



REVIEW ARTICLE

ANTICANCER EFFICACY OF SOME SELECTED VEGETABLES

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Abstract: The global cancer epidemic is rising continuously, placing further strains on the individuals, the families and the societies (in which they live). The number of cancer cases and related deaths worldwide, estimated to double over the next 20-40 years. Research over the past several decades suggests that a high intake of vegetables decreases the risk of several cancers both in experimental animals and in humans. Epidemiological studies point to the fact that long-term consumption of diet rich in vegetables reduces the risk of chronic diseases especially cancer (Temple and Gladwin, 2003). Chemoprevention, by the use of natural products, that can reverse / suppress or prevent carcinogenic progression, has become an appealing strategy to combat the dogma associated with increasing cases of cancers worldwide. Such diets can minimize exposure to deleterious substances, activation of procarcinogens and can maximize the intake of certain beneficial nutrients like isothiocyanates, unsaturated fatty acids, polyphenolic terpenoids (PPT), selenium, terpenes, etc. Current evidence suggests that garlic, green tea, tomatoes and soy intake as part of the diet may be useful in preventing various cancers. A number of exciting researches suggest that vegetables, fruits, whole grains, herbs, nuts and seeds contain an abundance of polyphenolic compounds, terpenoids, sulphur compounds, pigments and other natural antioxidants, that have been associated with protection from or treatment of conditions such as cancer. Therefore, we can say that natural products have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer and regular consumption of vegetables is associated with reduced risk of cancers and additive/ synergistic effects of phytochemicals in these vegetables are responsible for their potent antioxidant / anticancer activities.

Keywords: Vegetables, Anticancer, Antioxidant, Polyphenols, Terpenoids

INTRODUCTION

There is strong, consistent evidence that high intake of vegetables protect against various cancers. These protective effects of high vegetable consumption are attributed to the active micronutrients (vitamins and minerals) and non-nutritive components (phytochemicals) that exhibit a potential for modulating human metabolism in a manner favorable for the prevention of cancer. In other words, we can say that vegetables consumed in our daily diet could be a solution to this deadly disease by providing chemoprotective and chemotherapeutic remedy. Therefore, efforts are still being made for the search of effective naturally occurring anticarcinogens that would prevent, slow or reverse cancer development. A comprehensive review was conducted to assess the safety and efficacy of some vegetables in an attempt to prevent various diseases including cancer. A seminal description of vegetables that have been selected is given in the following pages. The information within braces is in order as: Family, English name, Hindi

name. This system has been followed throughout while describing the particular vegetable.

Abelmoschus esculentus [Malvaceae, Okra / Lady's Finger, Bhindi]

Okra is one of the popular nutritious vegetables of North-East African origin. It is valued for its edible green seed pods. Raw okra is 90% water, 2% protein, 7% carbohydrates and negligible in fat in a 100 gm amount and is rich in dietary fibre, vitamin A, vitamin C, vitamin K with moderate contents of thiamin, folate and magnesium. The genus *Abelmoschus* has been reported to be used for several ethno medicinal practices and have also demonstrated diverse pharmacological activities and possesses several phytochemical and nutritional properties as well as having no adverse effects on living cells. Pods / seeds and leaves of the plant are reported to be used as food in pharmaceutical industries and as traditional remedy all over the world (Liu, 2004). In a study conducted on the action of pectic rhamnogalacturonan (RG-1) obtained by hot buffer extraction of okra pods, results showed that okra RG-1 induces apoptosis in melanoma cells

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by interacting with galectin-3, thereby preventing cancer cell proliferation (Sengkhamparn *et al.*, 2009). The pectin inhibited the proliferation of highly metastatic mouse melanoma cells (B16 F10) by 75% after 48 hours of treatment and also increased the rate of programmed cell death (apoptosis) by nearly 23-fold (Vayssade *et al.*, 2010). A newly discovered component – lectin, obtained from water extraction from bhindi seed was studied for its anti-tumor effects against human breast cancer and skin fibroblast cells. The results showed that it induced significant cell growth inhibition (63%) in MCF-7 cells. The expression of pro-apoptotic caspase-3, caspase-9 and p21 genes was increased in MCF-7 cells treated with okra seed extract, compared to those treated with controls. In this study flow cytometry also indicated that cell death (72%) predominantly occurred through apoptosis. Thus, bhindi in its native form promotes selective antitumor effects in human breast cancer cells and may represent a potential therapeutic to combat human breast cancer (Monte *et al.*, 2014). In an elaborate study based on the green synthesis of gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) using pulp extract of *A. esculentus*, it has been demonstrated that pulp synthesized Au NPs (*via* green route) showed *in vitro* efficacy against Jurkat cells. Results of the study clearly showed that the IC₅₀ dose of Au NPs and Ag NPs is capable of significantly elevating intracellular reactive oxygen species and diminishing mitochondrial membrane potential, indicating the effective involvement of apoptosis in cell death (Mollick *et al.*, 2014).

***Brassica rapa* [Brassicaceae, Turnip, Shalgam]**

The turnip is a highly nutritious / starchy, root vegetable. It is one of the world's ancient vegetables having been cultivated for more than 4,000 years. The turnip's root is high in vitamin C (a natural antioxidant) and the turnip leaves sometimes eaten as "turnip greens" are a good source of vitamin A, folate, vitamin C, vitamin K and calcium. Turnip greens are also high in lutein (8.5 mg / 100 g). The vegetable is also a good source of carbohydrates, fibre, calcium, phosphorous, potassium and magnesium. This vegetable is strongly associated with a lower risk of developing numerous cancers due to the presence of phytochemicals which exhibit strong antioxidant activity (Amri, 2014). β -Phenylethylisothiocyanate is abundant in the peel, showed the highest content in turnip and inhibited the growth of human-derived hepatoma cell line (HepG₂) in a concentration-dependent manner (IC₅₀ value of 24.5 μ M), assessed by the MTT method (Hong and Kim, 2008). In another research, cellular viability of shalgam-treated cells compared to untreated controls was observed to vary in a dose-dependent manner, decreasing to 97.7 % at the lowest (50 μ g/mL) and to 59.3 % at the highest concentration (6400 μ g/mL) of shalgam extract. Viability of Caco-2 cells in the presence of black

carrot extract under same conditions and respective concentrations was 96.7 and 62.1%. Shalgam juice revealed higher inhibition on Caco-2 cells compared to untreated control group at the concentrations 3200 and 6400 μ g/mL ($p < 0.05$). In a previous study, anthocyanins associated with black carrot juice (2000 μ g/mL) were shown to display antiproliferative effect on HT-29 colorectal carcinoma cells (Netzel *et al.*, 2007) suggesting that the potential antiproliferative activity of shalgam juice might be associated with its black carrot-associated anthocyanins. Nevertheless, in this study, shalgam juice exhibited statistically higher antiproliferative activities than black carrot juice at 3200 μ g/mL ($p = 0.029$) (Ekinci *et al.*, 2016). Anticancer activity of turnip was also examined in the human lung (A-549) cancer cell line (ATCC#CCL-185) and it produced a considerable anticancer effect and moderate antioxidant effects (Saeed *et al.*, 2012; Farag and Motaal, 2010). The cytotoxic effect of aqueous extract of *B. rapa* roots was also studied in three types of cancer cell lines-Hep-2, AMN-3 and HeLa *in vitro*. The results showed that the cytotoxic effect of the extract dependent on type of cells, amount of dose and exposure time. The concentration 1250 μ g/ml gave higher growth inhibition (63 and 42%) against ANM-3 and Hep-2 respectively. The inhibition rate of 10000 μ g/ml crude roots extract against HeLa cells was 64% after 24 hours exposure (Barkat *et al.*, 2010). An antifungal peptide (9.4-kDa) designated as campesin was isolated from seeds of the plant. It inhibited proliferation of HepG₂ and MCF-7 cancer cells with an IC₅₀ of 6.4 μ M and 1.8 μ M respectively (Linn *et al.*, 2009).

***Capsicum annuum* [Solanaceae, Capsicum or Sweet/Colored Pepper, Shimla Mirch]**

Capsicum annuum is generally considered in culinary context to be vegetable and has both nutritional and nutraceutical importance (Govindarajan, 1986). Capsicum is rich source of vitamin C, E and bioactive compounds particularly polyphenols, β -carotene and zeaxanthin (Daood *et al.*, 1996; Hervert *et al.*, 2010). Capsicum is the only genus having the potential to produce capsaicinoids, with capsaicin and dihydrocapsaicin accounting for up to 90% of the total pungency of pepper fruits (Backonja *et al.*, 2010). Capsicum is widely used in traditional medicine and capsaicin was shown to be a potent angio inhibitory compound *in vitro* and *in vivo*. Capsaicin was also found to repress the growth of various immortalized or malignant cell lines through the induction of apoptosis and the inhibition of angiogenesis (Jung *et al.*, 2001; Kim *et al.*, 2004; Min *et al.*, 2004). Capsaicin is the major constituent that accounts for the pharmaceutical properties of pepper. It has analgesic effects and is used against arthritis pain and inflammation (Lara *et al.*, 2008). It also showed anticancer activity, protective effects against high cholesterol levels, obesity and activity against neurogenic inflammation (Moore and Moore

2003; Palenius and Ochoa-Alejo, 2005). The studies showed growth inhibition of human breast cell lines *in vitro* using different pepper extracts by slowing down the cell cycle progression through phase G1-S (Molnar *et al.*, 2004; Dou *et al.*, 2011). Researchers correlated significant growth arrest and apoptosis with the capsaicin content and its accepted mechanism of anticancer activity through the generation of reactive oxygen species (ROS) especially hydroxyl radicals (Yang *et al.*, 2009). Capsaicin and dihydrocapsaicin are the most abundant capsaicinoids in pepper fruits (Bernal and RosBarcelo, 1996; Walpole *et al.*, 1996). Diverse studies have shown that capsaicin has antiproliferative effect on several human cell lines derived from multiple myeloma (Bhutani *et al.*, 2007), gastric cancer (Kim *et al.*, 1997), pancreatic cancer (Zhang *et al.*, 2008), breast cancer (Chou *et al.*, 2009) and prostate cancer (Mori *et al.*, 2006). Capsaicin also produces reactive oxygen species in cells with resultant induction of apoptosis and cell cycle arrest, which is beneficial for cancer chemoprevention with inhibitory effects on cancer development in multiple organs such as stomach, lung and liver (Kundu and Surh, 2009). Furthermore, capsaicin had strong apoptotic activity in B16-F10 cells *via* the down-regulation of Bcl-2 (Jung *et al.*, 2007). These results suggest that capsaicin could have an effective role in the management of melanoma cancer patients (Shin *et al.*, 2008).

***Chenopodium album* [Amaranthaceae, Bathua, Bathu]**

Chenopodium album is a herbaceous vegetable plant usually cultivated as pot-herb or grown in gardens. Besides alkaloids (trigonelline and chenopodine), the plant contains essential oils, potassium & vitamin C (Sikarwar *et al.*, 2013). *C. album* is an important medicinal plant with diverse pharmacological spectrum and possesses various activities like anticancer, hepatoprotective, antioxidant, antibacterial and anti-inflammatory (Shaneza *et al.*, 2016). The cytotoxic and antioxidant properties of lipophilic compounds extracted from different parts of four *Chenopodium* species (*C. album*, *C. hybridum*, *C. rubrum* and *C. urbicum*) were evaluated. Large amounts of free polyphenols were observed in herb extracts of *C. album* (3.36 mg/g). The cytotoxic activities of the extracts were assessed against human lung carcinoma (A-549), ovarian carcinoma (TOV-112D) and normal human fibroblast cell lines. This study demonstrated that the extracts from herb and seeds of *C. album* showed the significant antiproliferative effect on the TOV-112D cell line. Toxicity of the extract of *C. album* to skin fibroblasts was also high. The mortality of cells after 72 h amounted to 95% in *C. album* and in seeds (0.2 mg/cm³) lower cytotoxic activity toward cells of metastatic ovarian carcinoma (55%) was observed. Moreover, 30% activity of this extract to human cells of pulmonary carcinoma was demonstrated, which

was likely to exert cytopathic effects (65%) on skin fibroblasts (Nowak *et al.*, 2016). In another study aimed to investigate the effect of *C. album* (leaves) on the growth of estrogen dependent (MCF-7) and estrogen independent (MDA-MB-468) human breast cancer cell lines, the different solvent extracts (petroleum ether, ethyl acetate and methanol) were assessed for their cytotoxicity using Trypan Blue exclusion and MTT bioassay. Among the various extracts studied for two cell lines, methanolic extract of *C. album* exhibited maximum anti breast cancer activity having IC₅₀ value 27.31 mg/ml against MCF-7 cell line. Significant percent inhibition (94.06%) in the MeOH extract of *C. album* at 48 h of exposure and concentration 100 mg/ml (p < 0.05) against MCF-7 indicates the presence of some structural moiety responsible for this observed antiproliferative effect (Khoobchandani *et al.*, 2009).

***Cucurbita moschata* [Cucurbitaceae, Pumpkin, Kaddu]**

Pumpkin is a popular and nutritious vegetable consumed worldwide and consists of many beneficial nutrients such as phytoestrogen, selenium, fiber, cucurbitacin E, calcium, zinc, other vitamins, minerals that are not only beneficial for cancer prevention, but also for curing many diseases. The pumpkin plant is considered as a super food for many diseases including cancers, especially gastrointestinal cancer (Wang *et al.*, 2012). The cucurbitacins are the most important compounds in cucurbitaceae family. These compounds are anticancer natural triterpenoids and have free radical scavenging effect / antioxidant activity (Jafarian *et al.*, 2012). Cucurbitacin E, is a tetra cyclic triterpenoid (C₃₂H₉₉O₈) that is extracted from plants of cucurbitaceae family. Cucurbitacin E prevents cancer by inhibiting the action of JAK₂ and STAT₃ phosphorylation. Cucurbitacin E has anti-proliferating effect *via* its effect on actin filament in endothelial cells. Actin is an important intermediate in signalling pathways of cell division control, so cucurbitacin E has an inhibitory effect on cell growth (Colagar and Souraki, 2012). Recently, a number of studies have indicated that cucurmosin from pumpkin has cytotoxic properties and induces apoptosis in a number of human tumor cells. Thus, cucurmosin was extracted from pumpkin and *in vitro* studies have shown that it inhibits the proliferation of murine melanoma B16, lung adenocarcinoma A-549, human chronic myelogenous leukemia K562 and human pancreatic PANC-1 cancer cells (Hou *et al.*, 2008; Xu *et al.*, 2009). Cucurmosin induces apoptosis of human PANC-1, HL60 and K562 cells and induces the differentiation of B16 cells (Xie *et al.*, 2006). Based on its cytotoxic activity against multiple human cancer cells through the induction of apoptosis / differentiation, it was hypothesized that cucurmosin is a candidate agent for human hepatoma treatment / chemoprevention. The results of the *in vitro* and *in vivo* studies have demonstrated that it is

a promising agent in inhibiting the growth potential of hepatoma HepG₂ cells (Xie *et al.*, 2012). In another research treatment with cucurbitacins B and E, showed growth inhibition accompanied by apoptosis and cell cycle arrest in breast cancer cell lines (MDA-MB- 231 and MCF-7) (Sun *et al.*, 2005). These compounds also modulated the expression of proteins involved in cell-cycle regulation in both of the estrogen-independent (MDA- MB-231) and estrogen-dependent (MCF-7) in human breast cancer cell lines (Blaskovich *et al.*, 2003). Growth inhibition and cytotoxic effect of cucurbitacin B on breast cancer cell lines- SKBR-3 and MCF-7 were attributed to G2/M phase arrest and apoptosis. Cucurbitacin B treatment inhibited Cyclin D₁, C-Myc and β -catenin expression levels, translocation to the nucleus of β -catenin and galectin-3(Dakeng *et al.*, 2012).

***Lagenaria siceraria* [Cucurbitaceae, Bottle Gourd, Lauki]**

Lagenaria siceraria, an annual herbaceous climbing plant, can be found in the forests of India, Moluccas and Ethiopia. Its aerial parts / fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, Brazil, Hawaiian Island and European Countries for its cardio tonic, general tonic and diuretic properties (Tyagi *et al.*, 2012). The plant has been suggested to possess antioxidant, antihelmintic, antihypertensive, cardioprotective, hepatoprotective, central nervous system stimulant and free radical scavenging activity. The fruit is reported to contain vitamins (B, C), the triterpenoid cucurbitacins (B, D, G, H), two sterols (fucosterol and campesterol), aerpe nebyonolic acid (an allergic compound), flavone-C glycosides (a ribosome inactivating protein) and lagenin (Minocha, 2015). A study was carried out to evaluate the anti-cancer activity of methanol extract of aerial parts of *L. siceraria* on Ehrlich's Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with aerial parts (200 and 400 mg kg⁻¹) and standard drug 5-Fluorouracil (20 mg kg⁻¹) was continued for 9 days. Evaluation of the effect of drug response was made by the study of tumor growth response including increase in life span, study of hematological parameters, biochemical estimations and antioxidant assay of liver tissue. Experimental results revealed that bottle gourd possesses significant anticancer activity which may be due to its cytotoxicity and antioxidant properties. The anticancer activity of methanolic extract was assumed probably due to its flavonoid content (Saha *et al.*, 2011). In another study, the anti-cancerous properties of bottle gourd peel extract and gold nano particles synthesized from bottle gourd peel extract were evaluated *in vitro* against A-431 (skin carcinoma, p53mutant) and A-549 (lung carcinoma, p53 wild type) cells at different concentrations by MTT assay. In A-549 cancer cells, with harbour wild type p53 protein, gold nano particles showed only

marginal cytotoxicity up to 75 μ g/mL concentration. However, treatment of A-549 cells with gold nano particles at 100 μ g/mL caused around 25% survival loss while bottle gourd peel extract did not show any significant anti-cancerous property regarding to A-549 cell line. Although for A-431 cell line, results showed that extract exposure caused some appreciable loss in cell survival. The cells treated with 12.5 μ g/ml of gold nano particles decreased the cell viability by 40%. At 75 μ g/ml the percentage cell survival was 32.24% compared to 20.12% at 100 μ g/mL. The results showed that the cytotoxicity of gold nano particles in cancerous cell is quite effective which suggested that nano-gold possesses great selectivity to cancer cell and can display potential application in cancer chemoprevention (Kumara *et al.*, 2015).

***Momordica charantia* [Cucurbitaceae, Bitter gourd, Karela]**

Momordica charantia, also known as balsam pear, commonly consumed as vegetable, is widely cultivated in Asia, Africa and South America and extensively used in folk medicines as a remedy for diabetes, specifically in India, China and Central America. The fruit is oblong and resembles a small cucumber; young fruit is emerald green that turns to orange-yellow when ripe (Grover *et al.*, 2002). In India, various medicinal properties are claimed for *M. charantia* that include antidiabetic, abortifacient, antihelmintic, contraceptive, antimalarial, laxative and is used for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism, scabies. (Tomar, 2009). *M. charantia* is known to contain glycosides such as momordin, vitamin C, carotenoids, flavonoids and polyphenols (Anila and Vijayalakshmi, 2000; Raj *et al.*, 2005). Multiple types of extracts from bitter gourd had *in vivo* (Chiampanichayakul *et al.*, 2001; Nagasawa *et al.*, 2002; Kohno *et al.*, 2004) and *in vitro* (Yasui *et al.*, 2005) anticancer activity. Eleostearic acid (α -ESA), which is a conjugated linoleic acid that makes up 60% of bitter gourd seed oil, can block breast cancer proliferation and induce apoptosis through a mechanism that may be oxidation dependent (Grossmann *et al.*, 2009). *In vitro* studies using pure α -ESA have reported anticancer activity as α -ESA significantly reduced viability of transformed NIH-3T3 mouse fibroblast (SV-T2) and monocytic leukaemia (U-937) cells (Suzuki *et al.*, 2001). In additional reports, DLD-1 colorectal adenocarcinoma cells treated with α -ESA *in vitro* were growth inhibited and underwent DNA laddering indicative of apoptosis (Tsuzuki *et al.*, 2004). Both Caco-2 and HT-29 colon cancer cells had decreased viability and increased DNA fragmentation when treated with α -ESA (Yasui *et al.*, 2006). Human breast cancer cells (MCF-7 and MDA-MB-231) were used to assess the efficacy of bitter gourd extract as an anticancer agent and it was

found that the extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis (Ray *et al.*, 2010). MCP₃₀, a protein isolated from bitter gourd seeds selectively induces prostate cancer apoptosis (Xiong *et al.*, 2009). *M. charantia* was found effective on highly metastatic PC-3M prostate cancer cell line (Rao *et al.*, 2004). Fruit and leaf extracts (50% methanol) from *M. charantia* possess chemopreventive potential on dimethyl benz(a)nthracene (DMBA) induced skin tumorigenesis, melanoma tumor and cytogenicity (Agrawal and Beohar, 2010). Methanolic extract as well as momordin of bitter gourd showed cell toxicity against human cancer cell lines (Lee *et al.*, 1998). Chronic treatment with hot water extract of karela inhibited uterine adenomyosis and mammary tumor growth in mice (Nagasawa *et al.*, 2002). It was demonstrated that maximal anticarcinogenic activity is found in the peel of *M. Charantia* (Singh *et al.*, 1998). The crude aqueous extract from bitter gourd showed *in vivo* antitumor activity (Jilka *et al.*, 1983). Alcoholic extract from the leaves of bitter gourd have an anti-metastatic effect against rat prostate cancer progression both *in vitro* and *in vivo* (Pitchakaran *et al.*, 2010). 9, 11, 13-octadecatrienoic acid (α -eleostearic acid), a major linolenic acid in bitter gourd seeds strongly inhibited the growth of some cancer and fibroblast cell lines including those of HL-60 leukemia and HT-29 colon carcinoma (Kobori *et al.*, 2008).

***Portulaca oleracea* [Portulacaceae, Purslane/Littlehogweed, Kulta]**

Portulaca oleracea is a warm-climate, herbaceous succulent annual plant with a cosmopolitan distribution. It is grown extensively as a potherb and added in soups and salads around the Mediterranean and Tropical Asian countries. Diverse compounds have been isolated from the plant such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. It contains more omega-3 fatty acids (alpha-linolenic acid in particular) than any other leafy vegetable. It also contains vitamins-A, B, C, E, carotenoids and dietary minerals such as magnesium, calcium, potassium, iron (Simopoulos *et al.*, 1992). *P. oleracea* possesses a wide spectrum of pharmacological properties such as neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic and anticancer (Zhou *et al.*, 2015). An investigation was designed to study the anticancer activity of seed extracts of kulta on the human hepatocellular carcinoma cells (HepG₂). The HepG₂ cells were exposed with 5-500 μ g/ml of vegetable extracts for 24 h. The results showed that extracts significantly reduced the cell viability of HepG₂ in a concentration dependent manner. The cell viability was recorded to be 67%, 31%, 21%, and 17% at 50, 100, 250, and 500 μ g/ml of kulta respectively by MTT assay and 91%, 62%, 27%, and 18% at 50, 100, 250 and 500

μ g/ml respectively by NRU assay. Results also showed that plant exposed cells reduced the normal morphology and adhesion capacity of HepG₂ cells. HepG₂ cells exposed with 50 μ g/ml and higher concentrations of kulta lost their typical morphology, became smaller in size and appeared in rounded bodies (Farshori *et al.*, 2014). In another research, cytotoxic effects of seed oil of *P. oleracea* against human liver cancer (HepG₂) and human lung cancer (A-549) cell lines have been investigate. Both cell lines were exposed to various concentrations of *P. oleracea* seed oil for 24 h. The results showed a concentration-dependent significant reduction in the percentage cell viability and an alteration in the cellular morphology of HepG₂ and A-549 cells. The percentage cell viability was recorded as 73%, 63%, and 54% by MTT assay and 76%, 61%, and 50% by NRU assay at 250, 500, and 1000 μ g/ml, respectively in HepG₂ cells. Percentage cell viability was recorded as 82%, 72%, and 64% by MTT assay and 83%, 68%, and 56% by NRU assay at 250, 500, and 1000 μ g/ml respectively in A-549 cells. The 100 μ g/ml and lower concentrations were found to be non cytotoxic to A-549 cells, whereas decrease of 14% and 12% were recorded by MTT and NRU assay respectively in HepG₂ cells. Both HepG₂ and A-549 cell lines exposed to 250, 500, and 1000 μ g/ml of *P. oleracea* seed oil, lost their normal morphology, cell adhesion capacity, become rounded and appeared smaller in size (Al-Sheddi *et al.*, 2015). A subclass of homo isoflavonoids from the plant also showed *in vitro* cytotoxic activities towards four human cancer cell lines (Yan *et al.*, 2012).

***Raphanus sativus* [Brassicaceae, Radish, Mooli]**

Raphanus sativus is an essential vegetable crop in India and is thought to have originated in Southern China from where it has spread to Japan and other parts of Asia. Its roots and leaves are edible and it contains glucosinolates which release isothiocyanates by the action of myrosinase. Thus, the major glucosinolate of radish is glucoraphanin which after enzymatic hydrolysis by myrosinase, produces 4-methylthio-3-butetyl isothiocyanate (raphasatin). This compound which accounts for some of the strong taste of radish, has powerful antioxidant activity and shows selective cytotoxic activity towards some human cancer cell lines (Rakhmawati *et al.*, 2009). The plant also possesses antidiabetic (Rakhmawati *et al.*, 2011), diuretic (Saganuwari, 2010), antifertility (Mishra *et al.*, 2011), hypertensive (Talha *et al.*, 2011), antimicrobial (Shukla *et al.*, 2011), nephroprotective (Kumar *et al.*, 2013), gastroprotective (Alqasoumi *et al.*, 2008) and hepatoprotective (Anwar and Ahmad, 2006) efficiency. A study (*in vitro*) showed that *R. sativus* sprout extracts inhibited cell proliferation and induced apoptosis in cancer cells (Papi *et al.*, 2008). The study also confirms that ethanol extract of the aerial parts of radish is capable of inducing apoptosis in MDA-MB-231 human breast cancer

cells. These results suggested that radish leaf may be a useful antitumor agent because it directly inhibits the growth of tumor cells and induces apoptosis (Kim *et al.*, 2011). In another study, the anticarcinogenic effect of radish in combating chemically induced colon cancer was evaluated. Results showed that radish significantly reduced serum CEA ($p<0.01$) and CA19-9 ($p<0.01$) as evidence of anticarcinogenic effect thus proving that the galactan polysaccharide has pronounced cytotoxic effects on colon cancer cell line and might be a suitable candidate as chemopreventive and adjuvant therapy for colon cancer (Mohamed *et al.*, 2013).

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REVIEW ARTICLE

HARNESSING MUTATION BREEDING FOR SUSTAINABLE CROP DEVELOPMENT: FROM PRINCIPLES TO SUCCESS STORIES

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Abstract: Mutation breeding has emerged as a powerful tool in crop improvement, offering a means to generate novel genetic variation beyond what is available in natural germplasm. By inducing mutations through physical and chemical mutagens, breeders have successfully developed improved varieties with enhanced yield, quality, stress resistance, and adaptation traits. Cereals, legumes, oilseeds, horticultural crops, and industrial crops have benefited extensively, with rice, barley, groundnut, soybean, grapefruit, cotton, and vegetatively propagated crops contributing notable success stories. The integration of modern molecular platforms such as TILLING, Mut Map and next-generation sequencing has transformed mutation breeding from a largely random process into a targeted and high-throughput strategy, enabling rapid allele mining and gene discovery. Despite challenges related to large-scale screening, epigenetic instability, linkage drag, and perception issues, advancements in high-throughput phenotyping, predictive breeding, and genomic selection are significantly improving the efficiency of mutant detection and deployment. With growing emphasis on climate resilience and sustainable agriculture, mutation breeding remains a complementary approach to genome editing, capable of creating unique alleles and offering regulatory advantages in many regions. This review highlights the principles, technological innovations, limitations, and success stories of mutation breeding, underscoring its enduring relevance in developing future-ready, sustainable crop varieties.

Keywords: Mutation, Breeding, Crop improvement

INTRODUCTION

Mutation breeding has emerged as a pivotal approach in modern crop improvement, enabling the creation of new genetic variations that are often difficult to obtain through traditional breeding methods. By inducing heritable changes in the DNA sequence using physical, chemical, or biological mutagens, mutation breeding accelerates the development of improved crop varieties with desirable traits such as enhanced yield, disease resistance, abiotic stress tolerance, and superior nutritional quality. The suitability of crops for mutation breeding depends on several factors, including their reproductive behaviour, genetic

background, and mutation frequency. Both genetic and epigenetic mechanisms play crucial roles in determining the stability and expression of induced mutations, influencing the success of trait development.

Advancements in molecular biology, high-throughput screening, and genomic tools have significantly strengthened mutation breeding by facilitating precise detection, characterization, and selection of beneficial variants. Applications of mutation breeding are widespread across cereals, pulses, oilseeds, fruits, and ornamentals, contributing notably to global food and nutritional security. Numerous success stories—such as improved rice, wheat, barley, banana, and groundnut cultivars—

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highlight the practical relevance and commercial impact of this technology. As climate change intensifies stress conditions and threatens agricultural productivity, mutation breeding continues to be a valuable and sustainable strategy for delivering innovative crop improvements to meet the needs of a growing population.

Since the first reports of induced mutations in the 1920s and the post-World War II expansion of radiation facilities, mutation breeding has produced a rich catalogue of mutant phenotypes used directly as cultivars or as sources of useful alleles in conventional breeding programs (Maluszynski *et al.*, 2000). As of recent counts, thousands of mutant varieties (across cereals, legumes, oilseeds, horticultural and industrial crops) have been officially released worldwide and are recorded in the FAO/IAEA Mutant Variety Database (MVD). Mutation breeding remains especially relevant for crops with narrow genetic bases, vegetatively propagated crops, and traits that are difficult to access through conventional recombination. In addition, the complementarity of induced mutations with molecular techniques (TILLING, sequence-based allele mining, and targeted genome editing) has revitalized interest in induced mutagenesis as part of integrated breeding pipelines (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023).

This review presents a comprehensive synthesis of methods, crop suitability, genetic and epigenetic determinants of mutant phenotypes and stability, applications, and case studies of successful mutant varieties, followed by practical recommendations for modern mutation-based programs.

METHODS OF INDUCING MUTATIONS

Physical Mutagens

Physical agents—primarily ionizing radiations (gamma rays from $^{60}\text{Co}/^{137}\text{Cs}$ sources, X-rays, fast neutrons, ion beams) are the most widely used mutagens in plant breeding. Gamma irradiation historically produced the bulk of mutant varieties, while newer techniques (ion beams) generate different mutation spectra (large deletions, chromosomal rearrangements) that can be useful for functional genomics and creating loss-of-function alleles (Mba, 2013). Ion beam mutagenesis is increasingly used for higher mutation density and diverse lesion types (Ghanim, 2024).

Chemical Mutagens

Chemical mutagens such as ethyl methane sulfonate (EMS), ethyl nitrosourea (ENU), sodium azide (NaN_3), and nitrosomethylurea introduce point mutations (mostly $\text{GC} \rightarrow \text{AT}$ transitions with EMS) and are valuable for generating allelic series and point mutations useful in reverse genetics (TILLING platforms). Chemical mutagenesis is favored when subtle allelic variation (e.g., enzyme activity modulation) is desired (Mba, 2013; Nerkar *et al.*, 2022).

Biological and Insertional Mutagenesis

Transposable elements, insertional mutagenesis using T-DNA or transposons, and somaclonal variation from tissue culture provide alternative sources of novel variation, often with tagging advantages for gene cloning.

Targeted and Precision Mutagenesis

While traditional random mutagenesis remains important, modern targeted technologies (CRISPR/Cas and other genome editors) provide precise allelic variants. Although CRISPR is not "mutation breeding" in the classical sense, it complements mutation breeding by enabling precise recreation of beneficial induced alleles or rapid functional validation of mutants (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023).

Significance of Mutation Breeding

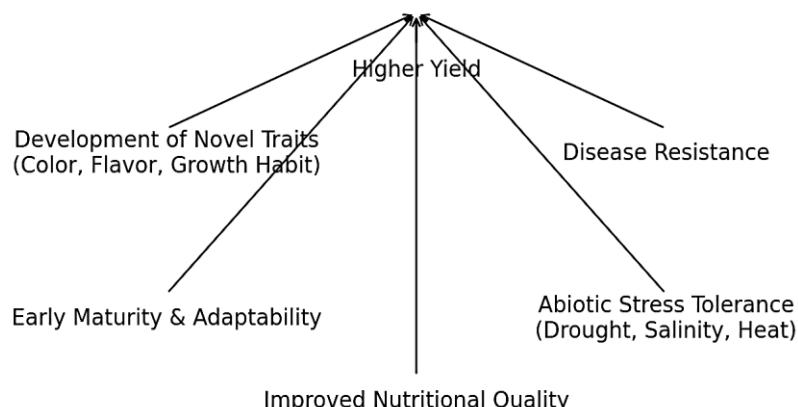


Figure 1: Importance of Mutation Breeding in Crop Improvement for Sustainable Agriculture. Mutation breeding contributes significantly to crop improvement by enhancing yield, strengthening disease resistance, increasing tolerance to abiotic stresses, enabling early maturity, improving nutritional quality, and generating novel traits for better adaptability and performance.

Screening, Selection and Validation Strategies

A major bottleneck in mutation breeding is the efficient identification of desirable mutants within large M1/M2 populations. To overcome this, several strategies are adopted, including phenotypic screening under controlled and stress environments such as drought, salinity, and disease inoculation, allowing the selection of visible beneficial traits. Molecular screening approaches like TILLING and targeted amplicon sequencing help detect specific point mutations in candidate genes, improving precision in mutant detection. High-throughput phenotyping (HTP) technologies-including imaging tools, UAV-based monitoring, and automated screening platforms-further enhance the rapid assessment of physiological and architectural traits across large populations. Additionally, backcrossing and trait validation steps ensure the genetic stability and agronomic suitability of selected mutants for commercial cultivation (Ghanim, 2024). Advances in next-generation sequencing (NGS) alongside TILLING have significantly accelerated allele discovery in chemically mutagenized populations, ultimately reducing the time required for trait-specific variety development and release (Mba, 2013; Nerkar *et al.*, 2022).

Crops Most Suitable for Mutation Breeding

Mutation breeding is applicable across diverse crop groups, but its success and adoption vary depending on biological and agronomic characteristics. Cereals such as rice, barley, and wheat have historically seen the highest number of mutant varieties, mainly due to large cultivation area, easy seed propagation, and the presence of distinct phenotypic traits like semi-dwarfism, maturity period, and grain quality. Rice alone contributes a major portion of the mutant variety database (Mba, 2013). Similarly, legumes and oilseeds including groundnut, soybean, and rapeseed have benefitted significantly from mutation breeding, leading to improvements in oil quality, disease resistance, and growth duration. Horticultural crops such as grapefruit, ornamental plants, and fruit trees have also been improved through induced mutations, particularly for traits like fruit colour, seed lessness, and modified flowering behaviour. Well-known examples include the grapefruit mutants 'Star Ruby' and 'Rio Red' (FAO/IAEA; Mba, 2013). In vegetatively propagated crops like cassava, banana, potato, and sugarcane, mutation breeding combined with in vitro techniques has contributed to the development of early-maturing, virus-resistant, and modified starch-type cultivars (Ghanim, 2024). Additionally, industrial crops such as cotton and other fibre crops have seen fibre quality improvements through induced mutants, benefitting the textile industry. Overall, the suitability of mutation breeding is strongly influenced by crop biology including life cycle duration, mode of

propagation, feasibility of mutagenesis, and availability of efficient screening techniques.

GENETIC BASIS OF MUTANT TRAITS

Types of Mutations and Effects

Induced mutations range from single nucleotide polymorphisms (SNPs) to large deletions, inversions, and chromosomal rearrangements. The phenotypic effect depends on mutation type (loss-of-function vs gain-of-function), gene redundancy, and interaction with the genetic background. EMS tends to produce point mutations (useful for TILLING), whereas gamma rays and neutrons often cause larger structural changes (Mba, 2013).

Dominance, Epistasis and Background Effects

Mutant phenotypes may be recessive, dominant or partially dominant. Many useful traits (e.g., semi-dwarfism) are often semi-dominant and readily selected in early generations, while complex traits (yield, tolerance) involve epistasis and require multi-generation evaluation and backcrossing to elite backgrounds (Figure 1) (Mba, 2013).

Mutation Rate and Spectrum

Mutation spectrum is mutagen-specific. EMS produces high density of point mutations (good for saturation mutagenesis), whereas ion beams provide diverse lesion types. Mutation rate optimization (dosage, exposure time) balances survival and mutation frequency; LD50 estimation is standard practice (Shahwar *et al.*, 2023).

Epigenetic Factors: Role and Application in Mutation Breeding

Epigenetics in Plants-Overview

Epigenetic modifications (DNA methylation, histone modifications, chromatin remodelling, small RNAs) modulate gene expression without altering DNA sequence and can be heritable across generations in plants (Tonosaki *et al.*, 2022; Tirnaz & Batley, 2019). Environmental stress, tissue culture, and mutagenic treatments can induce epigenetic changes that affect phenotype.

Epigenetic Variation as a Breeding Resource

Epigenetic variants (epialleles) provide additional heritable variation that can be harnessed for traits such as stress tolerance, flowering time, and metabolic traits. The concept of "epibreeding" proposes selection of stable epialleles or inducing beneficial epigenetic states through treatments (e.g., demethylating agents) or selection after tissue culture (Gupta *et al.*, 2022; Tonosaki *et al.*, 2022).

Interaction Between DNA Mutation and Epigenetics

Mutagenesis can induce both DNA sequence changes and epigenetic modifications. Furthermore, some induced mutations may affect genes involved in epigenetic regulation (methylases, chromatin factors), resulting in transgenerational epigenetic

effects. Conversely, epigenetic changes can modulate the expression of induced mutant alleles, altering penetrance and expressivity (Cao *et al.*, 2024; Varotto *et al.*, 2020).

Success Stories and Representative Varieties

Successful mutant varieties documented in the FAO/IAEA Mutant Variety Database (MVD) and literature. These examples illustrate the range of traits and crops improved via mutation breeding (Table 1).

Table 1. Representative mutant varieties released

S. No.	Crop	Variety / Mutant Line	Key Improved Trait(s)	Mutagen Used	Country (Year)	References
1	Grapefruit (<i>Citrus × paradisi</i>)	Rio Red	Deep red flesh, improved juice quality	Thermal neutron / irradiation	USA (1976)	FAO/IAEA
2	Groundnut (<i>Arachis hypogaea</i>)	TG-26	Disease resistance, improved agronomy	Gamma rays / EMS	India	RCA brief
3	Rice (<i>Oryza sativa</i>)	Multiple commercially released mutants	Semi-dwarfism, yield, quality traits	Gamma rays / EMS	Asia, worldwide	Ma <i>et al.</i> 2021
4	Barley (<i>Hordeum vulgare</i>)	Multiple mutants (e.g., malt quality)	Quality improvement, lodging resistance	Gamma irradiation	Europe/Asia	Maluszynski <i>et al.</i> , 2000 IAEA
5	Wheat (<i>Triticum aestivum</i>)	Dwarf & rust-resistant mutants	Semi-dwarfism, disease resistance	Gamma rays / chemicals	Global	FAO/IAEA; Ma 2021
6	Satsuma Mandarin (<i>Citrus unshiu</i>)	Gwonje-early	Early maturity	Gamma irradiation	Korea (2024)	Eun <i>et al.</i> , 2024
7	Sugarcane	Mutant clones	High sugar yield, ratooning ability	Physical / chemical (Radiation, EMS)	Various	Barrientos-Alfaro <i>et al.</i> , 2025
8	Banana	Pisang SPM-130	Fusarium wilt resistance	EMS	Malaysia	Oladosu <i>et al.</i> 2016
9	Chickpea	Pusa 408 / Pusa 547	Disease resistance	Gamma ray / EMS	India	Oladosu <i>et al.</i> 2016

Successful Mutant Varieties

Rio Red and Star Ruby Grapefruit

Rio Red and Star Ruby (red-fleshed grapefruit cultivars) exemplify the horticultural impact of induced mutation. Thermal neutron and other radiation treatments produced fruit with deeper red flesh and improved juice quality, traits that became commercially valuable in the US and global markets. These cultivars illustrate how bud sports and irradiated nucellar lines can produce commercially dominant phenotypes (Da Graça *et al.*, 2004; Louzada & Del Rio, 2021).

Mutant Rice Varieties

Rice has the highest number of mutant entries in MVD. Mutant selections produced semi-dwarf phenotypes, early-maturing lines, and grain quality variants. These mutants have been used directly or as parents in breeding programs to improve plant architecture and adaptation (Mba, 2013).

Groundnut Mutants (TG-26 and Others)

Groundnut mutant varieties (e.g., TG-26) developed in India improved disease resistance and agronomic performance; multiple mutant groundnut cultivars have been released and cultivated widely. These cases demonstrate mutation breeding utility in improving legumes with narrow genetic bases.

Integration with Molecular Tools and Modern Breeding

Modern molecular tools have significantly enhanced the precision and efficiency of mutation breeding. TILLING (Targeting Induced Local Lesions in Genomes) is a widely used reverse genetics approach that screens mutant populations to identify allelic variations in specific genes without requiring transgenic methods, thus making it valuable for functional genomics and breeding programs (Mba, 2013). Additionally, high-throughput sequencing-based strategies such as MutMap and MutMap-G enable rapid identification of causal polymorphisms

in mutant lines, allowing breeders to associate mutations directly with target traits. Further advancement comes through integrated hybrid pipelines, where induced mutations generate novel alleles that are subsequently introgressed into elite cultivars using marker-assisted selection (MAS) or genomic selection, ensuring both efficiency and precision in trait improvement (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023). Collectively, these tools shorten the breeding cycle, accelerate the path from mutation induction to varietal release, and facilitate targeted allele mining for complex quantitative traits.

Achievements of Mutation Breeding

Higher yield, earliness, stress resilience, salt tolerance, waterlogging tolerance, and bold seed size are some of the benefits of mutant breeding. In wheat, rice, and barley, improved varieties have been generated by mutation breeding. In addition to high yield, improved quality, earliness, dwarfness, disease resistance, and reduced toxin content have all been created in numerous crop varieties. Mutation has also been employed to induce male sterility, which lowers the cost of hybrid seed production, increases the range of genetic variety, and aids crop adaptation. There have been 2252 mutant variants developed in plant species across the world. 1585 have been released directly, while 667 have been released using mutants in hybridization. 1700 mutant variants have been introduced in seed propagated crops and 552 in vegetatively propagated species, out of a total of 2252. Rice (434 mutant variants) has the most mutant varieties created among seed propagated species, followed by barley (269), and wheat (222). Radiations have resulted in the development of maximum varieties.

EMS resulted in the production of maximal mutant variations among chemical mutagens [the most effective strategies for creating genetic variation and identifying critical regulatory genes for economically relevant features in crop development. Recent developments in genomics technology have resulted in a proliferation of genomic techniques in applied breeding, notably mutational breeding. Plant breeding has employed mutagenesis, or the act of creating mutations within an organism's genome. Induced mutagenesis and associated breeding tactics can improve quantitative and qualitative qualities in crops in a fraction of the time it takes to do so with traditional breeding. The global effect of mutation breeding derived agricultural types highlights mutation breeding's promise as a versatile and practical approach to any crop.

Challenges and Limitations

Despite its wide application and notable achievements, mutation breeding is accompanied by several challenges that constrain its full potential. One of the major limitations lies in the need for intensive screening, as selecting promising mutants from vast M2 populations demands substantial time, labor, and advanced phenotyping capacities. Another

concern is linkage drag and pleiotropy, where radiation-induced large chromosomal deletions may inadvertently remove beneficial linked genes or cause undesirable effects. Moreover, epigenetic instability arising from somaclonal variation or stress-related epigenetic changes can lead to inconsistent trait expression across generations. Public perception also plays a role, as mutation breeding, although non-transgenic, is sometimes misunderstood and may face resistance in certain regions. Additionally, intellectual property complexities and benefit-sharing issues can limit the accessibility of mutant-derived germplasm in global markets. Overcoming these limitations will require greater adoption of high-throughput phenotyping, strategic integration of genomic and molecular tools, and improved communication to build trust and awareness regarding the safety and advantages of induced mutants (Varotto *et al.*, 2020).

Practical Recommendations for Breeders

For effective implementation of mutation breeding, breeders should adopt strategic approaches that enhance mutation efficiency, screening accuracy, and stability of selected variants. First, conducting dose-response (LD50) assays is essential to determine the optimal mutagen concentration for each crop and genotype, ensuring sufficient mutation induction while minimizing lethal effects. Integrating random mutagenesis techniques such as EMS or gamma irradiation with targeted identification tools like TILLING and next-generation sequencing can greatly improve the ability to discover beneficial alleles, particularly for gene-specific improvement. High-throughput phenotyping systems and multi-environment evaluations should be utilized to efficiently detect mutants for complex and quantitative traits. Additionally, continuous monitoring of epigenetic stability is crucial, especially for mutants developed through tissue culture, where somaclonal variation can influence trait expression. Finally, breeders are encouraged to utilize resources such as the FAO/IAEA Mutant Variety Database (MVD) to locate existing mutants, prevent duplication of work, and facilitate the use of validated donor material for breeding pipelines (IAEA MVD, 2024).

Future Prospects

Mutation breeding is expected to remain a valuable and complementary approach alongside modern genome editing techniques. Its continued relevance is rooted in its ability to generate novel alleles that may not exist in natural gene pools, providing a unique resource for breeding programs, particularly in regions where stringent regulations limit the use of gene editing technologies. When combined with advanced molecular tools such as deep sequencing, TILLING, and MutMap methods, induced mutations can be identified, characterized, and deployed much faster than in traditional workflows. Emerging strategies like epigenetic selection, or epibreeding,

further expand the potential of mutation breeding by exploiting heritable epigenomic variation for traits related to stress tolerance and adaptability (Tonosaki *et al.*, 2022; Tirnaz & Batley, 2019). Moreover, the integration of mutation breeding with high-throughput phenotyping platforms, predictive breeding approaches such as genomic selection, and precision mutagenesis tools like ion beam irradiation offers a forward-looking path for accelerating the development of climate-resilient cultivars (Ghanim, 2024).

CONCLUSIONS

Mutation breeding has a proven track record and continues to be a practical and cost-effective approach to expand breeding variation. Its strengths lie in generating novel alleles, improving vegetatively propagated and under-resourced crops, and complementing molecular breeding. The future will see deeper integration of induced mutagenesis with high-resolution genomics, epigenomics, and phenomics, enabling faster delivery of resilient, high-performing varieties for food security and climate adaptation.

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RESEARCH ARTICLE

FLORISTIC DIVERSITY OF KOLLERU LAKE (RAMSAR SITE), ANDHRA PRADESH, INDIA

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Abstract: The present study focusses on the current status of the floristic diversity (Angiosperms) of Kolleru lake, Andhra Pradesh, India. The Kolleru Lake is a dynamic freshwater ecosystem with high ecological value and socio-economic importance for the region. It faces threats from anthropological activities but remains a critical site for biodiversity and a source of sustenance for its surrounding communities. In this contest the floristic studies have been conducted past few years during the filed explorations a total of 256 species plants belonging to 191 genera and 55 families were reported of these 167 herbs, 14 climbers, 15 twiners, 26 shrubs 26, and 34 trees were reported. According to IUCN criteria, Endangered 1, Data Deficient 2, and Least Concern 109. The present documentation will help to better understand the botanical diversity in this unique protected area for further conservation and management of the sanctuary.

Keywords: Wetland ecosystem, Kolleru Lake, Ramsar Site, Floristic Diversity, Conservation, Management.

INTRODUCTION

Wetlands are areas that serve as a bridge between land and water ecosystems, typically characterized by their water table being close to the surface or having shallow water cover (Mitsch & Gosselink, 1986). These ecosystems play a vital role in supporting various aquatic, semi-aquatic, and moisture-loving plants and animals. Particularly in tropical and subtropical regions, wetlands are known for the abundant growth of aquatic vegetation. This vegetation functions as a natural biofilter, absorbing significant amounts of both organic and inorganic nutrients from nutrient-rich waters. As a result, wetlands are often referred to by experts as the 'Kidney of the Landscape' or the 'Biological Supermarket.' These areas, where the soil remains saturated with water, are vital incubators that foster high levels of species diversity (Allen-Diaz *et al.*, 2004). The value of the world's wetlands is increasingly receiving due attention as they contribute to a healthy environment in many ways. They retain water during dry periods, thus keeping the water table high and relatively stable. During periods of flooding, they mitigate floods and trap suspended solids and attached nutrients. Thus, streams flowing into lakes by way of wetland areas will transport fewer suspended solids and nutrients to the lakes than if they flow directly into the lakes. The wetlands are also important for their role in birds'

migratory routes, as well as for providing habitat for resident birds. The removal of wetland systems due to urbanization or other factors typically causes lake water quality to deteriorate. In addition, wetlands are important feeding and breeding areas for wildlife and provide a stopping place and refuge for waterfowl. As with any natural habitat, wetlands are important in supporting species diversity and have a complex of wetland values. This study provides a comprehensive account of the current floristic diversity within the Kolleru Lake wetland ecosystem. Earlier, a few related studies have been conducted on Kolleru Lake by (Rasingam & Harikrishna 2024), and (Vijayalakshmi *et al.*, 2018).

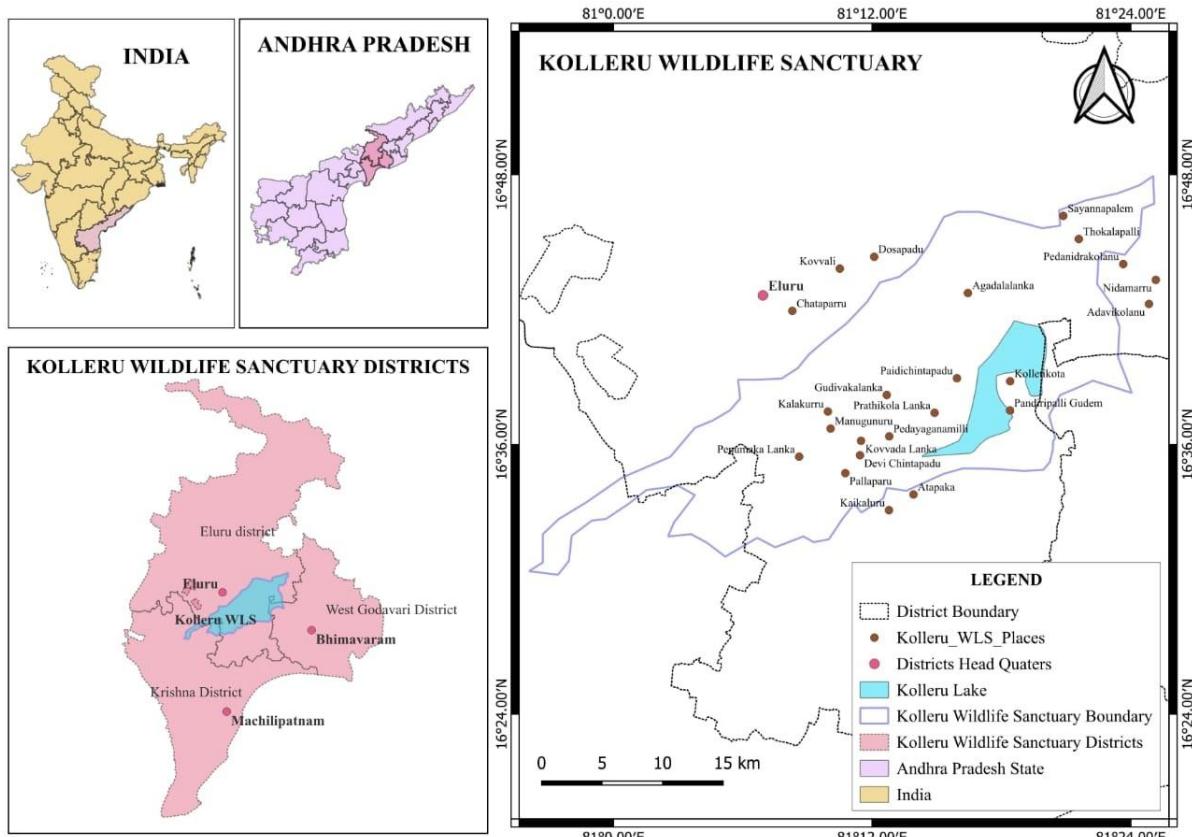
Study area

Kolleru lake (**Fig- 1**) is an important freshwater body located in the state of Andhra Pradesh, India. It is positioned between the Krishna River to the west and the Godavari River to the east, encompassing the Krishna, Eluru, and West Godavari districts of Andhra Pradesh. The lake comprises around 904 km² and has 144 villages, with 308 km² officially declared as a wildlife sanctuary. The area covers 260 km² in Krishna District's Kaikaluru and Gudivada taluks and the rest in Eluru, Bhimavaram, and Tadepalligudem taluks of West Godavari and Eluru Districts (Seshavatharam *et al.*, 1982). The geographical coordinates of the lake range from 16°24'14" to 16°48'40" N latitude and 80°55'17" to 81°27'32" E

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longitude, with an elevation ranging from 0 to 5 meters above mean sea level. Functioning as a natural flood-balancing reservoir for the Krishna and Godavari delta systems, Kolleru receives inflows from approximately 30 natural drains and channels, in addition to being fed primarily by two seasonal rivers, the Budameru and the Tammileru. The lake is connected to the Bay of Bengal via the Upputeru River, located approximately 32 km downstream, which facilitates the periodic drainage of excess water. The Kolleru wetland ecosystem comprises diverse habitats, including coastal freshwater lagoons and seasonal freshwater marshes or pools. The vegetation of the sanctuary mainly consists of emergent, submerged, and free-floating aquatic plants. Kolleru is recognized as a critical habitat for avifauna, supporting more than 200 species of resident and migratory birds, including notable species such as the grey pelican (*Pelecanus philippensis*), painted stork (*Mycteria leucocephala*), black-headed ibis (*Threskiornis melanocephalus*), and the spot-billed pelican

(*Pelecanus philippensis*). Due to its ecological significance, Kolleru Lake was designated as a Wildlife Sanctuary in 1999 and Ramsar site (Site No. 1209) on 19 August 2002, under the criteria outlined in Recommendation 4.7 of the Conference of the Contracting Parties (Anonymous, 2023). The establishment of the Kolleru Bird Sanctuary and the Atapaka Bird Sanctuary by the Government of Andhra Pradesh within the Ramsar site boundaries provides partial protection to both migratory and native avifauna. In addition to its ecological functions, the lake also plays a vital role in sustaining the livelihoods of the local population, serving as a source for fishing, agriculture, and ecotourism. The purpose of this research article is to explore and document the floristic diversity of the Kolleru Lake, Andhra Pradesh. It aims to analyze the spatial distribution of aquatic and terrestrial plant species, assess the influence of human activities on the Lake's ecosystem, and also assess human impacts and suggest conservation measures for sustainable wetland management.



METHODOLOGY

Extensive and systematic field explorations were carried out from 2023 to 2025 to assess the floristic diversity of the Kolleru Lake (Fig. 2), a significant freshwater wetland in Andhra Pradesh, India. Plants were photographed using a Nikon camera during the data collection and prepared as a digital herbarium. The collected specimens were processed

and mounted following the standard herbarium techniques outlined by Jain & Rao (1977), and Tomar (2024). These authenticated specimens were subsequently deposited in the Herbarium of Yogi Vemana University (YVU) for long-term preservation and future reference. The identification of plant species was carried out using the floristic literature, including Hooker's "The Flora of British India" (1872–1897), "The Flora of

the Madras Presidency" by Gamble & Fischer, (1915–1935), and "Flora of Andhra Pradesh" by Pullaiah *et al.*, (2018). The updated binomial nomenclature and author citations of all species

were validated using online taxonomic databases such as Plants of the World Online (POWO) and the International Plant Names Index (IPNI).



Figure 2: Different views of Kolleru Wildlife Sanctuary

RESULTS AND DISCUSSIONS

The present work enumerates 256 taxa (Fig: 3-5), representing 191 genera and 56 families. Dicotyledons are represented by 216 species belonging to 151 genera under 46 families, while monocotyledons are represented by 10 families, 40 genera, and 45 species. Out of the total 256 species, dicotyledons represented 82.75 % and monocotyledons 17.24 % respectively. An analysis of floristic composition reveals that Fabaceae is represented by the highest number of species 41 followed by Poaceae 20, Malvaceae 17,

Euphorbiaceae 17, Convolvulaceae 16, Amaranthaceae 13, Asteraceae 10, Acanthaceae 9, Apocynaceae 8, and Lamiaceae 8. According to IUCN criteria, 1 Endangered species, Data Deficient 2, and Least Concern 109. Of these 256, herbs represent 167, climbers 14, twiners 15, shrubs 26, and Trees 34. Among these 256 species, 190 are native to India, while 66 are from outside the country. A detailed description, common name, habitat, distribution, and IUCN status were provided for all species from the study area. Families have been arranged alphabetically in the list to facilitate ease of reference.

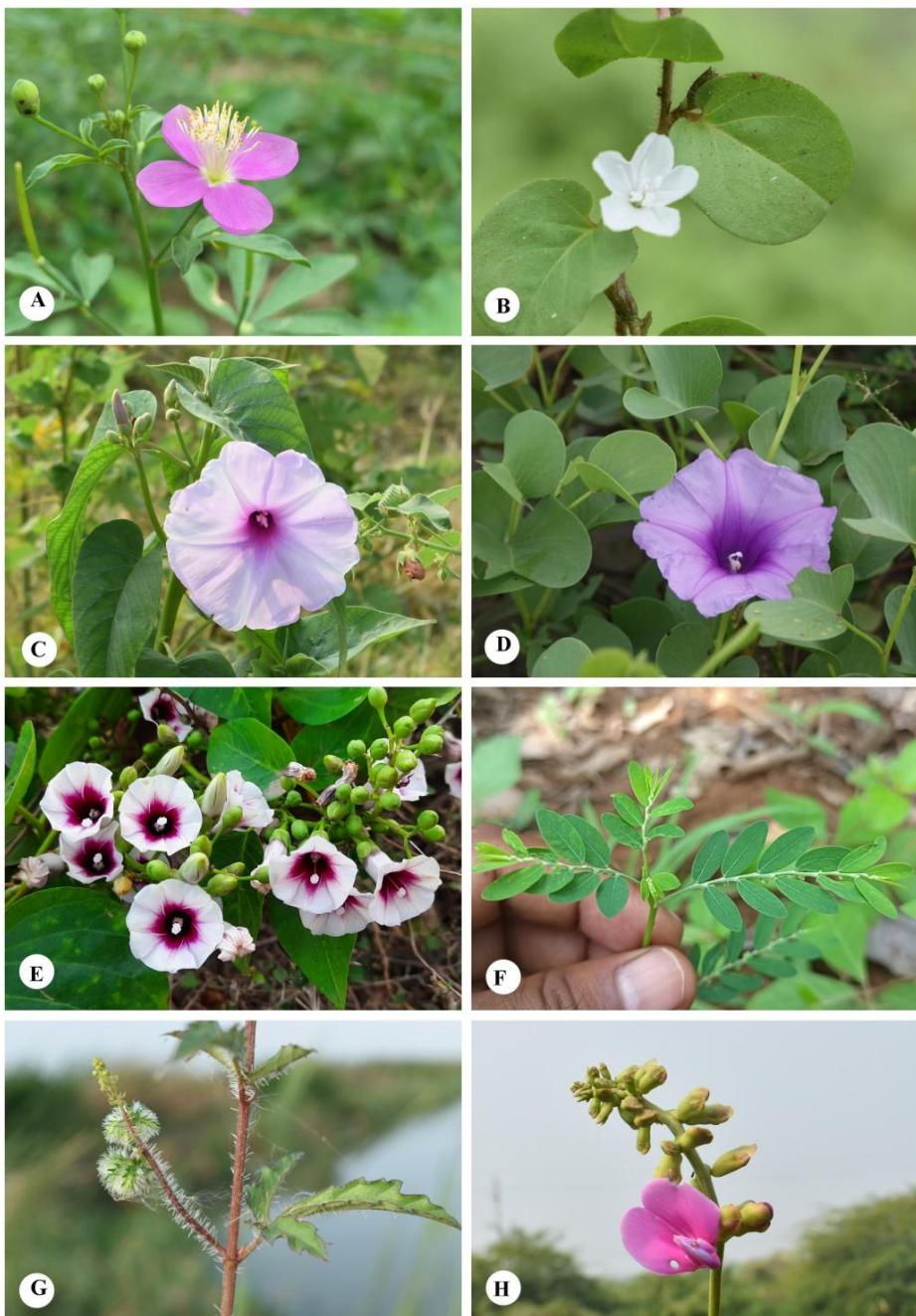


Figure 4: A. *Cleome chelidonii*; B. *Evolvulus nummularius*; C. *Ipomoea carnea*; D. *Ipomoea pes-tigridis*; E. *Ipomoea staphylina*; F. *Phyllanthus debilis*; G. *Tragia gallabatensis*; H. *Canavalia gladiata*

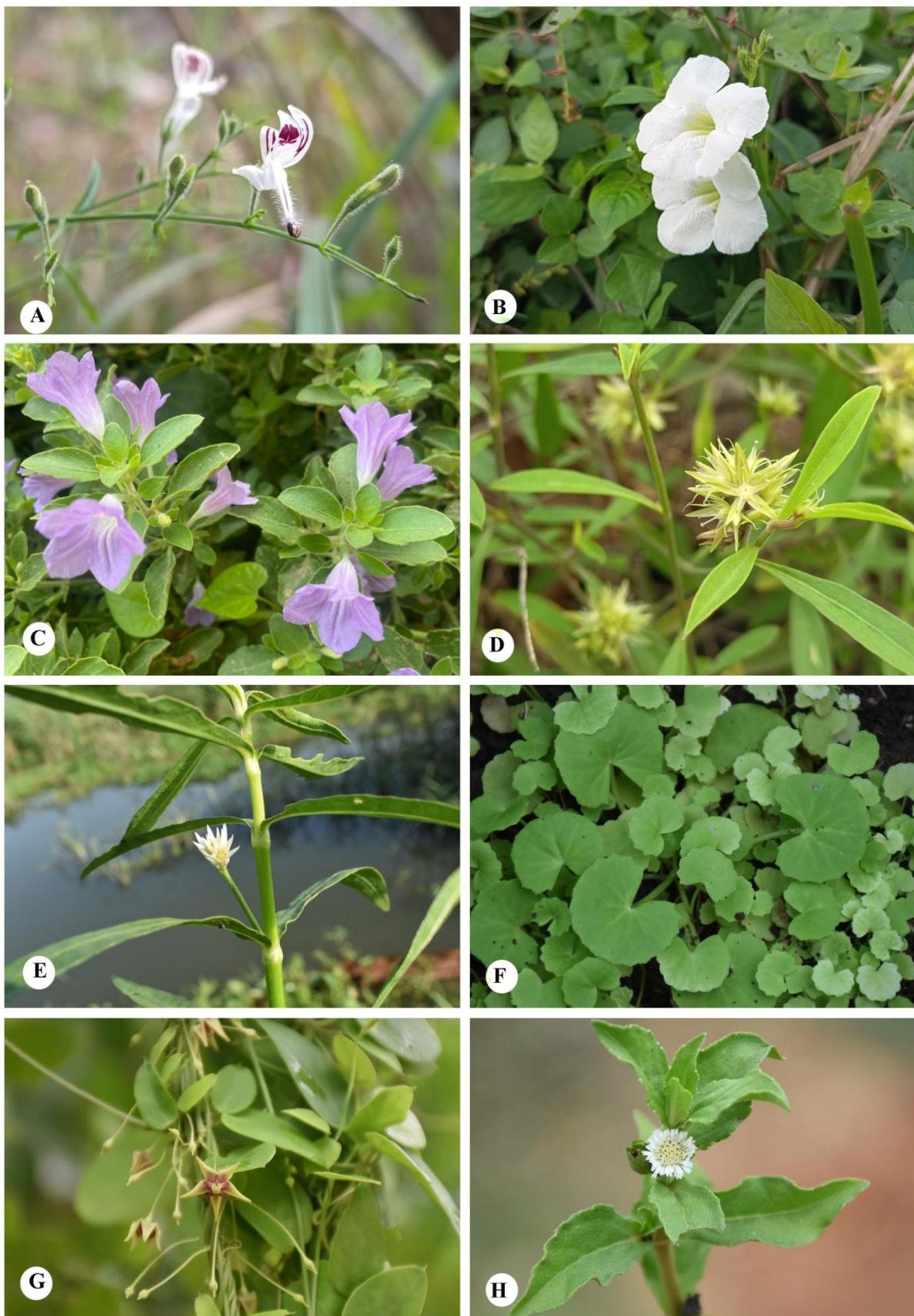


Figure 3: A. *Andrographis paniculata*; B. *Asystasia gangetica*; C. *Ruellia patula*; D. *Allmania nodiflora*; E. *Alternanthera philoxeroides*; F. *Centella asiatica*; G. *Pentatropis capensis*; H. *Eclipta prostrata*

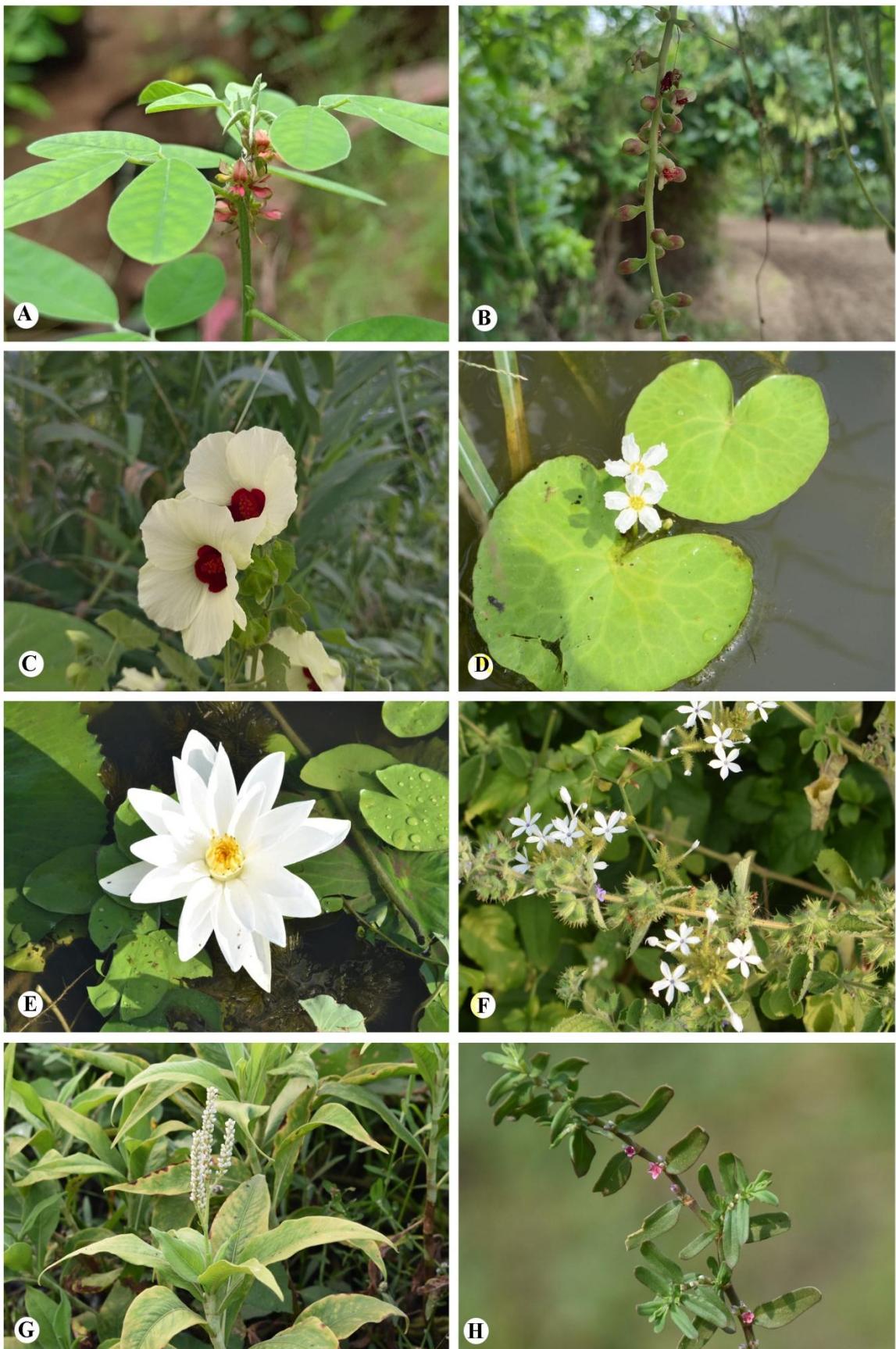


Figure 5: A. *Indigofera trifoliata*; B. *Barringtonia acutangula*; C. *Hibiscus vitifolius*; D. *Nymphoides indica*; E. *Nymphaea alba*; F. *Plumbago zeylanica*; G. *Persicaria barbata*; H. *Polygonum plebeium*

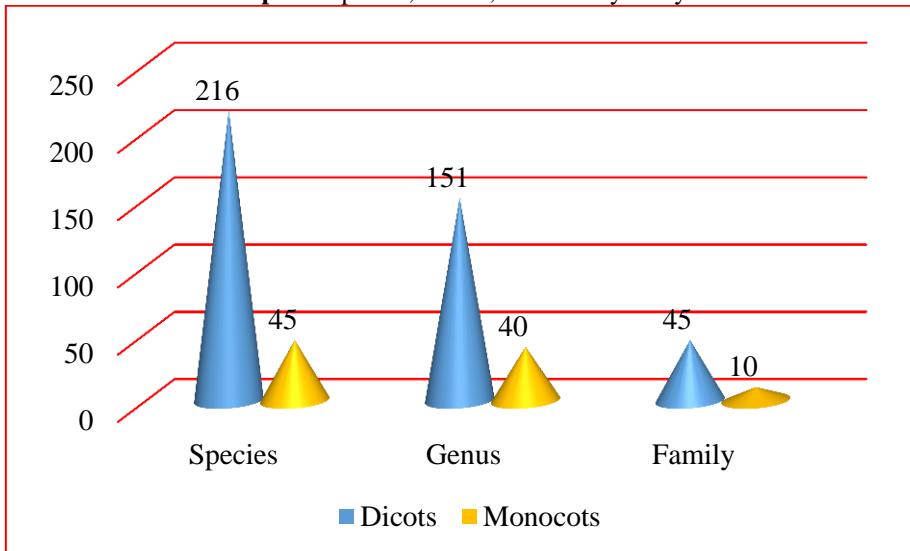
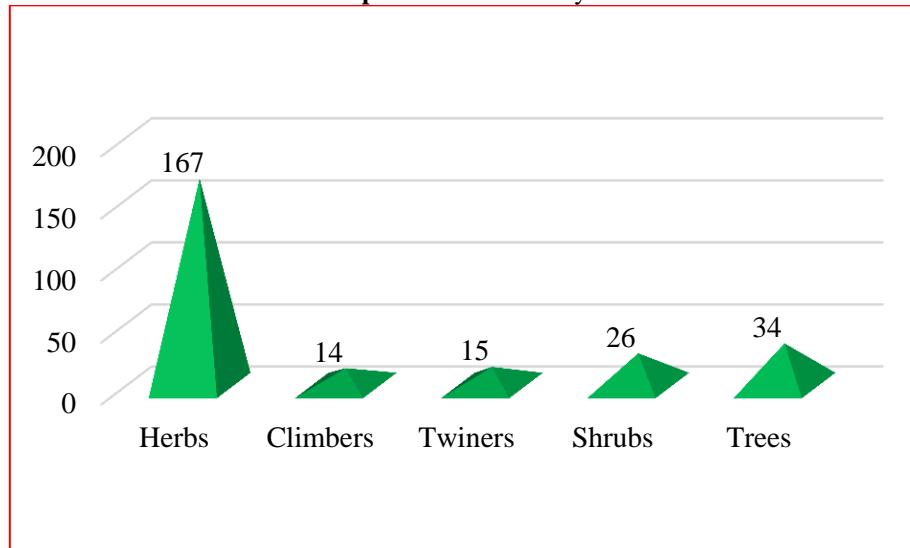
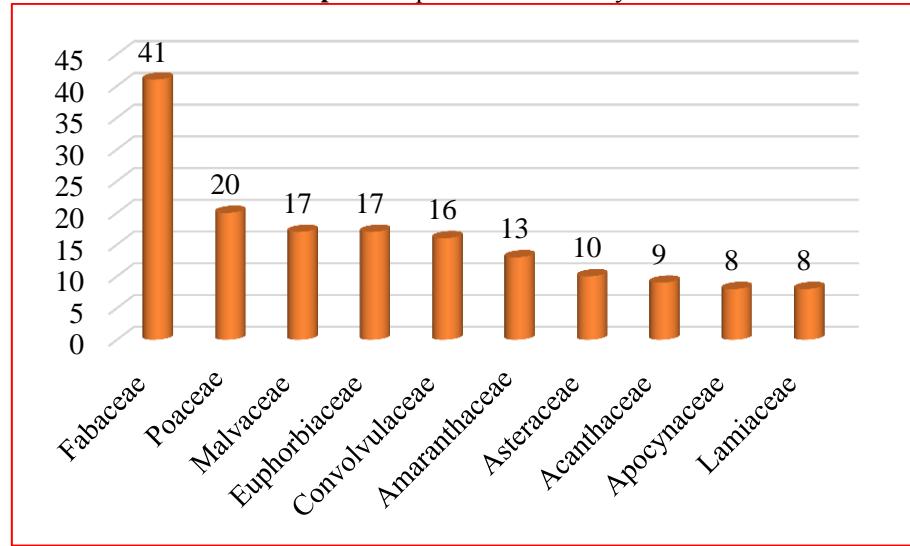
Graph 1: Species, Genus, and Family analysis**Graph 2:** Lifeform analysis**Graph 3:** Top 10 families' analysis

Table 1: Representing the floristic diversity of Kolleru Lake.

S.No	Name of the species	Habit	Common name	Native/ Introduced	IUCN status	Voucher No
Dicots						
Acanthaceae						
1	<i>Andrographis echooides</i> (L.) Nees	Herb	Aku pootha	Native		5606
2	<i>Andrographis paniculata</i> (Burm.f.) Wall. ex Nees	Herb	Nela vemu	Native		5681
3	<i>Asystasia gangetica</i> (L.) T.Anderson	Herb	Poda beera	Native		5455
4	<i>Barleria prionitis</i> L.	Herb	Mulla gorinta	Native		5623
5	<i>Elytraria acaulis</i> (L.f.) Lindau	Herb	Nelamarri	Native		5682
6	<i>Hygrophila auriculata</i> (Schumach.) Heine	Herb	Guddi Kamanchi	Native	LC	5697
7	<i>Ruellia patula</i> Jacq.	Herb	Vedichchedi	Native	LC	5671
8	<i>Ruellia prostrata</i> Poir.	Herb	Nela Neelambaram	Native		5431
9	<i>Ruellia tuberosa</i> L.	Herb	Jurbula gadda	Introduced		5473
Aizoaceae						
10	<i>Trianthema portulacastrum</i> L.	Herb	Galijeru	Native		5412
Amaranthaceae						
11	<i>Achyranthes aspera</i> L.	Herb	Uttareni	Native		5468
12	<i>Aerva javanica</i> (Burm.f.) Juss. ex Schult.	Herb	Pedda pindi	Native		5512
13	<i>Allmania nodiflora</i> (L.) R.Br. ex Wight	Herb	Gurugu Koora	Native		5696
14	<i>Alternanthera ficoidea</i> (L.) P.Beauv.	Herb	Adavi ponnaganti	Introduced		5411
15	<i>Alternanthera paronychioides</i> A.St.-Hil.	Herb	Ponnaganti kura	Introduced		5402
16	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Herb	Neeru ponnaganti	Introduced		5501
17	<i>Alternanthera sessilis</i> (L.) DC.	Herb	Ponnaganti kura	Native	LC	5459
18	<i>Amaranthus hybridus</i> L.	Herb	Dhantukura	Introduced		5672
19	<i>Amaranthus viridis</i> L.	Herb	Chilaka thotakura	Introduced		5502
20	<i>Digera muricata</i> (L.) Mart.	Herb	Chenchalaku	Native		5439
21	<i>Gomphrena celosioides</i> Mart.	Herb	Nela Rudraksha	Introduced		5532
22	<i>Ouret lanata</i> (L.) Kuntze	Herb	Kondapindi	Native		5474
23	<i>Suaeda maritima</i> (L.) Dumort.	Herb	Revu kadalu	Native		5543
Anacardiaceae						
24	<i>Mangifera indica</i> L.	Tree	Mamidi	Introduced	DD	5607
Apiaceae						
25	<i>Centella asiatica</i> (L.) Urb.	Herb	Saraswathi	Native	LC	5622
Apocynaceae						
26	<i>Calotropis gigantea</i> (L.) W.T.Aiton	Shrub	Tella jilledu	Native		5600
27	<i>Calotropis procera</i> (Aiton) W.T.Aiton	Shrub	Erra Jilledu	Native	LC	5599
28	<i>Catharanthus roseus</i> (L.) G.Don	Herb	Billa ganneru	Native		5624
29	<i>Hemidesmus indicus</i> (L.) R.Br.	Twiner	Sugandipala	Native		5505

30	<i>Oxystelma esculentum</i> (L.f.) Sm.	Twiner	Dudhi Pala	Native	LC	5410
31	<i>Pentatropis capensis</i> (L.f.) Bullock	Twiner	Chitapataku	Native		5585
32	<i>Pergularia daemia</i> (Forssk.) Chiov.	Twiner	Dustapuchettu	Native	LC	5492
33	<i>Stephanotis volubilis</i> (L.f.) S.Reuss, Liede & Meve	Twiner	Dudipala	Native		5577
Asteraceae						
34	<i>Ageratum conyzoides</i> L.	Herb	Pumpullu	Introduced	LC	5548
35	<i>Blumea obliqua</i> (L.) Druce	Herb	Makadmari	Native		5560
36	<i>Chromolaena odorata</i> (L.) R.M.King & H.Rob.	Shrub	Gandhuri	Introduced		5578
37	<i>Cyanthillium cinereum</i> (L.) H.Rob.	Herb	Gariti Kamma	Native		5418
38	<i>Eclipta prostrata</i> (L.) L.	Herb	Gunta galagara	Introduced	LC	5417
39	<i>Grangea maderaspatana</i> (L.) Desf.	Herb	Machi-patri	Native	LC	5594
40	<i>Parthenium hysterophorus</i> L.	Herb	Vayyari bhama	Introduced		5670
41	<i>Sphagneticola trilobata</i> (L.) Pruski	Herb	Galagara	Introduced		5542
42	<i>Tridax procumbens</i> L.	Herb	Gaddi chamanti	Introduced		5506
43	<i>Xanthium strumarium</i> L.	Herb	Marulutige	Native		5533
Boraginaceae						
44	<i>Cordia sebestena</i> L.	Tree	Virigi	Introduced	LC	5556
45	<i>Heliotropium curassavicum</i> L.	Herb	Nugu danti	Introduced	LC	5654
46	<i>Heliotropium indicum</i> L.	Herb	Nagadanti	Introduced		5442
Cleomaceae						
47	<i>Cleome chelidonii</i> L.f.	Herb	Kukkavavinta	Native		5463
48	<i>Cleome gynandra</i> L.	Herb	Tella vaminta	Native		5588
49	<i>Cleome viscosa</i> L.	Herb	Vaminta	Native		5541
Ceratophyllaceae						
50	<i>Ceratophyllum demersum</i> L.	Herb	Neeti Sambraani	Native	LC	5507
Combretaceae						
51	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Tree	Tella maddi	Native	LC	5669
52	<i>Terminalia catappa</i> L.	Tree	Badam	Native	LC	5621
Convolvulaceae						
53	<i>Cressa cretica</i> L.	Herb	Uppu Gaddi	Native	LC	5568
54	<i>Cuscuta chinensis</i> Lam.	Twiner	Amaravalli	Native		5582
55	<i>Evolvulus alsinoides</i> (L.) L.	Herb	Vishnukranthamu	Native		5683
56	<i>Evolvulus nummularius</i> (L.) L.	Herb	Eluka chevi	Introduced		5428
57	<i>Ipomoea aquatica</i> Forssk.	Herb	Thutikura	Native	LC	5508
58	<i>Ipomoea carnea</i> Jacq.	Shrub	Pedda thutaaku	Introduced		5461
59	<i>Ipomoea obscura</i> (L.) Ker Gawl.	Twiner	Nalla teega	Native		5435
60	<i>Ipomoea pes- carrae</i> (L.) R.Br	Twiner	Balabantatige	Native		5684
61	<i>Ipomoea pes-tigridis</i> L.	Twiner	Puritikada	Native		5695
62	<i>Ipomoea sagittifolia</i> Burm.f.	Twiner	Tellatuti	Native		5537
63	<i>Ipomoea staphylina</i> Roem. & Schult.	Twiner	Theendra Teega	Native		5608

64	<i>Ipomoea cairica</i> (L.) Sweet	Twiner	Kosta teega	Native	LC	5685
65	<i>Merremia emarginata</i> (Burm.f.) Hallier f.	Herb	Elikajemudu	Native	LC	5509
66	<i>Operculina turpethum</i> (L.) Silva Manso	Twiner	Tegada	Native		5579
67	<i>Xenostegia tridentata</i> (L.) D.F.Austin & Staples	Herb	Seetamma savaram	Native		5587
Cucurbitaceae						
68	<i>Coccinia grandis</i> (L.) Voigt	Climber	Dhonda	Native		5558
69	<i>Cucumis maderaspatanus</i> L.	Climber	Nugudosa	Native		5546
70	<i>Cucumis melo</i> L.	Climber	Karbuja	Native		5642
71	<i>Trichosanthes pilosa</i> Lour.	Climber	Adavi Potla	Introduced		5451
Elatinaceae						
72	<i>Bergia capensis</i> L.	Herb	Neeru Paavila	Native	LC	5540
Euphorbiaceae						
73	<i>Acalypha indica</i> L.	Herb	Kuppintaku	Native		5510
74	<i>Acalypha malabarica</i> Müll.Arg.	Herb	Nugu Kuppinta	Native		5429
75	<i>Chrozophora rottleri</i> (Geiseler) Spreng.	Herb	Erra miriyamu	Native		5496
76	<i>Croton bonplandianus</i> Baill.	Herb	Galivana chettu	Introduced		5433
77	<i>Euphorbia hirta</i> L.	Herb	Reddivari nanubalu	Introduced		5436
78	<i>Euphorbia hypericifolia</i> L.	Herb	Dudhi	Introduced		5476
79	<i>Euphorbia prostrata</i> Aiton	Herb	Nanabala	Introduced		5610
80	<i>Euphorbia serpens</i> Kunth	Herb	Matted Sandmat	Introduced		5511
81	<i>Euphorbia thymifolia</i> L.	Herb	Nelaparimalamu	Introduced		5525
82	<i>Ricinus communis</i> L.	Shrub	Amudamu	Native		5516
83	<i>Tragia gallabatensis</i> Prain	Herb	Duradaguntaku	Native		5406
84	<i>Jatropha gossypiifolia</i> L.	Shrub	Verri Amudamu	Introduced	LC	5454
Phyllanthaceae						
85	<i>Phyllanthus amarus</i> Schumach. & Thonn.	Herb	Nela usiri	Introduced		5551
86	<i>Phyllanthus debilis</i> J.G.Klein ex Willd.	Herb	Uchiny usirika	Native		5566
87	<i>Phyllanthus maderaspatensis</i> L.	Herb	Rcha usiri	Native	LC	5586
88	<i>Phyllanthus reticulatus</i> Poir.	Herb	Nalla purugudu	Native	LC	5598
89	<i>Phyllanthus virgatus</i> G.Frost.	Herb	Uchi usirika	Native	LC	5513
Fabaceae						
90	<i>Abrus precatorius</i> L.	Climber	Gurivinda	Native		5527
91	<i>Aeschynomene indica</i> L.	Herb	Jeeluga	Native	LC	5514
92	<i>Albizia lebbeck</i> (L.) Benth.	Tree	Chinduga	Native		5499
93	<i>Albizia lebbeck</i> (L.) Benth.	Tree	Dirisenamu	Native	LC	5562
94	<i>Alysicarpus monilifer</i> (L.) DC.	Herb	Amera	Native		5611
95	<i>Alysicarpus vaginalis</i> (L.) DC.	Herb	Baramatal-chettu	Native		5494
96	<i>Canavalia gladiata</i> (Jacq.) DC.	Climber	Thamba	Native		5456
97	<i>Chamaecrista absus</i> (L.) H.S.Irwin & Barneby	Herb	Thamba	Native	LC	5657

98	<i>Chamaecrista mimosoides</i> (L.) Greene	Herb	Chanupala-vittulu	Native	LC	5497
99	<i>Chamaecrista pumila</i> (Lam.) V.Singh	Herb	Nela ponna	Native		5641
100	<i>Clitoria ternatea</i> L.	Twiner	Sankupuvvu	Introduced		5471
101	<i>Crotalaria hebecarpa</i> (D.C) Rudd.	Herb	Majjiginta	Native		5686
102	<i>Crotalaria ramosissima</i> Roxb.	Herb	Potti-Gilligicha	Native		5693
103	<i>Desmanthus virgatus</i> (L.) Willd.	Shrub	Konda mamidi	Introduced	LC	5584
104	<i>Guilandina bonduc</i> L.	Shrub	Gachchakaya	Native	LC	5667
105	<i>Indigofera tinctoria</i> L.	Shrub	Neelimandu	Native		5619
106	<i>Indigofera trifoliata</i> L.	Herb	Baragadam	Native		5650
107	<i>Indigofera tsiangiana</i> Metcalf	Herb	Erra palleru	Native		5569
108	<i>Leucaena leucocephala</i> (Lam.) de Wit	Tree	Kainti	Introduced		5515
109	<i>Mimosa hamata</i> Willd.	Shrub	Undrakampa	Native		5528
110	<i>Mimosa pudica</i> L.	Herb	Attapatti	Introduced	LC	5529
111	<i>Neltuma juliflora</i> (Sw.) Raf.	Tree	Karra tumma	Introduced	LC	5625
112	<i>Neptunia oleracea</i> Lour.	Herb	Niru thalavapu	Native	LC	5581
113	<i>Parkinsonia aculeata</i> L.	Tree	Simatumma	Native	LC	5446
114	<i>Peltophorum pterocarpum</i> (DC.) Backer ex K.Heyne	Tree	Konda Chinta	Introduced	LC	5545
115	<i>Pithecellobium dulce</i> (Roxb.) Benth.	Tree	Sima chinta	Introduced	LC	5552
116	<i>Polhillides velutina</i> (Willd.) H.Ohashi & K.Ohashi	Shrub	Chiva-madu	Native		5595
117	<i>Pongamia pinnata</i> (L.) Pierre	Tree	Kanuga	Native	LC	5596
118	<i>Prosopis cineraria</i> (L.) Druce	Tree	Jammi	Native		5651
119	<i>Rhynchosia minima</i> (L.) DC.	Herb	Adavi chikkudu	Native	LC	5493
120	<i>Senna alata</i> (L.) Roxb.	Shrub	Seema Tangedu	Introduced	LC	5465
121	<i>Senna occidentalis</i> (L.) Link	Shrub	Kasindha	Introduced	LC	5458
122	<i>Senna uniflora</i> (Mill.) H.S.Irwin & Barneby	Herb	Oneleaf Senna	Introduced		5431
123	<i>Sesbania bispinosa</i> (Jacq.) W.Wight	Shrub	Errajiluga	Native	LC	5640
124	<i>Sesbania sesban</i> (L.) Merr.	Herb	Samintha	Native	LC	5549
125	<i>Tephrosia purpurea</i> (L.) Pers.	Herb	Vempali	Native		5570
126	<i>Tephrosia villosa</i> (L.) Pers.	Herb	Nugu vempali	Native	LC	5517
127	<i>Teramnus mollis</i> Benth.	Twiner	Karu minumullu	Native		5557
128	<i>Vachellia nilotica</i> (L.) P.J.H.Hurter & Mabb.	Tree	Nalla Tumma	Native	LC	5453
129	<i>Vigna stipulacea</i> (Lam.) Kuntze	Herb	Nelavankuda	Native		5413
130	<i>Vigna trilobata</i> (L.) Verdc.	Climber	Pilli pesara	Native		5495
	Lamiaceae					
131	<i>Leucas aspera</i> (Willd.) Link	Herb	Tummi	Native		5658
132	<i>Mesosphaerum suaveolens</i>	Shrub	Sima tulasi	Introduced		5467

	(L.) Kuntze					
133	<i>Ocimum gratissimum</i> L.	Herb	Nimma tulasi	Native		5504
134	<i>Ocimum tenuiflorum</i> L.	Herb	Krishna tulasi	Native		5441
135	<i>Tectona grandis</i> L.f.	Tree	Teku	Native	EN	5674
136	<i>Vitex negundo</i> L.	Tree	Vavili	Native	LC	5635
137	<i>Vitex trifolia</i> L.	Tree	Chiruvavil	Native	LC	5667
138	<i>Volkameria inermis</i> L.	Shrub	Takkolamu	Native		5648
Leceithidaceae						
139	<i>Barringtonia acutangula</i> (L.) Gaertn.	Tree	Kanapa	Native	LC	5520
Lentibulariaceae						
140	<i>Utricularia aurea</i> Lour.	Herb	Golden Bladderwort	Native	LC	5659
141	<i>Utricularia stellaris</i> L.f.	Herb	Nakshatrapu Budaga Mokka	Native		5639
Malvaceae						
142	<i>Abelmoschus ficulneus</i> (L.) Wight & Arn.	Shrub	Parupu benda	Native	LC	5524
143	<i>Abutilon indicum</i> (L.) Sweet	Shrub	Tuttura benda	Native		5443
144	<i>Abutilon pannosum</i> (G.Forst.) Schiltl.	Shrub	Indian mallow	Native		5534
145	<i>Ceiba pentandra</i> (L.) Gaertn.	Tree	Buruga	Introduced	LC	5665
146	<i>Corchorus aestuans</i> L.	Herb	Nela beera	Native		5535
147	<i>Corchorus olitorius</i> L.	Herb	Goninara	Native		5660
148	<i>Guazuma ulmifolia</i> Lam.	Tree	Bhadraksha	Introduced	LC	5627
149	<i>Hibiscus tiliaceus</i> L.	Tree	Yetagogu	Native	LC	5547
150	<i>Hibiscus vitifolius</i> L.	Shrub	Adavi pathi	Native		5550
151	<i>Malachra capitata</i> (L.) L.	Herb	Konda mamidi	Introduced		5475
152	<i>Malvastrum coromandelianum</i> (L.) Garcke	Herb	Garugudu	Introduced		5521
153	<i>Sida acuta</i> Burm.f.	Herb	Nela benda	Native		5447
154	<i>Sida cordata</i> (Burm.f.) Bors. Waalk.	Herb	Naga bala	Native		5536
155	<i>Thespesia populnea</i> (L.) Sol. ex Corrêa	Tree	Gangareni	Native	LC	5638
156	<i>Triumfetta rotundifolia</i> Lam.	Herb	Banki-tuthuru	Native		5491
157	<i>Urena lobata</i> L.	Shrub	Nalla Benda	Native	LC	5539
158	<i>Waltheria indica</i> L.	Herb	Nalla benda	Introduced	LC	5523
Martyniaceae						
159	<i>Martynia annua</i> L.	Herb	Garuda mukku	Introduced		5538
Meliaceae						
160	<i>Azadirachta indica</i> A.Juss.	Tree	Vepa	Introduced	LC	5628
Menispermaceae						
161	<i>Tinospora sinensis</i> (Lour.) Merr.	Climber	Tippatiga	Native		5661
Menyanthaceae						
162	<i>Nymphoides hydrophyllum</i> (Lour.) Kuntze	Herb	Anthara thaamara	Native	LC	5437
Molluginaceae						
163	<i>Glinus lotoides</i> L.	Herb	Chandras koora	Native	LC	5522
164	<i>Muntingia calabura</i> L.	Tree	Cherry chettu	Introduced	LC	5553
165	<i>Paramollugo</i>	Herb	Nakedstem	Native		5637

	<i>nudicaulis</i> (Lam.) Thulin		Carpetweed			
166	<i>Trigastrotheca pentaphylla</i> (L.) Thulin	Herb	Verri chararsi	Native		5675
Moraceae						
167	<i>Ficus arnottiana</i> (Miq.) Miq.	Tree	Kallaravi	Native		5617
168	<i>Ficus benjamina</i> L.	Tree	Konda golugu	Native	LC	5662
169	<i>Ficus hispida</i> L.f.	Tree	Bomma-medi	Native	LC	5629
170	<i>Ficus virens</i> Aiton	Tree	Adavi juvvi	Native	LC	5647
Nyctaginaceae						
171	<i>Boerhavia diffusa</i> L.	Herb	Atika mamidi	Native		5489
172	<i>Boerhavia erecta</i> L.	Herb	Punarnava	Introduced		5497
Nymphaeaceae						
173	<i>Nymphaea alba</i> L.	Herb	Nalla kaluva	Introduced	LC	5555
174	<i>Nymphaea nouchali</i> Burm.f.	Herb	Neeti Tamara	Native	LC	5554
175	<i>Nymphaea pubescens</i> Willd.	Herb	Kaluva	Native	LC	5567
Nelumbonaceae						
176	<i>Nelumbo nucifera</i> Gaertn.	Herb	Tamara	Native	DD	5559
Onagraceae						
177	<i>Ludwigia adscendens</i> (L.) H.Hara	Herb	Neeru bachhali	Native	LC	5416
178	<i>Ludwigia hyssopifolia</i> (G.Don) Exell	Herb	Nirubaccala	Introduced	LC	5464
Passifloraceae						
179	<i>Passiflora foetida</i> L.	Climber	Tellajumiki	Introduced		5427
180	<i>Turnera ulmifolia</i> L.	Herb	Cheravathali	Introduced	LC	5563
Plantaginaceae						
181	<i>Bacopa monnieri</i> (L.) Wettst.	Herb	Brahmi	Native	LC	5571
182	<i>Limnophila indica</i> (L.) Druce	Herb	Ambujam	Native	LC	5612
Plumbaginaceae						
183	<i>Plumbago zeylanica</i> L.	Herb	Chitramulam	Native		5440
Pedaliaceae						
184	<i>Pedalium murex</i> L.	Herb	Enugu palleru	Introduced		5630
Portulacaceae						
185	<i>Portulaca oleracea</i> L.	Herb	Pedda payali	Introduced	LC	5526
Polygonaceae						
186	<i>Antigonon leptopus</i> Hook. & Arn.	Climber	Teega roja	Introduced		5470
187	<i>Persicaria barbata</i> (L.) H.Hara	Herb	Kondamalle	Native	LC	5503
188	<i>Persicaria pulchra</i> Soják	Herb	Knotweed	Native	LC	5409
189	<i>Polygonum plebeium</i> R.Br.	Herb	Chimati Kura	Native	LC	5500
Rubiaceae						
190	<i>Dentella repens</i> (L.) J.R.Forst. & G.Forst.	Herb	Tella karaka	Native	LC	5572
191	<i>Leptopetalum biflorum</i> (L.) Neupane & N.Wikstr.	Herb	Rendu Pula Gaddi	Native		5430
192	<i>Morinda pubescens</i> Sm.	Tree	Maddi	Native		5573
193	<i>Oldenlandia umbellata</i> L.	Herb	Chiru vepa	Native		5583
194	<i>Spermacoce latifolia</i> Aubl.	Herb	Madana	Introduced		5576
Rhamnaceae						
195	<i>Ziziphus mauritiana</i> Lam.	Tree	Regu	Native	LC	5589
Sapindaceae						
196	<i>Cardiospermum</i>	Climber	Jyotishmati	Native		5575

	<i>corindum</i> L.					
197	<i>Cardiospermum halicacabum</i> L.	Climber	Budda kakara	Native	LC	5591
Sapotaceae						
198	<i>Mimusops elengi</i> L.	Tree	Bogada-manu	Native	LC	5645
Solanaceae						
199	<i>Datura metel</i> L.	Shrub	Nalla ummetta	Introduced		5614
200	<i>Datura stramonium</i> L.	Shrub	Tella Ummetta	Introduced		5444
201	<i>Physalis angulata</i> L.	Herb	Budda busada	Introduced	LC	5593
202	<i>Physalis peruviana</i> L.	Herb	Busarakaaya	Introduced	LC	5597
203	<i>Solanum nigrum</i> L.	Herb	Kakamachi	Native		5644
204	<i>Solanum trilobatum</i> L.	Shrub	Kondavuchinta	Native		5488
Urticaceae						
205	<i>Pouzolzia zeylanica</i> (L.) Benn.	Shrub	Eddu-mutte dumpa	Native		5574
Verbenaceae						
206	<i>Lantana camara</i> L.	Shrub	Akshinta pulu	Introduced		5601
207	<i>Lippia alba</i> (Mill.) N.E.Br. ex Britton & P.Wilson	Herb	juanilama	Introduced		5432
208	<i>Phyla nodiflora</i> (L.) Greene	Herb	Jala pippali	Native	LC	5604
Vitaceae						
209	<i>Causonis trifolia</i> (L.) Mabb. & J.Wen	Climber	Kanupu Tige	Native		5632
210	<i>Cissus vitiginea</i> L.	Climber	Adavi Draksha	Native		5590
Zygophyllaceae						
211	<i>Tribulus terrestris</i> L.	Herb	Palleru	Native	LC	5615
Monocots						
Alismataceae						
212	<i>Limnophyton obtusifolium</i> (L.) Miq.	Herb	Gurrapu dekka chettu	Native	LC	5634
Aponogetonaceae						
213	<i>Aponogeton crispus</i> Thunb.	Herb	Wavy Aponogeton	Native	LC	5655
Araceae						
214	<i>Lemna perpusilla</i> Torr.	Herb	Neela bendu	Introduced	LC	5564
215	<i>Pistia stratiotes</i> L.	Herb	Antara thamara	Native	LC	5565
Arecales						
216	<i>Borassus flabellifer</i> L.	Tree	Thati	Native	LC	5580
217	<i>Cocos nucifera</i> L.	Tree	Kobbari	Introduced		5643
218	<i>Phoenix sylvestris</i> (L.) Roxb.	Tree	Eetha	Native		5544
Commelinaceae						
219	<i>Commelina benghalensis</i> L.	Herb	Amruta kaada	Native	LC	5418
220	<i>Commelina clavata</i> C.B.Clarke	Herb	Jalapippali	Native	LC	5472
221	<i>Cyanotis axillaris</i> (L.) D.Don ex Sweet	Herb	Golla Gundu	Native	LC	5530
222	<i>Cyanotis cristata</i> (L.) D.Don	Herb	Netha kina	Native	LC	5653
Cyperaceae						
223	<i>Abildgaardia ovata</i> (Burm.f.) Kral	Herb	Flatspike Rush	Native		5609
224	<i>Bulbostylis barbata</i> (Rottb.) C.B.Clarke	Herb	Hairsedge	Native	LC	5620
225	<i>Cyperus</i>	Herb	Green Water Sedge	Native	LC	5673

	<i>brevifolius</i> (Rottb.) Hassk.					
226	<i>Cyperus rotundus</i> L.	Herb	Tunga	Native	LC	5656
227	<i>Fimbristylis quinquangularis</i> (Vahl) Kunth	Herb	Konda gaddi	Native	LC	5633
228	<i>Schoenoplectiella articulata</i> (L.) Lye	Herb	Nalla gaddi	Native	LC	5652
229	<i>Schoenoplectus litoralis</i> (Schrad.) Palla	Herb	Nameru	Native	LC	5668
	Hydrocharitaceae					
230	<i>Blyxa octandra</i> (Roxb.) Planch. ex Thwaites	Herb	Eight-Stamen Blyxa	Native	LC	5626
231	<i>Hydrilla verticillata</i> (L.f.) Royle	Herb	Punachu	Native	LC	5519
232	<i>Nechamandra alternifolia</i> (Roxb. ex Wight)	Herb	Indian Oxygen-Weed.	Native	LC	5649
233	<i>Ottelia alismoides</i> (L.) Pers.	Herb	Neeru veniki.	Native	LC	5666
234	<i>Vallisneria spiralis</i> L.	Herb	Nara mokka	Native	LC	5618
	Poaceae					
235	<i>Acrachne racemosa</i> (B.Heyne ex Roth) Ohwi	Herb	Tella karaka	Native		5616
236	<i>Apluda mutica</i> L.	Herb	Morum gaddi	Native		5692
237	<i>Arundo donax</i> L.	Herb	Kakiveduru	Native	LC	5663
238	<i>Chloris barbata</i> Sw.	Herb	Uppu Gaddi	Native		5636
239	<i>Cynodon dactylon</i> (L.) Pers.	Herb	Garika	Native		5676
240	<i>Dactyloctenium aegyptium</i> (L.) Willd.	Herb	Ganuku gaddi	Native		5680
241	<i>Dichanthium annulatum</i> (Forssk.) Stapf	Herb	Marvel grass	Native		5691
242	<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	Herb	Asian crabgrass	Native		5613
243	<i>Digitaria ciliaris</i> (Retz.) Koeler	Herb	Carb grass	Native		5687
244	<i>Echinochloa colona</i> (L.) Link	Herb	Othagaddi	Native	LC	5646
245	<i>Enteropogon dolichostachyus</i> (Lag.) Keng	Herb	Tikativva	Native		5679
246	<i>Eragrostiella bifaria</i> (Vahl) Bor	Herb	Indian love grass	Native		5698
247	<i>Eragrostis viscosa</i> (Retz.) Trin.	Herb	Sticky love grass	Native		5690
248	<i>Panicum repens</i> L.	Herb	Torpedo grass	Native	LC	5688
249	<i>Paspalum distichum</i> L.	Herb	Rellugaddi	Introduced	LC	5401
250	<i>Saccharum spontaneum</i> L.	Herb	Kaki ceruku	Native	LC	5678
251	<i>Setaria pumila</i> (Poir.) Roem. & Schult.	Herb	Yellow foxtail	Native		5699
252	<i>Sporobolus coromandelianus</i> (Retz.) Kunth	Herb	Medagascar dropseed	Native		5689
253	<i>Urochloa distachyos</i> (L.) T.Q.Nguyen	Herb	Arm-grass millet	Native		5677
254	<i>Urochloa ramosa</i> (L.) T.Q.Nguyen	Herb	Eduru gaddi	Native	LC	5631

	Pontederiaceae					
255	<i>Pontederia crassipes</i> Mart.	Herb	Pisachi tamara	Introduced		5664
	Typhaceae					
256	<i>Typha angustifolia</i> L.	Herb	Dabbu Jammu	Native	LC	5407

CONCLUSION

Kolleru Lake is a harbor for a diverse floristic assemblage, encompassing luxuriant aquatic hydrophytes, extensive reed beds, and a variety of medicinal aquatic and terrestrial plants along its margins. This rich vegetation forms the ecological backbone of the lake, sustaining high biodiversity and providing critical habitat for waterfowl, fish species, and other wildlife. However, its ecological integrity is increasingly threatened by anthropogenic pressures. Habitat degradation, unsustainable resource use, and the rapid encroachment of invasive alien species—such as *Nelumbo juliflora*, *Parthenium hysterophorus*, *Typha angustifolia*, and *Pontederia crassipes*, particularly along bunds and banks, pose serious risks to native plant communities. Given Kolleru Lake's status as a Ramsar-designated wetland, immediate and effective conservation measures are essential. Prioritizing habitat protection, controlling invasive species, and promoting sustainable management practices will be crucial to preserving the lake's biodiversity and ecological function.

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RESEARCH ARTICLE

DEVELOPMENT OF MICROPROPAGATION PROTOCOL FOR LITTLE GOURD (*COCCINIA GRANDIS* L.) UNDER IN VITRO CONDITIONS

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Abstract: An efficient protocol was developed for plant regeneration, multiplication and rooting under in vitro condition in *Coccinia grandis*. Seed setting is low and seeds are pseudo due to parthenocarpic nature. So far, farmers in India have been cultivating tindora traditionally through the age-old asexual cuttings, which are often infected by diseases and pests such as thrips and aphids, and therefore give low yields. Micro propagated tindora offers clean disease-free planting material and thereby giving as much as 10 times higher yield than traditional propagules. The present experiment was conducted at Center for Advance Research in Plant Tissue Culture, Anand Agricultural University, Anand during 2018 - 2019. Combination of bavistin (2000 ppm) for 18 min., cefotaxime (1000 ppm) for 14 min., kanamycin (1000 ppm) for 14 min., streptocyclin (1000 ppm) for 18 min. and 0.1% HgCl₂ for 8 min. for hard node explants, bavistin for 16 min., cefotaxime for 12 min., kanamycin for 12 min., streptocycline for 16 min. and 0.1% HgCl₂ for 7 min. for soft nodal explants and bavistin for 10 min., cefotaxime for 6 min., kanamycin for 6 min., streptocyclin for 8 min. and 0.1% HgCl₂ for 2 min. was found best for surface sterilization in the term of fungal contamination, bacterial contamination and drying of the all explants. Half strength of MS was the best initiation medium for all the explants. Treatment T₆ took lesser number of days to sprouting and gave higher number of sprouted explants and response percentage at 7, 14 and 21 days after initiation. Half strength of MS supplemented with 0.1 BA mg⁻¹ was optimum for maximum multiplication of shoots. Half strength of MS supplemented with 0.1 mg⁻¹BA+1.0 mg⁻¹GA₃ was optimum for maximum length of shoots. Half strength of MS supplemented with 1.0 IBA mg⁻¹ was optimum for maximum root length. Half strength of MS supplemented with 2.0 IBA mg⁻¹ was optimum for maximum number of roots. Coco peat was best suited for overall development of plants during primary hardening.

Keywords: Micro propagation, Parthenocarpic, Hardening

INTRODUCTION

Coccinia grandis, commonly known as ivy gourd, baby watermelon, or little gourd, belongs to the family Cucurbitaceae, which comprises nearly 960 species. Most cucurbits are annual climbing vines, and *C. grandis* is an aggressive perennial climber capable of forming dense mats that readily smother shrubs and young trees. The leaves are simple and alternately arranged, variable in shape from heart-shaped to pentagonal, and may grow up to 10 cm in length. The upper leaf surface is green and glabrous, whereas the lower surface is pale green with fine hairs. Tendrils are simple. The plant bears solitary white flowers, with a calyx of five recurved lobes, ovate corolla lobes, three stamens, and an inferior ovary. The fruit is slimy, pulpy and varies from ovoid to ellipsoid or cylindrical, typically exhibiting about ten white longitudinal stripes on the posterior region. It is green when immature and turns scarlet red upon ripening, measuring 2.5–6.0 cm in length.

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and containing numerous oblong seeds (6–7 mm) (Pekamwar et al., 2013).

The chemical composition of *C. grandis* includes 12.62% carbohydrates, 15% total protein, 11.25% water-soluble protein, 4.0% lipids, 61.92 mg/100 g total phenols, 25.55 mg/100 g vitamin C, 70.05 mg/100 g β-carotene, and appreciable amounts of minerals such as potassium (3.38 mg/100 g), phosphorus (1.15 mg/100 g), sodium (0.95 mg/100 g), iron (2.23 mg/100 g), and calcium (3.79 mg/100 g). The fruits contain a diverse range of bioactive compounds including steroids, saponins, ellagic acid, terpenoids, lignin, alkaloids, tannins, flavonoids, glycosides, phenols, β-amyrin acetate, lupeol, taraxerol, carotenoids, lycopene, cryptoxanthin, xyloglucan and β-sitosterol (Khatun et al., 2012).

Phytochemical analyses indicate the presence of saponins, flavonoids and polyphenols that contribute to the antioxidant and anti-inflammatory properties of the leaves (Umamaheswari & Chatterjee, 2008). Traditionally, *C. grandis* is

widely used in indigenous medicine, particularly for the management of diabetes, and has been described as the “Indian substitute for insulin.”

Reproductive biology of *C. grandis* poses challenges for large-scale cultivation. Seed setting is extremely low, and most seeds formed are pseudo-seeds due to its parthenocarpic nature. In India, farmers with limited resources continue to multiply the crop through age-old vegetative methods using vine cuttings, which are often infected with pests such as thrips and aphids as well as systemic diseases, resulting in reduced yield. Micro propagated plants, in contrast, provide disease-free planting material and have been reported to yield nearly ten times more than traditional propagules. Although *C. grandis* is dioecious, parthenocarpy leads to the cultivation of female plants only under field conditions. This selective bias has contributed to a gradual reduction in natural populations of little gourd.

Considering the declining natural population, poor seed fertility, and the urgent need for clean planting material, development of an efficient in vitro micropropagation protocol is essential for large-scale multiplication and conservation of this important medicinal cucurbit. Therefore, the present study was undertaken to standardize a reliable protocol for mass propagation of *C. grandis* using shoot tip and nodal explants.

MATERIALS AND METHODS

The present investigation was conducted at the Center for Advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. The experimental site is located at 22.35° N latitude and 72.55° E longitude, at an elevation of 45.1 m above mean sea level. Explants were collected from elite *Coccinia grandis* plants maintained in the farmer's field and in the greenhouse of the Center. All experiments were laid out in a Completely Randomized Design (CRD).

The basal medium used for culture was Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar, and appropriate concentrations of plant growth regulators (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes at 15 psi.

Nodal segments and shoot tips were excised from healthy plants and initially washed under running tap water to remove surface debris and microorganisms. They were then treated with 2–3 drops of Tween-20 for 7–8 minutes with continuous swirling, followed by thorough rinsing with sterile distilled water. Surface sterilization was carried out using a combination of antibiotics (Streptomycin, Kanamycin, Cefotaxime), a fungicide (Bavistin), and a heavy metal disinfectant ($HgCl_2$) for varying durations. After treatment, explants were rinsed

several times with sterile distilled water. All procedures were performed aseptically inside a laminar airflow cabinet.

For culture establishment, explants showing initial bud break were transferred to MS medium containing different combinations of auxins and cytokinins after 21 days of incubation. Multiple shoot induction was achieved on MS medium supplemented with varying concentrations of BA, GA₃ and other growth regulators. The most effective medium for shoot multiplication was identified through repeated subculturing and observation of shoot proliferation. All culture vessels were handled under aseptic conditions and incubated in the growth room under controlled environmental conditions.

For rooting, individual healthy shoots measuring approximately 4–6 cm in length were excised and transferred to half-strength MS medium containing suitable concentrations of root-promoting auxins, primarily indole-3-butyric acid (IBA).

Hardening of tissue culture-raised plantlets was carried out in the greenhouse using different hardening substrates such as cocopeat alone and in combination with soil. Plantlets were regularly supplemented with a nutrient solution and protected from infections using mild antifungal applications until acclimatization was completed.

RESULTS AND DISCUSSION

The effect of different surface sterilization treatments (T₁–T₆) on fungal contamination percentages in hard node, soft node, and shoot tip explants of *Coccinia grandis* is presented in Table 1. Each treatment consisted of a sequential combination of fungicide (Bavistin), antibiotics (Cefotaxime, Kanamycin, Streptocycline), and a disinfectant ($HgCl_2$), with variations in exposure time. The results clearly indicate that the duration and combination of sterilants significantly influenced contamination levels across all explant types.

Across treatments, fungal contamination ranged from 0.0 to 10% in hard nodes, 1.0 to 9.0% in soft nodes, and 1.0 to 10% in shoot tips. The highest fungal contamination was recorded in T₁, with 10% in hard nodes, 9% in soft nodes, and 8% in shoot tips. This suggests that the exposure durations applied in T₁ were insufficient to eliminate fungal spores, particularly in explants with higher surface microbial load such as hard nodes.

In contrast, T₆ was the most effective sterilization treatment, yielding 0% contamination in hard nodes, and only 1% in both soft node and shoot tip explants. This treatment involved the longest cumulative exposure to Bavistin (18 min), Streptocycline (18 min), and slightly extended exposure to $HgCl_2$ compared to other treatments. The improved sterilization efficiency in T₆ can be

attributed to the synergistic action of the fungicide and antibiotics, combined with adequate $HgCl_2$ exposure, which collectively inhibit a wide spectrum of fungal contaminants.

Intermediate treatments (T_2 , T_3 , T_4 , and T_5) showed progressively reduced contamination as exposure durations increased, but none were as effective as T_6 . For example, T_5 recorded low contamination levels (2% in hard nodes, 2% in soft nodes, 1% in shoot tips), indicating partial effectiveness, but still higher fungal presence compared to T_6 . Notably, shoot tips consistently showed slightly lower contamination than hard nodes in all treatments except T_1 , likely due to their younger, less lignified tissue which harbors fewer fungal propagules.

Overall, the data demonstrate that longer and sequential exposure to combined Bavistin-antibiotic- $HgCl_2$ treatment (T_6) is essential for achieving near-complete elimination of fungal contaminants in *C. grandis* explants. These findings align with previous studies in cucurbit tissue culture, where extended sterilant exposure significantly improved aseptic culture establishment (Devi et al. 2017).

Overall Response of Explants to Sterilization Treatments

Table 2 summarizes the influence of six sterilization treatments on bacterial contamination in hard node, soft node and shoot tip explants of *Coccinia grandis*. The results show that the percentage of bacterial contamination varied considerably among treatments, indicating that the duration and combination of fungicides, antibiotics and disinfectants had a significant impact on the efficiency of surface sterilization. Hard and soft node explants generally exhibited higher levels of bacterial contamination compared to shoot tips, which is expected because nodes are physiologically older tissues with more crevices and internal microbial load.

Performance of Lower-Duration Treatments (T_1 - T_3)

The first three treatments, which involved comparatively shorter exposure times to sterilants, were less effective in controlling bacterial contamination. In treatment T_1 , bacterial contamination reached 4.0% in hard nodes, 6.0% in soft nodes and 2.0% in shoot tips. These values suggest inadequate penetration and microbial suppression by antibiotics, likely because of insufficient exposure to Cefotaxime, Kanamycin and Streptocycline. Treatment T_2 showed slight improvement, with contamination levels decreasing to 1.0% in hard nodes, 3.0% in soft nodes and 1.0% in shoot tips. However, T_3 again exhibited increased bacterial contamination in hard nodes (6.0%) and in shoot tips (3.0%), indicating that the moderate variation in exposure time in T_2 and T_3 was not uniformly effective. Similar variations in contamination with shorter sterilization durations

have been reported in cucurbits such as *Momordica dioica* and *Momordica cymbalaria*, where inadequate sterilant exposure leads to partial survival of endophytic bacteria (Devi et al., 2017).

Performance of Intermediate-Duration Treatments (T_4 and T_5)

The intermediate treatments, T_4 and T_5 , showed considerably improved control of bacterial contamination. In T_4 , contamination levels were reduced to 5.0% in hard nodes, 3.0% in soft nodes and 4.0% in shoot tips. The improved performance is mainly attributable to the extended exposure to Bavistin, Cefotaxime and Streptocycline, which appeared to suppress surface-residing bacteria more effectively. Treatment T_5 performed even better, with contamination levels of 2.0% in hard nodes and only 1.0% in soft nodes and shoot tips. The higher efficiency in T_5 can be linked to the balanced combination of moderate $HgCl_2$ exposure and extended antibiotic treatment. Comparable improvements with prolonged sterilant exposure have been documented in *Trichosanthes dioica* (Kumar et al., 2003), demonstrating that intermediate exposure times often achieve a suitable balance between microbial suppression and tissue viability.

Effectiveness of the Longest-Duration Treatment (T_6)

Treatment T_6 was the most effective sterilization regime, yielding only 1.0% bacterial contamination in both hard and soft node explants and complete elimination (0.0%) of bacterial contamination in shoot tip explants. The superior performance of T_6 is attributed to the longest exposure durations to Bavistin (18 min), Streptocycline (18 min) and slight extension in $HgCl_2$ treatment, along with consistent exposure to Cefotaxime and Kanamycin. This extended and synergistic antibiotic-fungicide-disinfectant combination appears to neutralize both surface-associated and endophytic bacteria. Shoot tips, being physiologically young tissues with smoother epidermal surfaces, responded exceptionally well to T_6 , resulting in zero contamination. These results strongly agree with earlier reports in cucurbits where prolonged antibiotic/sterilant exposure yielded near-sterile explants (Devi et al., 2017).

Comparative Trend Across Explant Types

A consistent trend is clearly visible across explant types. Hard nodes showed the highest bacterial contamination in treatments with shorter exposure durations, due to their higher microbial load and thicker tissues. Soft nodes exhibited intermediate contamination levels, while shoot tips consistently showed the lowest contamination across treatments. This pattern matches observations in other cucurbitaceous species where meristematic tissues are more responsive to sterilization than nodal tissues (Sarowar et al., 2003). The gradual decrease in contamination from T_1 to T_6 reflects the

cumulative effectiveness of longer sterilization durations and stronger combinations of antibiotics and disinfectants.

General Response of Explants to Sterilization Treatments

Table 3 presents the drying (desiccation) percentage observed in hard node, soft node and shoot tip explants of *Coccinia grandis* subjected to six surface sterilization treatments. Drying of explants is a common physiological response to chemical stress induced by sterilants, particularly heavy metals such as mercuric chloride ($HgCl_2$), detergents and high concentrations of antibiotics. The percentage of drying across treatments ranged from 0.0% to 6.0%, indicating differential sensitivity of the explants depending on their tissue type and the intensity of sterilization. Shoot tips consistently showed higher drying percentages than hard and soft nodes due to their delicate meristematic nature, a trend also reported in cucurbit tissue cultures by Devi et al. (2017).

Response Under Lower Sterilization Exposure

In treatment T_1 , drying was highest across explants, with 2.0% in hard nodes, 3.0% in soft nodes and 6.0% in shoot tips. This heightened dryness can be attributed to the rapid osmotic shock and membrane injury produced by shorter but more abrupt exposure to multiple sterilants, particularly $HgCl_2$. The initial shock of sterilant exposure often leads to dehydration of explants, especially in species where epidermal tissues are thin. Treatment T_2 showed reduced drying, with only 1.0% in hard and soft nodes and 3.0% in shoot tips, suggesting that the slight increase in Streptocycline exposure provided better microbial control without markedly increasing tissue stress.

Response Under Moderate Sterilization Durations

Treatments T_3 and T_4 maintained relatively low drying percentages in hard and soft nodes (0–1.0%), demonstrating that these tissues tolerated moderate exposure durations without significant desiccation. Soft nodes exhibited slightly higher drying (1.0%) compared to hard nodes, likely due to a thinner epidermis and higher water content, making them more sensitive to osmotic stress. Shoot tips, however, consistently showed drying around 3.0% in T_3 , T_4 and T_5 , indicating that they remained the most vulnerable tissue type. Research by Kumar et al. (2003) in *Trichosanthes dioica* confirms that shoot meristems are highly sensitive to $HgCl_2$ and prolonged antibiotic treatments, which disrupt membrane integrity and accelerate dehydration.

Treatment T_5 demonstrated improved tolerance in soft nodes (0.0% drying), which suggests that the balance between antibiotic exposure and $HgCl_2$ treatment was optimal in minimizing tissue stress. However, shoot tips still exhibited 3.0% drying, reflecting their inherent sensitivity.

Best Performance Under Longest Sterilization Duration

Treatment T_6 was the most effective in minimizing drying across all explant types. Both hard nodes and soft nodes recorded 0.0% drying, indicating that these tissues tolerated the extended sterilization duration without physiological dehydration. Shoot tips also showed minimum drying at 1.0%, demonstrating that the combination of prolonged antibiotic exposure and slightly extended $HgCl_2$ treatment provided sterilization efficiency while minimizing tissue stress. These results align with the findings of Devi et al. (2017), who reported that optimized exposure to Bavistin and antibiotics reduced drying and browning in *Momordica cymbalaria* explants. Similarly, Sarowar et al. (2003) noted that controlled exposure to $HgCl_2$ prevents excessive plasmolysis and dehydration in cucurbits, supporting the present findings.

Comparative Sensitivity of Explant Types

A clear pattern emerges from the data: shoot tips exhibited the highest drying across treatments, followed by soft nodes and hard nodes. Meristematic shoot tips contain higher water content, thinner cell walls and more tender tissues, making them more susceptible to osmotic injury, as noted in earlier studies on cucurbit micropropagation. Hard nodes, being more lignified, consistently showed the least drying across all treatments. The almost negligible drying observed in T_6 suggests that the sequential sterilization strategy successfully minimized physiological stress in all explant types.

Overview of Shoot Multiplication Response at Second Subculture

Table 6 summarizes the influence of different MS-based media combinations supplemented with varying concentrations of BA, GA_3 , NAA, IAA and kinetin on shoot multiplication in *Coccinia grandis* at 42 days after culture initiation. All media supported shoot induction, as indicated by 100% shooting percentage, demonstrating that the species responds well to cytokinin-rich medium even at half-strength MS. Similar universal shoot induction in cucurbits has been reported in *Momordica cymbalaria* (Devi et al., 2017) and *Trichosanthes dioica* (Kumar et al., 2003).

Effect of BA-Supplemented Media on Shoot Number

The treatments containing BA alone (M1 to M5) exhibited marked differences in shoot multiplication. The highest number of shoots (2.75) was observed under medium **M4** ($\frac{1}{2}$ MS + **0.1 mg L⁻¹ BA**), indicating that BA at moderate concentrations promotes optimal cytokinin-mediated cell division and axillary bud break. Lower or higher concentrations of BA (e.g., 0.01 or 0.2 mg L⁻¹) produced fewer shoots, suggesting a threshold beyond which BA does not further stimulate axillary proliferation due to hormonal

imbalance. Similar concentration-dependent responses to BA have been documented in *Momordica dioica* and *Coccinia abyssinica* (Bekele et al., 2013).

Effect of BA and GA₃ Combination on Shoot Multiplication and Elongation

Media containing combinations of BA with GA₃ (M7–M10) showed varied responses. Moderate proliferation occurred in M₈ ($\frac{1}{2}$ MS + 0.05 mg L⁻¹ BA + 1.0 mg L⁻¹ GA₃) with two shoots per explant, whereas the highest shoot length (5.35 cm) was recorded in M₉ ($\frac{1}{2}$ MS + 0.1 mg L⁻¹ BA + 1.0 mg L⁻¹ GA₃), indicating the synergistic role of gibberellin in promoting cell elongation. The increased elongation in BA + GA₃ combinations aligns with reports in *Momordica charantia* (Verma et al., 2014), where GA₃ enhanced internode elongation without substantially increasing shoot number. Higher BA combined with GA₃ (M10) reduced shoot length (3.45 cm), suggesting possible hormonal antagonism at elevated cytokinin levels.

Effect of GA₃ Alone on Shoot Development

Media M11 and M12 contained GA₃ either alone or combined with very low BA. These treatments resulted in poor shoot multiplication (1.00 shoot per explant) but moderate shoot elongation (3.37–4.50 cm). This supports the physiological role of GA₃ as primarily an elongation promoter rather than a shoot multiplication regulator. Similar findings were reported by Devi et al. (2017), where GA₃ increased shoot height but did not substantially influence axillary bud proliferation.

Effect of BA + NAA Combination on Shoot Response

Treatments M₁₆ to M₁₈, containing BA with low NAA (0.05 mg L⁻¹), resulted in minimal shoot proliferation (1.00–1.75 shoots) and short shoot length (1.52–2.15 cm). The presence of auxin along with cytokinin likely influenced apical dominance and callus induction at the basal region, thereby reducing shoot multiplication efficiency. Similar inhibitory effects of BA–NAA combinations on shoot proliferation have been reported in *Cucumis sativus* and *Momordica cymbalaria* (Devi et al., 2017).

Effect of IAA + Kinetin Combination on Shoot Characteristics

In media M19–M25, IAA was combined with kinetin (Kn), producing generally low shoot numbers (1.00–2.00) and short shoot lengths (1.32–2.17 cm). This cytokinin type was less effective than BA for shoot proliferation, confirming earlier reports that kinetin is comparatively weaker than BA in cucurbits for stimulating axillary bud activity (Kumar et al., 2003). The minimum shoot length (1.32 cm) in M25 supports the observation that high kinetin relative to IAA does not enhance elongation. Treatments such as M19 and M23 produced moderate multiplication (2 shoots), but overall, the

IAA + kinetin combination was significantly inferior to BA-based treatments.

Overall Performance of Media in Second Subculture

Two media formulations exhibited clear and complementary advantages during the multiplication phase. Medium M4 ($\frac{1}{2}$ MS supplemented with 0.1 mg L⁻¹ BA) proved most effective for maximizing shoot proliferation, producing an average of 3.00 shoots per explant. In contrast, Medium M9 ($\frac{1}{2}$ MS containing 0.1 mg L⁻¹ BA and 1.0 mg L⁻¹ GA₃) supported superior shoot elongation, with shoots reaching an average length of 4.57 cm. These results reinforce the distinct functional roles of the growth regulators: BA serves as the primary cytokinin responsible for inducing multiple shoot buds, while GA₃ promotes internodal elongation and overall shoot extension. Similar synergistic responses between BA and GA₃ have been reported in related cucurbit micropropagation systems, further validating the observed patterns (Devi et al., 2017; Verma et al., 2014).

Overall Response During the Third Subculture

Table 7 presents the performance of different MS-based media formulations on the number of shoots, shoot elongation and shooting percentage in *Coccinia grandis* at the 63-day stage (third subculture). All treatments recorded 100% shooting response, indicating that once established, *C. grandis* maintains a stable regeneration capacity through successive subcultures. A similar high survival percentage in subsequent subcultures has been reported in *Momordica cymbalaria* (Devi et al., 2017) and *Trichosanthes dioica* (Kumar et al., 2003), confirming that cucurbits are resilient during in vitro proliferation.

Effect of BA-Based Media on Shoot Multiplication

The BA-only treatments (M1–M5) again displayed a clear concentration-dependent response. Among these, M4 ($\frac{1}{2}$ MS + 0.1 mg L⁻¹ BA) produced the highest number of shoots (3.00), maintaining its superiority from the second subculture. This demonstrates that 0.1 mg L⁻¹ BA is the optimum concentration for repeated axillary bud induction in *C. grandis*. Lower (0.01 mg L⁻¹) or higher (0.2 mg L⁻¹) BA concentrations produced only one shoot per explant, suggesting that either hormonal insufficiency or supra-optimal cytokinin levels inhibited shoot proliferation. A similar optimum for BA (0.1–0.5 mg L⁻¹) has been documented for shoot multiplication in *Momordica charantia* (Verma et al., 2014) and *Coccinia abyssinica* (Bekele et al., 2013).

Effect of BA + GA₃ Combination on Shoot Numbers and Elongation

In treatments with BA + GA₃ (M7–M10), shoot numbers remained moderate (1.00–2.00), with M₇ and M₁₀ producing two shoots per explant. Meanwhile, shoot elongation was pronounced in M₉,

(4.57 cm) and M₁₄ (4.25 cm), indicating that GA₃ showed a strong synergistic effect with BA for promoting internodal elongation. The role of GA₃ in enhancing shoot length is consistent with studies in *Momordica cymbalaria* (Devi et al., 2017), where GA₃ significantly improved shoot extension without substantially increasing multiplication.

Effect of GA₃ Alone and Combined with Low BA

GA₃ alone (M₁₁) produced fewer shoots (1.25) but reasonable shoot elongation (2.97 cm), confirming its elongation-dominant role. When GA₃ was combined with very low BA (M12), elongation improved slightly (3.57 cm), but multiplication remained low. This pattern mirrors similar results in cucurbits where GA₃ alone is insufficient for bud proliferation but enhances elongation (Sarowar et al., 2003).

Effect of BA + NAA on Shoot Development

Auxin-cytokinin mixtures (M₁₆–M₁₈) produced weak shoot responses, with only **one shoot per explant** and short shoot length (1.47–1.97 cm). The presence of NAA likely enhanced apical dominance or callus formation, thereby suppressing axillary bud development. Previous reports in *Cucumis sativus* and *Momordica* species show that NAA in combination with BA often reduces multiplication rate by shifting the developmental pathway toward callusing rather than shoot organogenesis (Devi et al., 2017).

Effect of IAA + Kinetin on Shoot Multiplication and Growth

Treatments M19–M25 containing IAA + Kn produced generally lower performance in both shoot number and length. Although M20 and M21 generated two shoots per explant, the shoot length remained inferior (2.40–2.44 cm). The lowest shoot length values (1.47 cm) were observed in M24 and M25. These results confirm that kinetin is less effective than BA for stimulating shoot multiplication in cucurbits, consistent with findings in *Trichosanthes dioica* (Kumar et al., 2003).

Comparison and Identification of Best Media

Two media formulations exhibited clear and complementary advantages during the multiplication phase. Medium M₄ (½ MS supplemented with 0.1 mg L⁻¹ BA) proved most effective for maximizing shoot proliferation, producing an average of 3.00 shoots per explant. In contrast, Medium M₉ (½ MS containing 0.1 mg L⁻¹ BA and 1.0 mg L⁻¹ GA₃) supported superior shoot elongation, with shoots reaching an average length of 4.57 cm. These results reinforce the distinct functional roles of the growth regulators: BA serves as the primary cytokinin responsible for inducing multiple shoot buds, while GA₃ promotes internodal elongation and overall shoot extension. Similar synergistic responses between BA and GA₃ have been reported in related cucurbit micropagation

systems, further validating the observed patterns (Devi et al., 2017; Verma et al., 2014).

Root Induction Response in *Coccinia grandis*

The results presented in Table 8 show the influence of different concentrations of indole-3-butyric acid (IBA) on the rooting behaviour of *Coccinia grandis* shoots cultured on half-strength MS medium. A clear variation was observed in the number of days required for root induction, root length, and number of roots, indicating a strong concentration-dependent physiological response to IBA application.

Days Required for Root Induction

The data show that R5 (½ MS + 2.0 mg L⁻¹ IBA) required the longest period for root initiation (10.25 days), which was statistically at par with R1 and R4. The delay at higher IBA concentrations suggests that supra-optimal auxin levels may temporarily suppress root initiation due to auxin-induced tissue browning or cellular stress. In contrast, the earliest root induction (8.75 days) occurred in R2 (0.5 mg L⁻¹ IBA), indicating that lower auxin concentrations favor quicker physiological activation of root primordia. Similar observations were reported by Sarowar et al. (2003), where 1.0 mg L⁻¹ IBA significantly enhanced rooting efficiency in interspecific *Cucurbita* hybrids without delaying root formation.

Root Length Response

Root elongation also exhibited marked differences among treatments. The highest mean root length was recorded in R3 (6.02 cm), indicating that **1.0 mg L⁻¹ IBA** is the most effective concentration for promoting vigorous root elongation in *C. grandis*. This coincides with findings in *Momordica charantia*, where 1.0 mg L⁻¹ IBA produced the longest roots and best anchorage quality (Verma et al., 2014). The minimum root length (3.50 cm) was observed in the hormone-free medium R1, demonstrating that endogenous auxin alone is insufficient for achieving optimum rooting in this species.

Number of Roots Formed

A similar trend was observed for root number, with R3 and R5 both producing the highest number of roots (7.00), indicating that moderate to moderately high levels of IBA effectively promote root proliferation. However, R5 produced many roots but shorter initiation time and shorter initial length, suggesting a compensation mechanism commonly observed under high auxin concentrations. The lowest number of roots (1.0) was recorded in R1, reinforcing the necessity of exogenous auxin for robust root induction. Reports for *Coccinia abyssinica* (Bekele et al., 2013) also support that half-strength MS media supplemented with IBA significantly increases the number of roots and their elongation, with more than 90% success in rooting.

Rooting Percentage

All treatments resulted in 100% rooting, confirming that *C. grandis* has a strong inherent capacity for adventitious root formation once shoots reach a physiologically competent stage. This universal rooting response aligns with findings in *Cucurbita* hybrids (Sarowar et al., 2003) and *Momordica cymbalaria* (Devi et al., 2017), where well-developed shoots consistently rooted in IBA-enriched medium.

Summary of Rooting Phase

Collectively, the results demonstrate that **1.0 mg L⁻¹ IBA (R3)** provides the best overall rooting performance in *C. grandis*, producing the longest roots and highest root numbers within a reasonable induction period. The consistency of these results with earlier cucurbit micropropagation studies suggests that *C. grandis* follows the general cucurbit pattern of rooting enhanced by moderate IBA supplementation.

Table 4.9 describes the performance of tissue-cultured plantlets of *Coccinia grandis* during acclimatization under three different hardening substrates. The results demonstrate a clear impact of substrate composition on survival percentage.

Survival and Mortality Patterns During Hardening

Among the media tested, cocopeat alone (H1) provided the most favorable conditions for primary hardening, allowing two out of five plantlets to survive, corresponding to 40% survival (or 60%

mortality). This higher survival rate may be attributed to the excellent water-holding capacity, aeration properties, and pathogen-free nature of cocopeat, which together create a gentle transition environment from in vitro to ex vitro conditions. In contrast, the addition of soil at either 20% (H2) or 50% (H3) resulted in complete mortality of plantlets during primary hardening. This can be attributed to the sudden exposure to heterogeneous microbial populations, higher compaction, and reduced moisture stability typically associated with soil-based substrates.

These results are consistent with those reported by Devi et al. (2017) in *Momordica cymbalaria*, where cocopeat alone or in combination with inert substrates such as perlite yielded higher survival rates compared to soil-containing mixtures. The physiological fragility of newly rooted micropropagated plants makes cocopeat advantageous because it provides a non-stressful environment with adequate moisture without hypoxia.

Secondary Hardening

The two surviving plants from the cocopeat treatment (H1) were successfully transferred to secondary hardening, where both survived completely, reducing the final mortality to 0%. This indicates that once the initial acclimatization stage is overcome, *C. grandis* plantlets possess strong adaptability to ex vitro conditions.

Table 1: Effect of different concentrations on fungal contamination

Treatments code	Chemical Concentration	Time period (Min.)			Hard node (%)	Soft node (%)	Shoot tip (%)
		Hard node	Soft node	Shoot tip			
T1	Bavistin (2000 ppm)	14	10	06	10 ± 1.2	9.0 ± 1.0	8.0 ± 0.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	14	12	08			
	HgCl ₂ 0.1%	08	05	02			
T2	Bavistin (2000 ppm)	14	10	06	8.0 ± 0.0	7.0 ± 1.0	5.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	16	12	10			
	HgCl ₂ 0.1%	08	05	1.5			
T3	Bavistin (2000 ppm)	16	12	10	4.0 ± 0.0	3.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptocycline (1000 ppm)	16	12	08			

	HgCl ₂ 0.1%	08	05	02			
T4	Bavistin (2000 ppm)	18	16	10	3.0 ± 1.0	5.0 ± 1.0	2.0 ± 1.2
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	HgCl ₂ 0.1%	09	08	02			
T5	Bavistin (2000 ppm)	16	14	10	2.0 ± 1.2	2.0 ± 1.2	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	05			
	Kanamycine (1000 ppm)	14	12	05			
	Streptocycline (1000 ppm)	16	14	08			
	HgCl ₂ 0.1%	08	06	02			
T6	Bavistin (2000 ppm)	18	16	10	0.0 ± 0.0	1.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	HgCl ₂ 0.1%	08	07	02			

Table 2: Effect of different chemical concentrations on bacterial contamination

Treatments code	Chemical Concentration	Time period (Min.)			Hard node (%)	Soft node (%)	Shoot tip (%)
		Hard node	Soft node	Shoot tip			
T ₁	Bavistin (2000 ppm)	14	10	06	4.0 ± 0.0	6.0 ± 1.2	2.0 ± 1.2
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	14	12	08			
	HgCl ₂ 0.1%	08	05	02			
T ₂	Bavistin (2000 ppm)	14	10	06	1.0 ± 1.0	3.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	16	12	10			
	HgCl ₂ 0.1%	08	05	1.5			
T ₃	Bavistin (2000 ppm)	16	12	10	6.0 ± 1.2	3.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptocycline (1000 ppm)	16	12	08			
	HgCl ₂ 0.1%	08	05	02			
T ₄	Bavistin (2000 ppm)	18	16	10	5.0 ± 1.0	3.0 ± 1.0	4.0 ± 0.0
	Cefotaxime (1000 ppm)	14	12	05			

	Kanamycine (1000 ppm)	14	12	05			
	Streptocycline (1000 ppm)	18	16	08			
	HgCl ₂ 0.1%	09	08	02			
T ₅	Bavistin (2000 ppm)	16	14	10	2.0 ± 1.2	1.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	16	14	08			
	HgCl ₂ 0.1%	08	06	02			
T ₆	Bavistin (2000 ppm)	18	16	10	1.0 ± 1.0	1.0 ± 1.0	0.0 ± 0.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	HgCl ₂ 0.1%	08	07	02			

Table 3: Drying percentage of hard node, soft node and shoot tip explants (The values have been presented as a mean ± S.E.)

Treatments code	Chemical Concentration	Time period (Min.)			Hard node (%)	Soft node (%)	Shoot tip (%)
		Hard node	Soft node	Shoot tip			
T1	Bavistin (2000 ppm)	14	10	06	2.0 ± 1.2	3.0 ± 1.0	6.0 ± 1.2
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptomycycline (1000 ppm)	14	12	08			
	HgCl ₂ 0.1%	08	05	02			
T2	Bavistin (2000 ppm)	14	10	06	1.0 ± 1.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptomycycline (1000 ppm)	16	12	10			
	HgCl ₂ 0.1%	08	05	1.5			
T3	Bavistin (2000 ppm)	16	12	10	1.0 ± 1.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptomycycline (1000 ppm)	16	12	08			
	HgCl ₂ 0.1%	08	05	02			
T4	Bavistin (2000 ppm)	18	16	10	0.0 ± 0.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	05			
	Kanamycine (1000 ppm)	14	12	05			
	Streptomycycline (1000 ppm)	18	16	08			
	HgCl ₂ 0.1%	09	08	02			
T5	Bavistin (2000 ppm)	16	14	10	1.0 ±	0.0 ±	3.0 ±

	Cefotaxime (1000 ppm)	14	12	06		1.0	0.0	1.0
	Kanamycine (1000 ppm)	14	12	06				
	Streptomycline (1000 ppm)	16	14	08				
	HgCl ₂ 0.1%	08	06	02				
T6	Bavistin (2000 ppm)	18	16	10		0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	06				
	Kanamycine (1000 ppm)	14	12	06				
	Streptomycline (1000 ppm)	18	16	08				
	HgCl ₂ 0.1%	08	07	02				

Table 4: Sprouted plants and response (%) at 7, 14 and 21 days after initiation

Treatments	At 7 days		At 14 days		At 21 days		Number of days taken for sprouting
	Number of sprouted plants	Response %	Number of sprouted plants	Response %	Number of sprouted plants	Response %	
T1	5.00	20	10.00	40	12.50	50	20.75
T2	8.75	35	12.50	50	17.50	70	14.00
T3	8.00	32	15.00	60	17.00	68	11.75
T4	9.50	38	15.50	62	18.00	72	11.00
T5	10.75	43	16.25	65	21.25	85	9.00
T6	12.50	50	18.75	75	23.75	95	7.00
S.Em.	0.22		0.22		0.22		0.14
C.D. (p=0.05)	0.66		0.66		0.66		0.42
C.V.%	4.85		3.01		2.40		2.36

Table 5:

Media code	Media details	Number Of shoots	Length of Shoots(cm)	Shooting (%)
M1	½ MS	2.25	5.05	100
M2	½ MS + 0.01 mg l ⁻¹ BA	2.00	4.70	100
M3	½ MS + 0.05 mg l ⁻¹ BA	1.00	4.88	100
M4	½ MS + 0.1 mg l ⁻¹ BA	3.00	5.13	100
M5	½ MS + 0.2 mg l ⁻¹ BA	2.00	4.53	100
M6	½ MS + 1.0 mg l ⁻¹ GA ₃	1.75	4.45	100
M7	½ MS + 0.01 mg l ⁻¹ BA + 1.0 mg l ⁻¹ GA ₃	1.00	3.98	100
M8	½ MS + 0.05 mg l ⁻¹ BA + 1.0 mg l ⁻¹ GA ₃	1.25	4.70	100
M9	½ MS + 0.1 mg l ⁻¹ BA + 1.0 mg l ⁻¹ GA ₃	1.00	5.45	100
M10	½ MS + 0.2 mg l ⁻¹ BA + 1.0 mg l ⁻¹ GA ₃	1.75	3.28	100
M11	½ MS + 2.0 mg l ⁻¹ GA ₃	1.00	3.25	100
M12	½ MS + 0.01 mg l ⁻¹ BA + 2.0 mg l ⁻¹ GA ₃	1.25	4.13	100

M13	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	4.35	100
M14	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	3.05	100
M15	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	2.00	3.25	100
M16	$\frac{1}{2}$ MS+0.02 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.58	100
M17	$\frac{1}{2}$ MS+0.05 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.63	100
M18	$\frac{1}{2}$ MS+0.1 mg l^{-1} BA+0.05 mg l^{-1} NAA	2.00	2.78	100
M19	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.1 mg l^{-1} Kn	1.25	1.45	100
M20	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	1.98	100
M21	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	1.40	100
M22	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	2.18	100
M23	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.05 mg l^{-1} Kn	1.00	1.50	100
M24	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.05 mg l^{-1} Kn	1.00	1.35	100
M25	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.1 mg l^{-1} Kn	1.00	1.83	100
S.Em.		0.12	0.08	-
C.D. (p=0.05)		0.35	0.23	-
C.V.%		17.75	4.73	-

Table 6:

Media Code	Media details	Number of shoots	Length of Shoots(cm)	Shooting (%)
M1	$\frac{1}{2}$ MS	2.00	4.00	100
M2	$\frac{1}{2}$ MS + 0.01mg l^{-1} BA	2.00	4.70	100
M3	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA	1.00	3.25	100
M4	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA	2.75	5.00	100
M5	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA	1.25	4.32	100
M6	$\frac{1}{2}$ MS +1.0 mg l^{-1} GA ₃	1.00	4.57	100
M7	$\frac{1}{2}$ MS + 0.01 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	1.00	4.70	100
M8	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	2.00	4.85	100
M9	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	1.75	5.35	100
M10	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	2.00	3.45	100
M11	$\frac{1}{2}$ MS +2.0 mg l^{-1} GA ₃	1.00	3.37	100
M12	$\frac{1}{2}$ MS + 0.01 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	4.50	100
M13	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	2.00	3.50	100
M14	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	2.30	100
M15	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	3.09	100

M16	$\frac{1}{2}$ MS+0.02 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.52	100
M17	$\frac{1}{2}$ MS+0.05 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.65	100
M18	$\frac{1}{2}$ MS+0.1 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.75	2.15	100
M19	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.1 mg l^{-1} Kn	1.75	1.89	100
M20	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	1.97	100
M21	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	1.92	100
M22	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.00	2.17	100
M23	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.05 mg l^{-1} Kn	2.00	2.05	100
M24	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.05 mg l^{-1} Kn	1.00	1.50	100
M25	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.1 mg l^{-1} Kn	2.00	1.32	100
S.Em.		0.11	0.08	-
C.D. (p=0.05)		0.32	0.23	-
C.V.%		15.42	5.25	-

Table 7:

Media Code	Media details	Number of shoots	Length of shoots (cm)	Shooting (%)
M1	$\frac{1}{2}$ MS	1.00	3.62	100
M2	$\frac{1}{2}$ MS + 0.01 mg l^{-1} BA	1.00	3.40	100
M3	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA	1.00	2.52	100
M4	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA	3.00	2.45	100
M5	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA	1.00	2.25	100
M6	$\frac{1}{2}$ MS + 1.0 mg l^{-1} GA ₃	1.00	3.76	100
M7	$\frac{1}{2}$ MS + 0.01 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	2.00	3.72	100
M8	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	1.00	3.47	100
M9	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	2.00	4.57	100
M10	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA+1.0 mg l^{-1} GA ₃	2.00	3.55	100
M11	$\frac{1}{2}$ MS + 2.0 mg l^{-1} GA ₃	1.25	2.97	100
M12	$\frac{1}{2}$ MS + 0.01 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	3.57	100
M13	$\frac{1}{2}$ MS + 0.05 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.75	2.57	100
M14	$\frac{1}{2}$ MS + 0.1 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	2.00	4.25	100
M15	$\frac{1}{2}$ MS + 0.2 mg l^{-1} BA+2.0 mg l^{-1} GA ₃	1.00	2.86	100
M16	$\frac{1}{2}$ MS+0.02 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.47	100
M17	$\frac{1}{2}$ MS+0.05 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.82	100
M18	$\frac{1}{2}$ MS+0.1 mg l^{-1} BA+0.05 mg l^{-1} NAA	1.00	1.97	100

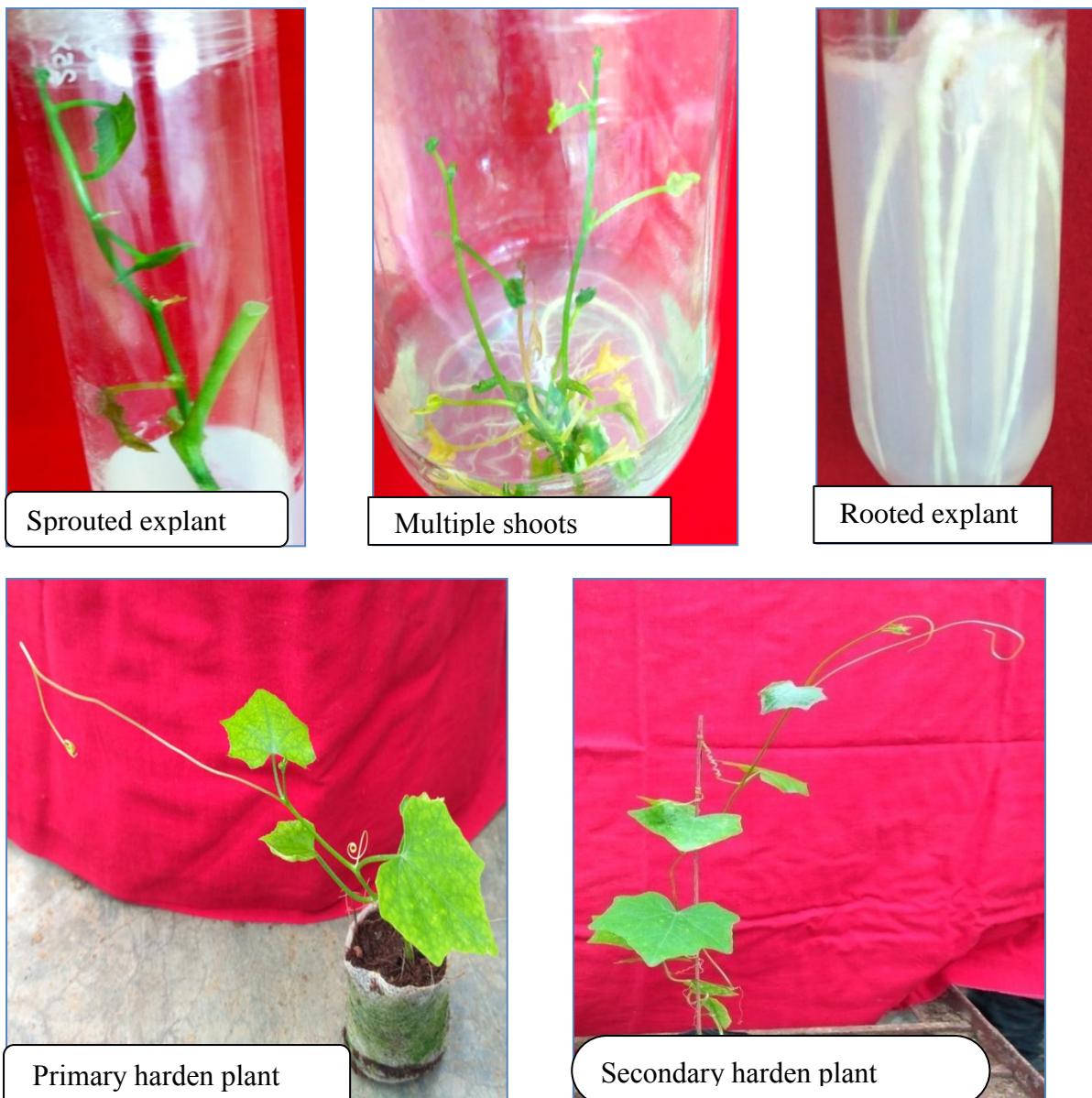
M19	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.1 mg l^{-1} Kn	1.75	1.90	100
M20	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.02 mg l^{-1} Kn	2.00	2.40	100
M21	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.02 mg l^{-1} Kn	2.00	2.44	100
M22	$\frac{1}{2}$ MS+0.1 mg l^{-1} IAA+0.02 mg l^{-1} Kn	1.25	2.06	100
M23	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.05 mg l^{-1} Kn	1.25	1.49	100
M24	$\frac{1}{2}$ MS+0.05 mg l^{-1} IAA+0.05 mg l^{-1} Kn	1.25	1.47	100
M25	$\frac{1}{2}$ MS+0.01 mg l^{-1} IAA+0.1 mg l^{-1} Kn	1.00	1.47	100
S.Em.		0.12	0.05	-
C.D. (p=0.05)		0.35	0.14	-
C.V.%		17.25	3.53	-

Table 8: Number of roots, No. of days taken for rooting, root length and rooting percentage at 21 days

Media Code	Media details	Days to root induction	Length of roots (cm)	Number of roots	Rooting (%)
R1	$\frac{1}{2}$ MS	9.75	3.50	1.00	100
R2	$\frac{1}{2}$ MS+ 0.5 mg l^{-1} IBA	8.75	4.25	3.50	100
R3	$\frac{1}{2}$ MS+ 1.0 mg l^{-1} IBA	9.25	6.02	7.00	100
R4	$\frac{1}{2}$ MS+ 1.5 mg l^{-1} IBA	9.50	4.37	2.50	100
R5	$\frac{1}{2}$ MS+ 2.0 mg l^{-1} IBA	10.25	5.25	7.00	100
S.Em.		0.26	0.11	0.18	-
C.D. (p=0.05)		0.78	0.34	0.55	-
C.V.%		5.44	4.79	8.69	-

Table 9: Mortality percentage at hardening

Treatments	Media details	Total number of plants taken for hardening	Number of plants survive	Mortality (%)
H1	Coco peat (100%)	5	2	60
H2	Soil : Coco peat (20:80)	5	0	100
H3	Soil : Coco peat (50:50)	5	0	100



CONCLUSION

The overall study establishes a robust, efficient, and reproducible micropropagation protocol for *Coccinia grandis*, with each phase—sterilization, establishment, multiplication, rooting, and acclimatization—clearly optimized for maximum success. The sterilization results confirm that Treatment T₆, comprising Bavistin, broad-spectrum antibiotics, and HgCl₂ with adequate exposure time, provides the most effective microbial control while minimizing tissue drying, thereby ensuring high explant survival during establishment. Shoot multiplication across multiple subcultures consistently demonstrated that BA at 0.1 mg L⁻¹, either alone or in combination with GA₃, is the most reliable cytokinin treatment for inducing multiple shoots and achieving superior elongation, while kinetin- and NAA-based treatments remained less effective. The repeated success of BA-centered

media across subcultures highlights its stability and suitability for large-scale propagation of this species. Rooting was maximized with IBA at 1–2 mg L⁻¹, producing vigorous and well-developed root systems. During acclimatization, cocopeat alone proved to be the best substrate for primary hardening, while soil-containing media caused high mortality due to early-stage stress—findings consistent with micropropagation behavior in other cucurbits. Collectively, the optimized protocol ensures high regeneration efficiency, genetic uniformity, and successful transfer of plantlets to ex vitro conditions, making it highly suitable for commercial-scale propagation and conservation of elite *Coccinia grandis* germplasm.

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SHORT COMMUNICATION

UNVEILING THE POTENTIAL: FRENCH BEAN (*PHASEOLUS VULGARIS* L.) PERFORMANCE IN CHAMPHAI DISTRICT MIZORAM

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Abstract: This study was designed in order to evaluate the yield performance of three French bean varieties namely Arka Anoop, Arka Suvidha and Arka Sarath in Champhai District Mizoram. It has been observed that variety Arka Anoop recorded maximum values with respect to Plant height Primary branches, Pod weight, Average No of pods per plant, and yield as compared with other two varieties namely Arka Suvidha and Arka Sarath.

Keywords: French Bean, Yield, *Phaseolus vulgaris*, Mizoram

INTRODUCTION

French bean (*Phaseolus vulgaris* L.; 2n=22) is an important leguminous vegetable crop which is grown popularly for its green pods and dry seeds. It is native to temperate region of Central America. It can be grown throughout the world and contribute nearly 30% of the total production of food legumes (Vasisht and Srivastava, 2012). It is known by various names such as snap bean, kidney bean, haricot bean and rajma in Hindi. It is believed to be derived from a wild vine originally evolved in the

hilly regions of Middle America and the Andes, which substantiates its evolutionary changes. In hilly regions inhabited by undernourished populations, these beans serve as a crucial protein source, often referred to as the "meat of the poor." A hundred grams of green pods boast high vitamin and mineral content, containing 1.7 grams of protein, 0.1 gram of fat, 4.5 grams of carbohydrates and 1.8 grams of fiber. Additionally, they possess medicinal properties beneficial in managing diabetes and certain cardiac issues.

Table 1: Growth and yield of French Bean varieties

Varieties	Plant height (cm)	Primary branches	Pod weight (gm)	Average no of pods per plant	Yield/ha (qtl)
Arka suvidha	41.43	4.35	13.05	19.37	76.77
Arka Sarath	37.58	3.78	11.1	16.56	67.81
Arka Anoop	43.78	5.70	14.70	23.14	81.71
S.E m \pm	0.60	0.21	0.10	0.94	1.09
C.D @5%	1.97	0.69	0.33	1.33	3.54

MATERIALS AND METHODS

The three varieties viz., Arka Sarath Arka Suvidha and Arka Anoop released by Indian Institute of Horticultural Research (IIHR), Bengaluru were used

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under On Farm Testing during Rabi 2022 at farmers field. The seeds were sown in the first fortnight of September following recommended spacing of 45 cm between rows and 30 cm between plants. The recommended FYM at the rate of 20 t/ha and

fertilizer at the rate of 30 kg N, 40 kg P2O5 and 20 kg K2O per ha were applied to the crop. The observations were recorded on five randomly selected plants per replication for each variety for most desirable character like plant height, no. of branches per plant, pod weight(gm), number of pods /plant, duration and yield (q/ha) in Table 1. The data on selected parameters of demonstration plots as well as control plots were collected on regular basis and continued till harvesting of crops. Regular field visits were made by the team of KVK scientists. The observations were recorded on number of primary branches per plant, number of green pods per plant, green pod length and green pod yield. The statistical analysis of the data was performed in randomized block design.

RESULT AND DISCUSSION

1. Plant height

The maximum plant height was observed in Arka Anoop (43.78 cm) followed by Arka Suvidha (41.43 cm) while lowest plant height was recorded in Arka Sarath (37.58 cm). The varietal differences in plant height were due to genotypic make up. Phookan et al. (1990) reported variations among the hybrids in plant height.

2. Number of primary branches per plant

The data (Table 1) showed that significantly higher number of primary branches per plant were recorded in Arka Anoop followed by Arka Suvidha. In Arka Anoop, number of primary branches per plant were 5.70, lowest primary branches were observed in Arka Sharath recorded 3.78. This finding was incongruity with those of Anjanappa et al. (2000),

3. Pod Weight

Significantly maximum pod weight (14.70gm) was noticed in Arka Anoop followed by Arka Suvidha where Pod weight was recorded 13.05 gm whereas lowest pod weight was noticed in Arka Sarath (11.10gm)

4. Average no of pods

Table 1 showed that maximum number of green pods per plant (23.14) was recorded in Arka Anoop followed by Arka Suvidha (19.37) and lowest in Arka

Sarath (16.56). This parameter is an important yield contributing trait and was in accordance with the results observed by Akhilesh et al. (2013) for number of green pods per plant.

5. Yield/ha

The yield parameters (Table 1) showed that significantly higher number of primary branches per plant, maximum green pods weight per plant and higher number of green pod per plant were recorded in Arka Anoop (81.71 qtl/ha) followed by Arka Suvidha (76.77 qtl/ha) while lowest was recorded in Arka Sarath (67.81). The pod yield variations amongst the genotypes under varying field conditions have been reported by several workers (Hariharan and Singh 1990).

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