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Chilli (*Capsicum annuum)* cultivation, diseases, breeding, advanced techniques in biotechnology- General review

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ABSTRACT

Biotechnology techniques are powerful tools that can supplement traditional breeding mode and improve crop cultivation. *In vitro* clonal propagation of chilli is relatively slower than other members of Solanaceae family because of its recalcitrant nature which makes it difficult to apply Recombinant DNA Technology. This review presents a consolidated data of cultivation pattern, diseases attacking chilli, breeding methods in chilli and advanced techniques in chilli Biotechnology.

Key words: Chilli, *Capsicum Annum*, cultivation, Disease, Tissue culture, Transformation, Molecular Breeding, yield data.

INTRODUCTION

Chilli, the universal spice named as "wonder spice" is one of the important commercial crop. Different varieties of chilli are cultivated and are used for various uses like vegetable, pickle, spice and condiments in daily life. Chilli is the most important ingredient in different cuisines all around the world. It adds flavor, aroma, texture, pungency, and color to the dishes. Indian chillies are famous for their color and pungency level. The world's hottest chilli "Naga Jolokia" is cultivated in Assam district in India.

Chillies were grown and cultivated from 500 B.C.[1, 2] Chilli was spread to the rest of the world by Christopher Columbus [3, 4] who discovered America in 1943. Chillies were cultivated by the farmers with a primary crop to protect the primary crop from any damage the birds could do. It gained popularity in America as a flavoring agent. In 16th century chilli was popular in Asia and native Asians started cultivating the crop. The south Asian climate suited the crop well. The concentration of production of chilli shifted to Asia. Today the most pungent varieties of chilli are grown in Asia. Chilli was brought to India by vasco-da-gama .There are more than 400 different varieties of chilli in the world. Chilli has many medicinal properties and is used in the traditional Indian medicine system "Ayurveda".[5]

Indian cuisine is unimaginable without this wonder spice India has become the largest producer and exporter of chilli in the world. It contributes to 25% of the world's total production of chilli. The scientific name of chilli is *Capsicum annum* and other capsicum species. The common name for chilli in English are chili pepper, chilli pepper, chili, Chile pepper In Dutch it is called Spaansepepper, chilepepper, rode pepper, cayennepeper, In German it is called chili, roter pFeffer, In Spanish it is called Chile , Chileaji, Chilepicante , Pimiento rojo. *Capsicum annum* is an annual, *capsicum frustescens* is a short lived perennial. Flowers develop about three months after planting; flowers are either white or greenish white. The lanceolate leaves are either glabrous or pubescent. *Capsicum annum* requires a warm tropical climate, cannot tolerate frost. It likes hot and dry weather for fruit ripening. The pollination is done by bees. It is a

herbaceous plant that reaches a height of one meter. It prefers well-drained, sandy or slit loam soil. The propagation is through direct seeding or transplanting. Chilli is being affected by various aphids, fruit fly and American bollworm (*Helicoverpa armigera*).[6]

Species

Capsicum genus consists of 20-27 species. C.*annum,* C.*baccatum,* C.*chinense*, C.*frustescens*, and c.*pubescens* are domesticated. Fruits of capsicum may vary in size, color and shape within species. Phylogenetic relationship between species was investigated.

Climatic conditions

Chilli is cultivated in tropical and sub-tropical climates, at up to 2000m altitude. They are mostly cultivated as a rain fed crop in India. The ideal rainfall and temperature requirement for chilli is 850-120 mm per annum and 20-25 degree centigrade respectively. The soil should be light loamy with rich organic matters necessary for the satisfactory growth of the plant. It also needs adequate soil moisture for growth.[7]

Seasonality

Chilli is grown as a seasonal and annual cash crop. Sowing of seeds starts in the first week of august that is after the commencement of south west monsoon. The total growth period of this crop is around 4-5 months. Harvesting is done mainly in December. After proper drying in sun the market starts from April.[7]

World scenario in chilli production

On the global front according to FAO statistics the total chilli production is 21.28 lakh tones. When comparing the history the highest production was around 26.4 lakh tones. This decline in production is due to unfavorable weather conditions in major chilli producing countries like India, china, Pakistan. The top 10 chilli producing countries are India, china Ethiopia, Myanmar, Mexico, Vietnam, Peru, Pakistan, Ghana and Bangladesh. India takes the major share of 36% in global production and china 11% Bangladesh (8%) Peru (8%) and Pakistan (6%). Malawi, Zimbabwe and Uganda export limited quantities of highly pungent “East African bird’s Eye”. China exports some high pungent type chilli as “Fukien”. Imports as whole chillies have fallen down as exports of chilli powder and oleoresin have grown. Number of factories in India, Pakistan and china has set up collaboration with multinational spice companies, which are now able to meet EC and ASTA standards. The world demand is expected to go up and there is a great scope for export of chillies. Demand is growing for products such as chilli paste, curry powders and chilli sauce which are more convenient for the food industry. To export these products strict hygiene and quality control must be maintained.[7]

Indian scenario in chilli production

India is the largest producer and consumer of chilli among other producers in the world. India has the largest area under chilli cultivation. It is nearly grown in all parts of the country. Chilli is grown throughout the entire year. The peak arrival of chilli in market is from February to April and extends till May[7].

South Indian scenario

In South India Andhra Pradesh is the leading producer of chilli and it also constitutes the maximum acreage for chilli cultivation in the country. It contributes to 49% of the total India’s production. It produces around 2.7 lakh tones of chilli. Karnataka follows Andhra Pradesh with 14%.Guntur is the Asia’s largest chilli market. The marketing season begins from March and reaches its peak during April and closes by mid of March. Rs.500 crore trade takes place in Guntur market during season. It is estimated that 25-30% of chilli is used for powder preparation.The major regions chilli is cultivated in India are Guntur, Warangal, Khammam, Krishna, Prakasham, Hyderabad, Pundur, Nizamabad, Cuddpah, Nellore, Dharwad , Mysore, Hasan, Bangalore ,Bellary, Ranibennur, Hubli, Gadag, Byadgi, Nasik, Coimbatore, Ramanathapuram, Tuticorn, Tirunelveli, Virudunagar, Kanyakumari, Madurai, Salem, Tiruchi , Villupuram, Cuddalore[7].

Cultivation pattern

Chilli is a simpler crop to cultivate. The soil is ploughed properly at the time of planting the crop. In India chillies are produced throughout the year. One in the dry season and other in the wet season .The seeds are sowed in April and harvested in august and for wet season seeds are sowed in

august and harvested in April, this season is accompanied by heavy rainfall. The watering and harvesting are the important things to be done in the cultivation of chilli. Regular and appropriate watering is needed when the seeds are in sprouting stage. Harvesting is done when the green chilli pod is green and matured[7].

Capsaicinoids

Chilli pepper gets its intensity from "capsaicin" (8-methyl-N-vanillyl-6-nonenamide) and several other related phytochemicals collectively called as "Capsaicinoids.[8, 9] capsaicin oleoresin is the main ingredient of the pepper spray while consuming chilli Capsaicinoids bind with the pain receptors in mouth and throat that are normally responsible for sensing heat, once the pain receptors are activated by the Capsaicinoids, these receptors send a message to the brain that the person has consumed something hot. The brain responds to the burning sensation by raising the heart rate, increasing perspiration and release of endorphins. Capsaicin alters energy produced by hydrolysis of ATP by body cells. In normal hydrolysis the SERCA protein uses this energy to move calcium ions into the sarcoplasmic reticulum. When capsaicin is present, it alters the conformation of the SERCA and thus reduces the ion movement as a result the ATP energy is instead released as heat.[10]

Nutritional value of chilli

Chilli comprises of the following nutrients Carotenoids, Proteins, Fats ,Vitamin A, Vitamin C, Provitamins E, P, B1, B2, B3, Steroidal alkaloidal glycosides and scopoletin. Chill's are low in saturated fat, cholesterol and sodium. It is high in Vitamin A, Vitamin C, Vitamin K, Vitamin B6, Potassium, Copper, Manganese, dietary fiber, Thiamin, Riboflavin, Niacin, Folate, Iron, Magnesium and Phosphorus. The nutritional values and health benefits of chilli are ideal for Maintaining optimum health and Weight loss.

Medicinal use

Chilli extracts have anticancer and anti mutagenic effects[11]. Carotenoids present in chilli extracts were found to have anti mutagenic and anti tumor promoting activity. Topical capsaicin has safe analgesic effect against many painful conditions .Capsaicin is responsible for cancer cell death in rats[12]. Capsaicin helps in weight loss for people suffering from obesity[13,14]. Capsaicin's from chillies are used to kill nerve cells in pancreases of mice with diabetes thus allowing the insulin producing cells to start producing insulin again[15, 16]. Chilli helps in the control of LDL or bad cholesterol, which is the cause for cardiovascular disease[17, 18]. If chilli pepper is consumed in the meal the amount of insulin required to lower blood sugar is reduced[19]. Chilli is used as a control to microbial contamination levels of foods in countries with minimal or no refrigeration[20]. Capsaicin could have an anti-ulcer protective effect on stomach infected with *H.pylori* by affecting the chemicals the stomach secretes in response to infection[21, 22, 23].

Health risks and precautions

Chronic ingestion of chilli products may induce gastro esophageal reflux[24]. Chilli may increase the number of daily bowel movements and lower pain threshold for people with irritable bowel syndrome[25]. Chilli should not be swallowed as whole; this may cause bowel obstruction and perforation. Consuming chilli's after anal fissure surgery may lead to post operative Symptoms[26].

Breeding

In chilli breeding[27] the choice of breeding method depends on the breeding objective and the plant material being used as parents. The aim of a chilli breeder is to give a better plant with superior genetic potential for yield, protection against production hazards and improved quality. The molecular breeding techniques are: (a) Molecular markers, (b) Transformation.

Molecular breeding

Modern plant breeding uses techniques of molecular biology to select, or in the case of genetic modification, to insert, desirable traits into plants.

Marker Assisted Selection

Different genes can influence a desirable trait in plant breeding. The use of tools such as molecular markers or DNA fingerprinting can map thousands of genes. Isozyme and molecular markers have been applied to chilli. A saturated Isozyme and Restriction Fragment Length polymorphism (RFLP) map of chilli has been

reported. [28] The map contains 192 chilli genomic DNA clones with 19 linkage groups with total coverage of 720cm. The specific map positions of 26 RFLP markers in 7 linkage groups were not determined and vast regions of chilli genome remain unmapped. This area of research is rapidly adding to our knowledge of chilli's and will undoubtedly be important in the future.

Tissue culture

In vitro clonal propagation is a key tool of biotechnology that exploits totipotent nature of plant cells, a concept proposed by Haberlandt (1902).[29] Tissue culture in combination with molecular techniques is used to incorporate specific traits through gene transfer. Highly efficient reproducible plant regeneration systems lack in chilli, so an array of research work has been conducted to explore regeneration potential of chilli. To certain extent regeneration has been achieved through organogenesis other modes of such as protoplast to plant regeneration are also explored. Gunay and rao (1978) [30] marked the clear influence of cotyledon as explants in the hormone combination BA (8.88µM) + IAA (2.85-5.71µM) for indirect somatic organogenesis in *Capsicum annum* and *Capsicum frutescens* species. Similarly same combination of hormone is used by Agrawal et al. (1989) [31] for explants hypocotyls, epicotyls, stem, leaf, root, shoot tip, embryo for direct and indirect organogenesis. Seeds were used as explants for organogenesis in the hormone combination BA(2.22µM) + NAA(0.54µM) by Ebida and Hu in 1993.[32] Direct and Indirect somatic embryogenesis of mature and immature zygotic embryo was tested by Harini and Lakshmi sita (1993) [33] and Buyukalaca and mavituna(1996) [34] for best results. Mahuri and Rajam(1993)[35] and Christopher and Rajam(1996)[36] used shoot tip as explant for axillary meristem regeneration with the supplement of BA to MS media. Silver nitrate was added as a supplement to MS media with other Auxin and Cytokinen for stem elongation was proposed by Hyde and Phillips in 1996[37] which recorded maximum percentage result. For direct as well as indirect somatic embryogenesis MS media supplemented with Auxin and Thidiozuran(TDZ) recorded green and shiny calli and maximum regeneration this combination was proposed by venkataiah et.al (2003)[38] . In *Capsicum frutescens* shoot tip was used as explants for axillary proliferation in the media composition BA (22.2µM) + Kin (4.6µM) by Sanatombi and Sharma in 2007. For organogenesis by cotyledon explants in *Capsicum annum* species Joshi and Kothari in 2007 used the media combination BA (22.2µM) + PAA (14.7µM). Again in 2008 Sanatombi and Sharma for organogenesis used leaf, cotyledon, hypocotyls explants in hormone combination BA (8.8µM) + IAA (11.4µM)

Transformation

A peculiar aspect of chillies is the inability to be regenerated. This limits the technique of genetic transformation. For unexplained reasons chilli has been recalcitrant to being regenerated.

Table 1: Showing production year wise

Production	Area (Ha)	Production (tons)
1999-00	977530	1056000
2000-01	884040	1046220
2001-02	881290	1113090
2002-03	827930	849250
2003-04	794080	1273860
2004-05	771240	1237781
2005-06	681612	1009481
2006-07	671543	1150486
2007-08	661892	1323045

Table 2: Showing area and production state wise

State	Area(Ha) (tons)	Production
Andhra Pradesh	171450	537710
Gujarat	31650	37840
Karnataka	69880	94500
Madhya Pradesh	46660	42480
Maharashtra	99300	51214
Orissa	75120	63290
Punjab	9882	15888
Rajasthan	17720	17530
Tamil nadu	49033	31830
West Bengal	51957	60727
Uttar Pradesh	17340	16119
Assam	14690	9490

once this perplexing problem is solved, Genetic Transformation will be available to introduce novel genes in chilli.

Traditional plant breeding techniques could not serve the increasing need for crop productivity but Genetic Transformation holds great promise to over come all the barriers of traditional methods. Genetic manipulation is an DNA technology which could transfer gene to alleviate a specific purpose. In general *Agrobacterium tumefaciens* has been used as vector for genetic transformation. *Agrobacterium* is the only vector so for transformation of chilli. Liu et al (1990)[39] used *Agrobacterium* strains A282 and C58 in binary vector pCV 3850 with GUS and nptII marker gene for transformation in chilli with explants hypocotyls, cotyledon, leaf disc which recorded positive expression GUS gene. *A.tumefaciens* strain LBA 4404 in vector p5T35 with GUS gene was treated with chilli cotyledon explants also resulted positive was tested in *Capsicum annum* species by Engler et al.(1993)[40.] Zhu et al. (1996)[41] used CMV-CP gene in *Capsicum* species with nptII marker in leaf explants resulted in the expression of CMV-CP gene. Hypocotyl and cotyledon explants were tested with *A.rhizogenes* strain K 599(GUS) by Li et al. (2003)[42] resulted in 40.8% transgenic plants. Mesophyll protoplast explants was tested with pCAMBIA 2300 vector containing TMV-CP , hpt, gus gene resulted in efficient transient expression and transformation was demonstrated by Jeon et al.(2007).[43] Hasnat et al.(2008)[44] studied the transformation efficiency of hypocotyls segments with binary vector pTCL5 in *Capsicum frutescens* resulted in positive transformation.

Insects and pests affecting chilli plant

Aphids (*aphis gossypii & myzus persicae*)

Aphids are generally seen in plants which are in reproductive phase. It appears on tender shoots and leaves which sucks sap from leaves. It secretes a sweet substance which attracts ants and because of this it develops a black pod which will lose quality.[45]

Mite (*polyphagotarsonimus latus*)

Mites are a major problem in chilli cultivation and it appears 40 days after sowing the seeds. Nymph and adult suck sap from leaves and the affected leaf curls to attain an inverted boat shape. Leaves turn dark grey in color, stops flowering and reduces yield. The fruits become hard and a white strip appear on the fruit.[45]

Thrips (*Scriptothrips dorsalis*)

Thrips are the major pest affecting chilli crop throughout its life period. Adults and nymphs lacerate the leaf tissue and suck the sap. Tender shoots, buds and flowers are attacked and as a result they become twisted and deformed.[45]

Fruit borers (i) tobacco caterpillar (*spodoptera litura*)

In chilli the caterpillar damages the fruits by boring in to it. The affected pods drop off or develops white color on drying.[45]

Gram caterpillar *(Helicoverpa armigera*)

Larvae damages by boring into fruits and feeds on inner contents of the pods. The entryhole are large and typically circular. The affected pod drops off.[45]

Ragi cut worm (*spodoptera exigua*)

It mostly damages leaves, flowers and tender pods. It feeds the fruit at the base of the calyx[45].

Root grub (*holotrichia consanguinea*)

Grub always eats the nodules the fine rootlets and also girdle on the main root. It generally prefers young plants. The attacked plants can be easily identified and pulled out easily[45].

Crop	Major pest	Scientific name	Insect order	%of crop loss
Chilli	Fruit borer	*Helicoverpa armigera*	Lepidopteran	35%
Chilli	Fruit borer	*Spodoptera litura*	Lepidopteran	35%

White fly(*bemisia tabaci*)

Nymphs and adults suck on under surface of leaves and excrete honey dew causing contamination on lint. Leaves appear sick and It transmits leaf curl virus.[45]

Major factors affecting chilli crop loss are Insects and pests, Virus and Herbicide tolerance. The major crop loss in chilli from insects and pests is 35%, virus 24%, herbicide is 2% and rest 39% Identity

Helicoverpa armigera is the single important pest in the agriculture of southern Asia. It attacks a wide range of crops.[46] .It has become resistant to a range of insecticides, resulting in increasing production costs and increased yield losses. It belongs to insect order Lepidoptera: noctuidae. The common names are, In English: Old African bollworm, corn earworm, cotton bollworm, In French: noctuelle des tomates, In German: Altweltlicher baumwollkapselwurm, In Spanish: oruga(gusano) de las mazorcas (Spanish).

Crop loss due to *Helicoverpa armigera*

The boll worm, *Helicoverpa armigera* is a polyphagous pest of worldwide occurrence inflict*ing* annual crop damage in India worth US $1billion.chilli yield loss due to *Helicoverpa armigera* on plants in green house and field conditions. The yield reduction is around 11.11q/ha respectively.

CONCLUSION

Plant tissue culture and Genetic transformation are powerful tools that can complement traditional breeding methods and can be used in the improved production of Capsicum. The popularity and demand for chilli are providing a boost to the chilli industry, but production is constrained to attack of pest restricting their potential yield. The most important aspect of chilli breeding is to incorporate pest, disease resistance varieties, while retaining high yield capacity. By involving the above contemporary methods pest, disease resistant variety of Capsicum can be produced, which will boost the economic status of our country in chilli production in the world market.

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