tropical cultivars for their rooting ability and leaf yield. They reported that “S36” exhibited more stable rooting ability among the tropical cultivars.

Bhat and Hittalmani S. [1992] studied clonal differences in mulberry root growth parameters in the selected 20 mulberry clones. The root growth parameter indicated significant differences with respect to shoot length, root length, number of roots per plant and shoot to root ratio by length and dry weight. The phenotypic and genotypic coefficient of variability, heritability and genetic advance revealed that shoot to root ratio by length and dry weight, number of roots per plant and volume of roots per plant are best characters for selecting mulberry genotypes for further improvement.

Dorcus and Vivekanandan [1992] reported that triploid mulberry cuttings start rooting within five days of plantation and diploid and tetraploid cuttings take up to ten days to initiate rooting.

Tikader et al. [1995] studied the survival potential of cuttings in different genotypes of different periods after planting. The maximum survival was noticed after 30 days of planting irrespective of the genotype and it decreased with increase in period. Survival percentage of different genotypes in different months varied from 15.28% to 71.36%, maximum being in October (71.36%) and minimum in June (15.28%).
Tikader et al. [1996] studied on propagation efficiency of mulberry (Morus spp.) at ploidy level and found that maximum survival percentage (71.81) was in triploids, followed by diploids (66.67) and tetraploids (59.17) The authors observed root shoot ratio by length to be highest (0.92) in triploids followed by tetraploids (0.73) and diploids (0.67). However, the root shoot ratio by weight was highest in tetraploids (0.58) followed by triploids (0.53) and diploids (0.20) on dry weight basis.

Vijayan and Chakraborti [1998] reported that mulberry, the most important crop plant in sericulture exhibits different ploidy levels. Among them, triploid forms are superior to other ploidy levels in almost all economically important characters.

Rahman et al. [1999] conducted experiments to evaluate the performance of mulberry variety “S_{1635}” under different system of planting in west Bengal. Negligible variants were noticed in leaf yield and cocoon yield indicating the easy adoption of this variety to different cultural practices.

Patil et al. [1999] worked out the genetical parameters in relation to leaf yield in six genotypes. Sufficiently high variability was reported between the mulberry genotypes for propagation parameters.

Phukan et al. [2000] studied growth and leaf yield of a few improved mulberry strains. “S_{1635}” and “BC-259” were found to be superior to other improved varieties such as “S-1” and “Jatinuni” [local] with respect to leaf yield, survival and growth parameters. Incidence of decease also found to be less in these varieties as compared to that of local.
Rao Eshwar et al. [2004] evaluated induced tetraploids and triploid mulberry genotypes for propagation, growth parameters and reported that the genotypes of different ploidy levels exhibited significant differences with respect to growth and yield parameter. Triploids performed better in all the parameters compared to other polyploid variants.

Rahman et al. [2004] studied on genetic variability and correlation of leaf yield and its contributing characters in mulberry varieties (Morus sp.). He observed that longest shoot height, nodes/meter and leaf area showed moderately high heritability and these characters were less influenced by the environment.

Mallikarjunappa et al. [2008] studied on genetic variability and correlation studies in mulberry germplasm and pointed out that fresh weight of leaves and rooting are the most important yield contributing components and respond well for selection as they have high heritability, besides these two characters number of branches/plant, plant height and internode length could also assist in effective selection for genetic improvement of leaf yield traits in mulberry.

2.3 PHYTOCHEMICAL INVESTIGATION

Biochemical analysis is one of the diagnostic techniques employed for evaluating plant nutrient status. Several investigations have been carried out to understand the variation in nutritional status of mulberry leaves with reference to leaf position and age. The varietal differences and seasonal variation in chemical composition has also been reported. The available works have been briefly reviewed as below.
Matsumara et al. [1955] observed wide range of variation in mulberry genotypes and discussed the importance of quality of mulberry leaves used as feed for silkworm.

Hamamura [1959] studied on food selection by silkworm larvae. Presence of the three stimulants is necessary for food selection by silkworms and the absence of any one of the stimulants inhibits feeding by the larvae.

Many workers experienced the nutritive values of several mulberry varieties [Hassanein and Shaarawy, 1962; Arai and Ito, 1963; Verma and Kushwaha, 1970]. They record moisture (75-80%), crude protein (24-36%), crude fat (3-4%), crude fiber (9-11%), ash (7-8%) and carbohydrates (12-20%) in healthy mulberry leaf. It is observed that the factors like nature of soil, season, temperature, frequency of irrigation, sunshine duration, varietal differences, ground water level, manurial application, pruning type, time of harvesting, transportation and preservation of leaves will largely determine the quality of leaves [Das and Sikdar, 1970; Kasiviswanathan et al., 1973; Basavanna et al., 1974; Kawase, 1975].

Abdullaev and Guseunova [1961] analyzed the chemical composition of mulberry leaves of some selected varieties and reported that, “Zarif” variety of Kura-Khachmos Zone had the highest content of proteins and total sugar. The Probed variety showed low amount of protein and total sugars.

Salmon et al. [1964] analyzed the chemical composition of mulberry leaves of tree and bush forms. The leaves of tree form had higher total nitrogen content than the bush form [OPH] Sulphur content was higher in bush form.
Abdullaev et al. [1966] studied chemical analysis of 10 promising mulberry varieties such as “Sikhgez” [control], “Zarif”, “Azari”, “Khanlar”, “Firudin”, “Amin”, “Tozlajan”, “Jakub”, “Kokuso70” and “Adrunli”. The highest content of protein Nitrogen and protein was noted in “Zarif”, “Khanlar”, “Jakub”, “Tozlajan”. Similarly, high content of soluble carbohydrates were recorded in “Zarif” and triploid variety “Khanlar” displayed the best growth development and yield.

Achundova [1966] studied the relationship between the nitrogen and protein content on the level of RNA in promising mulberry varieties. He showed young vigorously growing leaves had high RNA. Aging was accompanied with drop in RNA. Age related changes in RNA level was accompanied by changes in nitrogen and protein content. There was direct correlation between the RNA and protein contents in leaves.

Plaksina and Dzhafarov [1967] studied the chemical composition in mulberry leaf of some polyploid forms. The content such as nitrogen, sugars and carbohydrates were analyzed with respect to season. They reported significant changes in these mineral contents depending on the season.

Plaksina and Dzhafarov [1968] studied the total protein and nitrogen in the leaves of few polyploid forms of mulberry and reported that tetraploids of “Azerbaijan” showed high content of protein and nitrogen compared to diploid variety “Zarif”.

Plaksina et al. [1968] studied carbohydrate content in diploids and polyploids in 10 mulberry genotypes and reported that, differences were found in maltose and sucrose contents between polyploidy and diploids. However, Mannose content was high in polyploidy forms.
Vasuki and Basavanna [1969] studied the varietal differences in the content of total and soluble minerals of mulberry varieties grown in local condition. They reported that 26 varieties showed variation of 40-50% in soluble minerals. Total minerals widely varied from 13 to 22%.

Abdullaev et al. [1970] studied on biochemical properties of the leaves of triploid and tetraploid mulberries and reported that triploid varieties of mulberry among polyploids possessed high yield and nutritive leaves.

Mustafaev [1970] reported that triploid mulberry varieties performed better than diploids by giving higher leaf yield and higher contents of total nitrogen, protein, carbohydrate and vitamins in the leaves.

Alieva et al. [1971] studied the chemical composition of some central Asiatic forms of mulberry and reported that the polyploid forms frequently contain more phosphorus, carbohydrates and microelements than diploids.

Krishnaswami et al. [1971] reported that growth and development of silkworm larvae and ultimately the economic traits, such as yield, cocoon shell weight and silk percentage are greatly influenced by the nutritional level of the mulberry leaf which varies even among varieties of the same species.

Das and Prasad [1974] evaluated four polyploid mulberry varieties namely 2 tetraploids “T1” and “T20” and 2 triploids “Tr8” and “Tr10” against a diploid control for the leaf quality. Chemical analysis revealed a distinct higher protein content, higher total sugar content including reducing sugar and minerals in triploid varieties with higher
or equal moisture content and equal to less of fiber percentage.

Hotta [1975] stated that when mulberry leaves were stored, the starch will be broken down to sugars. He reported that the total chlorophyll content of fresh mulberry leaves ranges from 0.14% to 0.35% in weight. Top and bottom leaves contain lesser amount of chlorophyll compared to medium order ones.

Nakajima [1975] opined that the crude protein content was same in young and mature leaves but decreases with the age of leaf. He further reported that water content of leaves is large in young leaves [growing leaves] and it decreases gradually in mature leaves and increases again in declining leaves.

Anonymous [1975] opined that generally the nutritional status of mulberry leaves depends upon the levels of moisture, total protein, total carbohydrates and total minerals.

Horie [1978, 1980] reported that for better growth of silkworm larvae optimum protein level required is 20-25%.

Sudo, M. et al. [1979] opined that the quality of mulberry leaves varied with the leaf order (degree of maturity) and it influenced remarkably the larval growth and development.

Thangamani and Vivekanandan [1984] studied on physiological studies and leaf nutrient analysis in the evaluation of best seven mulberry varieties. They observed of all varieties tested, “MR₂” variety was found to be superior to others and also varieties. The total content of chlorophyll is higher in both “MR₂” and Japan varieties.
Li and Sano [1984] observed that high quantity of carbohydrates and lower levels of water and proteins in the feed resulted in slower larval growth, less body weight and cocoon weight.

Hanif and Islam [1987] reported the variation in nutrient content of mulberry leaves of different ages and their effect on cocoon characters.

Sreedhara et al. [1988] studied the changes in protein, sugar, and chlorophyll and moisture content level in developing mulberry leaves. They found that protein and moisture content were high in growing leaves.

Bose [1989] reported that chemical composition of mulberry leaves varies depending on the mulberry variety, season, temperature, length of sunshine, nature of the soil, kind of fertilizer used, level of ground water, method of raising etc.

Sathyanarayana Raju et al. [1990] recorded higher moisture percentage, carbohydrates, proteins, amino acids and mineral contents in the mulberry variety “S₄₁” when compared to “S₃₀”, “S₃₆” and “K₂”.

Bose et al. [1991] recorded the highest percentage of reducing and non-reducing sugars, starch, total carbohydrates, crude proteins and total free amino acids in variety “S₄₁”.


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They recorded significant variation in all biochemical constituents of leaves namely moisture content, total protein, sugars and chlorophyll content in tender leaves. Reported that top tender leaves are nutritionally rich compared to medium and mature leaves.

Machii and Katagiri [1991] studied on varietal differences in nutritive values of mulberry for rearing silkworm with special emphasis placed on nitrogen and amino acid contents. The result indicated that there were varietal differences in nutritive values of mulberry leaves.

Mogili et al. [1992] studied on physiological effects of stomatal characters in mulberry at three ploidy levels. A high yielding mulberry cultivar, “Kanva-2” of Morus alba is used in the present study after evolution of triploids and tetraploids. The comparison of different parameters such as stomatal dimension, chlorophyll content etc. revealed that they are higher in triploids tetraploids than in diploids.

Chikkanna et al. [1993] reported that the leaves in case of variety S54 contain more amounts of crude protein, free amino acids, total minerals and total soluble sugars than Kanva-2.

Bongale and Chaluvachari [1995] studied eight mulberry varieties namely “Mysore Local”, “Berhampore”, “S1”, “S36”, “Kanva-2”, “DD”, “RFS-175”, “KNG” and “English Black” for moisture content, total proteins, sugars and chlorophyll contents in tender leaves. Significant differences were recorded among varieties with respect to all the biochemical constituents studied.
Chaluvachari and Bongale [1995] worked on ten tropical varieties of mulberry along with four varieties of temperate origin from the Germplasm bank maintained at the Institute’s campus were evaluated [using tender leaves] employing leaf chemical analysis and bioassay moulting test. Significant differences were recorded among the varieties in respect of leaf biochemical components.

Bose et al. [1995] reported that succulent mulberry leaves with less fiber and higher mineral contents stimulate the metabolic activities in silkworm resulting in quantitative improvement of cocoon and silk. “S1635” appears to be the best in this respect.

Fotadar and Dandin [1997] carried out biochemical studies at Central Sericultural Research and Training Institute, Kashmir, to confirm and quantify the superiority of ten elite genotypes. Observations showed higher values of soluble proteins in “Ichinose”, “Chinese white”, “C4” and “Kairyoroso”. Moisture content and moisture retention capacity varied among the genotypes. Quantity of crude fiber and minerals was found maximum in “Goshoerami”.

Singhvi et al. [2000] conducted a study with ten mulberry genotypes “S34”, “S13”, “S54”, “S1”, “S799”, “S36”, “RFS135”, “K2”, “Mysore Local”, all diploids and “Tr10” triploid and found the varieties under study differed distinctly in respect of leaf nutrient composition.

Bose and Bindroo [2001] analyzed the biochemical constituent and leaf yield of seven promising mulberry (Morus alba L.) varieties and reported “Chak majra” as the best variety followed by “Chinese white” based on the nutritional and yield parameters. They also reported that, no single variety consists of all the nutrients at the highest level.
Kumar et al. [2002] has identified “V₁” as a superior genotype for cultivation as a tree. “V₁” was found to be on par with many other genotypes namely “S₁₃”, “S₃₆”, “S₆₄₂”, “Ber-S₁”, “BR₂”, etc., with respect to protein, chlorophyll content and leaf yield when grown as a tree.

Sinha et al. [2003] studied on leaves at different stages of maturity of the seven mulberry varieties “S₁₆₃⁵”, “V₁”, “SV₁”, “MR₂”, “RFS₁₇₅”, “JRH” and “Jatituni” grown under the agroclimatic conditions of Jorhat, Assam [India] for five biochemical constituents during spring, rainy and autumn rearing seasons. The results revealed that “S₁₆₃⁵” is the best among the seven mulberry varieties followed by “SV₁” and “JRH” in respect of the biochemical constituents studied. The analyses further indicated that moisture and protein content was decreased gradually with age from apical end of the branch and in all seasons. But the reverse trend was obtained in respect of total minerals, total carbohydrates and crude fiber content in all variety in all season.

Subramanian and Temkar [2004] studied on comparative biochemical constituents in tender, medium and bottom mulberry leaves in four mulberry varieties viz., “V₁”, “DD”, “S₃₆” and “K₂”. Irrespective to position of leaves maximum contents of moisture, total minerals and reducing sugars where registered in “S₃₆”. Similarly the highest content of crude protein, crude fiber, non reducing sugars and total sugars were observed in “V₁”. The tender leaves of “V₁” recorded the highest crude protein, non reducing sugars and total sugars.

Jalja et al. [2008] studied the leaf quality characters of seven mulberry genotypes “V1”, “V2”, “V4”, “K2”, “S13”, “S36” and “S54” under field conditions of CSR&TI, Mysore. The quality traits viz., leaf moisture, moisture retention (after 6 hrs of harvest), protein, nitrogen, carbohydrate, amino acid contents were found to be high in “V1” followed by “V4” and “S36”.

2.4 STUDIES ON LEAF MOISTURE CONTENT

In mulberry, leaf moisture plays a vital role in improving nutritive levels of leaves which intern improve the palatability of leaves of silkworm. Hence, the moisture content in the leaves may serve as one of the criteria in estimating their quality. Many scientist reported favorable effects of high moisture content of leaves on their palatability and digestibility by silkworm.

Sudo and Yammamota [1953] observed that the moisture content of mulberry leaves decreased gradually with growth of the mulberry. It is demonstrated that for successful rearing, maintenances/retention of sufficient moisture content in the leaves for prolonged duration is of immense importance [Ito and Arai, 1963].

Parpiev [1968] observed that high moisture and moisture retention capacity in the leaves has favorable effects on the palatability and assimilability of nutrients and serves as criteria in estimating the leaf quality.

Waldbauer [1968], Kasiviswanathan et al. [1973] and Paul et al. [1992] observed in their studies that availability of moisture content in the leaves enhances the feeding efficiency of the larvae, which in turn increases the growth rate.

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Thangamani [1986] estimated the moisture content in some indigenous mulberry varieties and reported that “MR2” variety contain higher moisture content [74.85] when compared to “K2” [71.38%] and “local” varieties [70.68%].

Dwivedi et al. [1986] observed 13.98% higher moisture content in the leaves of induced tetraploids of “RFS-135” over their control (diploids).

Sastry et al. [1988] reported that moisture content of the harvested leaf plays a vital role in cocoon crop production in tropical sericulture belt. After the harvest, the leaves lose their moisture gradually. The loss of moisture content was found to be less in “S30” and “S36” varieties, whereas the difference was not significant among other varieties.

Dwivedi et al. [1987] and Susheelamma et al. [1991] observed higher leaf water content in induced tetraploids of “S30”, “S36”, and “S41” genotypes when compared to their respective diploids.

Pathak and Vyas [1988] opinioned that the mulberry leaves contain significant amount of moisture during rainy season when compared to summer and winter seasons.

Fotadar et al. [1989] studied on evaluation and utilization of genetic variability in mulberry and reported that the percentage of moisture varied with the position of the leaf on the mulberry plant viz., tender, medium and coarse and with the three season’s spring, summer and autumn. He also observed that total minerals, reducing sugars, and total sugars go on decreasing from top to bottom.
Ninge Gowda [1990] reported the seasonal variation in moisture content among the Japanese mulberry varieties.

Sikdar [1993] studied on four each of high yielding evolved triploids and diploid clones of mulberry and observed that the leaves of the triploids are significantly superior for moisture, crude protein, sugar, starch and total carbohydrate contents and significantly inferior for total minerals than diploid leaves.

Vijayan et al. [1997] studied the leaf moisture of mulberry germplasm varieties and observed the variation in moisture content and moisture retention capacity among genotypes and season. They reported that moisture content and moisture retention capacity is under the influence of both genotypes and environmental factors.

Anilkumar and John [2001] studied the potential and prospects of utilization of agricultural by products for soil moisture conservation and also to evaluate the response of two varieties of mulberry to irrigate in relation to leaf quality. The effect of irrigation on leaf moisture content was significant over that due to no irrigation. Leaf moisture content was not significantly affected by soil moisture conservation practices. Varieties, levels of irrigation and soil moisture conservation practices influenced leaf protein content.

Patil et al. [2003] studied to identify the genotypes with better total moisture content and retention after 4, 8 and 24 hrs of harvesting in different order leaves at rearing room condition. Eleven mulberry varieties "V1", "S36", "S146", "S799", "S1635", "S1708", "TG", "C-20", "RFS-175", "Chinese white" and "M-5" were studied. The moisture content as well as retention was more in "S-1635", "RFS-175", "S-1708" as compared to other varieties.