Anatomy of a New Sepaloid Mutant Flower in Sugarbeet[1]

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ABSTRACT

A mutant sugarbeet plant with clusters of 12-20 flowers was discovered in progeny of a male sterile plant from the NC-7 collection of *Beta* crossed with inbred NB-1. Anatomical studies revealed that most flowers had 10-25 sepals instead of the normal 5. Anthers were not produced in any of the flowers. Pistil development was highly variable; some flowers had no ovules, some possessed exposed or naked ovules, and a few appeared normal. Even though plants were subjected to large amounts of pollen from several sugarbeet sources, no seed could be obtained, and inheritance of the trait could not be determined.

Additional Key Words: *Beta vulgaris*, male sterility, shoot differentiation, homeosis

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Flowers of the sugarbeet (*Beta vulgaris* L.), as in angiosperms in general, develop from groups of undifferentiated cells of the shoot apical meristem. Typically, flower structures originate in centripetal sequence, i.e., sepals, petals, stamens, carpels, and finally ovules (Popham, 1963). Sugarbeet flower development follows this typical differentiation pattern except that no petals are formed (Artschwager, 1927).

In many cases, modifications in flower structure occur in angiosperms, such as phylloidy (leaf-like floral parts), sepaloidy (excess sepals), petaloidy, stigmoidy, pistilloidy, and carpelloidy (Frankel and Galun, 1977, Johns et al., 1981). Sexual reproduction in angiosperms often depends upon the normal development of all floral parts. Abnormal development of one floral structure can disrupt development of subsequent parts, and may cause sterility (Johns and Palmer, 1982). In a review Meyer (1966) noted that all organ types of the flower are potentially capable of developing the form of any other organ present in the normal flower, and that petaloidy was the most common abnormality.

A stigmoid mutant flower was found by Kinoshita and Takahashi (1979) in M3 lines of a gamma-ray irradiated population of sugarbeet. Five sterile fleshy leaf-like structures with irregular lobed apicies developed instead of stamens. Papillae proliferated on the apical opening of these structures in a manner similar to the stigma in a normal flower but pollen grains failed to germinate on them. Pistils composed of three fused carpels developed normally (Chauhan et al., 1985). Jassem (1971) observed disturbed ovule development that reflected a monogenic recessive female sterility. Instead of the normal ovule, an undifferentiated tumor-like outgrowth devoid of an embryo sac was produced inside the ovary. In some of these intraovarian outgrowths, chambers resembling pollen-filled anther locules were observed.

In 1984 we observed a unique flowering sugarbeet plant with flowers tightly grouped in clusters. In this paper we describe the morphology and anatomy and report attempts to study the inheritance of this spontaneous mutant.

MATERIALS AND METHODS

This sepaloid mutant was discovered on a single plant from the cross of breeding line 8M16-2 X NB-1. Line 8M16-2 was one of a group of potentially new sources of cytoplasmic male sterility (CMS) which was found in the NC-7 collection of *Beta* germplasm. Specifically, 8M16-2 came from PI 141919, an introduction from Iran. NB-1 is an O-type (maintainer) inbred that was being used as a recurrent parent to develop isogenic

lines with potentially different CMS from many sources. Three other plants of the 8M16-2 X NB-1 cross were all normal in their flower characteristics except that they were pollen sterile.

The mutant plant was increased and maintained by *in vitro* shoot culture propagation (Saunders, 1982). Clones of the mutant plant were grown after 10 weeks photothermal induction in the greenhouse and in a controlled environment in growth chambers, with a 16-hr light and 8-hr dark period at 24°C. Inflorescence and flower development was monitored weekly. Clusters of flowers and stem sections were collected at various stages of growth for anatomical studies. Normal flowering plants, CMS plants, and stigmoid mutants also were sampled for comparative purposes.

Microtechnique Procedures. Collected samples were fixed in FAA (formalin-aceto-alcohol). Specimens for light microscopy were embedded in paraffin and sectioned at 12-15 μm with a rotary microtome. Longitudinal and transverse serial sections were stained with safranin O and fast green (Johansen 1940). Low magnification photomicrographs were taken with a Nikon SMZ-10 stereomicroscope, and high magnification photomicrographs were taken on a Zeiss Photomicroscope II.

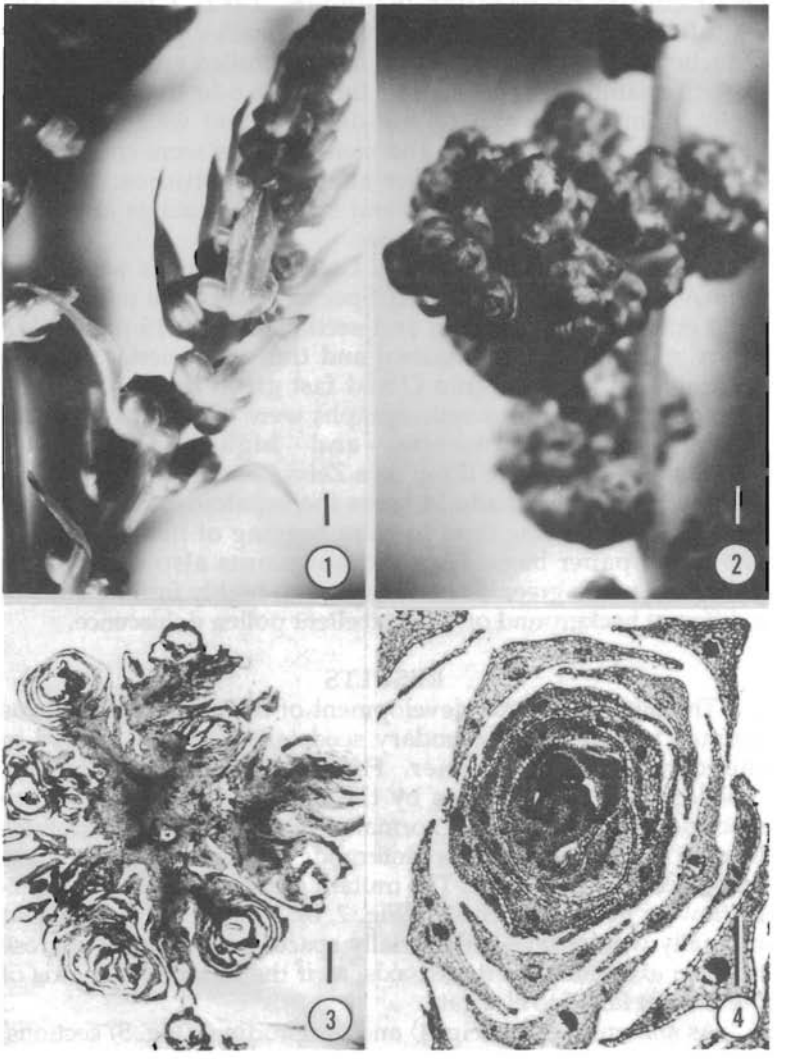
Attempts were made to cross the sepaloid mutant plants with several breeding lines by joint bagging of flower stalks in #20 white paper bags. Four mutant plants also were open-pollinated in the greenhouse with several highly fertile plants of a different background or with excellent pollen dehiscence.

RESULTS

The initial seedstalk development of the mutant plants was normal; primary and secondary seedstalk branches formed in the conventional manner. However, abnormal flower development was obvious by the time the floral buds were macroscopically visible. In normal plants, groups of 2-3 flowers formed sequentially at short internodes on an elongate tertiary inflorescence axis (Fig. 1). The mutant plant, in contrast, had 12-20 flowers in a single cluster (Fig. 2, 3). Multigerm flowers that normally would have been serially spaced along the stem arose from an extremely shortened axis, as if the inflorescence axis of the mutant failed to elongate.

As shown in cross-(Fig. 4) and longitudinal-(Fig. 5) sections, each mutant flower had about 10-25 sepals rather than the normal set of 5. The mutant flowers were devoid of stamens and pollen. The normal sugarbeet flower has a compound pistil of three carpels containing a single ovule. The three carpels are initiated as separate units that normally coalesce during development. In contrast, the female parts of the mutant flowers were extremely variable. Usually no ovule was present. In some cases there were three separate carpels (Fig. 6); in rare cases,

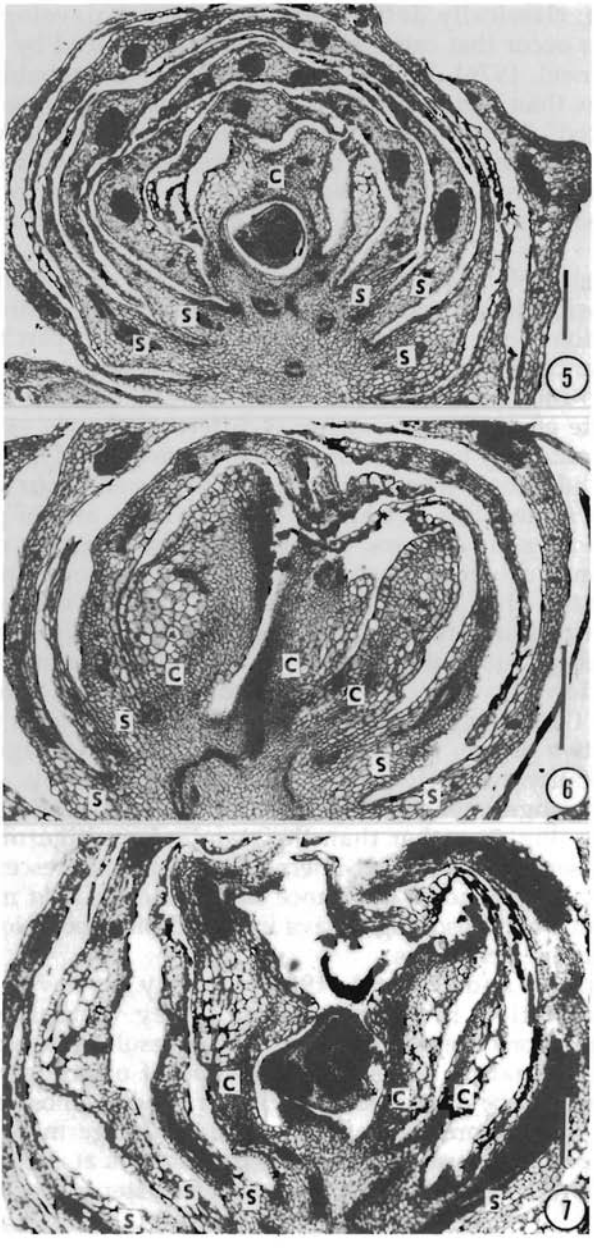
Figures 1-4. 1. Normal inflorescence of Beta vulgaris. **2.** Mutant cluster inflorescence. **3.** Transverse section through a mutant cluster. **4.** Transverse section through a sepaloid mutant flower. Scale bars = 1 mm (Fig. 1-3) or 250 μm (Fig. 4).



there was a naked ovule between them. A few of these naked ovules could be seen macroscopically. In other cases, the three carpels were fused to various degrees and sometimes possessed a normal-appearing pistil (Fig. 5.).

Even though the flower clusters were dusted heavily with pollen, all attempts to recover seed from the mutant plant failed. Anatomical studies revealed a low frequency of ovules on the

Figures 5-7. Longitudinal sections of sepaloid mutant flowers with different types of ovaries. S = sepals, C = carpels. **5.** Normal (closed) ovary. **6.** Three separate carpels, without ovule. **7.** Separate carpels with naked ovule. The ovule appeared to have been fertilized, but was immature. All scale bars = 250 µm



plant, and the female parts often were quite abnormal. A few immature seeds were observed in the sectioned material (Fig. 7; not evident at magnification shown).

DISCUSSION

This sepaloid mutant might be interpreted as a homeotic mutant, classically defined as one in which developmental changes occur that cause one organ to be replaced by another (Ouweneel, 1976). However, the situation is probably more complex than this. In the new sepaloid mutant, stamens were "replaced" by sepals but more sepals proliferated as well. In some cases, the three fused carpels were replaced with separate sepal-like carpels. There also appeared to be a change from a whorled to a spiral arrangement of an indefinite number of sepals.

Heslop-Harrison (1963) pointed out that auxins affect sex expression in a specific manner in flowering plants, and regulation of sex expression may be mediated in part through the balance of growth hormones in the developing buds. Auxins tend to increase femaleness whereas gibberellins have an opposite effect. Since centripetal differentiation in sugarbeet flower ontogeny begins with sepals (Artschwager, 1927), it may be that additional whorls of sepals are produced in our sepaloid mutant rather than of stamens in all flowers, and in place of carpels in many flowers. This may be due to action of plant hormones or growth regulator imbalance. Popham (1963) suggested that sepal initiation may occur when auxin concentrations are relatively high. Other flower parts are initiated at successively lower concentrations of auxin with formation of ovules at very low concentrations. Johns and Palmer (1982) suggest a shift in the balance of nutrients to some vegetative organs may cause a starvation or blockage in the translocation system to anthers and pollen mother cells.

This sugarbeet sepaloid mutant shows a tight cluster of flowers (Fig. 2) rather than the standard multigerm flower groups separated by short internodes on the inflorescence axis (Fig. 1). If a hormonal imbalance caused the sepaloid mutation in the flower, it also might have inhibited internode elongation of the inflorescence axis.

Durand and Durand (1984) recently reviewed sexual differentiation in higher plants. They concluded that differentiation of reproductive organs can result from expression of differentiation patterns. Each type of organogenesis is postulated to be controlled by regulator gene(s) whose action is effected by its products. Since normal multigerm sugarbeet plants produce clusters of three to five flowers at each node of the inflorescence, the 12-20 flowers per cluster in the sepaloid

mutant might be attributed to a change in the start-stop mechanism in the genetic code governing axis growth.

Kinoshita and Chauhan (1983) determined that a stigmoid mutant was governed by two recessive genetic factors with slight environmental modifications. Jassem (1971) discovered a female sterile character manifested by disturbed ovule development, that was inherited as a monogenic recessive. We would expect that the sepaloid mutant character also is governed by recessive genes, but we were unable to obtain crossed or self-pollinated seed to determine the inheritance. Removal of some sepals and careful pollination of individual ovules on the seedstalk or *in vitro* pollination of ovules under specific cultured conditions have not been attempted, but these might be a means of obtaining progenies for the determination of inheritance of this character.

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