

## Role of endogenous phytohormones and some macromolecules in regulation of sex differentiation in flowering plants

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**Abstract.** Endogenous levels of phytohormones like auxin, gibberellin, cytokinin, abscisic acid and ethylene appear to determine the fate of flower sex primordia. Higher levels of auxins, cytokinins and ethylene have been found to be correlated with female sex expression whereas a greater amount of gibberellins favours the differentiation of male sex organs. It appears that the phenotypic expression of flower sex is controlled by a balance between male-promoting and female-promoting hormones. Variations occurring with regard to amino acids, proteins and enzymes/isozymes during the reproductive phase may also have a prominent role in determining the nature of the flower sex.

**Keywords.** Phytohormones; macromolecular markers; sex expression.

### 1. Introduction

Initiation of flowering is one of the very important morphogenetic events in the life of a plant. Much work has been done to discern the factors responsible for initiation of flowering and several theories have been postulated concerning its mechanism (Lang 1952; Chailakhyan 1968; Thornley 1972; Charles-Edwards *et al* 1979). However, information regarding the factors of flower development and sex organ differentiation is insufficient. Exogenously applied phytohormones are known to change the sex of plants (Frankel and Galun 1977; Mohan Ram 1980). Chailakhyan and Khryanin (1979) have demonstrated that during transition from vegetative to reproductive phase, changes in the plant metabolism occur as a result of which certain new and specific macromolecules appear, disappear, or their levels change significantly. These molecules probably constitute molecular markers or probes of differentiation (Champault *et al* 1981). It is not necessary to examine many of these molecules but only the selected ones which are specific to different sexes.

In this review, the possible correlation amongst endogenous levels of phytohormones and some compounds like amino acids, proteins and enzymes/isozymes in relation to flower sex differentiation has been examined.

### 2. Role of endogenous hormones

#### 2.1 Auxins

Relatively high levels of endogenous auxins are correlated with female sex expression in most of the plants studied. Female plants/flowers of various species show greater

amounts of endogenous auxin than the male plants/flowers (Heslop-Harrison 1972; Frankel and Galun 1977).

Galun *et al* (1965) demonstrated that the quantity of auxin in hermaphrodite and andromonoecious cucumber plants, differed significantly, the hermaphrodite ones being richer.

## 2.2 Gibberellins

Among the 5 groups of plant hormones higher levels of endogenous gibberellins have been detected in the male than in the female plants (Vince-Prue 1975). Gibberellin has been found to be a major factor for enhancing male flower formation in cucumber—the most studied plant regarding the role of hormones on sex expression and a correlation between higher endogenous gibberellin level and male sex expression has been established (Frankel and Galun 1977).

Atsmon *et al* (1968) and Hayashi *et al* (1971) reported considerably higher levels of gibberellin-like substances in a monoecious line of cucumber (*Cucumis sativus* L.) than that in an isogenic gynoecious line. Greater gibberellin activity has also been recorded in leaves of male plants of hemp and spinach than in female ones (Chailakhyan and Khryanin 1979). However, Leshem and Ophir (1977) reported the presence of higher endogenous level of a gibberellin-like substance in female individuals of two dioecious species, viz. *Ceratonia siliqua* and *Phoenix dactylifera*. Recently, Ghosh and Basu (1983) have detected a high level of endogenous gibberellin-like substance in shoot tips of *Momordica charantia*, a monoecious cucumber, at the time when the plant had a minimum ratio of male to female flowers.

## 2.3 Cytokinins

Cytokinins have also been found in greater quantities in female plants than in male plants of various species studied. Zeatin was detected only in female flowers of *Mercurialis annua*, whereas its precursor nucleotides were observed in male plants also (Dauphin-Guerin *et al* 1980). Champault *et al* (1981) recorded the feminization of genetically male plants and postulated a hypothesis that female plants contain a larger pool of endogenous cytokinins than male ones.

Chailakhyan and Khryanin (1979) noted that in intact soil-grown plants of *Cannabis sativa* and *Spinacia oleracea*, cytokinin activity was greater in leaves as well as in primary root of female than in those of male plants. These authors recorded lower cytokinin activity in the leaves of derooted male plants of hemp and spinach and concluded that higher levels of natural gibberellins and low levels of cytokinins in the leaves of derooted plants were the major causes of the shift of sex expression in the male direction. Moreover, promotion of development of already differentiated pistils of *Cleome iberidella* grown *in vitro* by zeatin and benzylamino purine was shown by De Jong and Bruinsma (1974).

## 2.4 Abscisic acid

Higher levels of abscisic acid were recorded in the shoot tips of gynoecious plants of cucumber than in those of monoecious plants (Rudich and Halevy 1974; Friedlander *et*

al 1977). These investigators concluded that higher content of abscisic acid could be correlated with female flower differentiation and lower abscisic acid content with male flower differentiation.

### 2.5 Ethylene

It has been demonstrated that comparatively elevated levels of endogenous ethylene favour femaleness in most plants. In *C. sativus* and *C. melo* gynodioecious plants produce more ethylene than their monoecious counterparts. Andromonoecious plants were reported to evolve low amounts of ethylene as compared to monoecious plants and hermaphrodite flowers (Rudich *et al* 1976). The role of endogenous ethylene in femaleness was established in cucurbits by Byers *et al* (1972). They removed ethylene from female plants by ventilation and exposed them to ethylene inhibitors such as CO<sub>2</sub> and benzothiadiazole and observed the induction of hermaphrodite flowers. Gregg (1982) studied the light-enhanced ethylene content in several species of *Cycnoches* and *Catasetum* and reported that higher levels of ethylene were emitted by developing female flowers in plants grown in shade, whereas low levels of ethylene were evolved from male inflorescences grown in sunlight. Adams and Yang (1979) have demonstrated 1-aminocyclopropane-1-carboxylic acid (ACC) as the precursor of ethylene biosynthesis and the former has been shown to play its role during certain ethylene mediated activities such as fruit ripening (Hoffman and Yang 1980), senescence (Bufler *et al* 1980) and as actual factor for inducing femaleness in *Cucurbita pepo* (Hume and Lovell 1982).

Recently, it was also demonstrated that natural as well as ethephon-induced female flowers of dioecious *Cannabis sativa* evolve significantly higher amount of ethylene than the male flowers, specially at the early stages of development (Sriram and Mohan Ram 1984). These findings strengthen the concept that higher ethylene content is associated with female sex expression in plants.

### 3. Role of amino acids

Being a main form of soluble nitrogen within cells and playing a vital role in cell biosynthesis, amino acids are extensively studied molecules (Fowden 1973; Mifflin and Lea 1976; Umberger 1978). Nevertheless, reports on variation in the quantity/kinds of amino acids during transition from vegetative to reproductive stage or differences in male and female flowers are scanty.

During our studies on *Ricinus communis* by paper chromatography, we have shown that arginine appears only in male, leucine only in female and alanine, hydroxyproline and valine in flowers of both sexes but not in vegetative tissues (Jaiswal and Kumar 1980a). At the same time glutamic acid and serine, present in vegetative parts, disappear during the onset of flowering. Further, certain amino acids show differential presence or absence in vegetative and/or reproductive parts or in male and/or female flowers at a particular stage of development (Jaiswal and Kumar 1978; Kumar 1982). During transition from vegetative to reproductive phase, leucine and lysine appear in male flower primordia and lysine disappears in female flower primordia of *Morus nigra*. Hydroxyproline in female flower primordia of *Carica papaya*, and threonine and proline appear in similar tissues of *Coccinia indica*, but they are not observed in the vegetative tissues. However, in *C. indica*, tryptophan is recorded in the female vegetative

tissues but disappears in female flower primordia. Apart from these, certain other amino acids also showed variations with particular stage of flower development (Kumar 1982).

#### 4. Role of proteins

Earlier reports have shown differences in the protein profiles of vegetative and reproductive structures in a few plants, viz. tulip (Barber and Steward 1968), *Mercurialis annua* (Kahlem 1970; Kahlem *et al* 1973) and *C. papaya* (Jindal and Singh 1976). During flowering, alteration in the antigenic proteins in apical buds alongwith the disappearance of one protein species and increase in the concentrations of two others were reported in *Sinapis alba* (Pierard *et al* 1977).

The differences in the protein profiles of *M. nigra*, *C. papaya*, *C. indica* and *R. communis* were investigated and it was observed that male (as in *M. nigra* and *C. papaya*) or female (as in *C. indica*) flower buds had specific proteins during the reproductive phase that were not recorded at the vegetative stage. At the same time, certain protein species present in vegetative tissues of each plant were not found in the male and/or female flower buds (Kumar 1982).

Based on these observations, it may be conjectured that the development of reproductive structures requires some specific proteins varying from species to species. The proteins not found in a particular tissue (vegetative, male or female) may be inhibitory or not necessary for the development of that particular tissue.

#### 5. Role of enzymes and isoenzymes

There are certain enzymes whose activities and/or isozyme patterns differ from vegetative to reproductive phase or from male to female flowers in some species. Retig and Rudich (1972) reported that the activities of peroxidase and IAA oxidase were higher in gynoeious than in monoecious plants of cucumber. Champault (1973) found that the administration of IAA to genetically female plants led to the appearance of male flowers and male-specific isoperoxidases in *Mercurialis annua*. In tissue cultures of *M. annua*, the expression of maleness or femaleness was established by screening for two isoperoxidases which constituted the specific marker for the female strain. Champault *et al* (1981) subsequently called them 'sex-related markers'. Durand *et al* (1974) found certain isoesterases in female flowers of *Mercurialis*, whereas some isoperoxidases were specific to male flowers only. Bazin *et al* (1975) reported two isoesterases to be specific for the female flowers.

Analyses of the peroxidase activity and isoperoxidase patterns have been made in *M. nigra*, *C. papaya*, *C. indica* and *R. communis* (Jaiswal and Kumar 1980b, 1981, 1983; Kumar 1982). In *M. nigra*, *C. papaya* and *C. indica*, the level of peroxidase activity recorded was higher in female flower buds as compared to male flower buds and vegetative tissues. But in *R. communis*, male flower buds had a higher activity than the female flower buds. In *M. nigra*, one isozyme characteristic to vegetative tissues, disappeared in both male and female flower buds and a new band appeared in the male flower buds. Similarly, male flower buds of *C. papaya* had 3 new bands and female flower buds had one new band not recorded in the vegetative tissues. In *C. indica*, two new bands not encountered at the vegetative stage, appeared in male flower buds whereas in *R. communis* two new bands appeared in female flower buds which were not

present at the vegetative stage and in male flower buds. Recently, Sriram and Mohan Ram (1984) have demonstrated in *C. sativa*, that natural as well as ethephon-induced female flowers have a higher peroxidase activity than male flowers and that 3 isoperoxidases were specific to male flowers only. It is concluded that a higher total peroxidase activity in female flower buds of the dioecious plant indicates its involvement in differentiation of the latter, whereas the larger number of isozymes in male flowers may have a role in male sex expression. However, in *R. communis* just the opposite mechanism seems to be operative.

On the basis of differences in endogenous levels of hormones, discussed above, it may be concluded that control of sex expression operates through the production of specific hormonal substances, the continued synthesis of which is necessary to maintain the phenotypic expression of the genetic sex. Nevertheless, no single hormone has been recognized as solely responsible for regulating a particular sex so far. It is likely that the phenotypic expression of flower sex is controlled by a balance between male-promoting and female-promoting hormones. In several species, femaleness seems to be controlled by the endogenous level of ethylene and maleness by the endogenous level of gibberellins. The plants of either sex seem to be capable of synthesizing both hormones, but the determination of the ultimate sex depends on the balance between the two. If the level of gibberellin is relatively higher than that of ethylene, maleness would be expressed and if ethylene level exceeds that of gibberellins, the shift would be towards femaleness (Mohan Ram and Jaiswal 1974). However, Trewavas and Cleland (1983) have suggested that instances of poor correlation between concentrations of plant hormones found and changes in development may be due to changes in the sensitivity of the concerned tissues to the hormones. Nevertheless, some investigators are of the opinion that the sex expression in plants is controlled by a balance between floral stimulus or flower forming substance and phytohormones (Leonard *et al* 1981; Takahashi *et al* 1982).

Differentiation of organs involves selective gene expression. The right type of gene(s) must be expressed in the particular cells at the right time (Wareing 1982), which results in differences in quality and/or quantity of certain macromolecules of the differentiating cells. During initiation of flower primordia, cells responsible for the development of sex organs acquire biochemical capabilities that are profoundly different from those of the vegetatively growing apical meristems. Compounds such as amino acids, proteins and enzymes/isozymes, which have been found to vary during the reproductive phase, seem to play a prominent role in plant/flower sex differentiation and more information is required to further analyse their role.

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