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Interspecific hybrid between *Allium cepa* and *Allium sativum*

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Summary. Interspecific hybrids between *Allium cepa* and *Allium sativum* were obtained using the fertile clone *A. sativum* as the male parent. The nascent embryos which formed shortly in interspecific hybridization between *A. cepa* and *A. sativum* were rescued by ovule culture at an early stage. The zygotes or proembryos developed in Murashige and Skoog medium containing $5.7 \times 10^{-8} M$ indole-3-butyric acid (IBA). Once developed, the embryos were taken out of the ovule and cultured on embryo culture medium where they regenerated into whole plants. The hybridity of the plants obtained was examined by morphological observation, chromosome analysis, and ribosomal RNA gene analysis. The analyses proved that the plants were mature sexual hybrids between *A. cepa* and *A. sativum*. Each hybrid plant had keeled but fistulose leaves and formed a bulb resembling that of *A. cepa*. The hybrids produced not only *S*-propenyl-L-cysteine sulfoxide, which is the major flavor precursor in *A. cepa*, but also *S*-allyl-L-cysteine sulfoxide (alliin), which is characteristic of *A. sativum*.

Key words: Interspecific hybrid – Onion – Garlic – Nascent embryo rescue – Flavor precursors

Introduction

Onion (*Allium cepa*) and garlic (*Allium sativum*) are of economical importance in various parts of the world, and for many years plant breeders have wished to transfer desirable characteristics into this species. *A. sativum* had been observed to be sterile and never hybridized sexually, although fertile clones have recently been found in Central Asia (Etoh et al. 1988; Kotlinska et al. 1990). Many attempts have been made to use *A. cepa* for the production of interspecific hybrids in the subgenus *Rhizirideum* Wendelbo of *Allium* L., i.e., *A. cepa* × *A. fistulosum*, *A. cepa* × wild species (Saini and Davis 1967; McCollum 1971; Dolezel et al. 1980). The technique of in ovulo embryo rescue has been employed for hybridization because it helps embryo development by ovule culture. However, even embryo rescue has never been succeeded in obtaining hybridization between species of a different subgenus species (Gonzalez and Ford-Lloyd 1987). *A. cepa* and *A. sativum* have not been hybridized sexually because *A. cepa* is taxonomically far distant from *A. sativum*, being classified as the subgenus *Allium* (Hanelt 1990). Restriction enzyme analysis of the chloroplast genome has also shown that *A. cepa* is phylogenetically distant from *A. sativum* among *Allium* species (Havey 1991). All this suggests that sexual gene transfer between *A. cepa* and *A. sativum* will be difficult because species possessing differing chloroplast genomes may have already undergone many structural changes between the chromosomes. There may be interspecific hybrid inviability between these species in the early stages of embryo development. Nevertheless, it is expected that the biogenesis of the hybrid embryos does occur and is able to be restored to the natural state of development because the most critical time for successful hybridization is the stage at which embryo rescue is done. It was at this stage that we observed carefully the formation of the nascent hybrid embryo after pollination by the ovule clearing method

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(Herr 1982). This paper describes successful hybridization between *A. cepa* and *A. sativum* using this new technique, which is based on rescuing the nascent hybrid embryo.

Allium plants have *S*-alk(en)yl-L-cysteine sulfoxides as specific flavor precursors. Species-specific differences in *Allium* occur in the quantity and quality of these flavor precursors (Lancaster and Boland 1990). A study of the chemistry of these crops reveals that these flavors play a defensive role against animal pests and microorganisms (Block 1985; Carson 1987). *S*-propenyl-L-cysteine sulfoxide in *A. cepa* is the precursor of the major onion flavor and lachrimatory factor that is irritating and repugnant to certain animals (Block et al. 1980). Alliin in *A. sativum* is the precursor of allicin, which is not only the garlic flavor, but also an antibacterial and antifungal factor (Cavallito and Bailey 1944). No single *Allium* species has been found that produces both sulfur amino acids as the major flavor precursors. The aim of the study reported here was to assess the interspecific hybrids of *A. cepa* and *A. sativum* with respect to the production of both of the flavor and defense precursors.

Materials and methods

Plant materials

A. cepa cv 'Sapporoki' and clone no. 130 of fertile. *A. sativum* (Etoh 1983) were used as parents. The fertile garlic was propagated asexually by transplanting cloves in Japan, because its seed set (18 seeds per plant) and rate of seed germination (21.0%) were both very low (Etoh et al. 1988). Nevertheless, the plants did flower and underwent subsequent seed development after pollination.

Embryo rescue

Female plants were hand emasculated and isolated in the greenhouse. Pollination was initiated as soon as the stigmatic knob appeared. After 2–3 days, ovules were aseptically excised from flowers and put onto an ovule culture medium (Murashige and Skoog or Gamborg's B5 basal medium containing hormone, 6% or 8% sucrose, 200 mg/l myo-inositol, 500 mg/l casamino acid, and 0.2% gellum gum). The ovules were then kept at 25 °C under 2,000 lux at 12 h day/12 h night. The embryos were taken out of the ovule and further cultured on Murashige and Skoog or Gamborg's B5 basal medium containing 5.7×10^{-8} *M* indole-3-butyric acid (IBA), 4.6×10^{-7} *M* kinetin, 2% sucrose, 200 mg/l myo-inositol, 500 mg/l casamino acid, and 0.2% gellum gum. The embryos then developed to produce shoots and roots, and grew into plantlets. The plantlets were transplanted into vermiculite and kept for 3 weeks at 22 °C for acclimation and then potted into soil.

Microscopic observation

Pollen viability was determined by the fluorescein diacetate methods (Heslop-Harrison and Heslop-Harrison 1970). Pollen grains were stained with 10^{-6} *M* fluorescein diacetate in 0.5 *M* sucrose, and the fluorochromatic grains were counted by fluorescence microscopy (Zeiss photomicroscope).

Growing pollen tubes were observed by anilin blue staining according to the method of Kho and Baer (1968). After 24 h of cross-pollination, the styles were excised and treated with 1 *N* NaOH on a slide glass for 1 h at room temperature, washed with water, and stained with 0.05% aniline blue in 0.1 *M* K_3PO_4. The fluorescent pollen tubes were observed by fluorescence microscopy.

Nascent embryos were observed by the clearing method by Herr (1982). Ovaries were fixed in formalin/propionic acid/50% ethanol (5:5:90, v/v) for 24 h and stored in 70% ethanol. The ovules were then excised from the ovaries, dehydrated in absolute ethanol, and transferred to clearing fluid (lactic acid/chloral hydrate/phenol/clove oil/xylene/benzyl benzoate, 2:2:2:2:1:1, w/w). After 6 h of treatment, the cleared ovules were mounted under the slide using a supporting cover glass, and the embryos were observed by light (Nomarski) microscopy.

For the determination of chromosome number, root tips were pretreated with a 0.1% colchicine solution for 6 h. They were then fixed with ethanol/acetic acid (3:1, v/v) for 30 min, hydrolyzed in 1 *N* HCl for 2 min at 60 °C, and stained with 1% acetocarmine. The chromosomes were observed by light microscopy after the root tissue had been squashed.

Ribosomal RNA gene analysis

Total DNA was extracted from the leaf tissue (100 mg) according to the method of Honda and Hirai (1990). DNA (200 ng) was digested with 5 u *Xba*I for 3 h. The digests were applied to 0.7% agarose gel electrophoresis. After electrophoresis, the gel was treated with alkali and blotted onto a nylon membrane by the Southern method. The membrane was hybridized overnight at 55 °C in a hybridization buffer containing biotinylated rDNA (in plasmid pRR 217) as a probe (Uchimiya et al. 1983). The probe-target DNA hybrid was visualized using a detection kit from BRL.

Amino acid analysis

Twenty milligrams tissue was homogenized in 0.2 ml methanol/chloroform/water, 12:5:3 (v/v) to denature alliinase activity. The mixture was extracted with the organic solvent and 80% ethanol, and the extracts were then combined and phase separated by addition of chloroform and water. The aqueous phase was analyzed by an amino acid analyzer (Waters HPLC).

Results

Formation of hybrid embryos

Reciprocal hybridizations between *A. cepa* and *A. sativum* were carried out. Pollen viability was 90% in *A. cepa* and 58% in fertile *A. sativum*. Each pollen grain could germinate on either of the species stigmas and pollen tubes elongated through a style to the ovules in 12–24 h.

By using the ovule-clearing method we were able to observe a zygote or a proembryo in the fertilized ovule of *A. cepa* crossed with *A. sativum* until 4 days after cross-pollination. Figure 1 shows a nascent hybrid embryo and synergid in the embryo sac of onion; the development of the embryo was much slower than that in self-pollinated onion. The embryo and synergid then appeared to degenerate and

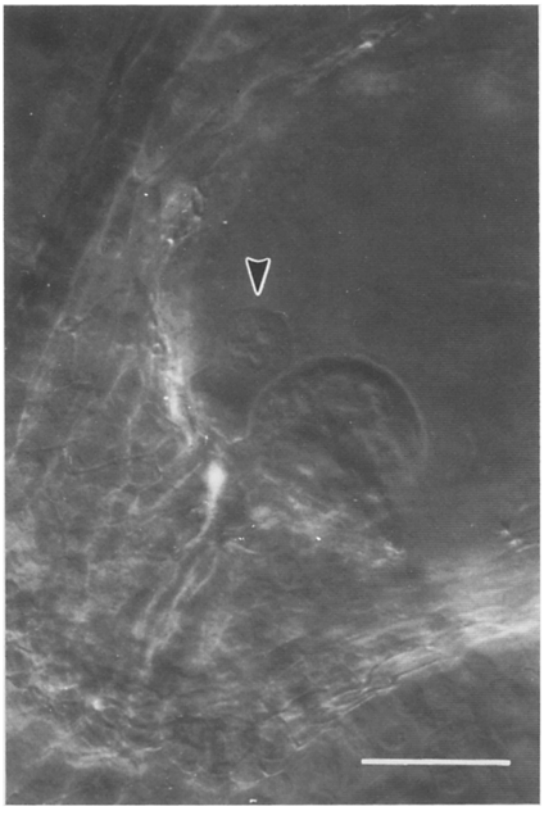

Fig. 1. Visualization of nascent hybrid embryo in the *A. cepa* embryo sac under light microscopy with Nomarski optics. The embryo (shown by *arrow*) and synergid (*right*) were observed. *Bar*: 50 μm

completely disappeared within 7 days (Table 1). In the case of the cross combination of *A. sativum* (♀) and *A. cepa* (♂), the embryo sac appeared to contain very little after cross-pollination. Since an egg cell merely occurred in the ovule of *A. sativum*, less embryos developed in the ovule crossed with *A. cepa*.

To rescue zygotes or hybrid proembryos, ovules were excised from each flower after 2–3 days of cross-pollination and cultured on ovule culture media. Four kinds of media were tried for ovule culture (Table 2). After 3–4 weeks 45 embryos were formed from 10,455 ovules of *A. cepa*, although no embryo was formed in the ovule of *A. sativum*. The embryos were about 2 mm long and cylindrical in shape. This high frequency of embryo formation was obtained in Gamborg's B5 medium and Murashige and Skoog medium containing $5.7 \times 10^{-8} M$ IBA. However, Gamborg's B5 medium induced polyembryonies, which represented 2–6 embryos formed in one ovule, and these could not develop shoots and roots.

The embryos were taken out from the ovule and further cultured on embryo culture medium as described above except for the addition of $4.6 \times 10^{-7} M$ kinetin and a decrease in the concentration of sucrose to 2%. The embryos which formed in ovule culture medium containing $5.7 \times 10^{-8} M$ IBA developed to the whole plants. Therefore, the frequency of plants obtained per ovule was higher on the Murashige and Skoog medium containing $5.7 \times 10^{-8} M$ IBA.

Table 1. Formation and degeneration of nascent embryos in the crosses between *A. cepa* and *A. sativum*

Cross combination ♀ ♂	Days after pollination	Number of ovules	Numbers of synergids	Numbers of embryos formed	
				n	%
A. cepa × *A. cepa*	4	10	10	5	50.0
	7	10	–[a]	5[a]	50.0
A. cepa × *A. sativum*	4	60	50	13	21.7
	7	100	0	0	0

[a] Developing embryos were too large to observe 7 days after pollination by the ovule-clearing methods, although they could be recognized as immature seeds

Table 2. Effect of culture medium on the development and regeneration of hybrid embryos of onion and garlic

Culture medium[a]		Number of cultured ovules	Embryos formed		Plants regenerated	
Basal	IBA($\times 10^{-8} M$)		Number	%	Number	%
MS	0	2,610	8	0.3	0	0
MS	5.7	2,667	13	0.5	3	23
B5	0	2,616	20	0.8	1	5
B5	5.7	2,562	4	0.2	1	25

[a] MS, Murashige and Skoog's basal medium containing 8% sucrose, 200 mg/l myo-inositol, 500 mg/l casamino acid, and 0.2% gellum gum; B5, Gamborg's B5 basal medium containing 6% sucrose, 200 mg/l myo-inositol, 500 mg/l casamino acid, and 0.2% Gellum gum; IBA, indole-3-butyric aicd

Hybridity

The somatic chromosome number in the hybrids was 16 and two satellited chromosomes were visible (Fig. 2). This is in full agreement with total chromosome numbers of *A. cepa* ($n = 8$) plus *A. sativum* ($n = 8$) by sexual hybridization. The karyotype of the fertile clone no. 130 consists of 10 long median chromosomes, 1 pair of short submedian chromosomes, and 2 pairs of satellited chromosomes (Etoh 1983), whereas *A. cepa* cv 'Sapporoki' does not contain any satellited chromosomes. Therefore, the two satellited chromosomes are probably derived from *A. sativum*. The results also suggests that chromosome eliminated did not occur in these hybrid plants.

*Xba*I digests of total DNA from *A. cepa* and *A. sativum* generated species-specific bands, thus producing appropriate profiles for the identification of the hybrid. *A. cepa* showed a 9.0-kb rDNA fragment, while *A. sativum* contained a 11.6-kb fragment. As shown in Fig. 3, the hybrid plant possessed both rDNA characteristics of their parents. This is genotypic evidence that a mature sexual hybrid plant has been obtained from *A. cepa* and *A. sativum*.

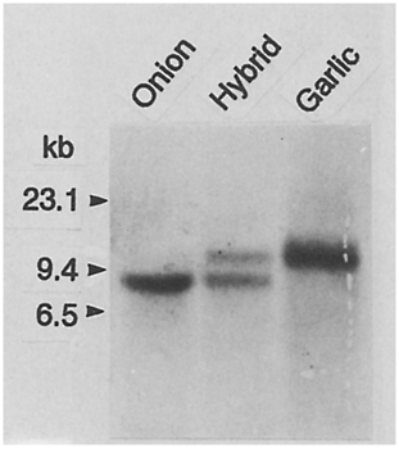

Fig. 3. Hybridization of biotinylated rDNA to *Xba*I digests of total DNA from leaf tissues of onion, hybrid, and garlic. *Numbers* represent molecular weight standards in kilobases

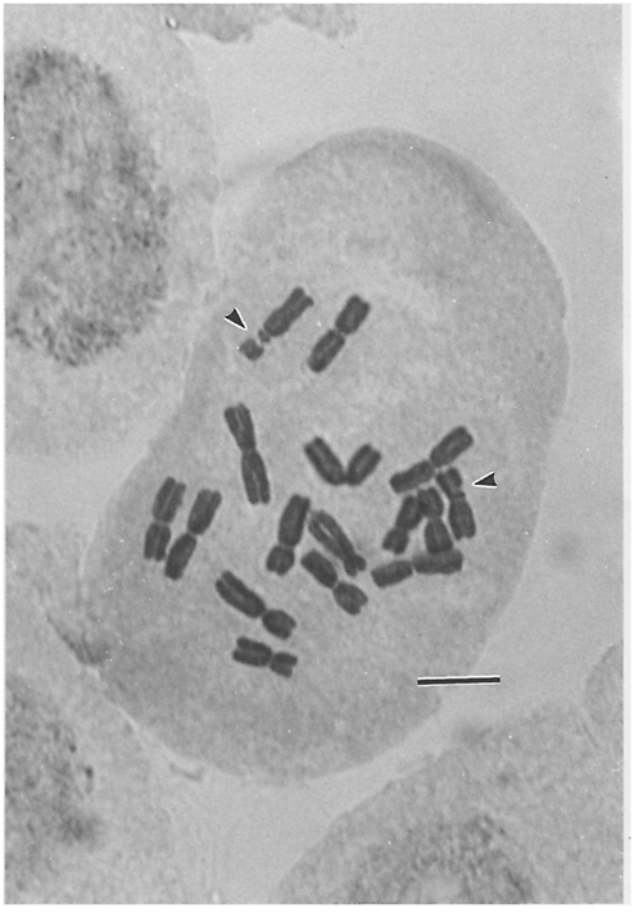

Fig. 2. Chromosomes of the interspecific hybrid in mitotic metaphase ($2n = 2x = 16$). Two satellited chromosomes (*arrows*) are visible. *Bar*: 10 μm

Morphology

The plants showed characteristics intermediate between *A. cepa* and *A. sativum*. Although the leaf of *A. cepa* is cylindrical and fistulose and that of *A. sativum* has a keeled leaf blade, the shape of the hybrid leaves was similar to the *A. sativum* leaf, but contained a schizogenous intercellular space, thus forming a keeled fistulose leaf (Fig. 4). The leaf sheaths showed a progressive increase in height from the first foliage leaf, characteristic of *A. sativum* leaf sheaths, whereas the leaf bases of *A. cepa* are all similar in length (Fig. 5). The shoot base of the plants was purple, characteristic of the *A. sativum* clone, whereas that of female plant is yellow. The bulbs of the plants resembled *A. cepa*, apparently consisting of scale leaves covered with dry leaf skins (Fig. 6A, B, D, and E), while the bulb of *A. sativum* is a clove composed of sprout and swollen storage leaves covered with leaf skin (DeMason 1990). Figure 6C and F shows a cluster of cloves: each bulb in *A. sativum* is much smaller than any one bulb in both *A. cepa* and the hybrid. Every year, the mother bulb of the hybrid plants can proliferate into 2–10 daughter bulbs.

Production of secondary metabolites

Amino acid analysis showed that the hybrid bulb as well as the leaf (data not shown) contained both *S*-propenyl-L-cysteine sulfoxide and alliin (*S*-allyl-L-cysteine sulfoxide) in addition to *S*-methyl-L-cysteine sulfoxide (Table 3). These flavor precursors always

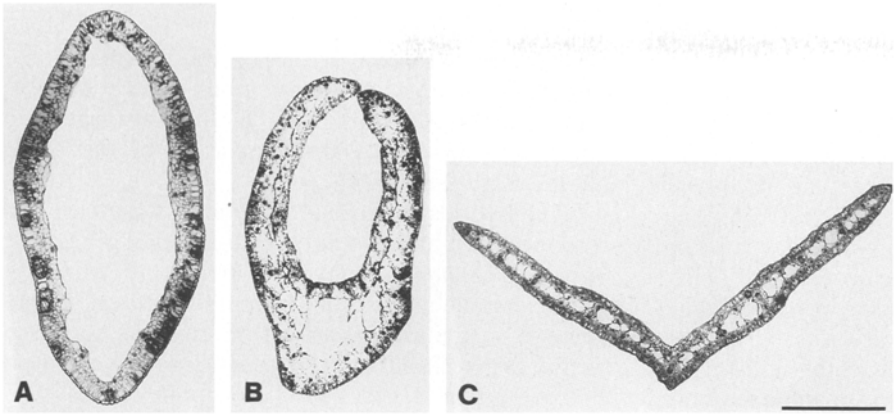

Fig. 4A–C. Comparison of leaf morphology of parents and hybrid. Transverse section of leaf is shown for *A. cepa* (**A**), hybrid (**B**) and *A. sativum* (**C**). *Bar*: 5 mm

Fig. 5. Interspecific hybrid of *A. cepa* and *A. sativum*. *A. cepa* (*left*), hybrid plant (*center*) and *A. sativum* (*right*) are shown

occurred in the leaf as well as in the bulb. In the hybrids, the production of total *S*-alk(en)yl-L-cysteine sulfoxide increased concomitant with a substantial net syntheses of *S*-propenyl-L-cysteine sulfoxide and alliin. The amino acids always occurred in each bulb at the same levels, but there were some changes in levels during growth.

Table 3. Amounts of flavor precursors in the bulbs of *A. cepa*, *A. sativum*, and their hybrids

Plant materials	Amounts of flavor precursor (mg/g fresh wt)[a]		
	MCSO	PeCSO	ACSO
A. cepa	0.34	1.86	ND
A. sativum	0.16	ND	1.00
Hybrid	0.27	0.73	1.30

[a] MCSO, *S*-methyl-L-cysteine sulfoxide; PeCSO, *S*-propenyl-L-cysteine sulfoxide; ACSO, *S*-allyl-L-cysteine sulfoxide (alliin); ND, not detected

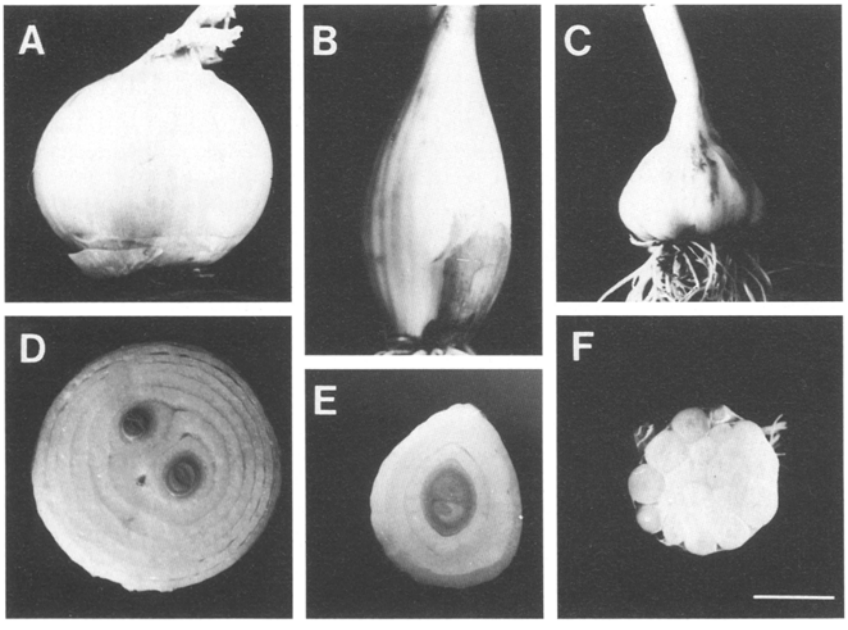

Fig. 6A–F. Comparison of bulb morphology of parents and hybrid. Whole bulb (**A–C**) and transverse section of bulb (**D–F**) are shown for *A. cepa* (**A** and **D**), hybrid (**B** and **E**) and *A. sativum* (**C** and **F**). *Bar*: 3 cm

Discussion

Using a nascent embryo rescue technique we have successfully obtained an interspecific hybrid between the distantly related species *A. cepa* and *A. sativum* that could not be obtained by conventional techniques (Gonzalez and Ford-Lloyd 1987). The development of a hybrid embryo could be confirmed (Fig. 1 and Table 1), but as the embryo disappeared 4 days after cross-pollination. Our success was most likely due to the earlier rescue of the embryo from the mother plant. This suggests that it is possible to obtain other hybrids of distantly related species by earlier rescue. We were not able to obtain a hybrid embryo with *A. sativum* as the female parent in the cross with *A. cepa*, this is probably due to the presence of only a few egg cells in the ovule of *A. sativum*.

Because nascent hybrid embryos probably require sufficient nutrition to develop in an ovule culture, several ovule culture media were examined. The production of hybrid plants by ovule culture was much better in Murashige and Skoog medium containing 5.7×10^{-8} *M* IBA (Table 2). The addition of IBA to the ovule culture medium increased the frequency of plant regeneration. It appears that IBA also overcomes interspecific hybrid inviability during ovule culture (Zhou et al. 1991).

The hybridity of the plants was confirmed by Southern blotting analysis of the gene coding for ribosomal RNA (rDNA) (Fig. 3). An analysis for rDNA has become available to examine the hybridity between *Allium* species. It appears that rDNA consists of highly conserved sequences even in *Allium* species-specific intergenic spacers.

The hybrids produced both of the major flavor precursors, *S*-propenyl-L-cysteine sulfoxide and alliin (Table 3). The formation of specific secondary metabolites in the hybrid not only helps identify the hybrid, such as the formation of volatile substances (Ninnemann and Juttner 1981), but may also be useful in assessing their degree of hybridity, such as the level of steroidal glycoalkaloid (Roddick and Melchers 1985). The sliced hybrid bulb had an odor that was intermediate between that of *A. cepa* and *A. sativum* because alliinase was active in the tissues. However, the properties of alliinase in *A. cepa* are different from those in *A. sativum*, i.e., pI, optimum pH, substrate specificity, molecular weight (Nock and Mazelis 1987). Further studies on the characterization of alliinase activity and the levels of flavors formed in hybrid plants are in progress.

The occurrence of alliin in bulbs would also improve the chemical defense of *A. cepa* by allicin against fungal attack (Table 3). The texture of the new vegetables (hybrid bulb) resembles that of a bulbing onion (Fig. 6), and as a spice, alliin would be expected to enhance the effect of monosodium glutamate in improving the taste quality of food (Ueda et al. 1990) and in aiding the formation of the antithrombotic compound ajoene by decomposition of the allicin (Block et al. 1984).

The hybrids between *A. cepa* and *A. sativum* may also serve as a bridge plant for transferring genes from subgenus *Allium* to subgenus *Rizirideum* Wendelbo and overcome crossability barriers in *Allium* species, because subgenus *Allium* will become the source of useful germ plasm for the genetic improvement of bulbing onion *A. cepa*. However, pollen viability of the hybrids was about 2%, and no seed was set by self-pollination. Although wide-cross hybrids usually show a high degree of sterility, fertility is normal restored by chromosome doubling of the sterile hybrid and by amphiploid production.

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References

Block E (1985) The chemistry of garlic and onions. Sci Am 106:94–99

Block E, Revelle LK, Bazzi AA (1980) The lachrymatory factor of the onion: an NMR study. Tetrahedron Lett 21:1277–1280

Block E, Ahmad S, Jain MK, Crecely RW, Apitz-Castro R, Cruz MR (1984) (E,Z)-Ajoene: a potent antithrombotic agent from garlic. J Am Chem Soc 106:8295–8296

Carson JF (1987) Chemistry and biological properties of onions and garlic. Food Rev Int 3:71–103

Cavallito CJ, Bailey JH (1944) Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J Am Chem Soc 66:1950–1951

DeMason DA (1990) Morphology and anatomy of *Allium*. In: Rabinowitch HD, Brewster JL (eds) Onions and allied crops, vol I. CRC Press, Florida, pp 27–51

Dolezel J, Novak FJ, Luzny J (1980) Embryo development and in vitro culture of *Allium cepa* and its interspecific hybrids. Z Pflanzenzuecht 85:177–184

Etoh T (1983) Germination of seeds obtained from a clone of garlic, *Allium sativum* L. Proc Jpn Acad 59:83–87

Etoh T, Noma Y, Nishitarumizu Y, Wakamoto T (1988) Seed productivity and germinability of various garlic clones collected in Soviet Central Asia. Memo Fac Agric Kagoshima Univ 24:129–139

Gonzalez LG, Ford-Lloyd BV (1987) Facilitation of wide-crossing through embryo rescue and pollen storage in interspecific hybridization of cultivated *Allium* species. Plant Breed 98:318–322

Hanelt P (1990) Taxonomy, evolution, and history. In: Rabinowitch HD, Brewster JL (eds) Onions and allied crops, vol I. CRC Press, Florida, pp 1–26

Havey MJ (1991) Phylogenetic relationships among cultivated

Allium species from restriction enzyme analysis of the chloroplast genome. Theor Appl Genet 81:752–757

Herr JM Jr (1982) An analysis of methods for permanently mounting ovules cleared in four-and-a-half type clearing fluids. Stain Technol 57:161–169

Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technol 45:115–120

Honda H, Hirai A (1990) A simple and efficient method for identification of hybrids using nonradioactive rDNA as probe. Jpn J Breed 40:339–348

Kho YO, Baer J (1968) Observing pollen tubes by means of fluorescence. Euphytica 17:298–302

Kotlinska T, Havranek P, Navratill M, Gerasimova L, Pimakhov A, Neikov S (1990) Collecting onion, garlic and wild species of *Allium* in central Asia, USSR. FAO/IBPGR Plant Genet Resources Newsl 83/84:31–32

Lancaster JE, Boland MJ (1990) Flavor biochemistry. In: Brewster JL, Rabinowitch HD (eds) Onions and allied crops, vol III. CRC Press, Florida, pp 33–72

McCollum GD (1971) Sterility of some interspecific *Allium* hybrids. J Am Soc Hortic Sci 96:359–362

Ninnemann H, Juttner F (1981) Volatile substances from tissue cultures of potato, tomato and their somatic fusion products-comparison of gas chromatographic patterns for identification of hybrids. Z Pflanzenphysiol 103:95–107

Nock LP, Mazelis M (1987) The C-S lyases of higher plants. Direct comparison of the physical properties of homogeneous alliin lyase of garlic (*Allium sativum*) and onion (*Allium cepa*). Plant Physiol 85:1079–1083

Roddick JG, Melchers G (1985) Steroidal glycoalkaloid content of potato, tomato and their somatic hybrids. Theor Appl Genet 70:655–660

Saini SS, Davis GN (1967) Compatibility in some *Allium* species. Proc Am Soc Hortic Sci 91:401–409

Uchimiya H, Ohgawara T, Kato H, Akiyama T, Harada H, Sugiura M (1983) Detection of two different nuclear genomes in parasexual hybrids by ribosomal RNA gene analysis. Theor Appl Genet 64:117–118

Ueda Y, Sakaguchi M, Hirayama K, Miyajima R, Kimizuka A (1990) Characteristic flavor constituents in water extract of garlic. Agric Biol Chem 54: 163–169

Zhou WM, Yoshida K, Shintaku Y, Takeda G (1991) The use of IAA to overcome interspecific hybrid inviability in reciprocal crosses between *Nicotiana tabacum* L. and *N. repanda* wild. Theor Appl Genet 82:657–661