

Study on DNA of Male or Female Specific PCR Products of Cannabis Plant

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Abstract – The cannabis plant is a tall yearly yield of monetary significance. It is generally dioecious, yet fiber hemp assortments have been reproduced to be monoecious. Isolating male and female plants at early formative stage is valuable because of the impact of sexual orientation at horticulturally critical attributes. A few examinations have been centered around fostering a solid atomic marker for sex assurance in cannabis plants. Our investigation analyzes three DNA markers for the identification of male genotype in absolutely twelve examples of modern hemp and therapeutic cannabis plants. Genotype scoring of SCAR119 marker had all the earmarks of being the most dependable, trailed by MADC2 and SCAR323, when contrasted with the noticed aggregate of plants. The outcomes affirmed the bits of knowledge given in past examinations. Examination and Polymerase Chain Reaction (PCR) investigation should proceed to discover further developed DNA markers for sex assurance of cannabis plants.

Keywords – Female, Plants, PCR Markers

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INTRODUCTION

Cannabis (advanced from Greek word Scythian or Thracian signifying "an approach to deliver smoke") has existed since antiquated occasions and is generally utilized as a fiber source, a food source, a medication, and an euphoriant. Cannabis (Marijuana) has been utilized to treat an assortment of sicknesses, including glaucoma, torment, queasiness, asthma, despondency, neuralgia, and a sleeping disorder. In the same way as other yields (e.g., wheat, corn), maryjane was initially a normally happening weed animal groups. Later it was reproduced and developed into a critical money crop for a multi-billion dollar illegal industry. Hemp of India (*Cannabis sativa*), considered as a medication, comprises of the dried blooming highest points of *Cannabis sativa*, which is a local of the inside of Asia, however developed in numerous pieces of the world. The plant is accepted to be begun in the sloping districts simply north west of the Himalayas. In India it chiefly fills around there or northern India and accomplishes a stature of 3-16 ft.

Taxonomy

The Cannabis genus was formerly placed with nettles in the family Urticaceae or mulberries in the family Moraceae, but is now considered along with hops (*Humulus* sp.) to belong to the family Cannabaceae, as highlighted in Table 1.1 (Shults et al., 2001).

Table: 1.1 Scientific classification of Cannabis

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Cannabaceae
Genus:	<i>Cannabis</i> L.
Species	<i>Cannabis sativa</i> L. <i>Cannabis indica</i> Lam. <i>Cannabis ruderalis</i> Janisch

Morphology

Cannabis is a yearly, dioecious, blossoming spice. The leaves are palmately compound, with serrate pamphlets (Figure 1.1). The primary pair of leaves typically have a solitary flyer, the number continuously expanding up to a limit of around thirteen handouts for every leaf (generally seven or nine), contingent upon assortment and developing conditions. At the highest point of a blooming plant, this number again reduces to a solitary flyer for every leaf. The lower leaf matches normally happen in a contrary leaf course of action and the upper leaf sets in a substitute course of action on the fundamental stem of a develop plant. Cannabis is normally diploid, having a chromosome supplement of $2n=20$, albeit polyploid people have been falsely created Cannabis ordinarily has blemished

blossoms with staminate "male" and pistillate "female" blossoms happening on isolated plants albeit androgynous blossoms here and there happen. Male blossoms are borne on free panicles, and female blossoms are borne on racemes. It isn't surprising for singular plants to bear both male and female blossoms in same strains, a condition called monoecy. On monoecious plants, blossoms of both genders may happen on discrete inflorescences, or on a similar inflorescence. All known strains of Cannabis are wind-pollinated (Clarke, 1991) and produce "seeds" that are actually called achenes.

Uses of Hemp (*Cannabis sativa*)

Hemp gives food and oil from its seeds, fiber for rope, texture from stem and psychoactive medications from its blossoms and leaves. Hemp development is considered as a natural well disposed movement that can uphold practical farming. It can possibly be utilized in phytomeration to eliminate hefty metal pollutants from the dirt. Moreover it very well may be utilized to forestall soil disintegration because of long tap root improvement, while improving soil through crop in pivot; anyway its development is stringently controlled because of its psychoactive nature and utilization in creating medications such hashish and weed. As a medication it ordinarily comes as dried buds or blossoms (cannabis), tar (hashish), or different concentrates by and large known as hashish oil. In the mid twentieth century, it got unlawful in the vast majority of the world to develop or have Cannabis for drug purposes.

Chemical constituents

Cannabis plants produce a special group of terpeno-phenolic compounds called cannabinoids, which produce the "high" one encounters from smoking weed. The ethicalness of cannabis lives principally in a resinous exudation, which is tossed out in blistering climate, upon the outside of the plant, delivering it moist and glue to the fingers. This is created considerably more to a great extent in the Indian than in the European plant, likely owing essentially to the distinction of environment. Cannabis contains an unpredictable combination of almost 60 distinctive individual synthetic compounds called cannabinoids, major of them are delta-9-Tetrahydro cannabinol (THC), Cannabinol (CBN), Cannabidiol (CBD) and so forth. The two cannabinoids ordinarily delivered in most noteworthy bounty are cannabidiol (CBD) and additionally Δ^9 - tetrahydrocannabinol (THC), yet just THC is psychoactive. Since the mid 1970s, Cannabis plants have been ordered by their compound aggregate or "chemotype," in view of the general measure of THC delivered, and on the proportion of THC to CBD (Small and Beckstead, 1973). The best measure of cannabinoids is found in the new development close to each apical tip (Kimura and Okamoto, 1970; Steinberg et al., 1975). Aside from this, Cannabis additionally contains flavonoids,

unstable oil, terpenoids and alkaloids. Every one of these substances are discharged by glandular trichomes that look hair-like development on the leaves, stem, and the blossoms of cannabis plant. These hair developments radiate a tacky substance that contains the dynamic opiate synthetic (THC). While there are no distributed reports of organs present on root surfaces, the majority of the ethereal parts have them, alongside non-glandular trichomes (De Pasquale et al., 1974). These trichomes happen most richly on the botanical calyxes and bracts of female plants (Dayandan and Kaufman, 1976). Roots contain just follow sums. Stalks, branches and twigs have more noteworthy amounts, albeit not as much as leaf material. Vegetative leaf contains fluctuating amounts relying upon its situation on the plant, lower leaves having less and upper ones more. Leaf organs are generally thick on the abaxial (underside) surface.

Cannabis is the lone plant to contain Δ^9 - tetrahydrocannabinol (THC) (Figure 1.2), one of the fundamental psychoactive constituents which is the rule compound liable for the vast majority of the trademark physiological impact of the cannabis item. It produces impact of elation from the outset followed by narcosis. The psychoactive impacts of Cannabis are known to have a biphasic nature. Essential psychoactive impacts incorporate a condition of unwinding, and less significantly, rapture from its fundamental psychoactive compound, THC. Optional psychoactive impacts, like an office for philosophical reasoning; thoughtfulness and metacognition have been accounted for, among instances of uneasiness and neurosis. At long last, the tertiary psychoactive impacts of the medication cannabis can remember an expansion for pulse and appetite, accepted to be brought about by 11-Hydroxy-THC, a psychoactive metabolite of THC delivered in the liver.

- The male plant generates lower amount of THC than female plant.
- The THC levels start to drop significantly, as the flowers of the female plants are fertilized to produce seeds.
- Three types of preparation of the plant are commonly used for euphoric purpose namely Bhang, Ganja and Charas.

Bhang

These are large green leaves and flowering shoots of either the male or the female plants. Bhang is not used for smoking. It is used to make beverages such as Thandai, Lassi, and Halwa.

Ganja

Ganja is made from the top leaves and unfertilized flower of young female plant. The leaves and

unfertilized flowers are dried and used. Ganja is used for smoking. Ganja has higher amount of narcotic chemical than Bhang.

Charas (Hashish)

Charas, that is, the separated resin, in whatever form, whether crude or purified, obtained from the cannabis plant and also includes concentrated preparation and resin known as hashish oil or liquid hashish. Charas is the resin extracted from the top leaves and unfertilized flower of young female plant. The leaves and flowers are rubbed in the hands. The resin stuck to the hand is collected and used as charas.

REVIEW OF LITERATURE

Sex assurance is the formative choice that happens during the vegetation cycle that prompts the separation of the two organs or cells that produce the two gametes. Darwin (1877) contemplated various types of blossoms on plants of same species. As indicated by him minority of plant species are 'explicitly polymorphic', including dioecious species, with discrete guys and females. He likewise showed that numerous dioecious species with bisexual family members have clear fundamentals of other gender structures in blossoms of plants of each sex, proposing ongoing development of unisexual blossoms. In numerous explicitly recreating plant species (and a few creatures) all people are basically indistinguishable in their sexual orientation condition. Some such 'explicitly monomorphic' species are androgynous.

Lloyd and Bawa (2012) gave the term 'cosexual'. This term was referred to those plants person which have both sex capacities, regardless of whether present inside each rose (bisexual), or in discrete male and female blossoms (monoecious). Charlesworth (2013) estimated that low recurrence and dispersed ordered dissemination of dioecy and sex chromosomes propose that cosexuality is the hereditary angiosperm state and Renner and Ricklefs (2012) expressed that dioecy develops from monoecy which has all the earmarks of being normal. Since, the species with male people and female people have developed over and over from androgynous begetters; the components for the control of sex assurance in blossoming plants are very different. The sex is primarily dictated by genotype in all species, yet the components territory from a solitary controlling locus to sex chromosomes bearing a few connected loci needed for sex assurance.

The hereditary control of sex assurance is getting surely known in a few creature frameworks, especially *Drosophila melanogaster*, *Caenorhabditis elegans* and warm blooded animals. In plants, understanding the sex assurance framework is firmly

associated with seeing how separate genders advanced. In plants, there is extraordinary variety in where, when and how this choice of sex separation happens (Christina and Jo, 2010).

De Meijer (2014) has revealed that Cannabis has been portrayed as prevalently dioecious a condition in which the male and female parts are on independent blossom on discrete plants, albeit some monoecious assortments (having separate male and female blossoms on a similar plant) have likewise been depicted. Angiosperms are likewise quite compelling for exact investigations of sex chromosome development, since they presumably advanced separate genders over and again and moderately as of late. Different plants, especially Bryophytes (Okada et al, 2001), additionally have intriguingly freely developed sex chromosomes.

Strittmatter et al. (2012) recommended that subdioecy is a condition where some dioecious populaces have propensity to deliver people that are not unmistakably male or female. This condition is thought to address a progress among hermaphroditism and dioecy. As per Ranalli (2004) some Cannabis assortments have likewise shown subdioecy. Cannabis show numerous sexual aggregates which can be portrayed as far as proportion of male to female blossoms happening in the person of ordinary in development.

OBJECTIVES

- 1) To Sequence the DNA of male or female Specific PCR Products of Cannabis plant.
- 2) To develop male or female specific PCR based marker for the identification of dioecious Cannabis plant at juvenile stages.

RESEARCH METHODOLOGY

Cannabis is normally happening weed species. It develops fiercely close to where the dirt is water bountiful. The testing of plants was done arbitrarily by utilizing arbitrary number table (proposed by Tippette). The zones were chosen keeping the power of the cannabis in thought. Since cannabis is discovered bounteously in the spaces connecting to Himachal Pradesh, Uttarakhand and Nepal (all exceptionally influenced by weed dealing), thus as needs be five area were chosen with due consent of State Excise Department. In each area three locales were settled for test assortment. The rundown of the multitude of locales of each region is appeared in Table 3.1. A sum of 75 Cannabis plant tests were gathered from the accompanying destinations of five region of Uttar Pradesh, India as demonstrated in Figure 1.1.



Figure 1.1: Map of Uttar Pradesh showing district selected for sampling

Table: 3.1 List of sampling sites

Districts	Name of collection Area	Male plants	Female plants
Allahabad	Meja	5	5
	Maheva	5	5
	Sarpatriya	5	5
Lakhimpur Kheri	Katuli	5	5
	Sonapur	5	5
	Lakhan	5	5
Saharanpur	Nagla	5	5
	Govindpur	5	5
	Phandpur	5	5
Gorakhpur	KajakpurDhala	5	5
	Pipraich	5	5
	Nauva Awal	5	5
Gonda	Pipri	5	5
	Naryanpur	5	5
	Chandpur	5	5
Total		75	75

RESULTS

Cannabis is a normally happening wild developing species. In India it is mostly found around there or northern India. Immense fields of cannabis (of high intensity) are found in the States of Uttarakhand, Himachal Pradesh, Jammu and Kashmir. It additionally fills fiercely in the bordering nation Nepal. As indicated by reports (The Hindu) supported observation has uncovered that cannabis is in effect unlawfully developed at Kullu in regions like Malana, Jana, Hanana, Tugla, Barsaini, Tosh, Baragarh, Bongu, Fozal Valley, Kharad, Kashtawad, Jarad and Lagh Valleys, Garihia, Gharhingcha, Kandi, Bali, Kathiari, Dhara Pari, Khanala, Tilapul, Tuncha, Chenur, Kasol, Vashisth, Jagat Sukh, Jerry, Anni and Dalash. Cannabis is additionally developed in different places in Mandi, Chamba, Shimla and Sirmour in Himachal Pradesh. In Jammu and Kashmir, this plant is filled in Anantnag, Baramulla, Srinagar, Pulwama, Kupwara, Doda and Kishtwar. Hashish fabricated from cannabis delivered here is viewed as of superior grade, actually like the one created in Nepal. Hashish (Charas), a cannabis item, is being produced in huge amounts in Himachal

Pradesh and Jammu and Kashmir notwithstanding endeavors by the Central enemies of opiates offices. These are the districts which are profoundly influenced by drug dealing of Charas and Ganja. Because of its psychoactive nature cannabis goes under NDPS Act, subsequently require permit from something similar for keeping up the species. In this way, strains of Cannabis species are not kept up by any private, government or National examination Institutes. Subsequently, with the assistance of State Excise Department, Uttar Pradesh, India, a study was directed to find and settle the destinations for test assortment (locales were chosen uniquely in Uttar Pradesh district since State Excise office, Uttar Pradesh can surrender consent for state as it were). Regions which were available bordering to the previously mentioned States and nation were settled. From each area three locales were chosen where plentiful fields of cannabis were available. In like manner, the leaves of cannabis were gathered from haphazardly chose (Proposed by Tippet) male and female plants in each site (populace) at time periods least 5 meters from the fields of each site of Allahabad, Lakhimpur Kheri, Gorakhpur, Gonda and Saharanpur locale with appropriate authorization of the State Excise Department of Uttar Pradesh. The leaves from realized male and female plants were gathered and put away in plastic sacks which close in zip-lock design at - 20°C. Leaves of adolescent plants were additionally gathered and kept in - 20°C. The adolescent plants were gathered and become further for sex ID.

CONCLUSIONS

- A single method for DNA isolation and purification was selected which was suitable for isolation of DNA from cannabis species leaf tissues. This was achieved by modifying the protocol of CTAB plant DNA extraction (Highpura) kit.
- The present optimized protocol for RAPD analysis may assist further in marker based studies on cannabis.
- The developed SCAR marker which showed consistency in the result with samples of various districts of Uttar Pradesh, India, thus confirming the reliability of the primer in determining sex of cannabis plant at juvenile stage.

The created SCAR marker can be of impressive assistance in the sex based assurance of cannabis in Uttar Pradesh. The current examination can help in investigating male and female plant and may help the law implementation organizations to perceive fields, where just the female plants are secluded to develop for creation of Ganja and Charas, before inflorescence and the cultivators may not assess ignorance of sex of plant to avert lawful ramifications. Thus the current investigation can be

exceptionally valuable in Forensic Science field, as in it might help in dissecting sex of cannabis plant before inflorescence at hereditary level and may help authorization organization to obliterate the intentionally developed charas and ganja fields and making a move against the people in question. The succession of female explicit RAPD section is distributed in NCBI (Pubmed) Gene bank having Accession No. JQ742432.1. Further, two additional arrangements of Cannabis (MADC2) has been distributed in NCBI Gene bank having promotion Nos. JF298280.1 and JN426768.1. (Addendum Publications)

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