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***Research Article***

# IMPACT OF PHYSICAL AND CHEMICAL MUTAGENS ON SEX EXPRESSION IN *CANNABIS SATIVA*

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## ABSTRACT
The phenomenon of sexual dimorphism was observed in all the plants growing under wild condition as well as in the cultivated fields of cannabis. But in the present study late-flowering plants (Winter condition) and the plants raised from the seeds treated with physical (gamma-rays with 1.0 KR dose) and physical and chemical mutagens in combination (pre-treatment with sodium azide and then with gamma rays) showed changed sex- expression under glass house condition. The female plants showed the presence of bisexual flowers, reduced female flowers and male flowers. Probably the changed conditions outside and inside the plant might have disturbed the balance of hormones to such an extent that in the female plants, male hormones also made their existence. Therefore, it may be inferred that like all morphogenetic processes, the development of the male and female flowers in the same or different plants is under the influence of hormones. The plants showing change in sex-expression showed bends in the stems. The thickened growth observed at these regions of bends is probably due to the accumulation of growth hormones at these regions.

## INTRODUCTION
Cannabis sativa is a dioecious plant. Morphologically, the male and the female plants do not differ much with regard to their vegetative growth and they can be identified only when the flowering occurs. At this stage, there is considerable difference in the inflorescence of the plant, female plants having generally compact and rarely lax inflorescence with flowers on the main shoot and short lateral branches, while male plants have inflorescence in the form of loose terminal and axillary panicles. This dimorphism regarding inflorescence and location of flowers is accompanied by a marked dimorphism in laminar shape (Heslop - Harrison and Hesclop – Harrison, 1958). According to Westergaard (1958) inheritance of sex in hemp is probably the most complicated one among dioecious plants. It was a major fibre crop in the past and the greatest amount of data on the physiology or sex expression of this plant is available, which has been reviewed by Heslop - Harrison (1957) and Napp - Zinn (1967). A great amount of work has also been done regarding the Sex reversal of this plant artificially (Heslop-Harrision, 1956; Jaiswal and Mohan Ram, 1974; Mohan Ram and Sett, 1979, 1982a, b; Sarath and Mohan Ram, 1979) and it has been concluded that both environmental factors and chemical agents affect sex-expression in hemp.

Auxins induce femaleness in male hemp plants (Heslop – Harrison, 1956). An effect of $GA_3$ on the stature of hemp was observed by Heslop – Harrison and Heslop – Harrison (1961) but no change in sex-expression was noticed by them. However Mohan Ram and Jaiswal (1972) observed male flowers in female plants after treating them with $GA_3$, $GA_{4+7}$ and $GA_9$. It was also found by them that the effect of Gibberellic acid could be stopped by simultaneous treatment of abscissic acid, while alone this chemical (abscissic acid) does not affect the sex – expression in Cannabis. The effect of various hormones (auxins, cytokinins, gibberellins) was also analysed on Cannabis tissues grown in vitro by Fisse *et al.,* (1982). Control of sex – expression by endogenous hormones (Galoch, 1978) and through exogenous growth regulators, especially in sexually dimorphic systems have also been reported (Heslop – Harrison, 1964).

Heslop – Harrison (1972) suggested that for real understanding of the sexual organs, it is instrumental to relate the effect of any environmental factor to a specific sensitive stage and tissue within the differentiating floral bud. According to him, once the initials of a particular floral organ are formed, they will develop into that particular floral organ (male or female) or stop their development, but Correns (1928) has cited many exceptions and has reported that sex – reversions may take place due to the change

in the normal development during ontogeny in some floral members. These modifications are not only restricted to dioecious plants but are also found in some monoecious plants and some of them are controlled genetically.

A perusal of literature reveals that the development of flowers of different sexes, whether in monoecious or in dioecious individuals is under the influence of hormones (Frankel and Galum, 1977). It was observed by Mohan Ram and Sett (1982) that the apical application of aminoethoxyvinylglycine (AVG) curtailed the feminizing effect of ethephon and when applied alone, it induced formation of fertile female flowers on the newly formed primary lateral branches in female plants.

## MATERIALS AND METHODS

Plants were raised in the glass house of Department of Bio-Sciences, H.P. University Shimla after giving different treatments to the seeds. About hundred seeds were used for each treatment and control. The uniform sized, healthy seeds (with 6.5 + 2.0% moisture content) were used throughout the study. Following treatments were given.

Treatment No1 – seed after socking in distilled water for 24 hours were treated with 1.0 KR dose of gamma rays (from $CO^{60}$ source in the Department of chemistry, H.P. University, Shimla).

Treatment No.2 – Seeds, after soaking for 24 hours in distilled water , were pre-treated with 0.2 M solution of sodium azide for two hours and then after washing thrice with distilled water they were treated with 1.0 KR done of gamma – rays.

No Treatment – These seeds were soaked in distilled water for 24 hours and no treatment was given to them.

Then, all the above seeds were kept for germination in the petridishes at $20^{+}.2\ ^{0}C$ temperature and finally transferred in to the earthen pots (diameter 80 cms). Three plants were accommodated in each pot and twenty pots of each treatment were placed in the glass house (including control). The observations were recorded right from the germination till the maturity of the plants.

## RESULTS

Sex reversal was observed in control plants (under glasshouse conditions) as well as in plants raised from the seeds after treatment of gamma rays (Treatment no-1) and sodium azide and gamma rays in combination form (Treatment -2). In nature also, hundreds of plants (approximately 2,000 in number at twenty different locations) were studied for morphological variations and all of them showed sexual dimorphism (Control).

Under glasshouse conditions, however, change in sex expression was observed in untreated as well as in plants raised from the seeds with treatment no.1 (gamma – rays, 1. O KR dose) and 2 (pre-treatment of 0.2 M sodium azide solution and then 1.O KR dose of gamma – rays).

It was found that this changed sex – expression appeared late in the growing season (November – December). Male flowers appeared directly on the female plants on the main stem (Figure – I) as well as on the lateral branches on the nodal regions (Figure – II). Thus, plants depicted both the sexes on the same plant. Male flowers appeared at both positions, just below the node (Figure-I) and above the node in the axil of the petiole (Figure –II, III). In addition to these observations, flowers with altered sex – expression appeared both on the main branch as well as on the lateral branches (Figure- IV, V).It is evident from Table (A) that plants with change in sex expression were shorter in height (67-87 cms.) as compared to unisexual plant (151 cms.) and were with lateral branches. The number of nodes was also less (8-12) as compared to control (15). Some Bisexual plants exhibited thickened stem tip and growth of the plant stopped for some period but was again resumed with the appearance of bent on that position (Figure – VI). On this newly developed portion, both male and female flowers were very close to each other as compared to the main branch.

In wild populations male flowers appeared in the month of June or July, while in plants showing change in sex –expression, it was found that male flowers appeared in the month of November – December.


**Figure (l) - A female plant showing sex-reversal with the appearance of male flowers at the nodes (m) below the leaves on the main branch**


**Figure (ll) - A female plant showing sex-reversal with male flowers (m) appearing on the axiliary branches and in the axils of the leaves**


**Figure (lll)- A branch from the bisexual plant showing both types of flowers, i.e. females (f , at the top ) and male flowers (m, below in the axils of the leaves)**


**Figure (lV) - A bisexual plant with flowers of altered sex (a) and female flowers (f) with long hairy stigmas**

**Figure (V) - A plant showing flowers with altered sex (a) on the main stem in the axils of the leaves and female flowers (f) with long stigmas**

**Figure (VI) – A bisexual plant with a bent stem. The bent stem is thickened before it gives off a branch with bisexual flowers**

**Table (A): Growth of the main shoot, primary lateral branches, no. of nodes and flower sex expression in female plants of *Cannabis Sativa.***

| | **Wild Population** | | | | **Under Glass House Condition** | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatments** | **Control** | | **Untreated** | | **Treatment No-1** | | **Treatment No.-2** | |
| | **Length (cms)** | **No. of nodes** | **Length (cms)** | **No. of nodes** | **Length (cms)** | **No.of nodes** | **Length (cms)** | **No.of nodes** |
| **Main Branch** | **151** | **15** | **87** | **12** | **67** | **8** | **68** | **9** |
| Primary lateral Branches | | | | | | | | |
| 1 | Absent | Absent | 22 | 9 | 43 | 15 | 15 | 15 |
| 2 | - | - | 16 | 6 | - | - | 12 | 11 |
| 3 | - | - | 13 | 3 | - | - | 8 | 5 |
| 4 | - | - | 20 | 3 | - | - | 5 | 5 |
| 5 | - | - | 11 | 3 | - | - | 4.5 | 5 |
| 6 | - | - | 7 | 2 | - | - | - | - |

These plants under glasshouse conditions without any treatment survived till the month of May of the next year and showed perennial nature instead of annual. It was found that fully male and bisexual flowers appeared only on the main branch (Treatment no-2) while in treatment no-1 bisexual flowers appeared on the main stem and one of the primary lateral branches. In treated plants the reduced female flowers with no stigmas appeared in all the primary lateral branches and main branch. Percentage of flowers with altered sex was higher on axillary branches in untreated plant as compared to plants raised from the seeds after some kind of treatment (Table – B).

**Table (B): Flower sex expression on the main and primary lateral branches in the female plants of *Cannabis Sativa***

| **Wild Population** | | | | | | | | **Under Glass House Condition** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control (No. of flowers)** | | | | | **Untreated (No. of flowers)** | | | | **Treatment-1(No.of flowers)** | | | | **Treatment-2(No.of flowers)** | | | |
| **Female Flowers** | **Bisexual Flowers** | **Redu-ced Flowers** | **Male Flowers** | **%age of al** | **Female Flowers** | **Bisexual Flowers** | **Redu-ced Flowers** | **Male Flowers** | **%age of al** | **Female Flowers** | **Bisexual Flowers** | **Redu-ced Flowers** | **Male Flowers** | **%age of al** | **Female Flowers** | **Bisexual Flowers** | **Redu-ced Flowers** | **Male Flowers** | **%age of al** |
| Female Flowers | tered flowers | | | | Female Flowers | tered flowers | | | | Female Flowers | tered flowers | | | | Female Flowers | tered flowers | | | |
| In Main Branch | | | | | | | | | | | | | | | | | | | |
| 206 | - | - | - | 0 | 48 | 9 | 30 | 7 | 48.93 | 10 | 42 | 136 | - | 94.76 | 131 | 127 | 15 | 6 | 55.06 |
| Primary lateral Branches | | | | | | | | | | | | | | | | | | | |
| 1. - | - | - | - | - | 6 | - | 35 | - | 85.36 | 45 | 167 | - | - | 78.77 | 14 | - | - | - | 0 |
| 2. - | - | - | - | - | 1 | - | 36 | - | 96.29 | - | - | - | - | - | 15 | - | - | - | 0 |
| 3. - | - | - | - | - | 1 | - | 21 | - | 95.45 | - | - | - | - | - | 3 | - | - | - | 0 |
| 4. - | - | - | - | - | 12 | - | 19 | - | 61.29 | - | - | - | - | - | 5 | - | - | - | 0 |
| 5. - | - | - | - | - | 15 | 10 | 12 | 22 | 74.56 | - | - | - | - | - | 5 | - | - | - | 0 |

In the plants, raised from the seeds after treatment no.l, branching was scarce and fully male or reduced female flowers were present on the main branch. The congregation of male flowers at each node with pedicel missing exhibited additional feature of modified female plants. Lateral branches showed only bisexual flowers in addition to the normal female flowers. Percentage of flowers with altered sex was more on the main branch as compared to primary lateral branches; (Table B). In plants raised from the seeds after treatment 2, the changed sex-expression appeared only on the main branch, inspite of the presence of many primary lateral branches. Percentage of flowers with altered sex was 53.6% and number of flowers with fully male character was only 2.15% (Table B).

Male flowers appeared larger on the plants after treatment 2 while those raised from the seeds with treatment no. 1, had smaller size as compared to unisexual plants. Female flowers present on the bisexual plants raised from the seeds after treatment 2 were larger in size (15.0 x 2.5mm) than the female flower on the normal unisexual plants (10.0 x 1.5 mm). In untreated sexually bisexual plants, female flowers were smaller (9.0 mm) but in width (1.5mm), there was not much difference when compared to normally existing unisexual plants. In plants raised from the seeds after treatment no. – 1, the female flowers were larger (14.0 x 2.0 mm) in bisexually plants as compared to the normal size of female flowers in unisexual plants (10.0 x 1.5 mm).Reduced female flowers ranged from 5-11 mm in length while their width range from 1.5-3.0 mm. Bisexual flowers were 8.0-11.0 mm long and 2.0-2.5 mm wide.

## DISCUSSION

During present observations the plant showed sexual dimorphism frequently in natural as well as cultivated hemp populations. Since the last fifty years, lots of information regarding the sex expression of Cannabis sativa and its modification has been gathered.

Heslop-Harrision (1956) reported that auxin treatment induces femaleness in male hemp plants, while Mohan Ram and Jaiswal (1970) observed that treatment of hemp plants with chemicals which release ethylene cause femaleness in male plants. It was very prominent in plants after treating them with 2-

chloroethylphosphoric acid spray under winter conditions. Winter conditions strengthen femaleness in hemp. Morphactin also affects the sex-expression within the flowers i.e. floral members (Mohan Ram and Jaiswal, 1971) but in present studies, it was observed that normally no change in sex expression occurs in the actual growing season (i.e., from March to October), but late in the season viz., November, some plants which were sown in early June (i.e., 2-3 months later than the time at which appearance of plants occurs in nature), show change in sex –expression l. In these surviving female plants, reduced female flowers, bisexual flowers and male flowers was observed and these plants survived till next May-June. From November to March there were severe winter and temperature was low with humidity and rainfall also comparatively low. Photoperiod was also less as compared to the normal growing season. These environmental factors further might have helped in modifying the sex experssion.The sex reversal observed during this period in our present observations can be explained due to low temperature or less photoperiod .But appearance of male flowers on the female plants under low temperature conditions is in contradiction with the early findings (Napp-Zinn, 1967; Harrision, 1972), which suggest that low temperature promote femaleness. It has also been reported by Tournois (1911) that short days increase the ratio of female plants to male plants in Cannabis sativa and this sex-expression cannot be explained on whole plant basis. Thus the present observations show that the during winter or short day conditions and low temperature, male flowers appeared on the female plants is in contrary to the earlier reports. Lange (1961) also reported femalization of male papaya trees due to low temperature.

Thus, it can be concluded that the only method for obtaining monoecious plants in otherwise dioecious plants, is not only by the direct application of chemical agents or hormones on the plants (Heslop – Harrision,1964; Mohan Ram and Sett,1979; Sarath and Mohan Ram, 1979; Sriram and Mohan Ram, 1984) but it can also be obtained by changing environmental conditions which can disturb the natural normal balance of hormones in plants.

In present observation, localized swelling or thickening of stems after irradiation are in agreement to those of Gunckel (1957) who suggested them probably due to the local mobilization of nutrients and auxins. It was significant to note that floral branches rising from this thickened portion were bisexual. Thus it appears that noticed change in sex expression is probably due to both hormonal imbalance and environmental conditions.

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