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Proposed Schemes for Mass-Extraction of Doubled Haploids of Cotton

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ABSTRACT

We propose (i) synthesis of hybrid-eliminating, haploid-inducing (HEHP) lines and populations homozygous for the Le_2^{dav} hybrid lethality allele and the *Se* allele for semigamous reproduction, (ii) cross pollination of HEHP plants with pollen of normal cotton (*Gossypium hirsutum* and *G. barbadense*) to form inviable true hybrids, but viable haploids, and (iii) bulk treatment of all seed or young viable seedlings with colchicine to recover doubled haploids en masse. Doubled paternal haploid plants and sectors will be phenotypically unique, as distinguished by fertility, size of plant parts, and expression of genetic marker(s). Inclusion of male sterility in segregating HEHP populations will facilitate manual or natural cross pollination, allowing large-scale usage. To convert cytoplasms of HEHP materials, available semigamous lines with the desired cytoplasm can be crossed with a HEHP pollen parent to obtain paternal haploid progeny that can be colchicine-doubled. The HEHP materials should facilitate all types of cytogenetic manipulations involving extraction of doubled (paternal) haploids. The basic concepts and methods proposed might be extrapolatable to other crops.

METHODS that promote the occurrence and/or identification of haploid progeny are known for many crops and other angiosperm species. The methods are correspondingly numerous and diverse in approach. Depending on the ease of their extraction and the particular crop and species involved, doubled haploids have been of varied importance for various tasks. For example, haploids have been used directly and indirectly for genetic analysis and mapping (Cipar et al., 1967; Simon and Peloquin, 1980; Chen et al., 1983), and for various cytogenetic analyses, e.g. determining genomic affinities (Beasley, 1942) and discerning cytogenetic architecture of diploids and polyploids (Beasley, 1942; Endrizzi, 1962; Brown, 1943; Peloquin et al., 1966). In tetrasomic tetraploids such as potato (*Solanum tuberosum* L.) and alfalfa (*Medicago sativa* L.), they have contributed significantly, albeit often indirectly, to analyses of quantitative genetics and the study of gene action (Mendiburu et al., 1974; Bingham, 1980). Moreover, haploids have had an increasingly important role in genetic improvement of the tetraploid cultivated species via unilateral and bilateral sexual polyploidization, via *2n* gametes (Peloquin, 1981). In diploids and disomic polyploids that are partly or completely allogamous, they can be valuable sources of pure lines, via chromosome doubling. Pure lines are the material of choice for many qualitative and quantitative genetic studies, and many pedigreed breeding projects, including, for example, current efforts to determine linkage relationships among restriction fragment length polymorphisms (Burr et al., 1988) and loci governing qualitative and quantitative traits. Furthermore, cytoplasmic substitution also can be expedited by extraction of doubled paternal haploids (Kermicle, 1969; Kasha and Kao, 1970; Turcotte and Feaster, 1974). Usefulness of haploids for genetic research and improvement of diploid and disomic polyploid organisms, however, has been limited. Two factors accounting at least in part for their limited use are (i) testing is more critical than inbred development per se, and (ii) methods for doubled-haploid extraction generally have been cumbersome, tedious, and/or expensive. To be useful as tools for applied breeding, doubled haploids must not be inferior to inbreds derived by traditional means, e.g., anther-derived doubled haploids of *Nicotiana* (Dhillon et al., 1983; De Paepe et al., 1982; Brown et al., 1983, Kasperbauer et al., 1983). Furthermore, the method of doubled-haploid extraction must be simple; applicable to diverse germplasm; and have low costs, commensurate with its role in the overall breeding program. Obviously, haploids must be produced at a relatively high frequency, and easily identified, selected, and doubled in chromosome number.

Our objective has been to bring previously known genetic information and materials to bear on the need for a useful means of rapid inbred development in cotton. Herein we describe evidence pertinent to the derivation of in vivo systems for mass extraction of doubled haploids of cotton. The principles described should be applicable to other crops and the literature indicates that in some crops the genetic tools to create similar systems may already be known. Thus, logic underlying the system described for *Gossypium* may constitute an example, or even a prototype of what might be devised elsewhere.

We propose to use (i) the allele Le_2^{dav} which is responsible for lethality of F_1 interspecific hybrids involving *G. davidsonii*, (ii) the mutant allele *Se* that causes semigamous apomixis, and (iii) bulk-treatment of seeds with colchicine without prior identification of haploids or haploid sectors, for extraction of doubled haploids of cotton. In combination, Le_2^{dav} and *Se* alleles will cause hybrid elimination and haploid production (HEHP), respectively. Brief overviews of these two genetic systems follow.

SEMIGAMY

Semigamy is a form of apomixis whereby syngamy occurs, but subsequent synkaryon formation fails (Maheshwari, 1950, p. 453). The mutant allele *Se*, which causes semigamy in cotton was first isolated by doubling the chromosome number of a spontaneous haploid of 'Pima S-1' cotton to produce line 57-4 (Turcotte and Feaster, 1963, 1974). In Arizona, line 57–4 produces from 31 to 60% haploid (*2n* = 26) seedlings after self-pollination. The frequency of haploid seedlings is lower, 1 to 10%, when *SeSe* plants are crossed with *sese* pollen parents. Progeny from self-pollinated *Sese* heterozygotes include about 10% haploid prog-

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eny. Frequencies of haploids among normal Pima cottons, *sese*, range from 1 in 8000 to 1 in 25000 (Turcotte and Feaster, 1974).

Haploid progeny from *SeSe* lines include maternal haploids and chimeric maternal/paternal haploids (Turcotte and Feaster, 1967, 1974). As a chimeric seedling grows, the apical meristem usually becomes exclusively maternal or paternal. Pre-emptive dominance of maternally vs. paternally derived tissue at the apical meristem occurs randomly (Chaudhari, 1978).

The *Se* allele allows syngamy, but prevents union of the sperm and egg nuclei in varying percentages of fertilized ovules (Turcotte and Feaster, 1967). The nonunited nuclei develop to form one embryo with respective maternal and paternal haploid sectors that can be identified as maternal or paternal in origin by using genetically marked parents. Identified haploids can be treated with colchicine to produce fertile, homozygous tetraploid sectors (Turcotte and Feaster, 1974).

The discovery and characterization of a semigametic mutant in Pima cotton by Turcotte and Feaster (1963, 1974) has provided means for rapidly producing pure lines of the tetraploid cultivated cottons *G. barbadense* (Turcotte and Feaster, 1982) and *G. hirsutum* L. ($2n = 4x = 52$). Via semigamy, pure lines can be quickly extracted from virtually any *Gossypium* taxon that can function as the pollen parent in crosses with *G. barbadense*. Moreover, the semigamy mutant allows efficient cytoplasmic substitution.

Le_2^{dav} HYBRID LETHALITY SYSTEM

The closely related American wild diploid taxa *G. davidsonii* Kell. and *G. klotzschianum* Anderss. (both $2n = 26$, D_3 genomes) ordinarily do not cross successfully with other taxa harboring an A and/or D genome, i.e., Asiatic diploids, and New World diploids and tetraploids. Lee and Smith (1970) crossed the *G. barbadense* Sea Island line AS-2 with a wild *G. barbadense* accession from Ecuador and selected an F_3 segregate, 15-4, that crossed readily with *G. davidsonii* to produce vigorous, although sterile, triploid plants. Using the technique of hexaploid bridging, Lee (1981a) transferred the complementary lethality factor of *G. davidsonii* to the tetraploid level, obtaining lines similarly isolated from all tested cultivars of *G. barbadense* and *G. hirsutum*.

Lee (1981b) elucidated the genetics of the D_3 complementary lethality system as follows: (i) the cultivated tetraploid cottons tested were homozygous for two alleles, Le_1 and Le_2; and (ii) *G. davidsonii*, and presumably also *G. klotzschianum*, are homozygous for an allele, Le_2^{dav}, that interacts with Le_1 and Le_2 to cause embryonic and/or seedling lethality. Thus, line 15-4 is of the genotype $le_1le_1\ le_2le_2$, whereas the derived tetraploid line carrying the D_3-genomic hybrid lethality factor is of the genotype $le_1le_1\ Le_2^{dav}Le_2^{dav}$. Several tetraploid cultivars were tested and found to be $Le_1Le_1\ Le_2Le_2$. In a larger survey, Rooney and Stelly (in press) found all of 52 *G. hirsutum* cultivars tested to be dimeric for the dominant alleles, too, indicating that the Le_2^{dav}-compatible alleles le_1 and le_2 are rare or absent in the USA breeding germplasm.

HEHP FOR SELECTIVE RECOVERY OF HAPLOIDS

Purely maternal or paternal haploids should be selectively recoverable from crosses of HEHP × normal cottons, i.e., *SeSe* $le_1le_1\ Le_2^{dav}Le_2^{dav}$ × *sese* $Le_1Le_1\ Le_2Le_2$ (Table 1). Progeny from this cross are expected to include mainly F_1 progeny, all of which, however, are expected to be inviable. Small percentages of viable progeny are expected, including various haploid and chimeric types. Because many haploid derivatives from *SeSe* plants are chimeric as seedlings, it is possible that all are chimeric at early stages of embryogenesis.

The fate of maternal-paternal haploid chimeras has not been empirically deducible, because the biochemical basis of the Le_2^{dav}-Le_1Le_2 lethality interaction has not been defined. It seemed possible that intercellular diffusates might mediate the lethality reaction, in which case seedlings chimeric for maternal and paternal haploid sectors expectedly would become necrotic and die, precluding facile recovery of haploids therefrom. The identification of translocatable and diffusable substances as the primary factor in some graft incompatibilities (Gur et al., 1968; Moore, 1986) certainly supports this notion. Conversely, if the lethality reaction were based exclusively on intracellular interactions, the chimeric haploids should remain unafflicted by necrosis, and survive. Circumstantial evidence that the latter hypothesis might be correct was provided by the fact that embryo/endosperm/maternal sporophytic interactions, per se, seem not to affect recovery of embryos of a given genotype. Rootstock and scion phenotypes were unaffected (Weaver, 1954) in reciprocal grafts involving incompatible genotypes of a different *Gossypium* hybrid lethality system (Gerstel, 1954). Furthermore, old index card file records at the Cotton Cytogenetics Laboratory from Dr. Meta Brown indicate that a paternal haploid was extracted from the cross of a semigamous female with *G. klotzchianum*, which also harbors the Le_2^{dav} allele (Lee, 1981a). Whether or not the seedling was initially chimeric, however, is not known, so the data were not definitive on the question at hand. Stelly and Rooney (in press) recently proved that the Le_2^{dav}«Le_1Le_2 reaction is in-

Table 1. Phenotypes of progeny or sectors of chimeric progeny expected from crosses between virescent hybrid-eliminating haploid-producing (HEHP) and normal plants of cotton (*Gossypium*), e.g., *SeSe* $le_1\ le_1\ Le_2^{dav}\ Le_2^{dav}\ v_7v_7$ × *sese* $Le_1Le_1\ Le_2Le_2\ V_7V_7$, after bulk treatment of seed with colchicine.†

	Phenotype			
	Viable	Ploidy	Color	Fertility
Nondoubled				
$F_1\ V_7v_7$	No‡	4x	–	–
Maternal haploid v_7	Yes	2x	Yellow	None
Paternal haploid V_7	Yes	2x	Green	None
Doubled				
8X (F_1) $V_7V_7\ v_7v_7$	No	–	–	–
Doubled maternal haploid v_7v_7	Yes	4x	Yellow	Fertile
Doubled paternal haploid V_7V_7§	Yes	4x	Green	Fertile

† Allele symbols: *Se* = mutant allele causing semigametic apomixis; le_1 and le_2 = alleles compatible with Le_2^{dav}; Le_2^{dav} = hybrid lethality factor (causes lethality when present with Le_1 and/or Le_2); v_7 = recessive allele causing abnormal virescence.

‡ Inviable F_1 genotype = $Le_1le_1Le_2Le_2^{dav}$.

§ Desired product, i.e., doubled paternal haploid plant or sector.

deed restricted to intracellular interactions. Progeny from a genetically marked cross *SeSe* Le_1Le_1 Le_2Le_2 Gl_2Gl_2 Gl_3Gl_3 v_7v_7 × *sese* le_1le_1 Le_2^{dav} Le_2^{dav} gl_2gl_2 gl_3gl_3 V_7V_7 were found to include two types: inviable and viable. Most progeny (1017 of 1026), all glanded, died within 2 to 3 d of emergence. The nine remaining seedlings included several types of nonchimeric haploid and chimeric seedlings with one or more sectors of genetically identifiable haploid tissue. Zygotic sectors, which occurred on some chimeric and all trichimeric seedlings, were readily distinguished by their glandedness, and the rapid onset of localized, nonspreading necrosis shortly after emergence. In all instances maternal and paternal haploid sectors remained unblemished by the necrotic reaction, manifesting the intracellular basis of the Le_2^{dav} hybrid lethality reaction. These results indicate that the biogenetic system proposed for HEHP will function as anticipated, and that the HEHP methods proposed are biologically and genetically sound.

SYNTHESIS OF HEHP MATERIALS

Four basic types of HEHP materials are proposed (Table 2). The following terminology applies below: "HEHP materials" collectively refers to HEHP lines and HEHP populations; "HEHP lines" are true-breeding for critical traits; "HEHP populations" are not true breeding in that they segregate for a male sterility allele or nuclear-cytoplasmic male sterility restoration allele.

Basic HEHP Line

Synthesis of HEHP lines is diagrammatically simple, but tedious. Fig. 1 is a diagram of two means of deriving the simplest type of HEHP line, *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$, i.e., from a sexual segregate, or from a chromosomally doubled-haploid segregate. The first cross combines compatibility alleles le_1 and le_2 with the semigamy allele *Se*, so that *Se* can subsequently be combined with Le_2^{dav}. The semigamous line 57–4 is homozygous for Le_1 and Le_2 and otherwise not directly hybridizable with Le_2^{dav} homozygotes. From the second cross, approximately one quarter of the hybrid seedlings will be viable (le_1le_1 $le_2Le_2^{dav}$), of which about one half will be *Sese* heterozygotes. Sexual (or doubled haploid) progeny from self-pollination of the *Se* heterozygotes will include segregates true breeding for HEHP traits. The desired *Se* le_1 Le_2^{dav} homozygotes can be identified by testcrossing with a semigamous virescent line that is Le_2^{dav}-incompatible, and selecting parents that form the largest number of viable haploid seedlings, but no viable 4*x* seedlings.

Table 2. Types of hybrid-eliminating haploid-producing (HEHP) cotton (*Gossypium*) materials.

1. Basic HEHP line:
   SeSe le_1le_1 $Le_2^{dav}Le_2^{dav}$†
2. HEHP lines with one or more genetic markers, e.g.,:
   SeSe le_1le_1 $Le_2^{dav}Le_2^{dav}$ v_7v_7
   or
   SeSe le_1le_1 $Le_2^{dav}Le_2^{dav}$ R_1R_1
3. HEHP populations with marker(s) and segregating (1:1) for male fertility:male sterility, e.g.:
   (ms_4ms_4:Ms_4ms_4) *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$ v_7v_7
   or
   (Ms_2ms_2:ms_2ms_2) *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$ v_7v_7
4. HEHP lines or populations with alternative cytoplasms, and, if necessary, a maintainer line, e.g.:
   [*G. harknessii*] *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$ v_7v_7,
   and maintainer,
   [*G. hirsutum*] *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$ v_7v_7

† Allele symbols: *Se* = mutant allele causing semigametic apomixis; le_1 and le_2 = alleles compatible with Le_2^{dav}; Le_2^{dav} = hybrid lethality factor (causes lethality when present with Le_1 and/or Le_2); v_7 = recessive allele causing abnormal virescence; R_1 = dominant allele causing anthocyanin pigmentation of plant parts; Ms_4 and ms_2 = dominant and recessive alleles, respectively, each causing complete male sterility; (*G. harknessii*) = cytoplasm causing male sterility, unless restored by nuclear allele *Rf*.

HEHP Lines with Markers

Genetic markers facilitate identification of haploids, as well as their parental origin (Turcotte and Feaster, 1967, 1974). Incorporating markers into HEHP lines that allow screening at several stages and among plant parts seems desirable, e.g., among seedlings, plants, branches, and even flowers and seeds. Virescence, glanding, and anthocyanin pigmentation are among the most useful seedling and plant markers in cotton. Glanding, anthocyanin pigmentation (petal spot), petal color, and pollen color are among the most useful floral markers. Lint color and naked seed traits are among the most useful mature fruit and seed markers, and

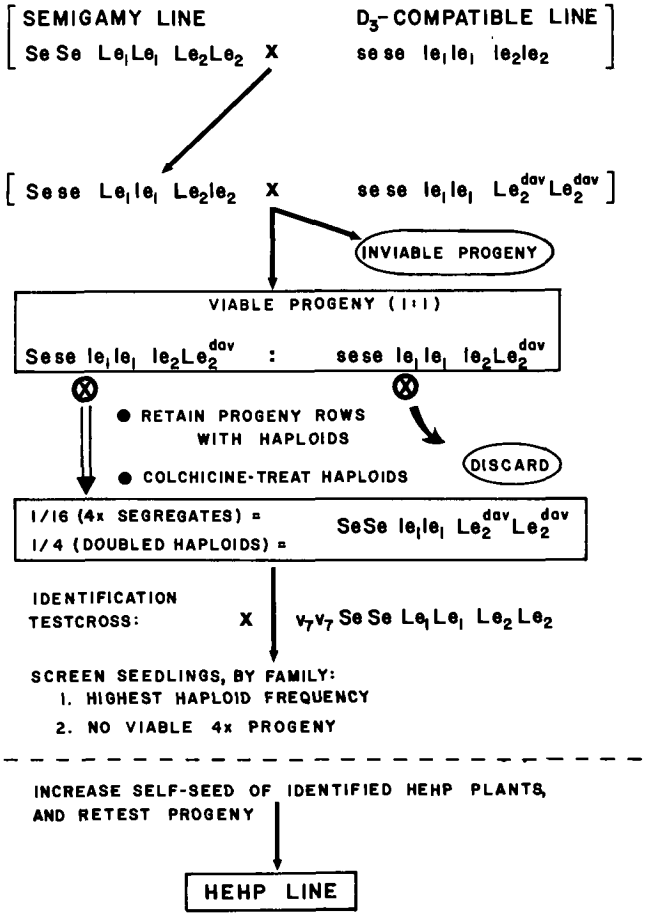

Fig. 1. Proposed derivation of a hybrid-eliminating haploid-producing (HEHP) line of cotton *SeSe* le_1le_1 $Le_2^{dav}Le_2^{dav}$. Allele symbols: *Se* = mutant allele causing semigametic apomixis; *se* = wild-type allele of *Se* locus; le_1 and le_2 = alleles compatible with Le_2^{dav}; Le_2^{dav} = hybrid lethality factor (causes lethality when present with Le_1 and/or Le_2); v_7 = recessive allele causing abnormal virescence.

could be used on a per seed basis, e.g., for sorting after bulk harvesting.

De novo synthesis of HEHP lines with genetic markers differs from synthesis of a simple HEHP line in that larger segregating populations and more progeny tests are required. Alternatively, markers can be more easily backcrossed into pre-existing HEHP lines, and thereby pyramided. In either case, a marker line must first be hybridized with the compatible le_1le_2 stock to be successfully hybridized with the Le_2^{dav} homozygote, because all existing marker lines are almost assuredly Le_1Le_2-homozygous.

HEHP Populations Segregating for Male Sterility

HEHP populations that segregate 1:1 for either dominant or recessive nuclear male sterility can be synthesized. Complete male sterility of cotton is caused by either of two monogenic dominant alleles Ms_4 or Ms_{11}, monogenic recessive ms_2, or either of two digenic recessive allele pairs ms_5 ms_6 or ms_8 ms_9 (Richmond and Kohel, 1961; Endrizzi et al., 1984). Deploying monogenic systems of male sterility will be simpler, so Ms_4 and ms_2 are cited as representative examples hereafter.

Strategies for developing a Ms_4-segregating HEHP population are limited by the fact that Ms_4 heterozygotes are male-sterile and can not be selfed to inbreed. A true-breeding HEHP line with the desired genetic markers should be obtained or synthesized, and then used as the recurrent backcross parent to derive a corresponding Ms_4ms_4 HEHP plant. Testcross progeny tests will be required to identify which segregates of a backcross family are homozygous for *Se*, Le_2^{dav}, and dominant marker alleles. Backcrossing the identified Ms_4ms_4 HEHP plant to the recurrent HEHP line parent will generate a HEHP population segregating 1:1 for male sterility. The population will be maintainable by crossing male-sterile segregates either to male-fertile segregates or to the recurrent HEHP line used previously. Plots isolating the HEHP populations from le_1le_2 pollen sources, and other HEHP materials should suffice for bulk maintenance and increase. Cytoplasmic substitutions for Ms_4-HEHP populations will be impossible by in vivo methods.

Development of HEHP populations segregating for a recessive male sterility allele, e.g., ms_2, will be complicated by the need for progeny testing to track the recessive allele in populations including only fertile genotypes, i.e., Ms_2Ms_2 and Ms_2ms_2. Male fertility of Ms_2ms_2 heterozygotes, however, will permit selfing for inbreeding and self-progeny testing, so prior synthesis of a HEHP line with all desired markers is not prerequisite to development of a ms_2-segregating HEHP population. Evaluation of seedlings from testcrosses will suffice to determine which male-fertile segregates were homozygous for *Se*, Le_2^{dav}, and dominant marker alleles. Evaluation of pollen shedding among self-progeny of the same male-fertile segregates will indicate which segregates were heterozygous for the ms_2 allele, because only their progeny would include male-sterile segregates. At the same time, male-sterile (ms_2ms_2) and male-fertile (Ms_2Ms_2 and Ms_2ms_2) sibs can be crossed to produce the initial HEHP population, which will segregate 1:2 male-sterile:male-fertile. Maintained similarly, the HEHP population will segregate 1:1 in subsequent generations. Bulk maintenance will be simple, because one need only avoid coplanting lines that produce le_1le_2 or $le_1Le_2^{dav}$ pollen.

HEHP Materials with Alternative Cytoplasms

Conversion of most HEHP materials to other cytoplasms will be simple. Semigamous, virescence-mutant lines, *SeSe* v_7v_7, with cytoplasms of various wild tetraploid and diploid *Gossypium* species are available, including cytoplasms of *G. barbadense*, *G. hirsutum*, *G. longicalyx*, *G. tomentosum*, *G. anomalum*, *G. herbaceum*, *G. harknesii*, and *G. arboreum* (Mahill et al., 1983). Analogous lines in development include cytoplasms of *G. capitis-viridis*, *G. davidsonii*, *G. trilobum*, *G. stocksii*, *G. mustelinum*, and *G. darwinii* (Umbeck and Stewart, 1985; and Stewart, personal communication). Viable seedlings from crosses between semigamous lines having wild cytoplasms and HEHP pollen parents will include only haploid seedlings. Paternal sectors of chimeras can be colchicine-doubled to obtain the cytoplasmically converted HEHP analogue. Cytoplasmic conversion of male sterility-segregating HEHP populations will be possible by in vivo methods when the male sterility is determined by a recessive allele(s). In some cytoplasms, HEHP materials will be partially or completely male-sterile, e.g. in the *G. harknessii* cytoplasm; in these instances, the original HEHP material can serve as a maintainer line.

EXTRACTION OF DOUBLED PATERNAL HAPLOIDS

HEHP lines and populations might be deployed in any of a wide variety of specific schemes to obtain doubled haploids of the desired genotype. In all instances, doubled paternal haploids will be sought, so all schemes must involve (i) cross pollination between HEHP plants, as female, and normal pollen parents; and (ii) chromosome doubling (Fig. 2). Although some true F_1 zygotes resulting from the cross pollination will abort during embryogenesis, resulting seed will include large numbers of F_1 embryos, and smaller numbers of maternal haploid, and chimeric maternal/paternal haploid embryos. Given prior bulk treatment of seed with colchicine, viable seedlings will include only undoubled and doubled haploids, including doubled paternal sectors (Table 2). Because most seedlings will be inviable, field planting densities can be extremely high, e.g., 10× to 100× the normal rate, depending on expected lethality from the colchicine treatment and expressivity of semigamy. Our experience to date with comparable crosses indicates that ca. 1000 seeds could be screened per flat of soil mix, from which one could expect to recover ca. 10 viable progeny.

Sizes of seedlings, plants, leaves and flowers, pollen shed, and boll-set at harvest will distinguish doubled from undoubled paternal haploid plants and sectors. Genetic markers will distinguish paternally and maternally derived plants and sectors. Given use of a recessive nuclear male-sterile in the HEHP population, all doubled maternal haploids will be male-ster-

ile. Thus, doubled paternal haploids and sectors will be readily identified and self-pollinated to establish homozygous lines. Sectors of chimeric plants could be tagged, or pruned accordingly.

APPLICABILITY OF THE HEHP PRINCIPLES

Regardless of the procedure used, extraction of doubled paternal haploids via HEHP females should be less cumbersome than via existing *SeSe* v_7v_7 lines. Bulk treatment of seed with dilute colchicine solutions (0.05–0.5%) should be especially advantageous because chromosome number is most easily doubled by this method in cotton (Gwyn and Stelly, unpublished). HEHP materials should prove maximally advantageous when large numbers of doubled haploids are sought. HEHP populations segregating for male sterility will be of greatest advantage when uncontrolled pollinations suffice, and when large numbers of doubled haploids are sought. Combining (i) male sterility, to enforce cross pollination, (ii) HEHP traits, to generate haploids exclusively, and (iii) mass treatment of seed, without prior manual screening, with colchicine, to double chromosome numbers nonlaboriously, should greatly facilitate extraction of large numbers of doubled paternal haploids. The overall system should lend itself especially well to large-scale operations, e.g., for doubled haploid-based recurrent selection schemes.

Genetie advantages of doubled haploid and recurrent selection breeding methods are maximized where large numbers of alleles are segregating, and where dominance epistatic effects otherwise interfere with evaluations. Hence, we envision these large-scale procedures to be especially promising for recurrent selection projects involving interspecific and unadapted germplasm. The HEHP materials may also prove invaluable for large-scale cytoplasmic conversions and

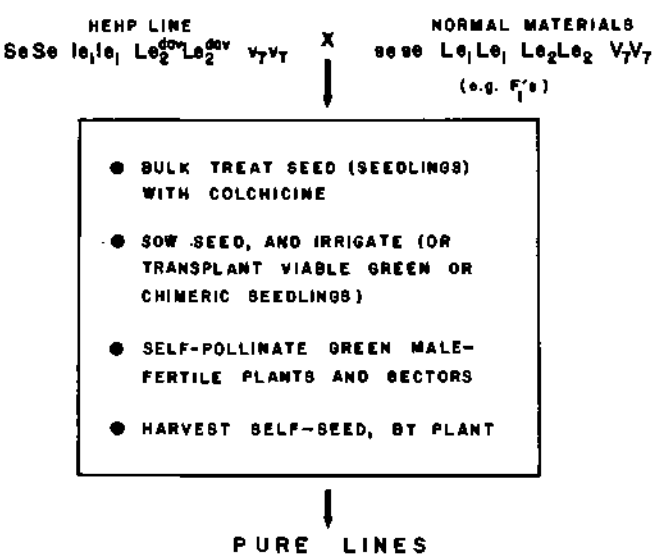

Fig. 2. Proposed method for extracting doubled haploids of cotton using hybrid-eliminating haploid-producing (HEHP) materials, e.g., a v_7-virescence mutant HEHP line. Allele symbols: *Se* = mutant allele causing semigametic apomixis; *se* = wild-type allele of *Se* locus; le_1 and le_2 = alleles compatible with Le_2^{dav}; Le_1^{dav} = hybrid lethality factor (causes lethality when present with Le_1 and/or Le_2); v_7 = recessive allele causing abnormal virescence; V_7 = dominant allele causing normal virescence.

inbreeding of assorted breeding materials, whieh might hasten identification of elite parental combinations for potential hybrid varieties.

Opportunities in *Gossypium* provided by the Le_2^{dav} hybrid lethality system and the ability to extract haploids by apomixis are not unique. Circumstantial evidence suggests that genetic materials and techniques analogous to those described herein can be developed for other crops. Evidence of hybrid lethality and semilethality systems among angiosperms is extensive. Several systems have been deseribed in *Gossypium* (Hutchinson, 1932; Stephens, 1950; Hutchinson and Silow, 1939; Gerstel, 1954; Stephens and Philhips, 1972; Phillips, 1976) of the Malvaceae. Furthermore, they have been detected in several families, including the Gramineae (Wiebe, 1934; Heyne et al., 1943; Caldwell and Compton, 1943; Oka, 1957; Chu and Oka, 1972), Compositeae (Hollingshead, 1930), Leguminoseae (Saunders, 1952; Stringham and Elling, 1966), Scrophulariaceae (Vickery, 1964; Christie and McNair, 1984), and seemingly also the Solanaceae (Sawant, 1956; East, 1935; Rick and Butler, 1956). Other examples presumably exist. Techniques for obtaining haploids abound. Hence, the potential utility of such systems to facilitate doubled-haploid extraction by in vivo or in vitro methods is more or less self-evident. For example, given appropriately broad distribution of interacting alleles or genes, incorporation of a hybrid lethality system into a parthenogenesis-inducing pollinator (Jorgensen, 1928; Hougas et al., 1964; Bingham, 1971) could obviate the need to manually screen for maternal haploid progeny; colchicine treatment could be applied to bulk embryos, seed or seedlings prior to ploidy identification, if doubled haploids are sought. Codeployment of hybrid lethality alleles with an allele(s) for a maternally or megagametophytically determined trait for paternal haploid formation, e.g., *ig* in maize (*Zea mays* L.) (Kermicle, 1969), could give results very similar to the $SeLe_2^{dav}$ combination of cotton. Somewhat different schemes of deployment of hybrid lethality alleles can be envisioned in conjunction with maternally determined formation of maternal haploids, as for *hap* in barley (*Hordeum vulgare* L.) (Hagberg and Hagberg, 1980). Interspecific crosses deploying complementary genes for hybrid necrosis can be used to facilitate screening for haploids of at least some strains of wheat (*Triticum aestivum* L.), because the haploids are green and readily distinguished from necrotic hybrids (Kihara and Tsunewaki, 1962). Similarly, Burk et al. (1979) have shown that the interspecific cross *N. tabacum* × *N. africana* facilitates recovery of maternal haploids among the viable progeny, because most F_1 progeny die before producing a true leaf, presumably due to a hybrid semilethality system. We infer that the *Gossypium* HEHP system will certainly have important ramifications in future cotton genetics researeh and improvement, and that the methods and approaches described herein should be extrapolatable and adaptable to other crops.

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